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GASCHROMATOGRAPHISCHE RETENTIONSDATEN UND STRUKTUR CHEMISCHER VERBINDUNGEN

II. METHYLVERZWEIGUNGEN UND DOPPELBINDUNGEN IN OFFENKETTIGEN KOHLENWASSERSTOFFEN

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EINLEITUNG

Das Ziel der vorliegenden Arbeit ist es, im Anschluss an Teil I[1] über α-verzweigte Carbonsäureester die Retentionsindices und die ΔI-Werte einiger wichtiger Substanzklassen zu messen und aus dem Datenmaterial strukturelle Inkremente abzuleiten, die die Voraussage unbekannter Indexwerte aus der Struktur erlauben und somit die Zuordnung von Chromatogrammen ohne Vergleichssubstanzen ermöglichen.

Wegen der komplexen Natur der intermolekularen Wechselwirkungskräfte ist besonders bei den Kohlenwasserstoffen damit zu rechnen, dass strukturelle Einflüsse auf die Dispersionswechselwirkung, die fast ausschliesslich das Retentionsverhalten der Kohlenwasserstoffe in apolaren Phasen bestimmt, sich nicht voneinander unabhängig auswirken und somit inkrementell erfassen lassen. Die zwischen den Molekülen wirksamen Kräfte vom van der Waals-Typ sind nämlich in starkem Masse von der Geometrie und von der durch diese bestimmten Symmetrie der Ladungsverteilung des Gesamtmoleküls abhängig. Darüber hinaus zeigen die ungesättigten Kohlenwasserstoffe als Folge der Polarisation der π-Elektronen in polaren stationären Phasen erhöhte Indexwerte und somit ΔI-Werte, deren Bedeutung für die strukturelle Zuordnung allerdings nicht sehr gross ist. Es lassen sich aber auch für die Indexwerte und nicht nur für die ΔI-Werte, wie KOVATS gezeigt hat[2,3], in vielen Fällen Inkremente ableiten, die zur Voraussage unbekannter Indexwerte geeignet sein können. In der vorliegenden Arbeit wurde ein grösseres Datenmaterial zusammengetragen und nach einem früher beschriebenen Verfahren, das zweidimensionaler Natur ist, zur Ableitung solcher Inkremente diskutiert, vgl. SCHOMBURG[4]. Man vergleicht zunächst die Indexwerte zweier strukturell verwandter Verbindungen in einer stationären Phase, die im vorliegenden Fall der Kohlenwasserstoffe vorzugsweise apolar ist, und setzt die beobachtete Indexdifferenz in Relation zu der strukturellen Variation zwischen den beiden Verbindungen. Die Gültigkeit des erhaltenen Inkrements wird dann in verschiedenen C-Zahlbereichen und an Molekülen, die weitere funktionelle Gruppen oder charakteristische Strukturmerkmale enthalten, geprüft. Besonders, wenn die untersuchten Verbindungen funktionelle Gruppen enthalten, die Bindungsmomente oder hohe Polarisierbarkeiten aufweisen, wird das von KOVATS schon ausführlich beschriebene

Verfahren der Interpretation der ΔI-Werte nutzbringend angewendet. Hierbei werden die Retentionsdaten einer Verbindung in zwei stationären Phasen verschiedener Polarität bestimmt, von denen eine apolar ist und somit keine Dipol–Dipol-Wechselwirkung mit den gelösten Verbindungen liefert.

Das vorstehend beschriebene Verfahren sei am Beispiel der isomeren Phenylalkane erläutert:

Die Indexwerte hängen ab:

1. von der Zahl der Kohlenstoffatome;
2. von der Art der funktionellen Gruppe;
3. von der Position der funktionellen Gruppe im Molekül;
4. von der Geometrie des Moleküls.

Enthält das Molekül z.B. eine Paraffinkette, so wird der Einfluss der Kohlenstoffzahl auf den Retentionsindex durch Subtraktion des Retentionsindex des entsprechenden n-Paraffins eliminiert:

$$I^A \text{ (Substanz)} - I^A \text{ (n-Paraffin)} = H^A \quad (A\text{: apolare stationäre Phase})$$

H^A wird der Homomorphiefaktor genannt. H^A-Faktoren anderer Art werden erhalten, wenn nicht der Index des n-Paraffins subtrahiert wird, sondern derjenige eines anderen Kohlenstoffgerüstes, z.B. bei verzweigten oder cyclischen Olefinen die entsprechenden gesättigten, verzweigten oder cyclischen Kohlenwasserstoffe. Auf diese Weise kann in erster Näherung der Einfluss eines strukturellen Merkmals auf den Retentionsindex einer isolierten Diskussion unterzogen werden. In zweiter Näherung ist natürlich mit einer Wechselwirkung zwischen den verschiedenen funktionellen Gruppen zu rechnen. Die H^A- und auch die H^P-Werte (P: polare stationäre Phase) sind also in 1. Näherung charakteristisch für eine bestimmte funktionelle Gruppe bzw. strukturelle Eigenheit des Moleküls. Bei den Phenylalkanen findet man z.B. eine starke Abhängigkeit der H^A-, aber auch der H^P-Werte von der Stellung der Phenylgruppe in der Paraffinkette. Nur unter Berücksichtigung der sterischen Verhältnisse in der Umgebung der funktionellen Gruppe kann für diese u.U. ein Indexinkrement ermittelt und benutzt werden. Aus diesem Grunde wird die strukturelle Variation innerhalb des Datenmaterials so durchgeführt, dass bei konstanter C-Zahl die Position der funktionellen Gruppe geändert und anschliessend auf Einflüsse der Kettenlänge auf die erhaltenen Inkremente untersucht wird. Ein ähnliches Verfahren ist auch auf die ΔI-Werte anwendbar, um den Einfluss der Position der funktionellen Gruppe und der Kettenlänge auf die Dipol–Dipol-Wechselwirkung zu untersuchen.

Beispiel

Einfluss der Stellung der funktionellen Gruppe auf H- und ΔI-Werte

	H^{Ap}	ΔI(Ap–OS 138)
1-Phenyldekan	694	158
2-Phenyldekan	616	149
3-Phenyldekan	573	137
4-Phenyldekan	550	134
5-Phenyldekan	537	133

Die H^{Ap}-Werte spiegeln die Veränderung der Symmetrie der Ladungsverteilung für die Dispersionswechselwirkung, die ΔI-Werte den Einfluss der Abschirmung durch die Alkylgruppen auf die Dipol–Dipol-Wechselwirkung wider.

Einfluss der Kettenlänge

	H^{Ap}	$\Delta I(Ap\text{–}OS\ 138)$
2-Phenylalkan C_7	620	149
2-Phenylalkan C_{10}	616	149
2-Phenylalkan C_{11}	615	146
2-Phenylalkan C_{12}	613	147
2-Phenylalkan C_{13}	611	147

METHYLVERZWEIGTE ALKANE

Das beschriebene Verfahren der Analyse von Indexwerten wurde zuerst auf einige Typen von verzweigten Paraffinen, überwiegend jedoch methylverzweigten, angewandt. Hierzu wurden sowohl die von MATSUKUMA[5] gemessenen als auch eine grössere Zahl eigener Werte benutzt.

Die Monomethylalkane zeigen die auffallende Eigentümlichkeit, dass ihre Indexwerte in apolaren Phasen in der Reihe

1, 3, 2, 4, 5- *n*-Methylalkan

abnehmen in völliger Äquivalenz zu den Dampfdrucken dieser Verbindungen. Diese Anomalie, die bei anderen Substituenten wie Äthyl, Cyclohexyl, Phenyl, Carboxymethyl, Acetoxyl und Trimethylsilyl an Paraffinketten vom Autor nicht beobachtet wurde — die Reihenfolge der Stellungsisomeren ist immer

1, 2, 3, 4, 5-Substituent-Alkan —

wird Gegenstand einer späteren Arbeit sein, vgl. hierzu SCHOMBURG[6]. Die Umkehrung zwischen dem 3- und dem 2-Methylalkan ist eine Folge der höheren Symmetrie der Ladungsverteilung des 2-Methylalkans gegenüber dem 3-Methylalkan. Bei dem ersteren stehen nämlich zwei gleiche (Methyl)-Gruppen an einem Kohlenstoffatom.

TABELLE I

	2-Methylalkane		*3-Methylalkane*		*4-Methylalkane*		*5-Methylalkane*	
	$HS_{80°}$	$HS_{70°}$*	$HS_{80°}$	$HS_{70°}$*	$HS_{80°}$	$HS_{70°}$*	$HS_{80°}$	$HS_{70°}$*
C_5	—29	—26						
C_6	—30	—30	—15	—15				
C_7	—32	—33	—22	—23				
C_8	—34	—36	—26	—28	—33	—34		
C_9	—36	—36	—30	—29	—38	—41		
C_{10}	—37		—31		—40		—42	—
C_{11}	—36		—30		—41		—46	—

* Eigene Messungen.

Tabelle I zeigt die Inkremente für Methylverzweigungen in Form der H^S-Werte (stationäre Phase Squalan), die aus den Daten von MATSUKUMA abgeleitet wurden. Erst von einer gewissen Mindestkettenlänge an, gerechnet von der Verzweigungsstelle aus, können konstante Inkremente verwandt werden, wie sich bei Variation der Kohlenstoffzahl zeigt. Die in dieser Arbeit angegebenen Retentionsindices sind an Isomerengemischen ermittelt worden, die aus den *n*-Paraffinen über die "methylene insertion reaction" (MIR) gewonnen wurden, vgl. SIMMONS, RICHARDSON UND DVORETZKY[7].

Vom Autor wurde darüber hinaus eine Reihe von zweifach methylverzweigten Kohlenwasserstoffen untersucht, die zum grössten Teil ebenfalls aus MIR stammten (Tabelle II). Gleichzeitig wurden mit Hilfe der Inkremente für eine einfache Verzweigung die Indexwerte dieser Kohlenwasserstoffe berechnet. Der Vergleich der berechneten mit den gemessenen Werten ergibt:

1. Sind die beiden Methylgruppen durch mindestens ein weiteres Kohlenstoffatom getrennt, ergibt sich bereits eine gute Additivität der Inkremente.

2. Für die 2,3-Dimethylalkane werden um 20–30 Einheiten zu niedrige Werte berechnet. Die Unsymmetrie der Ladungsverteilung, bezogen auf die drei räumlichen Achsen, ist wesentlich grösser als bei den 2,4-, 2,5- und 2,*n*-Alkanen und wird wahrscheinlich verstärkt durch gegenseitige Abstossung der Methylgruppen.

3. Für die 2,2-Dimethylalkane werden um 10 Einheiten zu hohe Indexwerte erhalten, wenn die Berechnung so durchgeführt wird, dass das Inkrement für die 2-Methylverzweigung doppelt eingesetzt wird. In diesem Falle liegt demnach eine höhere Symmetrie der Ladungsverteilung vor.

TABELLE II

	I^S *gefunden*		I^S *berechnet*	*Differenz*	*t* (°C)
2,2-Dimethylhexan	720	800 — 36 — 36 =	728	+ 8	70°
2,3-Dimethylhexan	761	800 — 36 — 28 =	736	—25	
2,4-Dimethylhexan	733	800 — 36 — 28 =	736	+ 3	
2,5-Dimethylhexan	729	800 — 36 — 36 =	728	— 1	
3,4-Dimethylhexan	772	800 — 28 — 28 =	744	—28	
2,2-Dimethylheptan	817	900 — 36 — 36 =	828	+11	80°
2,3-Dimethylheptan	856	900 — 36 — 29 =	835	—21	
2,4-Dimethylheptan	822	900 — 36 — 41 =	823	+ 1	
2,5-Dimethylheptan	833	900 — 36 — 29 =	835	+ 2	
2,6-Dimethylheptan	827	900 — 36 — 36 =	828	+ 1	
2,6-Dimethyldekan	1115	1200 — 36 — 45 = 1119		+ 4	120°

Durch zusätzliche Inkremente für die 2,3- und 2,2-Dimethylalkane werden auch die Indexwerte der Dimethylalkane vorausberechenbar. Selbst der Index des 2,2,3-Trimethylbutans, der zu 641 gefunden wird, lässt sich nach dem vorstehend Gesagten plausibel erklären.

2,3-Dimethylpentan	673		
2,2-Dimethylpentan	627	2,2,3-Trimethylbutan	641

Sowohl der Einfluss der symmetrievermindernden 2,3-Anordnung wie auch der Einfluss der symmetrieerhöhenden 2,2-Anordnung wirken sich auf den Retentionsindex des 2,2,3-Trimethylbutans aus. Führt man in das 2,3-Dimethylpentan eine weitere 2-Methylverzweigung ein, die etwa —30 IE ausmacht, so ergibt sich 673 — 30 = 643 und unter Berücksichtigung der Exaltation von —10 für 2,2-Dimethylalkane (vgl. Tabelle II), ein Wert von 633.

Mit Hilfe geschätzter und berechneter Indexwerte konnten in mehreren Fällen Hinweise auf die Struktur von unbekannten Kohlenwasserstoffen gegeben werden, die mit anderen physikalischen Methoden der Strukturaufklärung wie MNR und MS nicht auf einfache Weise erhalten werden konnten.

Für die Abschätzung von Indexwerten von Kohlenwasserstoffen sind natürlich die Siedepunkte, die in grossem Umfang in den API-Sammlungen enthalten sind, von grossem Nutzen. Verschiedentlich wurden vom Autor Indexwerte, die aus Siedepunktangaben stammen, extrapoliert auf höhere C-Zahlen und zur Identifizierung herangezogen. Doch ist schon bei relativ ähnlichen Isomeren Vorsicht geboten, weil sich nicht immer Siedepunktsunterschiede in den entsprechenden Indexdifferenzen wiederspiegeln. Nach der von KOVATS angegebenen Regel: $\delta I \sim 5 \cdot \delta t_S$ erhält man in einigen Fällen nicht die richtigen Indexdifferenzen. Ein wohlbekanntes Beispiel ist das Stoffpaar 2-Methylpentan/2,3-Dimethylbutan, dessen Trennung nur mit einer 100 m-langen Kapillarsäule mit Squalan als stationärer Phase erreicht werden kann. Aus der Siedepunktsdifferenz von 1.7° ermittelt man mit der Regel von KOVATS 8.5 Einheiten. Tatsächlich unterscheiden sich die Retentionsindices der beiden Isomeren in Squalan um eine Einheit und in *n*-Hexadekan um fünf Einheiten:

	I^S	δI^S	δI^S *ber.*	*Kp.*	δt_s
3-Methylpentan	585			63.3	
		15	15		3
2-Methylpentan	570			60.3	
		1	8.5		1.7
2,3-Dimethylbutan	569			58.0	

In der stationären Phase Squalan treten die Moleküle der beiden Isomeren mit den Squalanmolekülen in Wechselwirkung, während sie beim Verdampfungsvorgang nur mit gleichen Molekülen Dispersionswechselwirkung ausüben. Auf diese Weise ist leicht erklärbar, warum in *n*-Hexadekan, das keine Methylverzweigungen enthält, eine andere relative Flüchtigkeit für die beiden Isomeren gefunden wird ($\delta T = 5$). Ähnliche Verhältnisse liegen auch bei dem Isomerenpaar 1-Hexen/2-Methyl-1-penten vor:

	I^S	δI^S	*Kp.*	δt_s
1-Hexen	582		63.5	
		2		2.8
2-Methyl-1-penten	580		60.7	

Beiden Fällen gemeinsam sind Unterschiede in den Verzweigungsgraden der beiden Isomeren. Im folgenden wird auf weitere Fälle, in denen die Siededifferenzen

nicht die entsprechenden Indexdifferenzen ergeben, und somit zu falschen Zuordnungen führen können, hingewiesen.

Die verzweigten und unverzweigten *n*-Paraffine zeigen erwartungsgemäss keine ΔI-Werte.

UNVERZWEIGTE UND VERZWEIGTE ALKENE

Die Retentionsdaten der Alkene werden einerseits durch die π-Elektronen und deren Polarisation, andererseits aber auch durch die gegenüber den *n*-Paraffinen besonderen sterischen Verhältnisse, die sich aus der Aufhebung der freien Drehbarkeit an der Doppelbindung ergeben, bestimmt. α-Olefine haben ein Dipolmoment von 0.4 Debye, trotzdem liegen ihre Siedepunkte unterhalb denen der entsprechenden Alkane, obwohl bei den Kohäsionskräften zwischen den Molekülen ein Anteil Dipolwechselwirkung anzunehmen ist. In gaschromatographischen Systemen besteht wegen der starken Verdünnung nicht die Möglichkeit der Dipolwechselwirkung der Moleküle des gelösten Stoffes untereinander. Dennoch gehen die Retentionsindices der Olefine den Siedepunkten parallel. Über die Dispersionswechselwirkung unter Beteiligung von π-Elektronen scheinen bisher weder qualitative noch quantitative Vorstellungen vorzuliegen.

Tabelle III zeigt den Einfluss der Einführung einer oder zweier Doppelbindungen in die verschiedenen Positionen einer Paraffinkette. Es ergeben sich folgende von der C-Zahl im wesentlichen unabhängige Inkremente:

	H^S	$\Delta I(S\text{–}EMO)$
1-Alkene	—18	+41
α,ω-Alkadiene	—36	+83
trans-2-Alkene	— 2	+39
cis-2-Alkene	+ 3	+45

Während also die H^S-Werte bei der zweifach substituierten Doppelbindung der 2-Alkene um etwa 20 IE ansteigen, bleiben die ΔI-Werte nahezu konstant mit dem zu erwartenden Unterschied zwischen der *cis*- und der *trans*-Verbindung. Die *cis*-Verbindung hat das höhere Dipolmoment, bei der *trans*-Verbindung werden die π-Elektronen zusätzlich abgeschirmt. Auch für Alkadiene, die isolierte Doppelbindungen in α- und in β-Stellung enthalten, sind die angegebenen Inkremente gültig, vgl. Tabelle III, Gruppe 3. Das gleiche gilt für Cyclopropylderivate, wie sie bei der MIR anfallen, wenn eine isolierte Doppelbindung in das Molekül eingeführt wird. Wandert die Doppelbindung durch die Kohlenstoffkette, z.B. in der Kette des Undekans, aus der 1- in die 5-Stellung, so findet man folgende H^S-Werte für die resultierenden Stellungsisomeren:

1-Undecen	—18		
trans-2-Undecen	— 2	*cis*-2-Undecen	+ 3
trans-3-Undecen	—13	*cis*-3-Undecen	—12
trans-4-Undecen	—17	*cis*-4-Undecen	—17
trans-5-Undecen	—17	*cis*-5-Undecen	—17

Von der 3-Stellung an nähern sich die H^S-Werte demjenigen des 1-Olefins. Zugleich verschwinden die Unterschiede zwischen der *trans*- und der *cis*-Verbindung.

TABELLE III

	$I^S_{70°}$	$H^S_{70°}$	$I^{EMO}_{70°}$	ΔI		$I^S_{70°}$	$H^S_{70°}$	$I^{EMO}_{70°}$	ΔI
1-Penten	481	—19							
1-Hexen	582	—18	624	44	1,5-Hexadien	563	—37	648	85
1-Hepten	682	—18	724	42					
1-Octen	782	—18	822	40	1,7-Octadien	764	—36	847	83
1-Nonen	883	—17	923	40					
1-Decen	982	—18	1022	40	1,9-Dekadien	964	—36	1046	82
Mittelwert		—18		41			—36		83
trans-2-Penten	503	+ 3			*cis*-2-Penten	507	+ 7		
trans-2-Hexen	598	— 2			*cis*-2-Hexen	603	+ 3		
trans-2-Hepten	699	— 1			*cis*-2-Hepten	704	+ 4		
trans-2-Octen	798	— 2	837	39	*cis*-2-Octen	802	+ 2	847	45
trans-2-Nonen	896	— 4			*cis*-2-Nonen	900	0		
trans-2-Undecen	1099	— 1			*cis*-2-Undecen	1103	+ 3		
Mittelwert		— 2					+ 3		
trans-1,5-Heptadien	682	—18			*cis*-1,5-Heptadien	689	—11		
trans-1,7-Nonadien	880	—20			*cis*-1,7-Nonadien	885	—15		
Berechnet:	—18 —2	—20				—18 +3	—15		
			$I^{XF\,1150}_{70°}$					$I^{XF\,1150}_{70°}$	
trans-3-Hexen	593	— 7			*cis*-3-Hexen	593	— 7		
trans-3-Hepten	688	—12	728	40	*cis*-3-Hepten	691	— 9	743	52
trans-3-Undecen	1087	—13			*cis*-3-Undecen	1087	—12		
Mittelwert		—12					—13		
trans-4-Undecen	1083	—17			*cis*-4-Undecen	1083	—17		
trans-5-Undecen	1083	—17			*cis*-5-Undecen	1083	—17		

In den *verzweigten* Mono- und Diolefinen wirken sich die Inkremente für die Doppelbindung und für die Methylverzweigung additiv auf das Retentionsverhalten aus, wenn sie isoliert voneinander stehen. In allen anderen Fällen ist die Ermittlung neuer, charakteristischer Inkremente erforderlich. Im folgenden werden der Einfachheit halber zunächst nur 1- und 2-Alkene einschliesslich der entsprechenden Diene behandelt.

Auf die generelle Anwendung polarer stationärer Phasen, die ohnehin wegen der geringen Löslichkeit der Kohlenwasserstoffe schlechte Trenneffekte liefern, wurde verzichtet, da für die Trennung und Charakterisierung von Olefinen sterische und elektronische Einflüsse auf die Dispersionswechselwirkung von grösserer Bedeutung sind*.

Zur Isolierung des Einflusses einer Doppelbindung auf den Retentionsindex I^S wurde eine Homomorphiebetrachtung mit den entsprechenden gesättigten, verzweigten Kohlenwasserstoffen durchgeführt. Die gemessenen Werte sind nach C-Zahlen aufgegliedert in Tabelle IV aufgeführt. Die Retentionsdaten wurden bestimmt an Substanzen, die entweder in reiner Form vorhanden waren oder durch MIR aus folgenden Verbindungen erhalten worden waren:

1-Penten; 1-Hexen; 1-Hepten; 1-Octen;
1,4-Pentadien; 1,5-Hexadien; 1,7-Octadien; 1,9-Dekadien;
4-Methyl-1-penten; 2,3-Dimethyl-1-buten; 2-Äthyl-1-buten.

2-Methyl-1-alkene

Die Methylsubstitution an der α-Doppelbindung verursacht einen H^S-Wert von + 8 bis 14 IE mit einem Mittelwert von etwa + 11 IE, bezogen auf das entsprechende gesättigte, verzweigte Alkan. Gegenüber dem unverzweigten α-Olefin beträgt die Zunahme des I^S-Wertes dagegen + 29 IE. Das abgeleitete Inkrement ist auch für Kohlenwasserstoffe mit weiteren funktionellen Gruppen gültig, z.B.:

	H^S	*Homomorphes*:
2-Methyl-1,7-octadien	+ 10	7-Methyl-octen-1
2,4-Dimethyl-1-penten	+ 7	2,4-Dimethylpentan

Schwierigkeiten ergeben sich allerdings bei 2,3-verzweigten 1-Alkenen, s.u. Die Erhöhung des Retentionsindex bei den α-verzweigten Olefinen wird sowohl durch die Polarisation der Doppelbindung durch die Methylgruppe verursacht wie auch durch die Aufhebung der freien Drehbarkeit zwischen den Kohlenstoffatomen 1 und 2, also an der Stelle, an der sich die Methylverzweigung befindet (Entropieänderung). Bei den unsubstituierten α-Olefinen wird dagegen eine Erniedrigung des Index durch diesen Effekt beobachtet.

* Die Anwendung von $AgNO_3$-haltigen polaren Phasen führt zu einer gruppenweisen Eluierung der *trans*- und der *cis*-Olefine. Aus sterischen Gründen haben die *cis*-Verbindungen wesentlich höhere Retentionszeiten als die *trans*-Verbindungen. Vgl. z.B. die Trennung der isomeren Undecene von E. BENDEL *et al.*[8].

TABELLE IV

	I^S		I^S	H^S	t
2-Methylbutan	474	2-Methyl-1-buten	488	+14	22°
		3-Methyl-1-buten	449	−25	
2-Methylpentan	570	2-Methyl-1-penten	580	+10	22°
		4-Methyl-1-penten	549	−21	
		2-Methyl-1,4-pentadien	559	−11	
3-Methyl-pentan	585	3-Methyl-1-penten	551	−34	
		2-Äthyl-1-buten	593	+8	
		3-Methyl-1,4-pentadien	532	−53	
2,3-Dimethylbutan	569	2,3-Dimethyl-1-buten	558	−11	
2,2-Dimethylbutan	538	3,3-Dimethyl-1-buten	506	−32	
2-Methylhexan	667	2-Methyl-1-hexen	678	+11	60°
		5-Methyl-1-hexen	651	−16	
		2-Methyl-1,5-hexadien	665	−2	
3-Methylhexan	677	3-Methyl-1-hexen	645	−32	
		4-Methyl-1-hexen	659	−18	
		3-Methyl-1,5-hexadien	633	−44	
		2-Äthyl-1-penten	681	+4	
2,2-Dimethylpentan	627	4,4-Dimethyl-1-penten	606	−21	
2,3-Dimethylpentan	673	2,3-Dimethyl-1-penten	649	−24	
		3,4-Dimethyl-1-penten	638	−35	
		2-Äthyl-3-methyl-1-buten	658	−15	
2,4-Dimethylpentan	631	2,4-Dimethyl-1-penten	638	+7	
n-Butylcyclopropan	713	4-Cyclopropyl-1-buten	698	−15	
1,1-Diäthylcyclopropan	668				
1-Methyl-1-isopropyl-cyclopropan	642				
2-Methylheptan	764	2-Methyl-1-hepten	776	+12	70°
		6-Methyl-1-hepten	750	−14	
3-Methylheptan	772	3-Methyl-1-hepten	742	−30	
		5-Methyl-1-hepten	757	−15	
		2-Äthyl-1-hexen	779	+7	
4-Methylheptan	766	4-Methyl-1-hepten	748	−18	
2-Methyloctan	864	2-Methyl-1-octen	874	+10	80°
		7-Methyl-1-octen	847	−17	
		2-Methyl-1,7-octadien	857	−7	
3-Methyloctan	871	3-Methyl-1-octen	839	−32	
		6-Methyl-1-octen	853	−18	
		3-Methyl-1,7-octadien	823	−48	
4-Methyloctan	859	4-Methyl-1-octen	846	−13	
		5-Methyl-1-octen	845	−14	
		4-Methyl-1,7-octadien	830	−29	
n-Hexylcyclopropan	913	6-Cyclopropyl-1-hexen	897	−16	

3-Methyl-1-alkene

Wandert die Methylgruppe in die Allylstellung zur Doppelbindung, so ergibt sich genau der entgegengesetzte Einfluss wie bei den 2-Alkyl-1-alkenen, nämlich eine zusätzliche Indexerniedrigung. Man findet H^S-Werte von −32 gegenüber −18 bei den unverzweigten α-Olefinen. Es scheint, als ob die Methylgruppe in Allylstellung

eine günstige Koordination anderer Moleküle für die Dispersionswechselwirkung verhindert. Es ist allerdings auch nicht unwahrscheinlich, dass die in Allylstellung befindliche Methylgruppe einen elektronischen Einfluss auf die Doppelbindung ausübt.

4-Methyl-1-alkene

In der 4-Stellung zur Doppelbindung übt die Methylgruppe nur einen sehr geringen Einfluss auf die H^S-Werte aus. Man findet Werte von —14 bis —21 mit einem Mittelwert von —18. Je grösser also der Abstand zwischen Verzweigungsstelle und Doppelbindung ist, umsomehr gehen die H^S-Werte für Doppelbindung und Verzweigungsstelle additiv in die Retentionsindices ein.

Tabelle V zeigt den Vergleich von gemessenen und berechneten I^S-Werten von *n*-Methyl-1-alkenen. Für die Berechnung zugrunde gelegt wurden die Methylverzweigungsinkremente aus Tabelle I und der H^S-Wert für α-Olefine von —18.

TABELLE V

		I^S berechnet	I^S gefunden	Differenz	t
3-Methyl-1-buten	500 — 18 — 26 = 456		449	+ 7	22°
2-Methyl-1-buten	500 — 18 — 26 = 456		488	—32	
4-Methyl-1-penten	600 — 18 — 30 = 552		549	+ 3	22°
3-Methyl-1-penten	600 — 18 — 15 = 567		551	+16	
2-Methyl-1-penten	600 — 18 — 30 = 552		580	—28	
5-Methyl-1-hexen	700 — 18 — 33 = 649		651	— 2	60°
4-Methyl-1-hexen	700 — 18 — 23 = 659		659	0	
3-Methyl-1-hexen	700 — 18 — 23 = 659		645	+14	
2-Methyl-1-hexen	700 — 18 — 33 = 649		678	—29	
6-Methyl-1-hepten	800 — 18 — 36 = 746		750	— 4	70°
5-Methyl-1-hepten	800 — 18 — 28 = 754		757	— 3	
4-Methyl-1-hepten	800 — 18 — 34 = 748		748	0	
3-Methyl-1-hepten	800 — 18 — 28 = 754		742	+12	
2-Methyl-1-hepten	800 — 18 — 36 = 746		776	—30	
7-Methyl-1-octen	900 — 18 — 36 = 846		847	— 1	80°
6-Methyl-1-octen	900 — 18 — 29 = 853		853	0	
5-Methyl-1-octen	900 — 18 — 41 = 841		845	— 4	
4-Methyl-1-octen	900 — 18 — 41 = 841		846	— 5	
3-Methyl-1-octen	900 — 18 — 29 = 853		839	+14	
2-Methyl-1-octen	900 — 18 — 36 = 846		874	—28	

Erwartungsgemäss werden entsprechend dem oben Gesagten die grössten Abweichungen bei den 2-Methyl-1-alkenen und den 3-Methyl-1-alkenen beobachtet. Die berechneten Werte sind für die 2-Methyl-1-alkene im Mittel um 30 IE zu niedrig, diejenigen der 3-Methyl-1-alkene um etwa 14 IE zu hoch. Für die beiden Substanzklassen müssen daher zwei neue Inkremente festgelegt werden. Die Retentionsindices der 4-, 5-, 6- und 7-Methyl-1-alkene lassen sich ohne zusätzliche Inkremente mit ausreichender Genauigkeit berechnen, weil Verzweigungsstelle und Doppel-

TABELLE VI

		$I^S_{80°}$		I^S *berechnet*
1	8-Cyclopropyl-1-octen	1097	1100 + 13 — 18	= 1095
2	*trans*-1,9-Undekadien	1079	1100 — 2 — 18	= 1080
3	*cis*-1,9-Undekadien	1084	1100 + 3 — 18	= 1085
4	2-Methyl-1,9-dekadien	1057	1100 — 36 — 18 + 10	= 1056
5	3-Methyl-1,9-dekadien	1022	1100 — 29 — 18 — 32	= 1022
6	4-Methyl-1,9-dekadien	1025	1100 — 41 — 18 — 18	= 1023
7	5-Methyl-1,9-dekadien	1023	1100 — 45 — 18 — 18	= 1019
8	*trans*-2-Undecen		1099 — 18	= 1081
9	*cis*-2-Undecen		1103 — 18	= 1085
		berechnet		
10	2-Methyl-1-decen	200 + 874	1074 — 18	= 1056
11	3-Methyl-1-decen	200 + 839	1039 — 18	= 1021
12	4-Methyl-1-decen	200 + 846	1046 — 18	= 1028
13	5-Methyl-1-decen	200 + 845	1045 — 18	= 1027

bindung genügend voneinander isoliert sind. Zu ähnlichen Ergebnissen kommt man bei den MIR-Produkten der Alkadiene, deren Retentionsindices in Tabelle VI mit auf zwei Wegen berechneten Werten verglichen werden. Die Indexwerte der Substanzen 1–7 wurden bestimmt in einer Mischung die, durch MIR mit 1,9-Dekadien erhalten worden war, und stehen in Spalte 1. In der letzten Spalte stehen die inkrementell berechneten Werte. Alle sieben Verbindungen enthalten eine Doppelbindung in 1- bzw. 9-Stellung: —18.

Verbindung 1 enthält zusätzlich einen endständigen Dreiring: +13
Verbindung 2 und 3 enthalten eine Doppelbindung in 2-Stellung *cis* +3
und *trans*: und —2
Verbindung 4 hat eine Verzweigung in 2-Stellung: —36
eine methylverzweigte Doppelbindung in 2-Stellung: +10
Verbindung 5 hat eine Verzweigung in 3-Stellung: —29
eine unverzweigte Doppelbindung in 1-Stellung mit Methylsubstitution in 3-Stellung: —32
Verbindung 6 hat eine Verzweigung in 4-Stellung: —41
und eine unverzweigte Doppelbindung in 9-Stellung: —18
Verbindung 7 hat eine Verzweigung in 5-Stellung: —45
und eine unverzweigte Doppelbindung in 9-Stellung: —18

Die Vorausberechnung der Indexwerte der methylierten Dekadiene war auch auf folgendem Wege möglich:

Die Indexwerte der Substanzen 2 und 3 erhält man durch Einführung einer Doppelbindung in die 10-Stellung des *trans*- und *cis*-2-Undecen, deren Retentionsindices bekannt waren. Die Indexwerte der Substanzen 10 bis 13 wurden durch C-Zahl-Extrapolation aus den entsprechenden Nonenen bestimmt. Durch Einführung einer weiteren Doppelbindung kommt man zu den Verbindungen 4 bis 7. Die Übereinstimmung der gemessenen Werte mit den auf beiden Wegen berechneten ist für die Zuordnung ausreichend.

Weitere Beispiele für die Additivität der abgeleiteten H^S-Inkremente sind aus Tabelle V zu entnehmen:

	H^S	
2-Methyl-1-penten	+10	ähnliche Ergebnisse werden auch für die C-Zahlbereiche C_7 und C_9 erhalten
4-Methyl-1-penten	−21	
2-Methyl-1,4-pentadien	−11	−11
3-Methyl-1-hexen	−32	auch im C_9-Bereich
4-Methyl-1-hexen	−18	
3-Methyl-1,5-hexadien	−44	−50
4-Methyl-1-octen	−13	
5-Methyl-1-octen	−14	
4-Methyl-1,7-octadien	−29	−27

Enthält das Olefin eine doppelte Verzweigungsstelle neben einer Doppelbindung in Allylstellung oder in 4-Stellung, so wird der gleiche H^S-Wert beobachtet wie bei Olefinen mit einer einfachen Methylverzweigung in Allylstellung oder in 4-Stellung, z.B.:

	H^S
3,3-Dimethyl-1-buten	−32
3-Methyl-1-penten	−34
4,4-Dimethyl-1-penten	−21
4-Methyl-1-hexen	−18

Schwieriger liegen die Verhältnisse bei den 1- und 2-Alkenen mit benachbarter Methylverzweigung in 2,3- bzw. 3,4-Stellung. Im ersten Beispiel wird noch eine gute Additivität der Einflüsse auf die Doppelbindung beobachtet:

	H^S
3,4-Dimethyl-1-penten	−35
3-Methyl-1-penten	−34
4-Methyl-1-penten	−24

Das 3-Methyl-1-penten hat schon den gleichen H^S-Wert wie das 3,4-Dimethyl-1-penten. Der Einfluss der 4-Methylverzweigung auf die Doppelbindung macht höchstens −3 IE aus. Nur um diese drei Einheiten dürfte der H^S-Wert des 3,4-Dimethyl-1-pentens niedriger sein als der des 3-Methyl-1-pentens, da der Einfluss der C-Verzweigung schon bei der Bildung des H^S-Wertes eliminiert wird.

Bei der Substitution der Doppelbindung in 2-Stellung und der C-Kette in 3-Stellung ist zwar der gegenläufige Einfluss der beiden Verzweigungstypen erkenn-

bar, aber nicht quantitativ zu erfassen, da offenbar eine Wechselwirkung der beiden Strukturmerkmale des 2-Methyl-1-alkens und des 3-Methyl-1-alkens nicht auszuschliessen ist:

	H^S
2,3-Dimethyl-1-penten	−24
2-Methyl-1-penten	+10
3-Methyl-1-penten	−34

Geht man vom H^S-Wert des 2-Methyl-1-pentens aus, so macht der Einfluss einer zusätzlichen 3-Methylverzweigung −34 +18 = −16 IE aus, weil im H^S-Wert der Beitrag des unsubstituierten α-Olefins von −18 IE enthalten ist. Man kommt also auf einen Wert von +10 −16 = −6 IE und nicht von −24. Offenbar trägt die Doppelbindung des 3-Methyl-1-alkens bzw. des 2-Methyl-1-pentens nichts zur Erniedrigung des Index bei. Die sterischen Verhältnisse an der Doppelbindung allein genügen zur Erklärung der beobachteten Werte. In diesem Falle ergibt sich in

TABELLE VII

	I^S		I^S	H^S	t
2-Methylbutan	474	2-Methyl-2-buten	515	+41	22°
2-Methylpentan	570	2-Methyl-2-penten	599	+29	
		trans-4-Methyl-2-penten	563	−7	
		cis-4-Methyl-2-penten	556	−14	
3-Methylpentan	585	*trans*-3-Methyl-2-penten	603	+18	
		cis-3-Methyl-2-penten	613	+28	
2,3-Dimethylbutan	569	2,3-Dimethyl-2-buten	625	+56	
2-Methylhexan	667	2-Methyl-2-hexen	692	+25	60°
		trans-5-Methyl-2-hexen	661	−6	
		cis-5-Methyl-2-hexen	670	+3	
3-Methylhexan	677	*trans*-3-Methyl-3-hexen	685	+8	
		cis-3-Methyl-3-hexen	692	+15	
3-Äthylpentan	684	3-Äthyl-2-penten	697	+13	
2,3-Dimethylpentan	673	2,3-Dimethyl-2-penten	705	+32	
		cis-3,4-Dimethyl-2-penten	670	−3	
		trans-3,4-Dimethyl-2-penten	678	+5	
2,4-Dimethylpentan	631	2,4-Dimethyl-2-penten	641	+10	
n-Butan	400	1,3-Butadien	390	−10	22°
2-Methylbutan	474	2-Methyl-1,3-butadien	497	+23	
n-Pentan	500	*trans*-1,3-Pentadien	515	+15	
		cis-1,3-Pentadien	524	+24	
n-Hexan	600	*trans*-1,3-Hexadien	611	+11	
Cyclohexan	668	Cyclo-1,3-hexadien	655	−13	
2,3-Dimethylbutan	569	2,3-Dimethyl-1,3-butadien	612	+43	

TABELLE VIII

	H^S		H^S	*Zahl der C–C-Bindung an der Doppelbindung*
1-Hepten	—18	1-Hexen	—18	1
ø-2-Hepten	+ 2	ø-2-Hexen	0	2
2-Methyl-1-hexen	+11	2-Methyl-1-penten	+10	2
2-Methyl-2-hexen	+25	2-Methyl-2-penten	+29	3
ø-3-Methyl-3-hexen	+12	ø-3-Methyl-2-penten	+23	3
2,3-Dimethyl-2-penten	+32	2,3-Dimethyl-2-buten	+56	4

glänzender Übereinstimmung für den H^S-Wert des 2,3-Dimethyl-1-pentens +10 —34 = —24 IE.

2,4-Dimethyl-1-alkene

	H^S
2,4-Dimethyl-1-penten	+ 7
2-Methyl-1-penten	+10
4-Methyl-1-penten	—21

Die 4-Methylverzweigung trägt, wenn man von 2-Methyl-1-penten ausgeht. nur —3 Einheiten bei, weil die Doppelbindung im H^S-Wert des 2-Methyl-1-pentens schon erfasst ist. Sind die beiden Methylverzweigungen somit voneinander getrennt, ergibt sich wieder eine ausgezeichnete Additivität der Einflüsse.

Für die *mittelständig verzweigten Olefine* ist das Datenmaterial noch sehr unvollständig. Die Verschiebung der Doppelbindung und die Methylsubstitution an der Doppelbindung haben, wie Tabelle VII zeigt, indexerhöhende Wirkungen. Mit steigender Zahl der C–C-Bindungen an der Doppelbindung beobachtet man die in Tabelle VIII angeführten Indexzunahmen.

Es ergibt sich in erster Näherung, dass jede C-C-Substitution an der Doppelbindung der H^S-Wert um 15 bis 30 IE erhöht. Liegt in α-Stellung zur Doppelbindung eine Methylverzweigung vor, so führt das wie bei den 1-Alkenen zu einer Erniedrigung der H^S-Werte, z.B.:

	H^S		H^S
trans-2-Hexen	—2	*trans*-4-Methyl-2-penten	— 7
cis-2-Hexen	+3	*cis*-4-Methyl-2-penten	—14

Steht die Methylgruppe in 5-Stellung eines 2-Alkens, so ergibt sich wiederum gute Additivität der Inkremente; die richtige Reihenfolge der Elution der beiden Isomeren lässt sich allerdings nicht voraussagen.

	I^S *gef.*		I^S *ber.*
trans-5-Methyl-2-penten	661	699 — 30 — 3 =	666
cis-5-Methyl-2-penten	660	704 — 30 — 3 =	671

Der H^S-Wert des 2,4-Dimethyl-2-pentens wird plausibel, wenn man vom 2-Methyl-2-penten ausgeht:

	H^S
2,4-Dimethyl-2-penten	+ 10
2-Methyl-2-penten	+ 29
ø-4-Methyl-2-penten	— 11

In ϕ-2-Hexen wird durch Methylierung in 4-Stellung eine Indexerniedrigung von —15 beobachtet, so dass sich analog für das 2,4-Dimethyl-2-penten ein Wert von +14 ergibt.

Bei den *konjugierten Diolefinen* ist die Isolierung des Einflusses der Doppelbindungen auf den Retentionsindex schwieriger, weil sich die Geometrie des Kohlenstoffgerüstes beim Übergang zum Olefin so stark ändert, dass in den H^S-Werten ein grosser Beitrag der sterischen Veränderungen des Moleküls anzunehmen ist. Berechnet man die I-Werte der konjugierten Diene in der Weise, dass für jede Doppelbindung der H^S-Wert einer ungestörten α-Doppelbindung eingesetzt wird, so ist die Differenz zwischen den tatsächlich gemessenen Werten und den berechneten ein Mass für den Einfluss der Konjugation auf das Retentionsverhalten (Tabelle IX).

TABELLE IX

	I^S *ber.*	I^S *gef.*	
1,3-Butadien	364	390	+ 26
1,3-Pentadien *trans*	480	515	+ 35
1,3-Pentadien *cis*	491	524	+ 33
1,3-Hexadien *trans*	580	611	+ 31
1,3-Hexadien *cis*	585	—	—
2-Methyl-1,3-butadien	469	497	+ 28
2,3-Dimethyl-1,3-butadien	597	612	+ 15

Die Berechnung des I-Wertes des Isoprens erfolgte — ausgehend von 2-Methylbutan — durch Einführung einer unverzweigten und einer verzweigten α-Doppelbindung: 474 +14 —19 = 469. Der I-Wert des 2,3-Dimethylbutadiens ergibt sich aus 2,3-Dimethylbutan durch Einführung zweier α-verzweigter Doppelbindungen: 569 +14 +14 = 597. Beim Isopren ist der indexerniedrigende Einfluss der Methylgruppe auf die dreiständige Doppelbindung und beim 2,3-Dimethylbutadien der beiden Methylgruppen auf die jeweils in β-Stellung stehende Doppelbindung zu berücksichtigen. Hieraus erklärt sich insbesondere der niedrige δ-Wert des 2,3-Dimethylbutadiens.

Abschliessend seien noch einmal einige H- und ΔI-Werte von ungesättigten Kohlenwasserstoffen zusammengestellt (Tabelle X).

TABELLE X

	H^S	H^{EMO}	ΔI		H^S
1-Alkene	—18	+24	+42	2-Methyl-1-alkene	+11
α,ω,-Alkadiene	—36	+47	+83	3-Methyl-1-alkene	—32
Cyclopropylalkane	+13	+58	+45	4-Methyl-1-alkene	—18
trans-2-Alkene	— 2			5,6,7-Methyl-1-alkene	—17
cis-2-Alkene	+ 3			2-Methyl-2-alkene	+25
trans-3-Alkene	—12				
cis-3-Alkene	—12			Konjugation zweier Doppelbindungen erhöht den Index um weitere 30 bis 40 Einheiten.	
trans,cis-4,5-Alkene	—17				

Die folgende Arbeit, Teil III dieser Publikationsreihe, wird sich mit der Diskussion der Retentionsdaten von Cyclanen und Cyclenen sowie ihrer Alkylsubstitutionsprodukte beschäftigen.

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ZUSAMMENFASSUNG

Es wurden die Retentionsindices einer Reihe von verzweigten Alkanen und einer Auswahl der entsprechenden Mono- und Diolefine bestimmt. Die Substanzen stammten teilweise aus den chemischen Arbeiten des Max-Planck-Instituts für Kohlenforschung, teilweise wurden sie im Gemisch mit verwandten Isomeren durch Anwendung der "methylene insertion"-Reaktion auf Alkane und Alkene dargestellt. Aus dem Datenmaterial, das nur in Kapillarsäulen mit apolaren und polaren stationären Phasen gemessen wurde, lassen sich Indexinkremente für strukturelle Einheiten und eine Reihe von Regeln ableiten, die in vielen Fällen die Berechnung oder zumindest die Abschätzung von Indexwerten unbekannter Verbindungen ermöglichen. Neben den ΔI-Werten wurden die sog. H-Werte auf strukturelle Einflüsse hin interpretiert. Die H-Werte werden durch Vergleich des Retentionsindex einer Verbindung mit dem ihrer homomorphen erhalten und erlauben die Isolierung des Einflusses einzelner struktureller Merkmale auf den Retentionsindex. Auch bei den ΔI-Werten ist eine Homomorphiebetrachtung möglich, sie führt zu den $\delta(\Delta I)$-Werten, die aber für Teil III dieser Publikationsreihe von grösserer Bedeutung sind, weil die ΔI-Werte der offenkettigen Kohlenwasserstoffe klein und häufig uncharakteristisch sind oder nur durch etwaige Doppelbindungen im Molekül verursacht werden.

SUMMARY

The retention indices of a series of branched-chain alkanes and a selection of the corresponding mono- and di-olefins were determined. Some of the compounds were available from chemical work of the Max-Planck-Institut für Kohlenforschung, while the others were prepared, in admixture with related isomers, from alkanes and alkenes by methylene insertion reactions. The data were only obtained from measurements in capillary columns with non polar and polar stationary phases. Index increments for structural elements were derived from these data and rules were established, which in many cases make it possible to calculate, or at least estimate, the index values of unknown compounds. Besides the ΔI-values, the so-called H values were examined as regards structural influences. The H values are obtained by comparing the retention indices of two compounds that differ by only one structural element. It is thus possible to isolate the influence of single structural features on the retention index. The ΔI-values can be treated in the same way. This leads to $\delta(\Delta I)$ values, which are, however, of more importance for Part III of this series, since the ΔI values of open-chain hydrocarbons are only produced by double bonds in the molecule. They are small and not always characteristic.

LITERATUR

1 G. SCHOMBURG, *J. Chromatog.*, 14 (1964) 157.
2 E. KOVATS, *Helv. Chim. Acta*, 41 (1958) 1915.
3 A. WEHRLI UND E. KOVATS, *Helv. Chim. Acta*, 42 (1959) 2709;
P. TOTH, E. KUGLER UND E. KOVATS, *Helv. Chim. Acta*, 42 (1959) 2519.
4 G. SCHOMBURG, *Z. Anal. Chem.*, 200 (1964) 365.
5 I. MATSUKUMA, *J. Chem. Soc. (Japan)*, 84 (1963) 770.
6 G. SCHOMBURG, *Vortrag z. Wilkens Symp. Gaschromatog., Darmstadt, 1965.*
7 C. SIMMONS, D. B. RICHARDSON UND I. DVORETZKY, *Gas Chromatography, Edinburgh, 1960*, Butterworths, London, S. 211.
8 E. BENDEL, M. KERN, R. JANSSEN UND G. STEFFAN, *Z. Angew. Chem.*, 74 (1962) 905.

GASCHROMATOGRAPHISCHE RETENTIONSDATEN UND STRUKTUR CHEMISCHER VERBINDUNGEN

III. ALKYLVERZWEIGTE UND UNGESÄTTIGTE CYCLISCHE KOHLENWASSERSTOFFE

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Im folgenden werden die Retentionsdaten von cyclischen Kohlenwasserstoffen mit 3-, 5-, 6-, 7-, 8-, 10- und 12-Ringen sowie deren Methylsubstitutionsprodukte diskutiert. Sie wurden nur in Kapillarsäulen aus Stahl gemessen.

CYCLANE UND DEREN ALKYLSUBSTITUTIONSPRODUKTE

Die Interpretation der Retentionsdaten cyclischer Kohlenwasserstoffe, besonders aber der ungesättigten Derivate, ist in vielen Fällen schwierig wegen der Überlagerung sterischer und elektronischer Einflüsse auf die intermolekulare Wechselwirkung. Die Indexwerte der cyclischen Kohlenwasserstoffe unterscheiden sich in zwei Punkten wesentlich von denen der offenkettigen (vgl. auch WEHRLI UND KOVATS[1]):

1. Je nach Ringgrösse werden hohe ΔI-Werte beobachtet, und zwar für die Ringe mit 7 bis 12 Kohlenstoffatomen 80 bis 90 IE im System Squalan–Emulphor O. Für eine Doppelbindung findet man vergleichsweise 40 IE, für den Sechsring 48 IE. Die ΔI-Werte sind proportional der "Polarität" der polaren stationären Phase wie bei den Olefinen und Aromaten auch.

2. Da die Geometrie des Moleküls besonders bei den mittleren Ringen stark von der Ringgrösse abhängt, was mit der geringen Deformierbarkeit des Tetraederwinkels zusammenhängt, gibt es keinen inkrementellen Beitrag einer Ring-CH_2-Gruppe zum Retentionsindex. Gaschromatographisch liegt also keine homologe Reihe vor.

In Tabelle I sind die Retentionsdaten der Cyclane und einiger Methyl- bzw. Äthylcyclane wiedergegeben. Zum Unterschied von den offenkettigen Kohlenwasserstoffen werden auch die ΔI-Werte, die charakteristisch von der Struktur beeinflusst werden, behandelt.

Die *H^S-Werte** zeigen ein deutliches Maximum für den 9- und 10-Ring. Dieser Befund entspricht der für diese Ringe auch grössten Ringspannung, die sich in einem Enthalpieüberschuss äussert.

Unter Ringspannung wird im Sinne von DUNITZ UND PRELOG[2] die Summe aus Valenzwinkelenergie, Torsionswinkelenergie und der Energie der transanularen

* $H^S = I^S$ (Cyclan) — I^S (n-Paraffin).

TABELLE I

	I^S		I^{EMO}		ΔI		H^S	
	80°	120°	80°	120°	80°	120°	80°	120°
Cyclopentan	568	578	615		47		68	73
Cyclohexan	668	675	716		48		68	75
Cycloheptan	804	816	860	882	56	66	104	116
Cyclooctan	925	940	990	1016	65	76	125	140
Cyclononan		1049		1133		84		149
Cyclodekan		1147		1235		88		147
Cycloundekan		1235		1327		92		135
Cyclododekan		1325		1417		92		125
Methylcyclopentan	635		667		32		35	
Methylcyclohexan	733		767	782	34		33	
Methylcycloheptan	869		914	933	45		69	
Methylcyclooctan		999		1066		67		99
Methylcyclononan		1106		1180		74		106
Methylcyclodekan		1202		1280		78		102
Methylcycloundekan		1288		1370		82		88
Methylcyclododekan		1379		1463		84		79
Äthylcyclopentan	738						38	
Äthylcyclohexan	839		875		36		39	
Äthylcycloheptan	969						69	
Äthylcyclooctan	1092						92	

Wechselwirkung nicht gebundener Atome verstanden. Die Deformation der Valenzwinkel ist mit einer veränderten Polarisierbarkeit oder sogar mit Bindungsmomenten verbunden, sicher aber mit einer starken Verminderung der Symmetrie der Ladungsverteilung.

Die H^S-Werte der 5- und 6-Ringverbindungen unterscheiden sich nicht. Zu grösseren Ringen hin wird eine monotone Abnahme der H^S-Werte beobachtet, weil bei den hohen C-Zahlen erwartungsgemäss die Ringspannung stark abnimmt. Die gleichen Einflüsse treten auch bei den Methylcyclanen auf, nur, dass die H^S-Werte insgesamt um etwa 30 bis 50 % niedriger sind. Für eine Methylverzweigung in der Mitte einer Paraffinkette findet man, vgl. Teil II, Tabelle I, eine Abnahme von 40–45 IE. z.B.:

	H^{S*}			H^{S**}
Methylcyclodekan	88			
Cycloundekan	135	—47	5-Methylundekan	—46
Methylcyclooctan	99			
Cyclooctan	140	—41	4-Methyloctan	—38

* $H^S = I^S$ (Cyclan) — I^S (*n*-Paraffin).
** $H^S = I^S$ (Methylparaffin) — I^S (*n*-Paraffin).

Je kleiner und somit starrer die Ringe werden, um so weniger ist natürlich mit der Gültigkeit dieser Regel zu rechnen.

ΔI-Werte. Chemisch gesehen sind die gesättigten cyclischen Kohlenwasserstoffe "apolare" Verbindungen und unterscheiden sich nur unwesentlich in ihren Eigenschaften von den gesättigten offenkettigen Kohlenwasserstoffen. Der 3-Ring bildet in dieser Hinsicht allerdings eine Ausnahme, wie auch die gaschromatographischen Daten erkennen lassen. Gaschromatographisch sind die Cyclane und ihre Substitutionsprodukte "polare" Verbindungen, da sie *ΔI*-Werte haben, die in der Grössenordnung derjenigen einer Doppelbindung liegen. Nun wird sich weiter unten zeigen, dass die sterischen Einflüsse auf die Wechselwirkung eines ringförmigen Kohlenwasserstoffes durch die Einführung einer Doppelbindung etwa in der gleichen Grössenordnung liegen wie die Einflüsse der Polarisierbarkeit der π-Elektronen der Doppelbindung. Beim Vergleich der beiden Einflüsse ist die Polarität der polaren stationären Phase zu berücksichtigen.

Die *ΔI*-Werte der Cyclane steigen im Mittel stetig mit der C-Zahl an, nur bei den typischen mittleren Ringen gibt es die erwähnten Exaltationen. Die *ΔI*-Werte der Methylcyclane liegen in jeder C-Zahl um 10 Einheiten niedriger. Substitutionen mit längeren Alkylgruppen als Methyl führen zu keiner weiteren Verminderung oder Erhöhung der *I*-Werte, wenn diese keine Verzweigungsstellen enthalten. Sowohl bei den Cyclanen wie bei den Methylcyclanen unterscheiden sich der 5- und der 6-Ring kaum in ihrem H^S- und *ΔI*-Wert.

TABELLE II

	Kp. (°C)	$I^S_{70°}$	$I^{EMO}_{70°}$	$\Delta I_{70°}$	$H^S_{70°}$
Cyclohexan	80.7	668	710	42	68
Methylcyclohexan	100.9	730	764	34	30
Äthylcyclohexan	131.8	839	875	36	39
Isopropylcyclohexan	154.8	923	960	37	23
n-Propylcyclohexan	156.7	931	966	35	31
Isobutylcyclohexan	170.8	983	1010	27	—17
n-Butylcyclohexan	181.0	1029	1063	34	29
trans-1,2-Dimethylcyclohexan	123.4	807	837	30	7
cis-1,2-Dimethylcyclohexan	129.7	836	875	39	36
trans-1,3-Dimethylcyclohexan	124.4	810	844	34	10
cis-1,3-Dimethylcyclohexan	120.3	789	816	27	—11
trans-1,4-Dimethylcyclohexan	119.4	789	814	25	—11
cis-1,4-Dimethylcyclohexan	124.3	810	844	34	10
1,1-Dimethylcyclohexan	119.5	792	815	23	— 8
Cyclooctan		920	989	69	120
Methylcycloheptan		864	909	45	64
Äthylcyclohexan		839	875	36	39
Propylcyclopentan		834	865	31	34
Butylcyclobutan Pentylcyclopropan		813	858	45	13
Bicyclooctan-[5,1,0]		885	968	83	85
Bicyclooctan-[4,2,0] Bicyclooctan-[3,3,0] Bicyclooctan-[2,2,2]		870	936	66	70

Der Einfluss der Ringgrösse auf H^S- und ΔI-Werte bei gleicher C-Zahl ist an den Retentionsdaten in Tabelle II, Abschnitt 3, noch einmal deutlich zu erkennen. Geht man vom Cyclooctan aus und verengt den Ring bei konstanter C-Zahl, so sieht man sowohl bei den ΔI- wie bei den H^S-Werten einen starken Sprung beim Übergang zum 6-Ring, der allerdings teilweise durch die neu hinzukommende Methylsubstitution verursacht wird. Offenbar wird aber auch die Sonderstellung des 3-Rings. Das *n*-Pentylcyclopropan hat im Verhältnis zu seinem H^S-Wert einen sehr hohen ΔI-Wert. Der Quotient $\Delta I/H^S$ ist gleich 3.5, für Methylcycloheptan dagegen 0.7 und deutet auf den hohen Grad an π-Charakter der 3-Ringelektronen hin. Der Quotient ist gut geeignet, um in Kohlenwasserstoffgemischen Verbindungen mit 3-Ringen zu identifizieren.

Mono- und Dialkylcyclohexane

Mit steigender Länge der Paraffinkette am 6-Ring ergibt sich wieder eine monotone Zunahme der H^S-Werte, wobei der Äthylverbindung — wie so oft bei den Kohlenwasserstoffen — eine Sonderstellung zukommt. Die Abnahme des H^S-Wertes vom Cyclohexan zu den Alkylcyclohexanen um etwa 30 bis 35 IE entspricht etwa der Erniedrigung des Index von *n*-Paraffinen durch eine Methylverzweigung in der Kettenmitte, (vgl. wiederum Tabelle I in Teil II). Auffallend ist die geringe Abnahme des H^S-Wertes beim Übergang vom *n*-Propylcyclohexan zum Isopropylcyclohexan um etwa 9 Einheiten, während beim Übergang vom *n*- zum Isobutylcyclohexan der H^S-Wert um 46 Einheiten abnimmt, was über die Abnahme, die durch eine Methylverzweigung in einem Paraffin hervorgerufen wird, weit hinausgeht. Für den Übergang vom 1-Cyclohexyldekan zum 2-Cyclohexyldekan findet man eine Abnahme von 27 Einheiten.

Die ΔI-Werte liegen im System S–EMO bei etwa 35 Einheiten, nur für das Cyclohexan selbst wird ein grösserer Wert von 42 Einheiten gefunden.

Bei der Auftragung der I^S-Werte gegen die Siedepunkte erhält man nur sehr geringe Abweichung von der Linearität, so dass die Siedepunkte zur Zuordnung von Cyclohexanen herangezogen werden können.

Indexwerte von Alkylcyclohexanen mit wechselnder Stellung des 6-Rings in der Paraffinkette wurden in den C-Zahlbereichen von C_{11} bis C_{19} gemessen. Für diese Verbindungen kam natürlich Squalan als apolare Phase wegen seiner hohen Flüchtigkeit bei den erforderlichen Arbeitstemperaturen nicht in Frage. An seiner Stelle wurde Apiezon L benutzt. Da die Selektivität des EMO für Stellungsisomerien in Kohlenstoffketten nicht sehr ausgeprägt ist, wurde als polare Phase der Polyphenyläther OS 138 gewählt, dessen Polarität gegenüber dipolhaltigen Molekülen allerdings geringer ist als diejenige des EMO.

An dieser Stelle sei darauf hingewiesen, dass die Beurteilung der "Polarität" apolarer stationärer Phasen besonders schwierig ist, wie an den Beispielen in Tabelle III demonstriert sei: Die Zahlen bedeuten Indexdifferenzen zwischen den angegebenen Stoffpaaren.

Die Phase Siliconöl DC 200 ist gegenüber Benzol (Kohlenwasserstoff mit π-Elektronen) weniger polar als Apiezon L, gegenüber Hexancarbonsäureester und gegen Olefine stärker polar als Apiezon L. Vom Autor wird derjenigen Phase die grösste "Apolarität" zugeschrieben, die gegenüber Olefinen *und* sauerstoffhaltigen Verbindungen die niedrigsten Indexwerte liefert. Cyclische Kohlenwasserstoffe wie

TABELLE III

I	*Cyclooctan–n-Octan*	*1-Nonen–n-Nonan*	*Benzol–n-Hexan*	*Hexan-1-carbonsäuremethylester–n-Octan*
Squalan	+133	—18	+52	+64
Apiezon L	+156	—14	+82	+77
Siliconöl DC 200	+125	—12	+58	+104
OS 138	+225	+21	+225	+271
EMO	+202	+24	+257	+286

das Cyclooctan zeigen in Siliconöl DC 200 allerdings einen niedrigeren H^S-Wert als in Squalan. Da bei der Dispersionswechselwirkung zwischen apolaren Substanzen und apolaren Phasen sterische Faktoren die ausschlaggebende Rolle spielen, ist eine einheitliche Definition des Begriffes "Apolarität" ohne Berücksichtigung auch der sterischen Eigenheiten der beteiligten Moleküle nicht möglich. Beim Vergleich der polaren Phasen OS 138 und EMO, die im Sinne des definierten Polaritätsbegriffes ähnliche Polarität zeigen, fällt auf, dass das Cyclooctan in OS 138 einen extrem hohen H^S-Wert zeigt, zu dessen Deutung die sterischen Eigenheiten der stationären Phase (Phenylgruppen) herangezogen werden müssen.

Stellungsisomere Cyclohexylalkane

In den I^A- bzw. H^A-Werten, Tabelle IV, tritt die Cyclohexylgruppe mit einem konstanten inkrementellen Beitrag zum Retentionsindex in Erscheinung, der aber stärker als die ΔI-Werte von der Stellung in der Kohlenstoffkette und somit von der Geometrie des Moleküls abhängt (Tabelle V).

Der indexerhöhende Einfluss des Cyclohexanringes wird also durch den Effekt der Verzweigung kompensiert, jedoch ist die starke H^A-Abnahme zum 4-Isomeren damit allein nicht zu erklären. Hierzu müssen wiederum die sterischen Eigenheiten des gesamten Moleküls, besonders aber der Raumbedarf der Cyclohexylgruppe diskutiert werden.

Auch in den ΔI-Werten tritt der 6-Ring inkrementell in Erscheinung:

	ΔI
1-Cyclohexyldekan	49
2-Cyclohexyldekan	43
3-Cyclohexyldekan	36
4-Cyclohexyldekan	31

Schreibt man dem 6-Ring eine Polarität im Sinne einer Doppelbindung zu, so ist die Abnahme der ΔI-Werte durch verstärkte Abschirmung des 6-Ringes gegen die Dipole der Moleküle der polaren stationären Phase deutbar. Beim 4-Cyclohexyldekan wird die Cyclohexylgruppe z.B. durch einen Propyl- und einen Hexylrest, beim 2-Cyclohexyldekan durch eine Methylgruppe und einen Octylrest abgeschirmt. Von Butylresten an bleibt die Abschirmung einer polaren funktionellen Gruppe mit steigender Kettenlänge konstant, während sie von Methyl nach Butyl stark zunimmt, vgl. KOVATS[1] und SCHOMBURG[3].

TABELLE IV

	$I^{Ap}_{200°}$	$I^{OS}_{200°}$	ΔI
1-Cyclohexylheptan	1385	1423	38
2-Cyclohexylheptan	1362	1400	38
3-Cyclohexylheptan	1335	1373	38
4-Cyclohexylheptan	1321	1356	35
1-Cyclohexyldekan	1686	1735	49
2-Cyclohexyldekan	1659	1702	43
3-Cyclohexyldekan	1625	1661	36
4-Cyclohexyldekan	1592	1624	32
5-Cyclohexyldekan	1577	1608	31
2-Cyclohexylundekan	1760	1801	41
3-Cyclohexylundekan	1723	1759	36
4-Cyclohexylundekan	1689	1720	31
5-Cyclohexylundekan	1671	1702	31
6-Cyclohexylundekan	1666	1696	30
2-Cyclohexyldodekan	1859	1899	40
3-Cyclohexyldodekan	1822	1859	37
4-Cyclohexyldodekan	1787	1819	32
5-Cyclohexyldodekan	1768	1801	33
6-Cyclohexyldodekan	1760	1789	29
2-Cyclohexyltridekan	1959	2000	41
3-Cyclohexyltridekan	1921	1960	39
4-Cyclohexyltridekan	1886	1919	33
5-Cyclohexyltridekan	1866	1897	31
6-Cyclohexyltridekan	1856	1886	30
7-Cyclohexyltridekan	1854	1883	29

Ap = Apiezon L.
OS = Polyphenyläther OS-138.

Als praktische Folgerung aus dem Vergleich der strukturellen Einflüsse auf die H^A- und die ΔI-Werte ergibt sich, dass für die Charakterisierung solcher Isomerer H^A-Werte besser geeignet sind als ΔI-Werte. Trotzdem können die ΔI-Werte zumindest zur Charakterisierung der 1- und der 2-Cyclohexylalkane eine wertvolle Hilfe sein. Die Unterscheidung solcher Isomerer ist eine Stärke der gaschromatographischen Methodik und kann eine wertvolle Ergänzung für die NMR- und die MS-Spektroskopie sein.

TABELLE V

	H^{Ap}	$\delta(H^{Ap})$			*Inkrement für Methylverzweigung*		
1-Cyclohexylalkan	+ 86	26	63	98	— 36	— 30	— 40
2-Cyclohexylalkan	+ 60						
3-Cyclohexylalkan	+ 23						
4-Cyclohexylalkan	— 12						

Dimethylcycloalkane mit 5-, 6-, 7- und 8-Ringen (Tabelle VI)

Von diesen Verbindungen werden nur die I^S- bzw. H^S-Werte behandelt, da einerseits die vielen möglichen Isomeren in polaren stationären Phasen ohnehin nicht getrennt werden können und andererseits die ΔI-Werte keine besondere Information über die Struktur liefern. Von den Dimethylcyclohexanen wurden aber doch ΔI-Werte gemessen, die sich in Tabelle II, Abschnitt 2, befinden. Von den Dimethylcyclohexanen liegen *trans,cis*-Gemische einzelner Isomerer als Vergleichssubstanzen vor, die durch Hydrierung der entsprechenden Xylole dargestellt worden waren, während einzelne Dimethylcyclooctane aus stereospezifischen Dimerisationen von Isopren und Piperylen verfügbar waren. Sie wurden dem Autor von P. HEIMBACH zugänglich gemacht und entstammten Arbeiten von WILKE UND HEIMBACH[4]. Für alle anderen Isomeren wurden die Indexwerte an Gemischen aus MIR* mit den entsprechenden

TABELLE VI

	Kp. (°C)	$IS_{70°}$		*Kp.* (°C)	$IS_{70°}$
1,1-Dimethylcyclopentan	87.8	677	1,1-Dimethylcyclohexan	119.5	792
trans-1,2-Dimethylcyclopentan	91.9	693	*trans*-1,2-Dimethylcyclohexan	123.4	807
cis-1,2-Dimethylcyclopentan	99.5	725	*cis*-1,2-Dimethylcyclohexan	129.7	836
trans-1,3-Dimethylcyclopentan	91.7	690	*trans*-1,3-Dimethylcyclohexan	124.5	810
cis-1,3-Dimethylcyclopentan	90.8	686	*cis*-1,3-Dimethylcyclohexan	120.1	789
Äthylcyclopentan	103.5	738	*trans*-1,4-Dimethylcyclohexan	119.4	789
			cis-1,4-Dimethylcyclohexan	124.3	810
			Äthylcyclohexan	131.8	839
1,1-Dimethylcycloheptan		916.5	1,1-Dimethylcyclooctan		1046
trans-1,2-Dimethylcycloheptan		941	*trans*-1,2-Dimethylcyclooctan		1069
cis-1,2-Dimethylcycloheptan		961	*cis*-1,2-Dimethylcyclooctan		1075
trans-1,3-Dimethylcycloheptan		927	*trans*-1,3-Dimethylcyclooctan		1046
cis-1,3-Dimethylcycloheptan		920.5	*cis*-1,3-Dimethylcyclooctan		1044
trans-1,4-Dimethylcycloheptan		922	*trans*-1,4-Dimethylcyclooctan		1052
cis-1,4-Dimethylcycloheptan		926	*cis*-1,4-Dimethylcyclooctan		1054
Äthylcycloheptan		969	*trans*-1,5-Dimethylcyclooctan		1055
			cis-1,5-Dimethylcyclooctan		1049
			Äthylcyclooctan		1092

Methylcyclanen gewonnen. Hierbei fielen ausserdem die entsprechenden Äthylcyclane an. Die Zahl und die Verteilung der Isomeren in den MIR-Gemischen ergeben sich aus Tabelle VII. Hinzu kommt in jedem C-Zahlbereich das entsprechende Äthylcyclan.

Die Zuordnung der Peaks in den an den MIR-Mischungen erhaltenen Chromatogrammen war mit Hilfe von Vergleichssubstanzen, Siedepunktsangaben und über Massenspektren nur gesichert bei den Dimethyl-C_5-, C_6- und C_8-Verbindungen. Bei den Dimethylcycloheptanen bestehen für die Zuordnung noch gewisse Unsicherheiten. Bei der Zuordnung wurden folgende Schlüsse gezogen:

Die Äthylverbindungen haben jeweils den grössten Retentionsindex, die 1,1-Dimethylcyclane den niedrigsten.

* MIR = "methylene insertion reaction".

TABELLE VII

	DMCP	*DMCH*	*DMCHP*	*DMCO*
1,1	1	1	1	1
1,2 *trans*	2	2	2	2
1,2 *cis*	2	2	2	2
1,3 *trans*	2	2	2	2
1,3 *cis*	2	2	2	2
1,4 *trans*		1	2	2
1,4 *cis*		1	2	2
1,5 *trans*			1	2
1,5 *cis*			1	2
Isomerenzahl	5	7	9	9

DMCP = Dimethylcyclopentan; DMCH = Dimethylcyclohexan; DMCHP = Dimethylcycloheptan; DMCO = Dimethylclooctan.

Aus Siedepunktsangaben folgt: Die 1,2-Dimethylcyclane haben die höchsten Indexwerte, die Aufspaltung zwischen *cis*- und *trans*-Isomeren ist gross. Die Reihenfolge der isomeren Dimethylcyclopentane und -hexane (API-Werte).

Die Zuordnung der anderen Isomeren geschah bei den Dimethylcyclopentanen ausserdem massenspektrometrisch unter Verwendung von API-Spektren, bei den Dimethylcyclohexanen und -octanen mit Hilfe von Vergleichssubstanzen. Die Dimethylcycloheptane konnten nur durch Analogieschlüsse zugeordnet werden. Schliesslich wurde die statistische Isomerenverteilung bei der MIR zur Zuordnung herangezogen. Immer wurde die erwartete Verteilung quantitativ gefunden. Auf diesem Wege konnte besonders die Zuordnung der 1,1-Dimethylcyclane des 1,4-Dimethylcyclohexans und des 1,5-Dimethylcycloheptans sichergestellt werden. Auch bei den Dimethylcyclanen wurden bei der Zuordnung über die Siedepunkte Abweichungen von der Proportionalität zwischen Siedepunkt und Retentionsindex beobachtet (Tabelle VIII).

TABELLE VIII

	I^S	δI^S		*Kp.* (°C)	$\delta(Kp.)$
		gef.	*ber.*		
trans-1,2-Dimethylcyclopentan	693	3	~ 1	91.9	0.2
trans-1,3-Dimethylcyclopentan	690			91.7	
1,1-Dimethylcyclohexan	792	3	~ 0.5	119.5	0.1
trans-1,4-Dimethylcyclohexan	789			119.4	

Aufschlüsse über die Beweglichkeit der Ringsysteme gibt der Indexunterschied zwischen den *trans*- und den *cis*-Isomeren (Tabelle IX).

Die *cis*-1,3-Verbindungen und das *cis*-1,5-Dimethylclooctan haben eine höhere Flüchtigkeit als die *trans*-1,3-Verbindung. Vergleicht man die H^S-Werte der Dimethylcyclane mit denen der Monomethylcyclane, so stellt man fest, dass auch die Einführung der zweiten Methylgruppe in den Ring eine ähnliche Indexabnahme bewirkt wie eine Methylverzweigung in einer Paraffinkette (vgl. Tabelle X).

TABELLE IX

I^S (*cis*-ISOMERES) — I^S (*trans*-ISOMERES)

	Dimethylcyclane			
	1,2	*1,3*	*1,4*	*1,5*
5-Ring	+ 32	— 4		
6-Ring	+ 29	—21	+ 21	
7-Ring	+ 20	— 6	+ 4	
8-Ring	+ 6	— 2	+ 2	— 6

Jede Methylgruppe erniedrigt den H^S-Wert somit um etwa 40 IE. Für das 4-Methylheptan findet man eine Abnahme von 34 IE gegenüber dem *n*-Octan. Der Grund für diese Parallelität liegt in der Beweglichkeit der Ring-C-Atome.

TABELLE X

	H^S	*Abnahme* $\delta(H^S)$
Cyclooctan	140	
Methylcyclooctan	100	40
Dimethylcyclooctane	55–60	40–45
Cycloheptan	116	
Methylcycloheptan	70	46
Dimethylcycloheptane	25–30	40–45

CYCLANE UND ALKYLCYCLANE MIT EINER ODER MEHREREN DOPPELBINDUNGEN

Es ist zu erwarten, dass bei der Einführung von Doppelbindungen in Ringe nicht mit einer einfachen Additivität von Indexinkrementen für Ring und Doppelbindung gerechnet werden kann. Die komplizierte Geometrie und die spezielle Beweglichkeit der C-Atome besonders in den sog. mittleren Ringen wird durch die Aufhebung der freien Drehbarkeit zwischen den C-Atomen einer Doppelbindung durchgreifend beeinflusst.

Das Auftreten von *cis,trans*-Isomeren bei den Cyclanen ist nur von einer Mindestgrösse des Ringes an möglich. So gibt es zwar *trans*- und *cis*-Cycloocten, allerdings ist das erstere unbeständig und zeigt einen höheren Siedepunkt als die *cis*-Verbindung. Tabelle XI zeigt eine Zusammenstellung der I- und ΔI-Werte der Cyclene und Methylcyclene unter Berücksichtigung der *cis,trans*-Isomerie. Allerdings ist die Zuordnung der *cis,trans*-Isomeren bei den Methylcyclenen nicht ganz sicher.

Diskussion

1. Mit steigender C-Zahl ist die Indexzunahme pro C-Atom bis zum neuen Ring grösser als 100, um zu grösseren Ringen hin wieder abzunehmen, so dass sich sogar Werte unter 100 ergeben.

2. Die *trans*- und *cis*-Isomeren unterscheiden sich in apolaren Phasen im Index um etwa 5 bis 10 Einheiten. Bei den mittelständigen langkettigen Olefinen, wie z.B. dem Undecen-5, wird dagegen kein nennenswerter Unterschied gefunden.

TABELLE XI

	I^S		I^{EMO}		ΔI		H^S	
	80°	120°	80°	120°	80°	120°	80°	120°
Cyclopenten	552	559	635	649	83	90	52	59
Cyclohexen	681	689	769	784	88	95	81	89
Cycloheptan	785	800	878	900	93	100	85	100
trans-Cycloocten	899							
cis-Cycloocten	895	913	993	1019	98	106	95	113
trans-Cyclononen		1024		1146		122		124
cis-Cyclononen		1029		1147		118		129
trans-Cyclodecen		1122		1239		117		122
cis-Cyclodecen		1130		1251		121		130
trans-Cycloundecen		1214		1330		116		114
cis-Cycloundecen		1223		1346		123		123
trans-Cyclododecen		1306		1421		115		106
cis-Cyclododecen		1315		1440		125		115
1-Methylcyclopenten	647	660	724	737	77	77		60
1-Methylcyclohexen	769	781	853	865	84	84		81
1-Methylcycloheptan	863	874	947	969	84	95		74
1-Methylcycloocten	969	983	1059	1084	90	101		83
1-Methylcyclononen		1102		1214		112		102
trans-1-Methylcyclodecen / *cis*-1-Methylcyclodecen *		1200		1316		116		100
trans-1-Methylcycloundecen / *cis*-1-Methylcycloundecen *		1292		1409		117		92
		1295		1413		118		95
trans-1-Methylcyclododecen		1382		1500		118		83
cis-1-Methylcyclododecen		1392		1513		121		92

* Die Klammern sollen andeuten, dass die Zuordnung des *trans*- und des *cis*-Isomeren fraglich ist.

3. Die H^S-Werte (gegenüber *n*-Alkanen gleicher C-Zahl) zeigen wie diejenigen der Cyclane Maximalwerte für den 9- und 10-Ring, der auf einen Enthalpieüberschuss als Folge der grossen Beweglichkeit der Ring-C-Atome zurückzuführen ist.

4. Die ΔI-Werte steigen bis zum 9-Ring an, um mit steigender Ringzahl konstant zu werden. Sie spiegeln zwei Einflüsse wider, den des gesättigten Ringes und den der Doppelbindung. Ein hoher ΔI-Wert wird für das Cyclohexen beobachtet, der z.T. auf der ausgeprägten *cis*-Konfiguration der Doppelbindung, die sich im 6-Ring ausbildet, beruht.

Eliminiert man den Einfluss des Ringes auf die I-Werte, wie in Tabelle XII geschehen, so sieht man, dass eine Doppelbindung im Ring 25–35 IE zum ΔI-Wert beiträgt. Für die *cis*-Cyclene mit 5- bis 8-Ring-C-Atomen findet man 35, für die *trans*-Cyclene vom 9-Ring an 38 bis 23 Einheiten mit abnehmender Tendenz. *trans*-Cyclononen hat einen höheren ΔI-Wert als das *cis*-Cyclononen, was auf die geringe Symmetrie zurückgeht. Die ΔI-Werte mittelständiger, offenkettiger Olefine sind vergleichsweise höher und liegen bei etwa 40 IE. In der gleichen Tabelle finden sich auch die H^S-Werte, die sich durch Homomorphiebetrachtung zwischen den Cyclenen und den entsprechenden Cyclanen ergeben. Mit Ausnahme des Cyclohexens findet man bei den einfachen Cyclenen negative Werte, die in der Grössenordnung der α-Olefine bzw. der mittelständigen Olefine liegen. Höhere Werte werden nur für den 8-, 9- und 10-Ring gefunden.

TABELLE XII

		δH^S	$\delta(\Delta I)$	$t(°C)$
Cyclopenten	— Cyclopentan	—16	36	80
Cyclohexen	— Cyclohexan	+13	40	80
Cyclohepten	— Cycloheptan	—19	37	80
cis-Cycloocten	— Cyclooctan	—30	33	80
trans-Cyclononen	— Cyclononan	—25	38	120
cis-Cyclononen	— Cyclononan	—20	34	120
trans-Cyclodecen	— Cyclodekan	—25	29	120
cis-Cyclodecen	— Cyclodekan	—17	33	120
trans-Cycloundecen	— Cycloundekan	—21	24	120
cis-Cycloundecen	— Cycloundekan	—12	31	120
trans-Cyclododecen	— Cyclododekan	—19	23	120
cis-Cyclododecen	— Cyclododekan	—10	33	120
Methylcyclopenten	— Methylcyclopentan	+12	45	80
Methylcyclohexen	— Methylcyclohexan	+36	50	80
Methylcycloheptan	— Methylcycloheptan	— 6	39	80
Methylcycloocten	— Methylcyclooctan	—16	34	120
Methylcyclononen	— Methylcyclononan	— 4	38	120
cis-Methylcyclodecen	— Methylcyclodekan	— 2	38	120
trans-Methylcycloundecen	— Methylcycloundekan	+ 4	35	120
cis-Methylcycloundecen	— Methylcycloundekan	+ 7	36	120
trans-Methylcyclododecen	— Methylcyclododekan	+ 6	34	120
cis-Methylcyclododecen	— Methylcyclododekan	+12	37	120

Die *I*-Werte der Methylcyclene sind ein wenig niedriger als diejenigen der Cyclene, obwohl der Beitrag der Doppelbindung grösser ist als der des Ringes. Die *trans*- und *cis*-Isomeren zeigen wesentlich geringere Unterschiede in den ΔI-Werten als bei den Cyclenen. Die Reihenfolge der beiden Isomeren ist aber noch nicht sichergestellt.

Der Beitrag der Doppelbindung zum ΔI-Wert, vgl. Tabelle XII, vorletzte Spalte, ist bei den Methylcyclenen erwartungsgemäss etwas grösser. Ausserdem werden kaum noch Unterschiede zwischen den *trans*- und den *cis*-Isomeren beobachtet. Der Einfluss der Methylgruppe auf die intermolekulare Wechselwirkung ist offenbar stärker als derjenige der sterischen Anordnung der Ring-C-Atome. Die H^S-Werte der 1-Methylcyclene sind wesentlich höher als diejenigen der Cyclene, und zwar um etwa 25 IE. Eine Ausnahme stellt nur das 1-Methylcycloocten dar. Für β- und mittelständig methylverzweigte Doppelbindungen beobachtet man bei offenkettigen Kohlenwasserstoffen gegenüber den unverzweigten Typen eine Indexzunahme von 20 bis 25 Einheiten (Tabelle XIII).

TABELLE XIII

	$\delta(H^S)$
2-Methyl-2-hexen → 2-Hepten	23
3-Methyl-3-hexen → 3-Hepten	19
cis-Methylcyclodecen → *cis*-Cyclodecen	30
cis-Methylcycloundecen → *cis*-Cycloundecen	28
cis-Methylcyclododecen → *cis*-Cyclododecen	23

TABELLE XIV

	$I^S_{50°}$	$I^{EMO}_{50°}$	ΔI	H^S
Cyclopenten	554	631	77	—14*
Methylcyclopentan	633	661	28	0
Methylencyclopentan	650	723	73	+17
1-Methyl-1-cyclopenten	647	716	69	+14
3-Methyl-1-cyclopenten	607	668	61	—26
4-Methyl-1-cyclopenten	610	671	61	—23
Bicyclohexan (3,1,0)	670	745	75	
	$I^S_{70°}$	$I^{EMO}_{70°}$	ΔI	H^S
Cyclohexen	677	764	87	+15*
Methylcyclohexan	730	764	34	0
Methylencyclohexan	736	817	81	+ 6
1-Methyl-1-cyclohexen	766	847	81	+36
3-Methyl-1-cyclohexen	736	810	74	+ 6
4-Methyl-1-cyclohexen	739	813	74	+ 9
Bicycloheptan (4,1,0)	796	881	85	
Cyclohepten	782	869	87	—19*
Methylcycloheptan	864	909	45	0
Methylencycloheptan	861	949	88	— 3
1-Methyl-1-cycloheptan	862	941	79	— 2
3-Methyl-1-cycloheptan	847	924	77	—17
4-Methyl-1-cycloheptan	849	927	78	—15
5-Methyl-1-cycloheptan	851	930	79	—13
Bicyclooctan (5,1,0)	885	968	83	
	$I^S_{100°}$	$I^{EMO}_{100°}$	ΔI	H^S
Cycloocten, *cis*	905			—32*
Methylcyclooctan	992	1054	62	0
Methylencyclooctan				
1-Methyl-1-cycloocten	978			—14
3-Methyl-1-cycloocten	958			—31
4-Methyl-1-cycloocten	963			—29
5-Methyl-1-cycloocten	973			—19
Bicyclononan (6,1,0)	1003			

* Bezogen auf die entsprechenden Cyclane.

Mit weiter steigender Ringgrösse nähern sich die H^S-Werte der 1-Methyl-1-cyclene denjenigen der 2-Methyl-1-alkene, die positiv sind. Steht die Methylgruppe nicht an der Doppelbindung, sondern in 3-, 4- oder 5-Stellung — Retentionsdaten in Tabelle XIV — so ergibt sich der gleiche Einfluss der Substitution auf die H^S- und ΔI-Werte wie bei den entsprechenden offenkettigen Typen, vgl. Teil II, S. 16.

H^S-Werte

1. Gegenüber den 1-Methyl-1-cyclenen haben die 3-Methyl-1-cyclene einen wesentlich erniedrigten Index, und zwar:

beim 5-Ring um 40
beim 6-Ring um 30
beim 7-Ring um 15
beim 8-Ring um 20 IE.

2. Beim Übergang der Methylgruppe aus der 3- in die 4-Stellung ergibt sich eine geringe Zunahme des H^S-Wertes um 3 bis 5 IE. Bei den offenkettigen Olefinen des vergleichbaren Typs beträgt die Zunahme 12 bis 14 Einheiten.

3. Die 4- und 5-Methyl-1-cyclene unterscheiden sich nur noch wenig in ihren Eigenschaften, so dass kein eindeutiger Effekt der Stellung der Methylgruppe festzustellen ist.

4. Die exocyclischen Cyclene mit gleichem Kohlenstoffgerüst zeigen H^S-Werte, die beim 5- und 7-Ring denen der 1-Methyl-1-cyclene entsprechen, das Methylencyclohexan hat allerdings einen wesentlich niedrigeren Wert. Die H^S-Werte nehmen vom 5- zum 7-Ring in der Reihe $+17$, $+6$, -3 ab.

5. Die H^S-Werte der Bicycloverbindungen werden an anderer Stelle behandelt.

ΔI-Werte. Die ΔI-Werte lassen sich in erster Näherung als Summe der Beiträge der Ringe und der Doppelbindung auffassen, vgl. hierzu S. 27, und die Tabellen XII und XIV.

1. Der Anteil der Doppelbindung an den ΔI-Werten der 1-Methyl-1-cyclene liegt wie bei den entsprechenden offenkettigen Olefinen bei etwa 30 bis 40 IE, bei den anderen Methylcyclenen bei 35–45 IE.

2. Die ΔI-Werte der 3-, 4- und 5-Methyl-1-cyclene sind niedriger als die der 1-Methyl-1-cyclene, besonders beim 5- und 6-Ring.

3. Die exocyclischen Verbindungen haben die höchsten ΔI-Werte, obwohl die Doppelbindung nur zweifach C–C-substituiert ist. Der Grund hierfür liegt in der guten Zugänglichkeit der Doppelbindung für die intermolekulare Wechselwirkung mit der polaren stationären Phase.

4. Die ΔI-Werte der Bicyclen mit Dreiringen sind grösser als die der ungesättigten Cyclane. Auch dieser Befund spricht dafür, dass π-Elektronen nicht ausschliesslich als Ursache für die "Polarität" dieser Verbindungen angesehen werden dürfen. Es scheint, als ob die Retentionsdaten der Olefine eher durch die veränderten sterischen Verhältnisse interpretierbar sind.

Zusammenfassend kann man sagen, dass die bei den offenkettigen Olefinen gefundenen Regeln das Retentionsverhalten auch der vorstehend behandelten cyclischen Olefine, zumindest qualitativ, zu interpretieren erlauben.

In Tabelle XV sind die Retentionsdaten von Cyclenen mit längeren Alkylgruppen am Ring aufgeführt. Auch in diesen Fällen wird das Retentionsverhalten mit Hilfe weniger Regeln verständlich.

H^S-Werte. Die Werte sind jeweils auf die Indices der entsprechenden gesättigten cyclischen Kohlenwasserstoffe bezogen, *nicht* auf die der *n*-Paraffine.

1. Die Vinylgruppe am Cyclohexanring verhält sich wie bei den offenkettigen Olefinen.

	H^S	
1-Octen	−18	
Vinylcyclohexan	−21	
1-Vinyl-3-cyclohexen	−24	(gegen 1-Äthylcyclo-3-hexen)
Vinylbenzol	+27	(gegen Äthylbenzol)

TABELLE XV

	$I^S_{70°}$	$I^{EMO}_{70°}$	ΔI	$H^S_{70°}$
Äthylcyclohexan	839	875	36	0
Vinylcyclohexan	818	894	76	−21
Äthylidencyclohexan	859	942	83	+20
1-Äthylcyclohexen	861	942	81	+22
1-Äthylcyclo-3-hexen	848	926	78	+ 9
1-Vinylcyclo-3-hexen	824	940	116	−15
Äthylbenzol	840	1026	186	+ 1
Vinylbenzol	867	1118	251	+28
Äthylcyclopentan	738			
Äthylcyclopenten	749	821	72	+11
n-Propylcyclopentan	834	865	31	0
Propylidencyclopentan	853	929	76	+18
1-Propylcyclopenten	839	906	67	+ 5
	$I^S_{120°}$	$I^{EMO}_{120°}$	ΔI	$H^S_{120°}$
1-*n*-Propylcyclohepten	1056	1144	88	
1-*n*-Butylcycloheptan	1171	1227	56	0
1-*n*-Butylcycloheptan	1151	1234	83	−20
1-*n*-Butylidencycloheptan	1163	1254	91	− 8
1-*n*-Propylcycloocten	1166	1259	93	

Der H^S-Wert des 1-Vinyl-3-cyclohexens ist bezogen auf den Index von 1-Äthyl-3-cyclohexen. Der gegenüber 1-Octen um 6 IE erniedrigte H^S-Wert rührt von der Substitution durch die Vinylgruppe in der 4-Stellung zur Ringdoppelbindung her (vgl. 4-Methyl-1-alkene, Teil II). Am aromatischen 6-Ring erhöht sich dagegen der H^S-Wert wegen der Konjugation um 27 IE.

2. Steht die Doppelbindung im Ring, so sind die H^S-Werte denen der entsprechenden Methylverbindungen — und somit auch denen der offenkettigen Olefinen des gleichen Typs — ähnlich.

	H^S		H^S
1-Methyl-1-cyclohexen	+36	3-Methyl-1-cyclohexen	+6
1-Äthyl-1-cyclohexen	+22	3-Äthyl-1-cyclohexen	+9

Weitere Beispiele s. Tabelle XV.

3. Die Alkylidencyclane, also die an der Methylengruppe alkylsubstituierten Methylencyclane haben ähnliche H^S-Werte wie die 1-Alkyl-1-cyclane. Die Doppelbindung ist dreifach C–C-substituiert.

	H^S		H^S
1-Äthyl-1-cyclohexen	+20	1-Butyl-1-cycloheptan	−20
Äthylidencyclohexan	+22	Butylidencycloheptan	−8

ΔI-Werte. Ringe und Doppelbindungen verleihen den ungesättigten Alkylcyclanen eine "Polarität", die charakteristisch vom Substitutionsgrad der Doppelbindung, der Substitution in der Nähe der Doppelbindung und den sterischen Verhältnissen im Molekül, gegeben durch die Ringgrösse und die Konfiguration der Doppelbindung, beeinflusst wird. In diesem Falle haben auch die *ΔI*-Werte eine gewisse Bedeutung für die Identifizierung solcher Verbindungen. Bei der folgenden Diskussion von *ΔI*-Werten wurde der Anteil des gesättigten Ringsystems aus dem *ΔI*-Wert eliminiert. Die sich ergebenden *ΔI*-Differenzen werden als $\delta(\Delta I)$ bezeichnet.

1. Vinylgruppe:

	$\delta(\Delta I)$		*Homomorphes*
1-Vinylcyclohexan	76 — 36 =	40 IE	Äthylcyclohexan
1-Vinyl-3-cyclohexen	116 — 78 =	38 IE	1-Äthyl-3-cyclohexen
Vinylbenzol	251 — 186 =	65 IE	Äthylbenzol

2. In der Doppelbindung substituierte Cyclene haben höhere *ΔI*-Werte, die Alkylidencyclene die höchsten:

	$\delta(\Delta I)$		*Homomorphes*
Äthylidencyclohexan	83 — 36 =	47 IE	Äthylcyclohexan
1-Äthyl-1-cyclohexen	81 — 36 =	45 IE	Äthylcyclohexan
1-Äthyl-3-cyclohexan	78 — 36 =	42 IE	Äthylcyclohexan

3. Ein monosubstituierter aromatischer Ring liefert pro Doppelbindung einen Beitrag von 50 IE zum *ΔI*-Wert.:

	$\delta(\Delta I)$		*Homomorphes*
Äthylbenzol	186 — 36 =	150 IE	(drei Doppelbindungen)

AROMATISCHE KOHLENWASSERSTOFFE (TABELLE XVI)

Die Interpretation der HS-Werte, relativ zu den entsprechenden gesättigten Ringsystemen (Tabelle XVI), wird bei den Dialkylcyclohexanen durch den Einfluss der *cis–trans*-Isomerie erschwert. Für die vergleichenden Betrachtungen wird der Mittelwert der Indexwerte der *trans*- und *cis*-Verbindungen benutzt. Die Unterschiede in den sterischen Eigenheiten der Ringsysteme beim Übergang vom beweglichen des Cyclohexans zum starren des Benzols überlagern die drei Einflüsse, die sich an der Doppelbindung auswirken, nämlich diejenigen der:

1. ungestörten Doppelbindung
2. Konjugation
3. der Substitution.

H^S-Werte

Benzol	−24	
drei Doppelbindungen im Ring		– geringer Einfluss auf den H^S-Wert mittelständige *cis*-Doppelbindungen.
Konjugation		– indexerhöhender Einfluss.
starres Ringsystem mit hoher Symmetrie		– vermindernder Einfluss.
Toluol	+21	
Äthylbenzol	+ 1	
zusätzlich Alkylsubstitution		– indexerhöhender Einfluss.
Styrol	+28	
1,2-Dihydronaphthalin	+48	
eine Vinylgruppe zusätzlich		– vermindernder Einfluss.
Konjugation der Vinylgruppe mit dem aromatischen Ring		– stark erhöhender Einfluss.
o,m,p-Xylole +60 +53 +55		
zusätzlich zweifache Methylsubstitution		– Unterschiede in der Symmetrie der Ladungsverteilung sind die Ursache für die Abnahme der H^S-Werte vom *o*- über *m*- nach *p*-Xylol.

TABELLE XVI

	I^S		I^{EMO}		ΔI
	70°	120°	70°	120°	
Cyclohexan	668		710		42
Benzol	644		843		199
Methylcyclohexan	730		764		34
Toluol	751		942		191
trans-1,2-Dimethylcyclohexan	807		837		30
cis-1,2-Dimethylcyclohexan	836		875		39
o-Xylol	875		1073		198
trans-1,3-Dimethylcyclohexan	810		844		34
cis-1,3-Dimethylcyclohexan	792		816		24
m-Xylol	854		1036		182
trans-1,4-Dimethylcyclohexan	789		814		25
cis-1,4-Dimethylcyclohexan	810		844		30
p-Xylol	856		1040		184
Äthylbenzol	840		1026		186
Styrol	867		1118		251
trans-Dekalin		1081		1158	77
cis-Dekalin		1119		1212	93
9,10-Octalin		1123		1253	130
Tetralin		1150		1400	250
1,2-Dihydronaphthalin		1148		1500	352
1,4-Dihydronaphthalin		1167		1466	299
Naphthalin		1169		1527	358

ΔI-Werte. Strukturelle Einflüsse sind in den *ΔI*-Werten weit weniger charakteristisch wirksam. Auch hier wird der Beitrag des Grundgerüstes eliminiert. Die sich ergebenden $\delta(\Delta I)$-Werte sind Tabelle XXI, Spalte 3, zu entnehmen. Der Beitrag einer Doppelbindung im aromatischen System zum *ΔI*-Wert liegt zwischen 50 (Benzol) und 54 (*o*-Xylol) IE. Die Alkylsubstitution hat einen geringen erhöhenden Einfluss auf die $\delta(\Delta I)$-Werte. Der hohe Wert für das *o*-Xylol entspricht dessen Dipolmoment.

Ungesättigte Derivate des Dekalins

H^S-Werte

	H^S		H^S
9,10-Octalin	+23	2,3-Dimethyl-2-penten	+32
Tetralin	+50	*o*-Xylol	+60
1,2-Dihydronaphthalin	+48	Styrol	+28
1,4-Dihydronaphthalin	+67	Tetralin + Cyclohexen	+50 +12 +62

Auch bei den Naphthalinderivaten ergibt sich, dass Konjugation die H^S-Werte nicht erhöht. Die Ursache für den hohen Wert des 1,4-Dihydronaphthalins ist die gleiche wie beim 1,4-Cyclohexadien (Stabilisierung der Wannenform).

ΔI-Werte. Vgl. hierzu Tabelle XVI und Tabelle XXII oben.

Das Dekalin hat etwa den doppelten *ΔI*-Wert wie das Cyclohexan (85 gegen 42), so dass selbst in kondensierten Ringsystemen der Cyclohexanring bezüglich des *ΔI*-Wertes als strukturelle Einheit angesehen werden kann.

	$\delta(\Delta I)$		$\delta(\Delta I)$
9,10-Octalin	45	1-Methyl-1-cyclohexen + eine weitere C–C-Bindung	+47
Tetralin	165	*o*-Xylol	+163
1,2-Dihydronaphthalin	267	Styrol + eine weitere C–C-Bindung an der Doppelbindung	+215
		Tetralin + Cyclohexen	+165 +46
1,4-Dihydronaphthalin	214		+211

Bei den *ΔI*-Werten ergibt sich also eine gute Additivität der Inkremente. Konjugierte Vinylgruppen am aromatischen Kern zeigen einen charakteristischen Beitrag zum *ΔI*-Wert, während die H^S-Werte nur Einflüsse aufweisen, die die Geometrie des Moleküls verändern, vgl. z.B. 1,4-Dihydronaphthalin und 1,2-Dihydronaphthalin.

	1,4-Dihydronaphthalin	*1,2-Dihydronaphthalin*
H^S	+67	+48
ΔI	214	267

Stellungsisomere Phenylalkane

Zum Ende dieses Abschnitts seien noch die Retentionsdaten stellungsisomerer Phenylalkane diskutiert, die allerdings wegen ihrer höheren Molekulargewichte und entsprechend höherem Siedepunkt nur in dem System Apiezon L–OS 138 gemessen werden konnten. Die Daten sind in Parallele zu setzen zu denen der entsprechenden Cyclohexylderivate von Tabelle XVII. Für die Zuordnung der einzelnen Isomeren lagen

TABELLE XVII

	I^{Ap}		I^{OS}		$\partial I/\partial T$	ΔI 200°
	150°	200°	200°	215°		
1-Phenylheptan	1376	1396	1553	1557	0.4	157
2-Phenylheptan	1300	1320	1469	1473		149
3-Phenylheptan	1266	1285	1427	1431		136
4-Phenylheptan	1260	1278	1415	1419		137
1-Phenyldekan		1694	1851	1856	0.3	157
2-Phenyldekan		1616	1765	1769		149
3-Phenyldekan		1573	1710	1715		137
4-Phenyldekan		1550	1684	1688		134
5-Phenyldekan		1537	1670	1674		133
2-Phenylundekan		1715	1861	1864	0.3	146
3-Phenylundekan		1670	1808	1813		138
4-Phenylundekan		1645	1780	1785		135
5-Phenylundekan		1631	1765	1769		134
6-Phenylundekan		1625	1758	1763		133
2-Phenyldodekan		1813	1960	1960	0.2	147
3-Phenyldodekan		1767	1907	1911		140
4-Phenyldodekan		1741	1879	1881		138
5-Phenyldodekan		1725	1861	1864		136
6-Phenyldodekan		1718	1854	1856		136
2-Phenyltridekan		1911	2058	2059	0.1	147
3-Phenyltridekan		1865	2006	2007		141
4-Phenyltridekan		1838	1977	1978		139
5-Phenyltridekan		1821	1960	1962		139
6-Phenyltridekan		1813	1950	1950.5		137
7-Phenyltridekan		1813	1950	1950		137

Ap = Apiezon L.
OS = Polyphenyläther OS 138.

an Modellsubstanzen nur die vier Phenylheptane und das 1-Phenyldekan vor. Die H^A-Werte können sinnvoll nur gebildet werden unter Eliminierung des C-Zahleinflusses (Tabelle XVIII).

Bei gleicher Stellung der funktionellen Gruppe in der Kohlenstoffkette ist die Abhängigkeit der H^{Ap}-Werte von der Kettenlänge um so geringer, je länger die Alkylgruppen, von der Verzweigungsstelle an gerechnet, sind, vgl. die Werte der 4-Phenylheptane. An dieser Stelle soll nicht diskutiert werden, warum beim Übergang der funktionellen Gruppe aus der 1- in die 2- und aus der 2- in die 3-Stellung für die

TABELLE XVIII

$H^{Ap} = I^{Ap}$ (PHENYLALKAN) — I^{Ap} (n-ALKAN)

C-Zahl	*1-Phenylalkan*	*1-Cyclohexyl-alkan*	*2-Phenylalkan*	*2-Cyclohexyl-alkan*
7	696	685	620	662
10	694	686	616	659
11			615	660
12			613	659
13			611	659
	3-Phenylalkan	*3-Cyclohexyl-alkan*	*4-Phenylalkan*	*4-Cyclohexyl-alkan*
7	585	635	578	621
10	573	625	550	592
11	570	623	545	589
12	567	622	541	587
13	565	621	538	588

Phenyl- und die Cyclohexylgruppe so verschiedene Werte gefunden werden. Sie hängen mit der Geometrie des planaren Benzolrings und der Beweglichkeit des Cyclohexanringes zusammen. Bildet man die H^A-Werte (bezogen auf die entsprechenden Cyclohexanverbindungen), so findet man für die 1-Phenylalkane positive, für die 2-, 3- und 4-Phenylalkane negative Werte. Die negativen Werte sind eine Folge des Kettenverzweigungseffektes, der natürlich durch Art und Grösse (Raumbedarf) des Substituenten beeinflusst wird. Aus diesem Grunde findet man nicht die gleichen Verzweigungsinkremente wie bei den Methylverbindungen.

Die stellungsisomeren Phenylalkane wie auch Cyclohexylalkane werden in der Reihenfolge:

1,2,3,4,5-Phenylalkan,

und nicht wie die Methylalkane in der Reihenfolge:

1,3,2,4,5-Methylalkan

eluiert.

Unterschiede in den H^A-Werten zwischen Stellungsisomeren nehmen natürlich stark ab, je mehr die Phenyl- bzw. Cyclohexylgruppe in die Mitte der Kette wandert, so dass gleichlange Alkylketten an der Verzweigungsstelle stehen.

ΔI-Werte. An den ΔI-Werten ist die Abhängigkeit von der Stellung in der Kette

TABELLE XIX

$\Delta I = I^{OS} - I^{Ap}$

C-Zahl des Alkans	*1-Phenylalkane*	*2-Phenylalkane*	*3-Phenylalkane*	*4-Phenylalkane*
7	157	149	136	137
10	157	149	137	134
11		146	138	135
12		147	140	138
13		147	141	139

ein eindeutiger Abschirmungseffekt der Phenylgruppe gegen die intermolekulare Wechselwirkung mit der polaren stationären Phase erkennbar (Tabelle XIX).

Benzol hat einen ΔI-Wert von 151, Cyclohexan von 46 (80°). Im C_{10}-Bereich findet man ΔI-Werte von 49 für 1-Cyclohexyldekan, von 43 für 2-Cyclohexyldekan und von 36 für 3-Cyclohexyldekan. Die indexerhöhende Wirkung des Benzolrings bzw. des Cyclohexanrings tritt also in den Alkylbenzolen und Cyclohexylalkanen deutlich und quantitativ erfassbar in Erscheinung. Die Abschirmung des Phenylrestes nimmt natürlich zu den mittelständigen Isomeren hin zu, so dass die ΔI-Werte abnehmen. Die Identifizierung stellungsisomerer Verbindungen über den ΔI-Wert ist aber nur bei dem 1-, 2- und 3-ständigen Isomeren möglich. Von der Butylgruppe an ändert sich die abschirmende Wirkung der Alkylgruppen nämlich nicht mehr mit der Kettenlänge, vgl. hierzu KOVATS UND WEHRLI[1] und SCHOMBURG[3].

MEHRFACH UNGESÄTTIGTE CYCLANE MIT 6-, 8-, 10- UND 12-RING-C-ATOMEN

Die einfach ungesättigten Cyclane wurden bereits zu Anfang behandelt. Durch Einführung weiterer Doppelbindungen in die genannten Ringe wird deren Geometrie durchgreifend verändert. Die Beweglichkeit der Ringe nimmt ab, bei den grösseren Ringen bestimmt die Konfiguration der einzelnen Doppelbindungen die sterische Anordnung der Ring-C-Atome. Diese Einflüsse machen sich besonders stark bemerkbar in den H^S-Werten, weniger in den ΔI-Werten.

H^S-Werte. 6-Ring. Die beiden Cyclohexadiene unterscheiden sich stark in ihren H^S-Werten (Tabelle XX).

	H^S
Cyclohexen	+12
1,3-Cyclohexadien	−13
1,4-Cyclohexadien	+26
1,3,5-Cyclohexatrien	−24

Bei dem 1,3-Isomeren sind die Doppelbindungen in Konjugation, der H^S-Wert wird erniedrigt. Bei dem 1,4-Isomeren wird die Wannenform mit ihrer geringen Symmetrie der Ladungsverteilung fixiert, erhöhter H^S-Wert. Das Cyclohexatrien hat den niedrigsten H^S-Wert wegen der Konjugation bzw. der hohen Symmetrie und der Starrheit der C–C-Bindungen.

Der unterschiedliche Einfluss der Konjugation ist nur durch die Symmetrie der sich ausbildenden sterischen Anordnungen erklärbar.

8-Ring. Das Analogon zum 1,4-Cyclohexadien, das 1,5-Cyclooctadien, hat einen ähnlich hohen H^S-Wert und von den drei möglichen Dienen den höchsten. Auch hier haben die konjugierten Typen die niedrigsten H^S-Werte.

	H^S
1,3-Cyclooctadien	−36
1,3,5-Cyclooctatrien	−20
Cyclooctatetraen	−75

TABELLE XX

		I^S		I^{EMO}		ΔI
		70°	120°	70°	120°	
C_6	Cyclohexan	668		710		42
	Cyclohexen	677		764		85
	1,3-Cyclohexadien	655		796		141
	1,4-Cyclohexadien	694		840		146
	Benzol	644		843		199
		80°		80°		
C_8	Cyclooctan	925	940	990	1012	65
	cis-Cycloocten	896	1019	993	1019	97
	trans-Cycloocten	899				
	1,3-Cyclooctadien	889		1023		134
	1,4-Cyclooctadien	895		1034		139
	1,5-Cyclooctadien	915		1074		159
	1,3,5-Cyclooctatrien	905		1100		195
	Cyclooctatetraen	850		1078		228
	3,3,0-Bicyclooctan	870		936		66
	3,3,0-Bicyclo-2-octen	850		950		100
C_{10}	Cyclodekan		1147		1237	90
	trans-Cyclodecen		1122		1241	119
	cis-Cyclodecen		1130		1252	122
	1,6-Cyclodekadien *cis-cis*		1108		1258	150
C_{12}	Cyclododekan		1326		1417	91
	trans-Cyclododecen		1306		1421	115
	cis-Cyclododecen		1315		1440	125
	Cyclododekatrien					
	trans-trans-trans-1,5,9		1256		1428	172
	trans-trans-cis-1,5,9		1279		1464	185
	trans-cis-cis-1,5,9		1294		1490	196
	cis-cis-cis-1,5,9		1290		1493	203

Der Wert des 1,4-Cyclooctadiens von —30 und der geringe Unterschied zum 1,3-Cyclooctadien zeigt an, dass die Konjugation der π-Elektronen einen geringeren Einfluss auf die H^S-Werte hat als die resultierenden sterischen Verhältnisse.

10-Ring. Je grösser die Ringe werden, umsomehr sind auch die Diene mit den entsprechenden offenkettigen Typen gleicher C-Zahl vergleichbar, da durch die Einführung einer weiteren Doppelbindung die sterische Anordnung des Ringes immer weniger beeinflusst wird.

	H^S
1,6-Cyclodekadien *cis,cis*	—39 (2 Doppelbindungen)
4- bzw. 5-Undecen *cis*	—17 (1 Doppelbindung)

12-Ring. Überraschenderweise führt aber entgegen der Beobachtung beim Undecen mit mittelständiger Doppelbindung *trans*- und *cis*-Konfiguration zu unterschiedlichen H^S-Werten.

		H^S *gef.*	H^S *ber.*
Cyclododecen	*trans*	—20	
Cyclododecen	*cis*	—11	
Cyclododecatrien	*trans,trans,trans*	—70	—60
Cyclododecatrien	*trans,trans,cis*	—47	—51
Cyclododecatrien	*trans,cis,cis*	—32	—42
Cyclododecatrien	*cis, cis, cis*	—36	—33

In den isomeren Cyclododecatrienen findet man eine gute Additivität der Doppelbindungsinkremente. Man kann daraus schliessen, dass das Cyclododecatrien so beweglich ist, dass sowohl *trans*- wie auch *cis*-Konfiguration sich ungehindert einstellen können.

ΔI-Werte. Wird der Einfluss der Ringsysteme wiederum abgetrennt, so erhält man folgende *ΔI*-Inkremente für die Doppelbindung, vgl. Tabelle XXI und XXII.

TABELLE XXI

		H^S	$\delta(\Delta I)$	$\delta(\Delta I)$/ *Doppelbindung*
1-Octen	— *n*-Octan	—18	40	40
Cyclohexen	— Cyclohexan	+12	43	43
1,3-Cyclohexadien	— Cyclohexan	—13	99	50
1,4-Cyclohexadien	— Cyclohexan	+26	104	52
Benzol	— Cyclohexan	—24	157	52
1-Methylcyclohexen	— Methylcyclohexan	+36	50	50
Toluol	— Methylcyclohexan	+21	157	52
o-Xylol	— *trans*-1,2-Dimethylcyclohexan	+68	168	56
o-Xylol	— *cis*-1,2-Dimethylcyclohexan	+39	159	53
m-Xylol	— *trans*-1,3-Dimethylcyclohexan	+44	148	49
m-Xylol	— *cis*-1,3-Dimethylcyclohexan	+62	158	53
p-Xylol	— *trans*-1,4-Dimethylcyclohexan	+67	159	53
p-Xylol	— *cis*-1,4-Dimethylcyclohexan	+46	154	51
Vinylcyclohexan	— Äthylcyclohexan	—21	40	40
Äthylidencyclohexan	— Äthylcyclohexan	+20	47	47
1-Äthylcyclohexen	— Äthylcyclohexan	+32	45	45
1-Äthylcyclo-3-hexen	— Äthylcyclohexan	+9	42	42
1-Vinylcyclo-3-hexen	— Äthylcyclohexan	—15	80	40
Äthylbenzol	— Äthylcyclohexan	+1	150	50
Styrol	— Äthylcyclohexan	+28	215	54
Styrol	— Äthylbenzol	+27	65	65
1-Propylcyclopenten	— *n*-Propylcyclopentan	+5	36	36
1-Propylidencyclopenten	— *n*-Propylcyclopentan	+18	45	45
Methylencycloheptan	— Methylcycloheptan	—3	47	47
1-Methylcycloheptan	— Methylcycloheptan	—3	40	40

Temperatur: 70°.

40–50 IE für Doppelbindungen in offenkettigen Olefinen.
45–55 IE für eine Doppelbindung in Sechsringverbindungen.
65 IE für Vinylgruppe an aromatischen Kernen.
30 IE für Doppelbindungen in höheren Ringen.

Das 1,5-Cyclooctadien hat von den höheren Cyclenen den höchsten $\delta(\Delta I)$-Wert/Doppelbindung, das 1,3-Cyclooctadien vergleichsweise einen sehr niedrigen. Je mehr das Ringsystem eingeebnet wird, um so grösser werden die ΔI-Werte.

TABELLE XXII

		$t°$ (°C)	H^S	$\delta(\Delta I)$	$\frac{\delta(\Delta I)}{\text{Doppelbindung}}$
9,10-Octalin	— *trans*-Dekalin	120	+42	53	45
9,10-Octalin	— *cis*-Dekalin	120	+ 4	37	
Tetralin	— *trans*-Dekalin	120	+69	173	55
Tetralin	— *cis*-Dekalin	120	+31	157	
1,2-Dihydronaphthalin	— *trans*-Dekalin	120	+67	275	67
1,2-Dihydronaphthalin	— *cis*-Dekalin	120	+29	259	
1,4-Dihydronaphthalin	— *trans*-Dekalin	120	+86	222	53·5
1,4-Dihydronaphthalin	— *cis*-Dekalin	120	+48	206	
Naphthalin	— *trans*-Dekalin	120	+88	281	54·5
Naphthalin	— *cis*-Dekalin	120	+50	265	
cis-Cycloocten	— Cyclooctan	80	−29	32	32
trans-Cycloocten	— Cyclooctan	80	−26		
1,3-Cyclooctadien	— Cyclooctan	80	−36	69	35
1,4-Cyclooctadien	— Cyclooctan	80	−30	74	37
1,5-Cyclooctadien	— Cyclooctan	80	−10	94	47
1,3,5-Cyclooctatrien	— Cyclooctan	80	−20	130	43
Cyclooctatetraen	— Cyclooctan	80	−75	163	41
trans-Cyclodecen	— Cyclodekan	120	−25	−29	30
cis-Cyclodecen	— Cyclodekan	120	−17	−32	
1,6-Cyclodekadien *cis, cis*	— Cyclodekan	120	−39	60	30
trans-Cyclododecen	— Cyclododekan	120	−20	24	29
cis-Cyclododecen	— Cyclododekan	120	−11	34	
trans-trans-trans-1,5,9-Cyclododekatrien	— Cyclododekan	120	−70	81	27
trans-trans-cis-1,5,9-Cyclododekatrien	— Cyclododekan	120	−47	94	31
trans-cis-cis-1,5,9-Cyclododekatrien	— Cyclododekan	120	−32	105	35
cis-cis-cis-1,5,9-Cyclodekatrien	— Cyclodekan	120	−36	112	37

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ZUSAMMENFASSUNG

Die Retentionsindices einer grösseren Zahl von gesättigten und ungesättigten cyclischen Kohlenwasserstoffen mit 3, 5, 6, 7, 8, 9, 10, 11- und 12-gliedrigen Ringen wurden in Kapillarsäulen mit apolaren und polaren stationären Flüssigkeiten bestimmt

und die H- bzw. ΔI-Werte auf inkrementell erfassbare strukturelle Einflüsse untersucht. Wegen der besonderen sterischen Verhältnisse bei den Ringen sind allerdings die Möglichkeiten einer inkrementellen Berechnung oder Abschätzung von Retentionsindices gegenüber den offenkettigen Kohlenwasserstoffen eingeschränkt. Dafür haben — auch die gesättigten — cyclischen Kohlenwasserstoffe charakteristische ΔI-Werte, die jedoch vom Substitutionsgrad abhängen. Durch Einführung von Doppelbindungen in die Ringsysteme ergeben sich wegen der Verminderung der Beweglichkeit komplizierte sterische Verhältnisse, die einen nicht leicht zu übersehenden Einfluss auf die intermolekulare Wechselwirkung und damit auf die Retentionsdaten haben. Trotzdem lassen sich für die Abschätzung von Retentionsindices Regeln aufstellen, die die Zuordnung gaschromatographisch getrennter isomerer Mischungspartner mit Ringen im Molekül gestatten.

SUMMARY

The retention indices of a relatively large number of saturated and unsaturated cyclic hydrocarbons with 3, 5, 6, 7, 8, 9, 10, 11 and 12-membered rings were determined in capillary columns with non-polar and polar stationary liquid phases and the H and ΔI values were examined to find out whether the influence of structural elements produces increments. Because of the particular steric factors that are involved in the case of rings, the possibilities of incremental calculation or estimation of retention indices are limited as compared with open-chain hydrocarbons. On the other hand, cyclic hydrocarbons — also the saturated ones — have characteristic ΔI values, which, however, depend on the degree of substitution. Introduction of double bonds in the ring systems produces complicated steric relationships due to the decrease in mobility and these have a considerable influence on the intermolecular interaction and consequently on the retention indices. Nevertheless, rules for the estimation of retention indices can be established, which permit the characterization of isomeric cyclic compounds that have been separated by gas chromatography.

LITERATUR

1 E. KOVATS UND A. WEHRLI, *Helv. Chim. Acta*, 42 (1959) 2709.
2 J. D. DUNITZ UND V. PRELOG, *Angew. Chem.*, 72 (1960) 896.
3 G. SCHOMBURG, *J. Chromatog.*, 14 (1964) 157.
4 G. WILKE UND P. HEIMBACH, unveröffentlicht.

ÜBER DIE PROGRAMMIERTE GASCHROMATOGRAPHIE

I. ETAPPENMÄSSIGE PROGRAMMIERUNG DES TRÄGERGASES UNTER ISOTHERMISCHEN VERHÄLTNISSEN

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Die Gaschromatographie, diese bedeutende Errungenschaft der modernen chemischen Analyse, kann durch die vielversprechende Technik der Programmierung eine erhebliche, neuartige Erweiterung verbuchen. Als Vorläufer dieser neuen Technik sind LIPSKY, LANDOWNE UND LOVELOCK, weiterhin, WOLFF UND WOLFF zu betrachten, die 1959–60 bei der gaschromatographischen Analyse von Fettsäuren verschiedene Trägergasgeschwindigkeiten benutzten[1,2]. Ihre ursprünglichen Mitteilungen erschienen nicht in chromatographischen Fachblättern, und die Referate vernachlässigten die Hervorhebung der neuartigen Methodik weshalb diese wichtige Neuerung auch in Fachkreisen ziemlich unbekannt blieb.

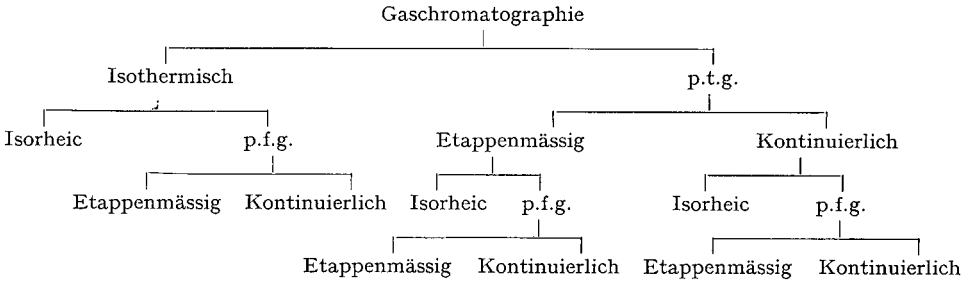
Ähnlich wiederfuhr es den wichtigen Veröffentlichungen von VALUSSI UND COFLERI[3], VERGNAUD[4] und von MORGANTINI[5] aus dem Jahr 1962 in welchen die theoretischen und praktischen Fragen der neuen Technik und die Möglichkeit ihrer mathematischen Behandlung erwogen und daneben eine einfache Einrichtung zur praktischen Verwirklichung beschrieben wurden. MORGANTINI benutzte zuerst die Benennung "Gaschromatographie mit programmierter Strömung".

Grösseres Interesse fand diese Technik nachdem PURNELL[6] in seinem 1962 erschienenen Buch auf die Möglichkeiten der Programmierung hinwies. Die theoretische und praktische Bearbeitung dieses Gebiets beschleunigte sich nun. Unter den Forschern die sich damit erfolgvoll befassten sind SCOTT[7], COSTA NETO, KÖFFER UND DE ALENCAR[8,9], CLARKE[10], weiterhin ZLATKIS, FENIMORE, ETTRE UND PURCELL[11] zu nennen.

Unter den praktischen und theoretischen Problemen der neuen Methodik schien es uns ratsam, zuerst die Frage der etappenmässigen Trägergasprogrammierung unter isothermischen Verhältnissen zu untersuchen, wobei wir uns auf eine frühere Arbeit stützten[12]. Es gelang uns dabei die Programmierung des Eintrittsdrucks des Trägergases unter isothermischen Verhältnissen auszuführen.

Bevor wir in die eingehende Besprechung eingehen soll versucht werden die verschiedenen technischen Möglichkeiten der Flüssigkeit–Gas Elutionschromatographie (im Folgenden Gaschromatographie) zusammenzufassen. In der Tabelle I wurden in Analogie zur Abkürzung der Chromatographie mit programmierter Temperatur p.t.g. (programmed temperature gas chromatography) die Bezeichnung p.f.g. für programmierte Strömungschromatographie (programmed flow gas chromato-

TABELLE I



graphy) angewandt, weiterhin die Bezeichnung "isorheic" für Vorgänge unter konstanter Strömung übernommen[8,9].

Die Trägergasprogrammierung unter isothermischen Verhältnissen kann entweder beschleunigend oder verlangsamend wirken. Ein Programm unter Voraussetzung $p_{ein_i} < p_{ein_{(i-1)}}$ gibt eine verlangsamende, ein Programm unter Voraussetzung $p_{ein_i} > p_{ein_{(i-1)}}$ eine beschleunigende Programmierung. Da bei der nur selten zur Anwendung kommenden verlangsamenden Programmierung keine Gefahr der Übereinanderprogrammierung der Komponenten besteht, erfordert die Ausarbeitung und Verwirklichung eines solchen Programms keine besondere Überlegung, allein die Analysezeitdauer muss berücksichtigt werden. Mit dieser Art der Programmierung befasst sich deshalb diese Mitteilung nicht.

Eine beschleunigende etappenmässige Trägergasprogrammierung unter isothermischen Verhältnissen, im folgenden kurz nur Trägergasprogrammierung genannt, lässt sich erfolgvoll anwenden wenn man über ein solches unter Normalumständen erhaltenes Chromatogramm verfügt, das abgesehen von der Analysezeitdauer entsprechend ist. Die Normalverhältnisse sind definiert durch T = konst., W_0 = konst. und j_0 = konst. Wir halten diese Definition glücklicher als die von uns[12] früher vorgeschlagene, da j_0 besser die Druckverhältnisse representiert als das dort vorgeschlagene p_{ein}.

Nach Auswahl der geeigneten Normalaufnahme bestimmt man bei der entsprechenden Temperatur T im Intervall $1.0 \leq (p_{ein}/p_{aus}) \leqq 3$ die Wertpaare jW-p_{ein} und man stellt sie in einem Koordinatensystem dar (Fig. 1).

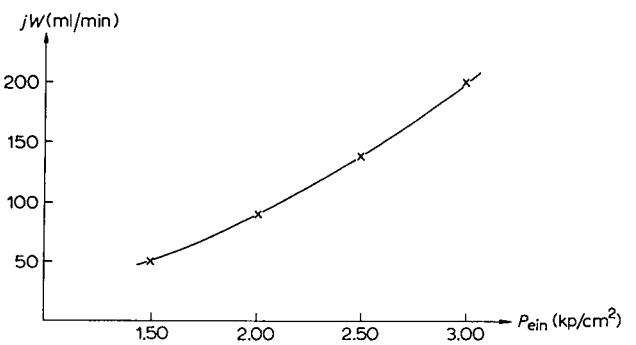


Fig. 1. Abhängigkeit von jW (ml/min) von dem Eintrittsdruck des Trägergases (kp/cm²).

TABELLE II

	Komponente	*Retentionszeit (min)*	*Netto Verzögerungsvolumen (ml Trägergas)*	*ΔV (ml Trägergas)*
1	Luft	0.44	—	—
2	Aceton	0.96	25.4	35.6
3	Benzol	1.68	61.0	35.5
4	Toluol	2.44	96.5	65.5
5	*m*- und *p*-Xylol	3.76	162.0	33.0
6	*o*-Xylol	4.47	195.0	45.5
7	Anisol	5.36	240.5	505.3
8	Nitrobenzol	15.70	745.8	307.9
9	Methylsalicylat	22.00	1053.7	293.2
10	*o*-Nitroaethylbenzol	28.00	1346.9	708.7
11	*p*-Nitroaethylbenzol	42.50	2055.6	2370.3
12	Methylphthalat	91.00	4245.9	

Aus dem Normalchromatogramme errechnet man folglich die Retentionszeiten, netto Verzögerungsvolumina, weiterhin die ΔV Werte der Komponenten, wo

$$\Delta V_{z;z-1} = V_{N_z} - V_{N(z-1)} \tag{1}$$

Die Daten einer als Modell dienenden Probe sind in Tabelle II zusammengestellt.

In Kenntnis der Retentionszeiten wählt man den Zeitpunkt des ersten Programmstarts ohne Berechnung, der Probe entsprechend. Unter Programmstart ist die während der möglichst kürzesten Zeit zu erfolgende Umstellung des Eintrittsdruckes des Trägergases vom Wert $p_{\text{ein}_{(i-1)}}$ auf den schon vorhergehend errechneten Wert p_{ein_i} zu verstehen. Im obigen Fall ist ratsam in der 7. Minute das erste Programm starten zu lassen (p_{ein_i}) da bis dahin alle eng aufeinanderfolgende leichtflüchtige Komponenten mit annehmbarer Retentionszeit schon von der Kolonne heruntergekommen sind, während die weiteren Komponenten erst bedeutend später erscheinen.

Das Trägergasprogramm ist mit Hilfe folgender Gleichung zu berechnen:

$$1 \leqq \frac{\Delta V_{\min}}{j_i W_i} \leqq 3 \tag{2}$$

In unserem Beispiel beträgt im Fall von $i = 1$, $\Delta V_{\min} = 293.2$ (ml Trägergas), folglich kann:

$$1 \leqq \frac{293.2}{j_1 W_1} \leqq 3$$

nur erfüllt werden, sofern $j_1 W_1 \leqq 293{,}2$, zu welchen Werten gemäss Fig. 1 die Werte 2.10 (kp/cm²) $\leqq p_{\text{ein}} \leqq$ 3.00 (kp/cm²) als höchste Druckgrenze des Geräts gehören. Die Minimalzeitdauer der Analyse d.h. diejenige Zeitdauer während welcher eine für uns interessante Komponente noch eine qualitativ und quantitativ auswertbare Spitze gibt, erhält man natürlich bei dem Maximalwert des errechneten p_{ein}. Die Gegebenheiten mancher Geräte gestatten nicht die Verwirklichung der Minimalzeitdauer. (Bei manchen Gaschromatographen beträgt der maximal einstellbare

und messbare Wert des Eintrittsdrucks des Trägergases 3.0 (kp/cm²).) Solchenfalls muss man bei diesem Wert arbeiten der relativ am nähesten zum errechneten Maximalwert von p_{ein} liegt. Ein zweites Trägergasprogramm $p_{ein_2} > p_{ein_1}$ kommt in diesem Fall, da die höchste Leistungsfähigkeit des Apparats schon beansprucht ist, sowieso nicht in Frage. Ein gutes Beispiel hiezu liefert die erwähnte Probe, wo die Minimalzeitdauer laut Rechnung bei $p_{ein} = 3.50$ (kp/cm²) erhalten worden wäre, hingegen jedoch nur $p_{ein} = 3.00$ (kp/cm²) eingestellt werden konnte.

Die unter Normalverhältnissen (a) und mit vorausgerechnetem Trägergasprogramm (b) erhaltenen Chromatogramme der Probe sind in Fig. 2 zu sehen. Es handelt sich um dieselben Komponenten wie in Tabelle II. Beide Chromatogramme wurden in einem Carlo Erba Gaschromatograph Model C ohne irgendwelche Änderungen des Geräts aufgenommen. Die Versuchsumstände waren wie folgt:

Detektor: Thermistor
Brückenstrom: 20 mA
Empfindlichkeit: 50 %
Kolonne: Kupferspirale, 2.0 m lang, innerer Durchmesser 5 mm
Füllung: 20 %iges Silikonöl auf Celit 550 Träger von Korngrösse 0.2 mm
Temperatur des Verdampfers: 300.0°
Temperierung: 160.0 ± 0.1°
Einwaage: mit Hamiltonscher Spritze 4.0 µl
Trägergas: Wasserstoff
Strömungsgeschwindigkeit des Trägergases: 56.5 ml/min (23.5°, 759.0 mm Hg)
Kompensograf: 2.5 mV (Vollausschlag)
Papiergeschwindigkeit: 1.25 (cm/min).

Das Chromatogramm (b) wurde unter gleichen Verhältnissen erhalten, nur mit dem Unterschied, dass in Minute 7 das Trägergasprogramm von $p_1 = 3.00$ (kp/cm²) gestartet worden ist.

Im allgemeinen ist es ratsam das Trägergasprogramm p_{ein_i} dann zu starten, wenn neben dem gerechneten Wert von $p_{ein_{(i-1)}}$ ein so ein ΔV Wert sich finden lässt, dessen Quotient mit $j_{(i-1)} \cdot W_{(i-1)}$ grösser ist als 3. Bestehen mehrere solche ΔV Werte, so sind für diese die Rechnungen ebenso auszuführen wie beim ersten Programmieren. Ist nur ein solcher ΔV Wert zu finden, so ist dieser in Gleichung 2 an Stelle von ΔV_{min} zu setzen. Bei der Planung weiterer Programmierungen ist jedoch die Rücksicht auf die Leistungsfähigkeit des Geräts nicht zu vernachlässigen.

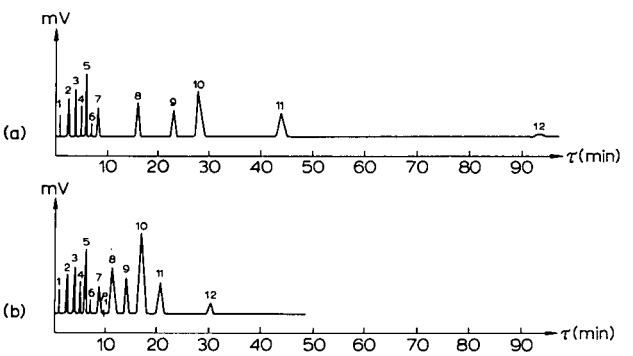


Fig. 2. Chromatogramm der Probe unter (a) Normalverhältnissen und (b) mit $p_{ein} = 3.00$ kp/cm² Trägergasprogramm.

Bei den Programmierungen muss sehr beachtet werden, dass nicht über die errechneten Werte programmiert wird, da solchenfalls verschiedene Spitzen leicht übereinander laufen. Zur Berechnung der Programmierung haben wir einige einfache Zusammenhänge gegeben[12], durch deren weitere Verfeinerung folgende Gleichungen erhalten wurden.

$$t_{Rp} = t_0 + \sum_{i=1}^{n-1} t_i + t_r \tag{3}$$

$$V_N = j_0 W_0 (t_0 - t_c) + \sum_{i=1}^{n-1} j_i W_i t_i + j_n W_n t_r \tag{4}$$

Wenn $n = 1$ so hat man für (3) und (4) einfach

$$t_{Rp} = t_0 + t_r \tag{5}$$

$$V_N = j_0 W_0 (t_0 - t_c) + j_1 W_1 t_r \tag{6}$$

Die programmierten Retentionszeiten der einzelnen Komponenten kann man mit Hilfe von Gleichungen 3 und 4 einfach erhalten und wenn nötig können auch die notwendigen Änderungen des Programms unternommen werden.

Als Beispiel wird die Errechnung der mit 5 verschiedenen Programmen modifizierten programmierten Retentionszeiten der 12 Komponenten der in Tabelle II gegebenen Probe dargestellt. In Tabelle III sind die wichtigeren Daten der Programmierung zusammengefasst.

Die entsprechenden Werte in Gleichungen 3 und 4 gesetzt erhält man

$$t_{Rp \cdot 12} = 1.0 + 0.5 + 2.5 + 2.5 + 4.0 + t_r = 10.5 + t_r \tag{7}$$

$$4425.9 = 48.9\,(1.0 - 0.44) + 0.5 \cdot 42 + 2.5 \cdot 37 + 2.5 \cdot 49 + 4 \cdot 140 + t_r \cdot 200 \tag{8}$$

Aus Gleichung 8

$$t_r = 18.01 \text{ min}$$

Setzt man diesen Wert in Gl. 7,

$$t_{Rp \cdot 12} = 28.51 \text{ min}$$

Statt der errechneten 28.51 min ergab sich die programmierte Retentionszeit der 12 Komponenten für 30.0 min. Ursache der Abweichung ist der Umstand, dass sich beim Starten der Programme das neue Gleichgewicht zwar schnell, doch nicht augenblicklich einstellt.

TABELLE III

$p_{ein}(kp/cm^2)$	j	W (ml/min)	t (min)	jW (ml/min)
$p_{be_0} = 1.30$	0.865	56.5	$t_0 = 1.0$	48.9
$p_{be_1} = 1.20$	0.921	45.6	$t_1 = 0.5$	42.0
$p_{be_2} = 1.10$	0.962	38.5	$t_2 = 2.5$	37.0
$p_{be_3} = 1.50$	0.789	62.2	$t_3 = 2.5$	49.0
$p_{be_4} = 2.50$	0.559	250.0	$t_4 = 4.0$	140.0
$p_{be_5} = 3.00$	0.500	400.0	$t_5 = t_r = ?$	200.0

Zum Kennzeichnen der Wirksamkeit des Trägergasprogrammes dient folgende Beziehung

$$h = \frac{\Delta t}{t_R} \cdot 100\,\%, \qquad \text{wo } \Delta t = |t_R - t_{Rp}| \tag{9}$$

Die Wirksamkeit der in Fig. 2 dargestellten Programmierung hinsichtlich der einzelnen Komponenten zeigt Tabelle IV.

TABELLE IV

	Komponente	*Wirksamkeit* (%)
8	Nitro-benzol	34.6
9	Methylsalicylat	43.2
10	*o*-Nitro-aethylbenzol	48.6
11	*p*-Nitro-aethylbenzol	54.0
12	Methyl-phthalat	64.2

Grundlage der quantitativen Auswertung ist das durch Gl. 4 definierte netto Verzögerungsvolumen. Der Gebrauch des netto Verzögerungsvolumens gestattet auch die unmittelbare Verwendung der unter Normalumständen erfolgten Identifizierungen.

Die quantitative Auswertung ist mit komplizierteren Problemen verbunden, da die die Spitzen des Normalchromatogramms beeinträchtigenden Parameter verschiedener Weise sich durch die Programmierung verändern. In erster Linie musste deshalb die Wirkung des Trägergasprogramms auf diese Spitzenparameter d.h. auf das Gebiet unter der Kurve untersucht werden.

Da die chromatographischen Glockenkurven durch die Gaussche Verteilungsfunktion annähernd beschreibbar sind und im Normalfall die dem Spitzenmaximum entsprechende Konzentration als direktes Mass der Stoffmenge betrachtet werden kann

$$\int_{-\infty}^{+\infty} c\,\mathrm{d}t = \sqrt{\frac{2\pi}{\lambda}}\, c_m$$

wurde zuerst die Änderung der der Höchstkonzentration entsprechenden Spitzenhöhe und des λ Verteilungsparameters auf Einwirkung des Trägergasprogramms unter sonst gleichen Umständen untersucht. Fig. 3 zeigt, dass λ linear dem wachsenden Trägergasprogramm zunahm, während die der Höchstkonzentration entsprechende Spitzenhöhe (m) dabei abnahm (Fig. 4). Für die Abnahme der Spitzenhöhe ist in entscheidendem Ausmass die Konzentrationsempfindlichkeit des Detektors verantwortlich, die sich bei wachsendem Trägergasprogramm stark verändert (Fig. 5).

Das Trägergasprogramm beeinträchtigt auch die Halbwertbreite bzw. die Grösse der durch die in die Inflexionspunkte gezogenen Tangenten aus der Grundlinien herausgeschnittenen Strecke; beide werden durch Erhöhung des Trägergas-

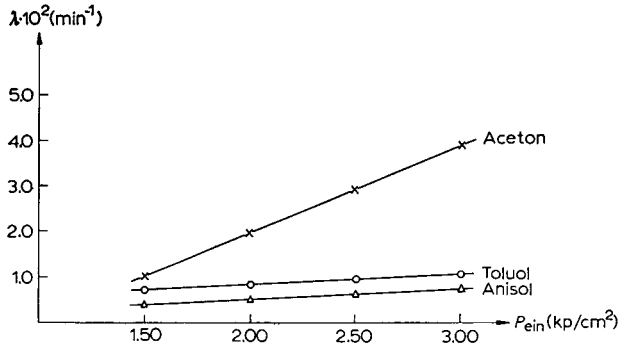


Fig. 3. Änderung des λ Verteilungsparameters in Funktion des Eintrittsdrucks vom Trägergas im Fall von Aceton, Toluol und Anisol.

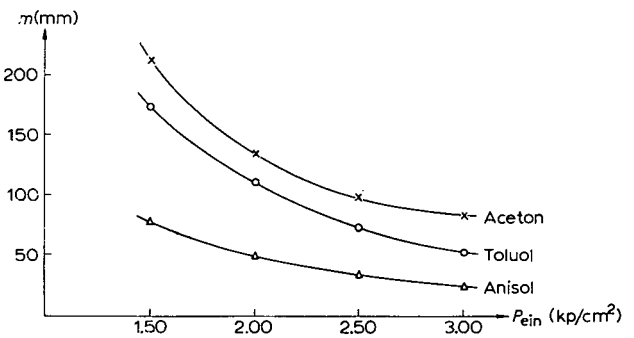


Fig. 4. Änderung der Spitzenhöhe in Funktion des Eintrittsdruckes von Aceton, Toluol und Anisol.

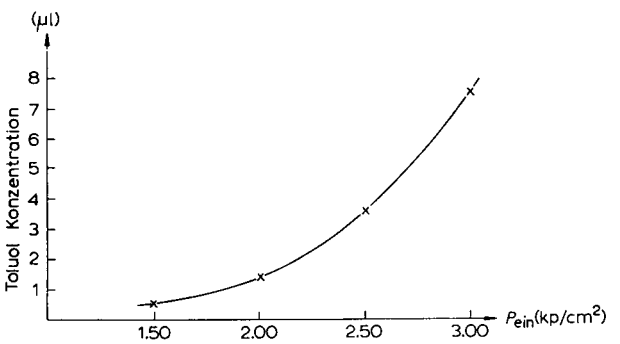


Fig. 5. Gleiche Spitzenhöhen gebende Toluolkonzentrationen in Funktion des Eintrittsdrucks des Trägergases übrigens unter gleichen Verhältnissen chromatographiert.

Eintrittdrucks vermindert. Diese Wirkung ist jedoch auf die Änderung des Verteilungsparameters rückführbar, da

$$1 = \frac{2.354}{\lambda} \tag{11}$$

$$d = \frac{4}{\lambda} \tag{12}$$

Unsere Ergebnisse zeigen, dass trotz zahlreicher in der Literatur beschriebener Versuche es kaum zu denken ist, dass man solche Korrektionsfaktoren finden könnte, mit welchen alle diese Wirkungen berücksichtigbar wären. Wir fanden, dass die Methode der inneren Normalisation zur quantitativen Auswertung gut dienlich ist, sofern die Gebiete mit Integrator, Planimeter oder durch Wägen des Gewichts der ausgeschnittenen Spitzen gemessen werden.

Bei der Prüfung von Gemischen von bekannter Zusammensetzung bewegte sich die relative Abweichung zwischen 0.1–3.0 %. Die Bestimmung der Komponente 10 des Modells in Fig. 2 ergab im Normalfall 16.00 Gewichtsprozente, mit zweifacher beschleunigender Programmierung 15.88 %.

BEZEICHNUNGEN

T = Analyse temperatur

W, W_0, W_i, W_{i-1}, W_n = Trägergasströmungsgeschwindigkeit im Auslauf der Kolonne (bei dortiger Messung von Druck und Temperatur) (ml/min) bei entsprechenden p_{ein}, p_{ein_i}, $p_{ein_{i-1}}$, p_{ein_n} Werten.

p_{ein}, p_{ein_0}, p_{ein_i}, $p_{ein_{i-1}}$, p_{ein_n} = Eintrittsdruck des Trägergases im allgemeinen und im Normallfall bzw. im i, i–1 $\cdots$ n-stem Fall (kp/cm²).

j, j_0, j_i, j_{i-1}, j_n = Korrektionsfaktore des Druckgefälles bei entsprechendem p_{ein_i}, $p_{ein_{i-1}}$, p_{ein_m}. Ihr Wert beträgt[13]

$$\frac{3}{2}\left[\frac{(p_{ein}/p_{aus})^2 - 1}{(p_{ein}/p_{aus})^3 - 1}\right]$$

p_{aus} = Austrittsdruck des Trägergases (kp/cm²)

$\Delta V_{z;z-i}$ = Differenz der netto Verzögerungsvolumina der z und $z-1$-sten Komponente (ml Trägergas)

z = Nummer der Komponente

i = Nummer

n = Zahl der Programmstarte bevor die zu untersuchende Komponente in Höchstkonzentration erscheint

V_N = netto Verzögerungsvolumen (ml Trägergas)

ΔV_{min} = der aus den netto Verzögerungsvolumen der programmierten Komponenten berechnete geringste ΔV Wert (ml Trägergas)

t_{Rp} = programmierte Retentionszeit der Komponente (min)

t_0 = Zeitdauer von der Einwaage bis zum Starten des ersten Trägergasprogramms (min)

t_i = i-ster Zeitabstand zwischen zwei unmittelbar aufeinander folgenden Trägergasprogrammstarten vor der Erscheinung der Höchstkonzentration einer betreffenden Komponente (min)

t_r = Zeitdauer zwischen der Erscheinung der Höchstkonzentration der betreffenden Komponente und dem unmittelbar vorhergehenden Trägergasprogrammstart (min) sofern $n > 0$. Sofern $n = 0$, d.h. es wurde nicht programmiert, so ist $t_r = t_R$.
t_R = Retentionszeit der Komponente in Normalfall (min)
t_c = Retentionszeit von Luft (Argon, Helium) in Normalfall (min)
h = Wirksamkeit des Trägergasprogramms (%)
c = Konzentration der Prüfsubstanz (Molbruch)
t = Zeit (min)
λ = Verteilungsparameter (min^{-1})
c_m = dem Spitzenmaximum entsprechende Konzentration (Molbruch)
l = Spitzenbreite in Halbhöhe (min)
d = die durch die in die Inflexionspunkte gezogenen Tangenten aus der Grundlinie herausgeschnittene Strecke
m = Spitzenhöhe (mm).

ZUSAMMENFASSUNG

Eine etappenmässige Programmierung des Eintrittsdruckes vom Trägergas unter isothermischen Verhältnissen gestattet eine weitgehende analytische Verbreitung der programmierten Strömungsmethodik, da sie es ermöglicht durch Errechnung der Minimalzeit der Analyse die Retentionszeit der Komponenten innerhalb der prinzipiell durch ΔV_{min}, bzw. durch die Leistungsfähigkeit des Geräts gegebenen Grenzen zu programmieren.

SUMMARY

Stepwise programming of the inlet pressure of the carrier gas under isothermal conditions permits a considerable analytical extension of the programmed flow technique. By calculation of the minimal time of analysis, the retention time of the components can be programmed within the limits governed in principle by ΔV_{min}, or by the output capacity of the apparatus.

LITERATUR

1 S. R. LIPSKY, R. A. LANDOWNE UND J. E. LOVELOCK, *Anal. Chem.*, 31 (1959) 852.
2 J. P. WOLFF, *Ann. Fals. Expert. Chim.*, 53 (1960) 318; G. WOLFF UND J. P. WOLFF, *Rev. Franç. Corps Gras*, 7 (1960) 73.
3 S. VALUSSI UND G. COFLERI, *Boll. Lab. Chim. Provinciali (Bologna)*, 13 (1962) 1; *Riv. Ital. Sostanze Grasse*, 39 (1962) 617.
4 J. M. VERGNAUD, *Bull. Soc. Chim. France*, (1962) 1914.
5 M. MORGANTINI, *Boll. Lab. Chim. Provinciali (Bologna)*, 13 (1962) 545.
6 H. PURNELL, *Gas Chromatography*, Wiley, New York, 1962, S. 387.
7 R. P. W. SCOTT, *Nature*, 198 (1963) 782.
8 C. COSTA NETO, J. W. DE ALENCAR UND J. T. KÖFFER, *Anais Acad. Brasil. Cienc.*, 36 (1964) 115.
9 C. COSTA NETO, J. T. KÖFFER UND J. W. DE ALENCAR, *J. Chromatog.*, 15 (1964) 301.
10 S. A. CLARKE, *Nature*, 202 (1964) 1106.
11 A. ZLATKIS, D. C. FENIMORE, L. S. ETTRE UND J. E. PURCELL, *J. Gas Chromatog.*, 3 (1965) 75.
12 L. MÁZOR, J. BALLA UND J. TAKÁCS, *J. Chromatog.*, 20 (1965) 221.
13 A. T. JAMES UND J. P. MARTIN, *Biochem. J.*, 52 (1952) 238.

GAS–LIQUID CHROMATOGRAPHY OF DERIVATIVES OF NATURALLY-OCCURRING MIXTURES OF LONG-CHAIN POLYISOPRENOID ALCOHOLS*

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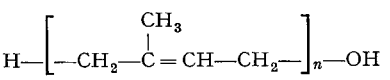
(Received November 22nd, 1965)

INTRODUCTION

There is present in nature a wide variety of polyisoprenoid alcohols (prenols) with the general structure:



These alcohols differ not only in the number of isoprenoid units present in the molecule, but also in the *cis-trans* nature of each of these units. Closely related compounds exist in which one or more of the isoprenoid units is saturated.

LINDGREN[1] has reported on the nature of the prenols present in the wood of *Betula verrucosa* (the silver birch). More recently[2] he has used gas–liquid chromatography to establish that the mixture isolated was of four alcohols containing six, seven, eight and nine isoprene units respectively, with the C_{35} and C_{40} compounds predominating. Using essentially the same experimental approach, the present authors have shown the presence of five polyisoprenoid alcohols containing nine, ten, eleven, twelve and thirteen isoprene units respectively in leaves of four higher plants. A mixture of alcohols isolated from *Saccharomyces cerevisiae* (baker's yeast) yielded three prenols containing fourteen, fifteen and sixteen isoprene units respectively.

EXPERIMENTAL

Chromatography

The acetates or hydrocarbons, dissolved in cyclohexane, were injected onto silanised Chromosorb W which had been coated with SE-30 (1 % w/w) and had been packed in a 4 ft. long silanised stainless-steel tube having an internal diameter of 0.125 in. A dual column F & M Model 810 gas chromatograph fitted with a flame ionization detector was used isothermally at a temperature of either 300 or 340°. The carrier gas was argon at a flow rate of 60 ml/min.

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Materials

A sample of the mixture of prenols from *Betula verrucosa* and referred to as betulaprenols, was provided by Dr. B. O. LINDGREN. The mixture of prenols from the leaves of *Aesculus hippocastanum* (the horse-chestnut) and referred to as castaprenols, was prepared by the authors[3]. The mixtures of prenols from the leaves of *Beta vulgaris* var. *crassa* (sugar beet) and of *Ficus elasticus* (rubber plant) were prepared in essentially the same manner in conjunction with Dr. J. F. PENNOCK. Mr. P. J. DUNPHY and Dr. J. F. PENNOCK provided the mixture of prenols derived from *Arum maculatum* (Lords and Ladies). The isolation of the prenols from *Saccharomyces cerevisiae* was carried out in conjunction with Drs. J. BURGOS and J. F. PENNOCK. Solanesol was provided by Hoffmann-La Roche.

A sample (10–20 μg) of solanesol or of the prenol mixtures appeared to chromatograph as a single spot on thin layers of silica gel G, using methanol in benzene (1:99, v/v) as developing solvent and staining the developed chromatogram with phosphomolybdic acid.

Acetylation and hydrogenation

Samples of solanesyl acetate and of the acetates of the prenol mixtures were prepared by mixing and leaving overnight, solutions of the alcohols (10–20 mg) in benzene (5 ml) with acetic anhydride (5 ml) and pyridine (3 drops). Ice cold 2 *N* HCl (10 ml) was then added and the acetates were extracted with ether.

Small portions of these acetates were hydrogenated at room temperature and pressure in a Towers microhydrogenation apparatus using platinum oxide as catalyst and cyclohexane as solvent. The resulting mixtures contained perhydroprenyl acetates and the corresponding saturated hydrocarbons resulting from elimination of the acetyl residue during hydrogenation. The hydrocarbons and acetates were separated by preparative thin-layer chromatography. Silica gel G was used as adsorbent and light petroleum (b.p. 40–60°) as developing solvent.

When samples of the perhydroprenyl acetates were required with minimal losses to hydrocarbon, it was found best to first hydrogenate the free prenols. The perhydroprenols were then acetylated.

Solanesyl trimethyl silyl ether

The trimethyl silyl ether of solanesol was prepared in essentially the same method as described by LUUKKAINEN *et al.*[4]. Solanesol (20 mg) was dissolved in tetrahydrofuran (2 ml) containing trimethyldichlorosilane (3 drops) as catalyst. Hexamethyl-disilazine (0.3 ml) was then added slowly, drop by drop, and the mixture allowed to stand at room temperature for 0.5 h. The precipitate produced was removed by centrifugation and the supernatant solution was taken to dryness. This material was triturated for 2 min with hexane and again centrifuged. The product in the supernatant solution was recovered by evaporating the solvent and it was dissolved in isooctane for gas chromatography.

Solanesyl trifluoroacetate

Solanesol was esterified with trifluoroacetic anhydride, using pyridine as catalyst, according to the method described by VANDENHEUVEL *et al.*[5]. The reaction was complete in only a few minutes at room temperature. Solvent (tetrahydrofuran) and excess reagent were removed by evaporation.

RESULTS AND DISCUSSION

Perhydroprenyl acetates

The chromatographic mobility of fully hydrogenated polyisoprenoid compounds is uncomplicated by problems arising from the *cis* or *trans* nature of the isoprene units. In this form one might expect the retention times of the series of perhydroisoprenologues to be related in the same way as are the retention times of a series of homologues. In fact LINDGREN[2] has produced good evidence that this does happen in the case of the perhydrohexa-, perhydrohepta-, perhydroocta- and perhydronona-prenyl acetates. Because the present authors required to investigate larger polyisoprenoid alcohols, they have run their gas chromatograph at higher temperatures than did LINDGREN. It was reassuring to find that the relationship between these perhydroprenyl acetates described by LINDGREN still held.

In Table I are recorded the retention times at 300° of the four components of the mixture of perhydrobetulaprenyl acetates. These retention times were reproducible over numerous runs on the same day but changed slightly from one day to the next. When a sample of perhydrosolanesyl acetate was chromatographed, either

TABLE I

GAS-CHROMATOGRAPHIC DATA FOR THE MIXTURE OF PERHYDROBETULAPRENYL ACETATES AND FOR PERHYDROSOLANESYL ACETATE AT 300°

Component	*Retention time (min)*	*Peak area (% of total)*
1	0.35	4.2
2	1.05	52.2
3	2.58	41.0
4	5.90	2.6
Perhydrosolanesyl acetate*	5.90	

* The perhydrobetulaprenyl acetates mixture was chromatographed both with and without added perhydrosolanesyl acetate.

with the mixture or separately but on the same day as the mixture, its retention time coincided exactly with that of the fourth peak in the chromatogram of the mixture (see Table I.) Fig. 1 shows that if the four components of the mixture are assumed to contain six, seven, eight and nine saturated isoprene residues respectively, then a plot of log retention time against the number of saturated isoprene residues gives four points, all of which fall on a straight line. This is precisely the situation described by LINDGREN[2] for lower temperatures. It is good evidence that the original mixture of prenols contained four compounds containing respectively six, seven, eight and nine isoprene residues. The C_{35} and C_{40} isoprenologues were the major components (see Table I). Mass spectrometry and nuclear magnetic resonance spectroscopy support this conclusion[2].

Most of the available evidence[3] indicated that "castaprenol" was a C_{60} prenol containing rather more *cis* than *trans* isoprene residues. However, mass spectrometry suggested that while the C_{60} prenol predominated, C_{55} and C_{65} prenols were also pre-

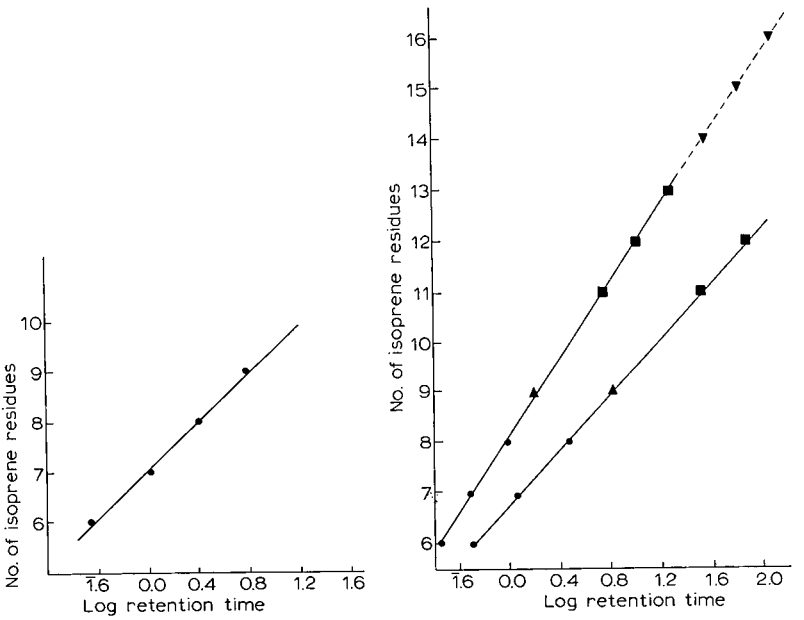

Fig. 1. Relationship between $\log_{10}$ retention time at 300° and the number of saturated isoprene residues per molecule of each component of the mixture of perhydrobetulaprenyl acetates.

Fig. 2. Relationship between $\log_{10}$ retention time at 300° (lower line) and 340° (upper line), and the number of saturated isoprene residues per molecule of each component of a mixture of perhydrobetulaprenyl acetates (—●—●—), perhydrosolanesyl acetate (—▲—), perhydrocastaprenyl acetates (—■—■—) and the perhydroprenyl acetates from *Saccharomyces cerevisiae* (--▼--▼--).

sent. As expected, the relative intensities of the three molecular ions varied with the temperature at which the mass spectrum was obtained and it was not possible to calculate the precise composition of the original mixture. Gas–liquid chromatography of the mixture of perhydrocastaprenyl acetates revealed three clear components, the middle peak of the three on the chromatogram being of largest area (see Table II). When a sample of the mixture was chromatographed together with samples of the mixture of perhydrobetulaprenyl acetates and of perhydrosolanesyl acetate (see Table II) it was clear that peak 1 corresponded in position to perhydroprenyl acetate made up of eleven isoprene residues, peak 2 to one made up of twelve isoprene residues and peak 3 to one of thirteen isoprene residues. This is made clearer in Fig. 2, in which a plot of log retention time against the number of saturated isoprene units gives points, all of which fall on a straight line at both 300 and 340°. As forecast by mass spectrometry, the major constituent of the mixture was the C_{60} isoprenologue. In some cases, when relatively large quantities of the mixture were applied to the column, a small peak corresponding to the C_{50} isoprenologue could be observed. This material accounted for less than 0.5 % of the mixture.

In Table III are collected together the chromatographic data for the perhydroacetates of the prenols derived from *Ficus elasticus*, *Beta vulgaris* and *Arum maculatum*. The retention times for the different components differed slightly from those obtained with the mixture of perhydrocastaprenyl acetates on different days.

TABLE II

GAS-CHROMATOGRAPHIC DATA FOR THE MIXTURES OF PERHYDROCASTAPRENYL ACETATES, PERHYDROBETULAPRENYL ACETATES AND PERHYDROSOLANESYL ACETATE CHROMATOGRAPHED TOGETHER AT 300 AND 340°

Component	*Retention time (min)*		*Peak area** (% *of total*)
	at 300°	*at 340°*	
Betula-1**	0.48	0.29	
Betula-2**	1.28	0.47	
Betula-3**	2.86	0.82	
Perhydrosolanesyl acetate**	6.47	1.47	
Casta-1	32.2	5.48	16.2
Casta-2	69.0	10.0	81.7
Casta-3	— ***	18.2	2.1

* These figures refer to the mixture of perhydrocastaprenyl acetates chromatographed at 340°.

** The mixture of perhydrocastaprenyl acetates was chromatographed with and without adding the mixture of perhydrosolanesyl acetate and perhydrobetulaprenyl acetates. The peak for Betula-4 was hidden under that for perhydrosolanesyl acetate.

*** Insufficient sample was applied to the column for this peak to be observed. A larger sample was applied at 340°.

TABLE III

GAS-CHROMATOGRAPHIC DATA FOR THE PERHYDRO-ACETATES OF THE PRENOLS ISOLATED FROM THE LEAVES OF *Ficus elasticus* AND *Beta vulgaris* AND THE SPADICES OF *Arum maculatum* AND CHROMATOGRAPHED AT 300° AND 340°

Probable isoprenologue	*Retention time (min)*		*Peak area (% of total)**		
	at 300°	*at 340°*	*Ficus*	*Beta*	*Arum*
C_{45}	5.9– 6.0	1.40–1.62	2.1	1.3	—
C_{50}	13.6–14.6	2.63–2.82	7.4	3.3	11.5
C_{55}	29.2–31.0	5.29–5.56	61.2	51.5	36.5
C_{60}	61.8–63.1	9.7 –10.1	28.3	42.6	41.4
C_{65}	— **	17.8 –18.1	1.0	1.3	10.6

* These figures refer to those samples chromatographed at 340°.

** Insufficient sample was applied to the column for this peak to be observed. A larger sample was applied at 340°.

TABLE IV

GAS-CHROMATOGRAPHIC DATA FOR THE MIXTURE OF PERHYDROPRENYL ACETATES ISOLATED FROM *Saccharomyces cerevisiae* AND CHROMATOGRAPHED AT 340°

Component	*Retention time (min)*	*Peak area (% of total)*
1 (C_{70})	36.1	13.5
2 (C_{75})	64.8	73.4
3 (C_{80})	119.1	13.1

However, when a further sample of each was chromatographed with a sample of mixed perhydrobetulaprenyl acetates and perhydrosolanesyl acetate retention times were observed which when plotted as in Figs. 1 and 2 gave points all of which fell on a straight line. This was taken as good evidence that the components of the mixtures had chain lengths as indicated in Table III. No other accurate evidence concerning the size of these compounds is available yet but thin-layer and paper chromatographic data indicate that the figures in Table III for the major components of each mixture are probably correct.

The relevant data for the fully hydrogenated acetates of the prenols isolated from *Saccharomyces cerevisiae* are recorded in Table IV. It can be seen in Fig. 2 that the retention times of the three components are consistent with the mixture containing a C_{70}, a C_{75} and a C_{80} perhydroprenyl acetate. It is clear that the three points on Fig. 2 corresponding to these three components fall remarkably close to the line extrapolated from a plot of the results reported in Table II, bearing in mind that the samples were chromatographed on different days and that the peaks in the *Saccharomyces* chromatogram were rather broad. The major component of the mixture proved to be the C_{75} compound (see Table IV) and this was accompanied by much smaller quantities (about one eighth of the total in each case) of the C_{70} and C_{80} compounds. This conclusion is supported by mass spectrometry and it is also relevant that the mean equivalent weight of the original mixture of alcohols, as determined by the specific activity of the acetates formed from [1-^{14}C] acetic anhydride, corresponded to a C_{75} polyisoprenoid monohydric alcohol[6].

Saturated hydrocarbons

It was indicated in the Experimental section that hydrogenation of the prenyl acetates resulted in the formation of saturated hydrocarbons produced by the replacement of the acetyl residue with a hydrogen atom. In most cases a small sample of the hydrocarbon(s) was recovered by thin-layer chromatography and this was subjected to gas chromatography under the same conditions as were the perhydroprenyl acetates.

The retention times of the components of each sample of hydrocarbons are recorded in Table V. Fig. 3 illustrates that if one assumes that these hydrocarbons correspond in chain length to the prenyl acetates present in the mixture from which

TABLE V

RETENTION TIMES FOR SATURATED HYDROCARBONS DERIVED FROM PRENYL ACETATE MIXTURES AND CHROMATOGRAPHED AT 340°

Component	*Retention time (min) and source*			
	Solanesol	*Aesculus*	*Ficus*	*Beta*
1	0.78		0.75	0.74
2		1.42	1.42	1.45
3		2.80	2.81	2.82
4		5.42	5.33	5.41
5		7.50	— *	7.50

* Insufficient sample was applied to the column for this peak to be observed.

they were derived, then a plot of the number of saturated isoprene residues against log retention time produces a good straight line. As expected the retention times of the hydrocarbons were considerably shorter than were those of the corresponding perhydro-acetates. The peaks on the hydrocarbon chromatograms were also sharper than those on chromatograms of the perhydroprenyl acetates when the recorder was operating under the same conditions.

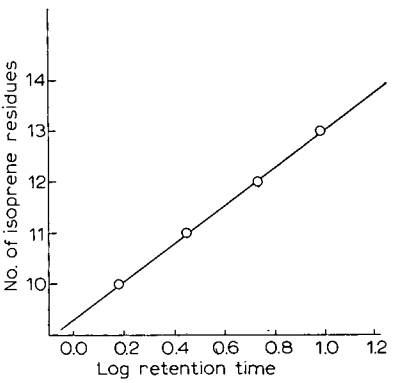

Fig. 3. Relationship between $\log_{10}$ retention time at 340° and the number of saturated isoprene residues per molecule of each component of the saturated hydrocarbons derived from the mixture of castaprenyl acetates.

The relative areas under each hydrocarbon peak of the different mixtures were essentially the same as for the corresponding perhydroprenyl acetates. The peak with a retention time of 1.42 min in the sample derived from *Aesculus hippocastanum* accounted for less than 0.5 % of the total mixture. Its presence suggests that the original mixture contained small quantities of a decaprenol. A hint of its presence was gained from some of the chromatograms of the castaprenyl acetates and of the perhydrocastaprenyl acetates.

Prenyl acetates

When the unsaturated prenyl acetates were chromatographed the same number of peaks was obtained as appeared in the chromatograms of the corresponding perhydroprenyl acetates and saturated hydrocarbons. It appeared that the order in which the unsaturated acetates left the column was the same as for the corresponding perhydro-acetates and saturated hydrocarbons. The relative areas under the respective peaks were essentially the same for the three types of compounds. The retention times for those mixtures containing a number of *cis*-isoprenoid residues were somewhat smaller than for the corresponding perhydroprenyl acetates but in the case of all-*trans*-solanesyl acetate the difference was less marked. The peaks were broadened when compared with those of the saturated hydrocarbons and the perhydroprenyl acetates and retention times varied a little from one chromatogram to the next. Nevertheless, this variation was relatively minor and it was quite obvious that marked *cis*:*trans* differences affected the retention times. It was no longer possible to fit all of the points to a straight line when the number of isoprene residues was plotted against log retention time. Retention times for a number of the mixtures are recorded

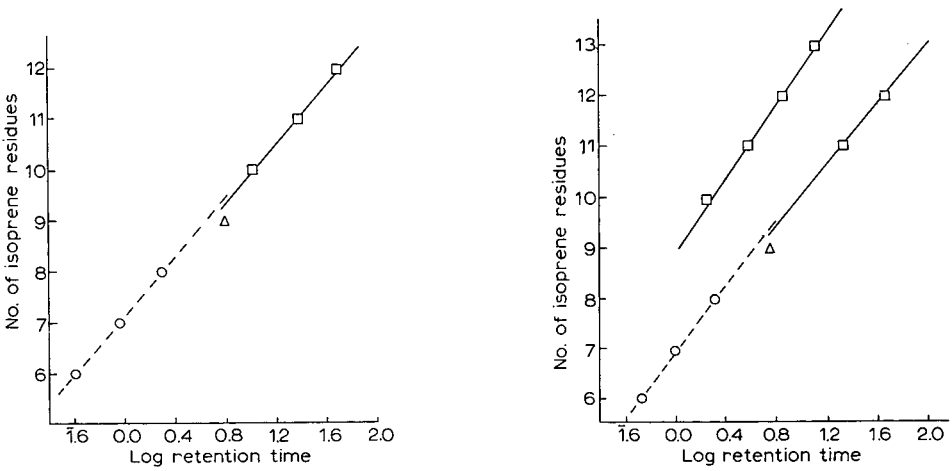

Fig. 4. Relationship between $\log_{10}$ retention time at 300° and the number of isoprene residues per molecule of each component of a mixture of betulaprenyl acetates (--⊙--⊙--), solanesyl acetate (△) and prenyl acetates from *Beta vulgaris* (—□—□—).

Fig. 5. Relationship between $\log_{10}$ retention time at 300° (lower lines) and 340° (upper line), and the number of isoprene residues per molecule of each component of a mixture of betulaprenyl acetates (--⊙--⊙--), solanesyl acetate (△) and castaprenyl acetates (—□—□—).

in Table VI. Log retention time against the number of isoprene residues for each component of the *Betula* plus *Beta* and *Betula* plus *Aesculus* plus solanesyl mixtures is plotted in Figs. 4 and 5, respectively.

Nuclear magnetic resonance studies indicate that in the betulaprenol mixture[1,2], in the castaprenol mixture[3] and in the mixture of prenols isolated from *Ficus elasticus* and *Beta vulgaris*[6] more than half of the isoprene residues are in the *cis* configuration. It is not known, however, if one component of the mixtures is predominantly *trans* and the others predominantly *cis* or if each component contains a small excess of *cis* over *trans* residues. Work is in fact progressing in this laboratory on the separation and

TABLE VI

RETENTION TIMES OF SOME PRENYL ACETATE MIXTURES AT 300 AND 340°

Number of isoprene residues	*Retention time (min) and source*					
	Solanesol (300°)	*Betula* (300°)	*Betula* + *Aesculus* + *Solanesol* (300°)	*Aesculus* (340°)	*Betula* + *Beta* (300°)	*Betula* + *Ficus* (300°)
6		0.47	0.57		0.41	— *
7		0.98–1.17	1.10		0.92	0.94
8		2.01–2.25	2.15		2.01	1.91
9	5.85–6.40	5.82–6.35	5.85		6.40	— *
10			— *	1.85	10.3	10.0
11			22.0	3.82	24.2	22.4
12			47.2	7.4	48.0	**
13			— *	13.1	— *	**

* Insufficient sample was applied to the column for this peak to be observed.
** Not recorded.

collection of the individual components of each mixture with a view to settling this point. Nevertheless, at this stage it is interesting to note that in Fig. 4 the points for the betulaprenyl acetates fall on a straight line as do the points for the prenyl acetates derived from *Beta vulgaris*, whereas the point for solanesyl acetate (all-*trans*-nonaprenyl acetate) is well clear of either line (see also Fig. 5). It is reasonable to argue that if one of the mixtures had contained a predominantly all-*trans* component then the point for this component also would not have fallen on the same straight line as that produced by the predominantly *cis* components. The evidence of Table VI and Figs. 4 and 5 would support the idea that the *cis*:*trans* ratio is not drastically different in each of the components of any one of the mixtures. It may also be that the prenols of *Aesculus hippocastanum* are similar, in this respect, to those of corresponding chain length derived from *Beta vulgaris* and *Ficus elasticus*.

It is important to the student of the biogenesis of these compounds to know, for instance, if the distribution of *cis* and *trans* residues in the C_{50} component of the *Beta vulgaris* mixture is precisely the same as in the ω-C_{50} portion of the C_{55} component and, further, if the distribution of *cis* and *trans* residues in the C_{55} component is precisely the same as in the ω-C_{55} portion of the C_{60} component. Such a situation would be a strong pointer to the shorter components being biogenetic precursors of the longer components (assuming *cis–trans* isomerases are not involved). Unfortunately, while the gas-chromatographic data do not rule this situation out, they cannot be quoted as evidence in favour of it. Although this technique has proved to be a powerful tool in studying long chain prenols, it cannot be used to supply useful information regarding relatively small differences in distribution of *cis* and *trans* isoprene residues.

Solanesyl trimethyl silyl ether and trifluoroacetate

It was found that the retention times (at 300°) of solanesyl trimethyl silyl ether and solanesyl trifluoroacetate were 8.9 min and 5.0 min compared with 5.9 min for solanesyl acetate. Since it appeared that the use of even the trifluoroacetates would offer no substantial advantage over the use of the acetates, no further work was carried out with these derivatives.

General

The foregoing results and discussion show that gas–liquid chromatography is a valuable tool not only for detecting the presence of small quantities of prenologues in natural mixtures of prenols, but also for forecasting the chain length of the components of these mixtures. In this way it has been possible to show that samples of prenol acetates that chromatographed on thin layers of silica gel as if one compound are in fact mixtures of prenol acetates of varying chain-length. The gas chromatograms can also be used to indicate the approximate percentage composition of the mixtures. The accurate composition of these mixture will be uncertain until the response of the flame ionisation detector to each component has been checked.

Previous reports have described the gas–liquid chromatography of alcohols varying in size from C_{16} (ref. 7) to C_{45} (ref. 2). It is perhaps remarkable that it has been found possible to extend the range to the acetate of C_{80} perhydroprenol. C_{100} and C_{110} prenols have also been isolated from natural sources[8,9]. It may be that, given a stationary phase of sufficient thermostability, it will be possible to chromatograph derivatives of these compounds.

SUMMARY

Gas–liquid chromatography has been used to confirm that the leaves of *Aesculus hippocastanum* contain prenols ranging from C_{45} to C_{65} with the C_{60} component predominating. Leaves of *Beta vulgaris* var. *crassa*, *Ficus elasticus* and *Arum maculatum* were shown to yield a mixture of prenols with the same range in chain length. The prenols from *Saccharomyces cerevisiae* were found to range from C_{70} to C_{80}. Retention times for prenol acetates, perhydroprenyl acetates and the derived saturated hydrocarbons are reported, together with the approximate quantitative composition of each mixture.

REFERENCES

1 B. O. LINDGREN, *Acta. Chem. Scand.*, 18 (1964) 836.
2 B. O. LINDGREN, *Acta. Chem. Scand.*, 19 (1965) 1317.
3 J. STEVENSON, A. R. WELLBURN AND F. W. HEMMING, in preparation.
4 T. LUUKKAINEN, W. J. A. VANDENHEUVEL, E. O. A. HAAHTI AND E. C. HORNING, *Biochim. Biophys. Acta*, 52 (1961) 599.
5 W. J. A. VANDENHEUVEL, J. SJÖVALL AND E. C. HORNING, *Biochim. Biophys. Acta*, 48 (1961) 596.
6 F. W. HEMMING AND J. F. PENNOCK, in preparation.
7 W. J. A. VANDENHEUVEL, W. L. GARDINER AND E. C. HORNING, *J. Bhromatog.*, 19 (1965) 263.
8 J. BURGOS, F. W. HEMMING, J. F. PENNOCK AND R. A. MORTON, *Biochem. J.*, 88 (1963) 470.
9 J. BURGOS, P. H. W. BUTTERWORTH, F. W. HEMMING AND R. A. MORTON, *Biochem. J.*, 91 (1964) 22P.

THE LOSS OF METHYL ESTERS OF POLYUNSATURATED ACIDS DURING GAS CHROMATOGRAPHY

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INTRODUCTION

It has been found in this laboratory that the gas chromatographic analysis of mixtures containing methyl stearate, methyl oleate and methyl linoleate on Celite columns coated with polydiethyleneglycol adipate using a ^{90}Sr detector gives quantitative results. However, as reported in a preliminary note by GERSON, SHORLAND AND McINTOSH[1], methyl linolenate in comparison with methyl stearate showed a loss of 51 %. Furthermore analysis of the methyl esters of shark liver oil before and after hydrogenation revealed a loss of 51 % C_{20}-unsaturated acids (largely pentaenoic) and of 86 % C_{22}-unsaturated acids (largely hexaenoic). Losses of methyl esters of polyunsaturated acids are implicit in the work of ACKMAN AND BURGHER[2], who recorded the use of correction factors as did SEHER[3].

The purpose of the present work was to test the effect of varying the nature of the liquid phase and of the solid support on the losses of polyunsaturated acids containing from three to six double bonds.

The solid supports tested included Celite 545, Chromosorb W, firebrick and glass microbeads. The effect of varying the polarity of Celite by precoating with dimethyldichlorosilane or with Epicote resin was also studied. The polar liquid phases examined were diethyleneglycol adipate polyester (DEGA), ethyleneglycol adipate polyester (EGA), butanediol succinate (BDS), ethyleneglycol succinate polyester (EGS), *o*-phthalic ethyleneglycol (PEG). In addition the non-polar Apiezon L (APL) was tested.

EXPERIMENTAL

Methyl stearate (18:0) was prepared by refractionation of concentrates obtained during ester fractionation analysis and purified by crystallization from acetone. When tested by gas chromatography it was found to be free from impurities. Methyl linolenate (18:3), methyl arachidonate (20:4), methyl eicosapentaenoate (20:5) and methyl docosahexaenoate (22:6) were obtained from the Hormel Institute, Austin, Minnesota, U.S.A., and were certified to be at least 90 % pure with the exception of (18:3), which was over 99 % pure.

Test mixtures

Test mixtures were prepared by weighing out known amounts of methyl stearate and the methyl esters of the polyunsaturated acids. The weight percentages of the polyunsaturated acids were checked by the iodine values (Wijs, 1 h). Agreement within 4 % was obtained and the accepted composition of the test mixture was based on the iodine value.

Solid supports

Celite 545 (30–80 mesh) and glass microbeads (approx. 80 mesh) were obtained from The British Drug Houses Ltd., Poole, England. Glass microbeads (74–53 microns) were obtained from the La Pine Scientific Company (Chicago, Ill., U.S.A.). Chromosorb W (60–80 mesh) was supplied by Johns Manville Corporation, New York, and firebrick (60–80 mesh) was obtained through the courtesy of the N.R.C. Prairie Regional Laboratory, Canada.

Liquid phases

APL was obtained from Edwards High Vacuum Ltd., Crawley, Sussex, England; DEGA from Cambridge Industries Ltd., Cambridge, Mass., U.S.A.; Epicote-resin from Shell Oil Company, New Zealand, and dichlorodimethylsilane from Dow Corning Corporation, Midland, Michigan, U.S.A. EGA was prepared as described by FARQUHAR *et al.*[4]. BDS, EGS and PEG were obtained through the courtesy of the Prairie Regional Laboratory, N.R.C., Canada.

Preparation of solid supports

Siliconized columns were prepared by treating the Celite with a 5 % solution of dichlorodimethylsilane in toluene for 2–3 h. Epicote coated columns were prepared by coating an acetone solution of the resin onto Celite in a rotary evaporator. The liquid phase was subsequently applied in toluene solution, in which Epicote is only sparingly soluble.

Coating the solid support with liquid phase

In general the solid supports were coated by mixing with a solution of the liquid phase and evaporating the solvent *in vacuo* on a rotary evaporator. For APL, light petroleum (b.p. 40–60°) was used. For other liquid phases, acetone was found to be more suitable. Column packings coated with EGA or with DEGA were spread in a thin layer on a tray and heated to 205° overnight before packing. One of the microbead columns was packed with solid support without the liquid phase. The liquid phase was then introduced in acetone solution and the solution allowed to drain. The solvent was afterwards removed by passing a stream of air through the column. In another experiment, a Celite column coated with 20 % w/w DEGA was prepared by mixing the Celite with a solution of DEGA in acetone and allowing the solvent to evaporate over a period of a week with occasional gentle stirring.

Packing the column

Small amounts of the packing, equivalent to about 6 in. in length were poured into the column. After each addition the column was rotated against a $^3/_4$ in. hexagonal shaft driven by an electric motor to pack the particles more closely.

Analytical methods

The separations were carried out on columns 225 cm long and 0.6 cm internal diameter. An argon ionisation detector as described by LOVELOCK, JAMES AND PIPER[5] was used. Except where otherwise stated the detector voltage was 1000 V. The introduction of the sample and measurement of the peak areas were carried out as described by GERSON[6]. The loss of methyl esters of polyunsaturated acids was estimated by comparing their measured peak area with that calculated from the area of the methyl stearate peak which was assumed to be correct.

RESULTS AND DISCUSSION

As already mentioned, GERSON *et al.*[1] using 20 % DEGA on Celite showed losses of 51 %, 51 % and 86 % for methyl linolenate, methyl C_{20}-polyunsaturated and methyl C_{22}-polyunsaturated esters, respectively. In the present work, a loss of 20 % only was found for methyl linolenate for this type of packing showing that the same type of packing varies in performance from column to column. It may be significant that the loss of 51 % was associated with an old column, whereas the smaller loss of 20 % was recorded with a new column. For the work now recorded the columns were freshly prepared. The results in the Tables are based on six determinations.

Tables I and II show that in the analyses of methyl linolenate and methyl arachidonate using Celite as the solid support, the losses are smaller with 20 % EGA than with 20 % DEGA, but nevertheless substantial. Although presiliconizing further reduced the loss of methyl linolenate no significant difference was noted for methyl arachidonate (see Tables I and II). The siliconized column was also tried with the methyl esters of more highly unsaturated acids but substantial losses (Table III) were incurred amounting to 42 % and 67 %, respectively, for methyl eicosapentaenoate and methyl docosahexaenoate.

On the other hand, the application of 10 % w/w Epicote to the Celite before the addition of 20 % w/w EGA minimised the losses and gave quantitative results for

TABLE I

LOSS OF METHYL LINOLENATE (18:3) ON GAS–LIQUID CHROMATOGRAPHIC COLUMNS

Mixture (wt. %)		*Solid support*	*Liquid phase*	*Temp.* (°C)	*Mean change* (%)	*Standard deviation* (±)	*Area correction factor*
18:0	*18:3*						
36	64	30–80 mesh Celite	20 % DEGA	205	—20	5.6	1.25
36	64	30–80 mesh Celite	20 % EGA	205	—12	5.0	1.14
38	62	30–80 mesh Celite	20 % EGA siliconized	205	— 3	2.5	—
57	43	30–80 mesh Celite	20 % EGA 10 % Epicote	185	— 2	5.5	—
57	43	30–80 mesh Celite	20 % EGA 10 % Epicote	205	— 2	6.3	—
38	62	80 mesh glass beads	2.5 % DEGA	205	— 7	2.4	1.08
38	62	80 mesh glass beads	1 % APL	205	— 9	6.4	1.10

TABLE II

LOSS OF METHYL ARACHIDONATE (20:4) ON GAS–LIQUID CHROMATOGRAPHIC COLUMNS

Mixture (wt. %)		*Solid support*	*Liquid phase*	*Temp.* (°C)	*Mean change* (%)	*Standard deviation* (±)	*Area correction factor*
18:0	20:4						
30	70	30–80 mesh Celite	20 % DEGA	205	—40	4.3	1.67
25	75	30–80 mesh Celite	20 % EGA	205	—31	7.2	1.45
33	67	30–80 mesh Celite	20 % EGA siliconized	205	—36	3.1	1.58
35	65	30–80 mesh Celite	20 % EGA 5 % Epicote	205	—26	11.0	1.35
35	65	30–80 mesh Celite	20 % EGA 10 % Epicote	205	+ 1	8.2	—
33	67	80 mesh glass beads	0.75 % EGA	205	— 6	4.2	1.06

TABLE III

LOSS OF METHYL EICOSAPENTAENOATE (20:5) AND METHYL DOCOSAHEXAENOATE (22:6) ON GAS–LIQUID CHROMATOGRAPHIC COLUMNS

Mixture (wt. %)		*Solid support*	*Liquid phase*	*Temp.* (°C)	*Mean change* (%)	*Standard deviation* (±)	*Area correction factor*
18:0	*20:5*						
29	71	30–80 mesh Celite	20 % EGA siliconized	206	—42	5.9	1.72
29	71	30–80 mesh Celite	20 % EGA 10 % Epicote	206	—22	4.6	1.28
36	64	60–80 mesh Chromosorb W	17 % BDS	207	—45	7.1	1.82
36	64	60–80 mesh Chromosorb W	17 % EGS	207	—46	3.6	1.85
		1000 V; 800 V			—46	5.9	1.85
36	64	60–80 mesh Firebrick	17 % PEG	207	—41	5.8	1.69
		1000 V; 800 V			—50	1.9	2.00
29	71	30–80 mesh Celite	5 % APL	206	—10	1.3	1.11
18:0	*22:6*						
53	47	30–80 mesh Celite	20 % EGA siliconized	205	—67	4.5	3.03
41	59	30–80 mesh Celite	20 % EGA 10 % Epicote	206	—57	6.0	2.33
41	59	30–80 mesh Celite	20 % EGA 10 % Epicote	185	—58	7.4	2.38
53	47	30–80 mesh Celite	5 % APL	205	—51	4.7	2.04

methyl linolenate and methyl arachidonate (Tables I and II). It was less effective for methyl eicosapentaenoate and for methyl docosahexaenoate (see Table III). The application of 5 % w/w Epicote to Celite was ineffective (see Table II).

The loss of methyl linolenate was considerably smaller with 1 % w/w APL on glass beads than with EGA on Celite but this trend was reversed with precoated Celite. A column using 0.75 % w/w EGA on 80 mesh glass beads gave results with methyl arachidonate (see Table II) which were almost as good as Epicote treated Celite, suggesting that this or other finely divided glass supports have potentialities in the quantitative analysis of methyl esters of polyunsaturated fatty acids.

It has been our observation that 80 mesh glass microbead columns are difficult to pack if the amount of liquid phase exceeds 2 % (w/w). Resolution on such columns was generally poor and varied with each column. No improvement was effected when using 74–53 micron glass beads.

The possibility that loss of polyunsaturated esters was due to the liquid films being destroyed during drying in the rotary evaporator was excluded by introducing the liquid phase, dissolved in acetone, into a column packed with uncoated glass beads, allowing the solution to drain off and then drying the column in a current of air. Losses on this column were similar to those on columns whose packing had been dried in the rotary evaporator. Celite columns in which the liquid phase was prepared by mixing the solid support with a solution which was allowed to evaporate slowly, with occasional gentle stirring, over a period of a week also gave similar results to those obtained by removal of the solvent on a rotary evaporator.

The effect of temperature on the losses of methyl esters of polyunsaturated acids was not extensively investigated. The losses of methyl docosahexaenoate on Celite coated with 10 % Epicote and 20 % EGA were shown to be similar at 206° and 185°.

ACKMAN AND BURGHER[2] from the correction factor (derived empirically by comparison of hydrogenated and unhydrogenated samples) of 1.5 for 22:6 determined on organosilicone columns of EGSS-X and EGSS-Y on siliconized Gas-chrom P (see ACKMAN AND JANGAARD[7]) indicated a loss of 33.3 % as compared with 57 % on Epicote–EGA columns used in the present work (see Table III).

The quantitative data presented in this paper as well as the discoloration near the inlet of the columns suggest that the losses of methyl esters of polyunsaturated acids are due to their polymerization on the column. The extent to which this occurs, however, depends on the nature of the solid support as well as of the liquid phase. The relatively good recovery of 22:6 by ACKMAN AND BURGHER[2] with EGSS-X and EGSS-Y suggests that these liquid phases are superior to those investigated in the present work.

Coating the Celite with Epicote reduced the loss of methyl esters of unsaturated acids without impairing resolution, while the substitution of Celite by glass beads also reduced the losses but generally decreased the resolving power. The object of producing columns of high resolving power for methyl esters of highly unsaturated acids without loss has been achieved in the present investigation for methyl linolenate, methyl arachidonate and to a less extent methyl eicosapentaenoate.

According to NOVITSKAYA[8] the response of the argon-ionisation detector was found to be proportional to the mass of the methyl esters of palmitic, stearic, oleic and linoleic acids while with methyl linolenate, the detector response was relatively

greater with increasing voltage within the range of 750–1500 V. In the present work the voltage generally used was 1000. Two tests were made, however, at 800 V with methyl eicosapentaenoate. The results suggest the possibility of increased response at the higher voltage but the difference was small in relation to the losses of the ester on the column.

This work indicates that each column should be tested with methyl esters of highly unsaturated acids and where necessary a correction factor applied. In addition it is considered advisable in the analysis of mixtures containing methyl esters of acids with more than three double bonds to check the fatty acid composition against that of a fully hydrogenated sample.

SUMMARY

Using Celite as a solid support it was found that the recovery of methyl esters of polyunsaturated acids during gas chromatographic analysis decreased with increasing unsaturation. Greatest losses were sustained with DEGA as the liquid phase. Limited tests indicated that BDS, EGS and PEG were also unsatisfactory. With linolenate, EGA on siliconized Celite proved to be satisfactory. Similarly improved results were obtained by precoating the solid support with Epicote resin, when 20 % EGA as the liquid phase gave quantitative results for methyl linolenate and methyl arachidonate. The loss of methyl eicosapentaenoate was diminished from 42 % to 22 % on Epicote precoated columns. On the other hand the loss of methyl docosahexaenoate was decreased from 67 to 57 % only. With this ester better results (51 % loss) were obtained with 5 % w/w APL on untreated Celite. Glass microbeads provided a more inert solid support than Celite but on account of lack of resolving power it was not examined closely.

REFERENCES

1 T. GERSON, F. B. SHORLAND AND J. E. A. MCINTOSH, *Biochem. J.*, 91 (1964) 11C.
2 R. G. ACKMAN AND R. D. BURGHER, *J. Fisheries Res. Board Can.*, 21 (1964) 319.
3 A. SEHER, Institut für industrielle Fettforschung, Münster, Germany, personal communication, 1965.
4 J. W. FARQUHAR, W. INSULL, P. ROSEN, W. STOFFEL AND E. H. AHRENS, *Nutr. Rev.*, 17, Suppl. 6, No. 8 (1959)
5 J. E. LOVELOCK, A. T. JAMES AND E. A. PIPER, *Ann. N.Y. Acad. Sci.*, 72 (1959) 720.
6 T. GERSON, *J. Chromatog.*, 6 (1961) 178.
7 R. G. ACKMAN AND P. M. JANGAARD, *J. Am. Oil Chemists' Soc.*, 40 (1963) 744.
8 G. A. NOVITSKAYA, *J. Chromatog.*, 18 (1965) 20.

GAS–LIQUID CHROMATOGRAPHY OF SOME TYPES OF HETEROCYCLIC COMPOUNDS

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INTRODUCTION

The importance of gas–liquid chromatography is quite extensively recognised, at present, for solving not only analytical problems in the broad sense, but also as a method permitting the resolution of some of the questions related to the characteristic parameters of a molecule. The number of investigations in which the behaviour of a substance under conditions of gas–liquid chromatography is linked with the structure and spatial image of a molecule, is, however, still quite limited.

In this respect, heterocyclic compounds hold a prominent place. In most cases these are polar substances in which, when passing from one compound to another (as, for instance, in the thiophene, furan and pyrrole series), it is possible to trace by the chromatographic behaviour the peculiarities due to the heteroatom as long as it conserves, at first approximation, the geometry of the ring. In addition, the analytical aspect of the matter has not, in the main, been investigated either.

This work describes the basic results of studying the gas–liquid chromatographic behaviour of heterocyclic compounds; these were mostly synthesized first at the Zelinsky Institute of Organic Chemistry of the U.S.S.R. Academy of Sciences. The results illustrate the complex of problems which can be solved by gas–liquid chromatography, for instance:

(1) Qualitative and quantitative analyses of multicomponent mixtures, including high-boiling mixtures (with a boiling point up to 450°) and highly polar (*e.g.* pyridine) compounds;

(2) Determination of thermodynamic parameters of interaction between a substance and a stationary phase, and interpretation of the thermodynamic data as a function of the structure of a molecule.

EXPERIMENTAL

The investigation was carried out on a gas–liquid chromatograph made at the Zelinsky Institute of Organic Chemistry of the U.S.S.R. Academy of Sciences. The chromatograph is equipped with a heat conductivity detector, a 2 mV recorder with a time range of 1–2 sec, and an air thermostat which makes it possible to maintain a temperature in the column and in the heat conductivity detector up to 300°. Helium was used as carrier gas.

In every case, except when specially stipulated, copper spiral columns were used, with an inner diameter of 4–6 mm. To distribute the filling evenly, it was charged into the columns with periodic tapping.

PYRIDINE BASES

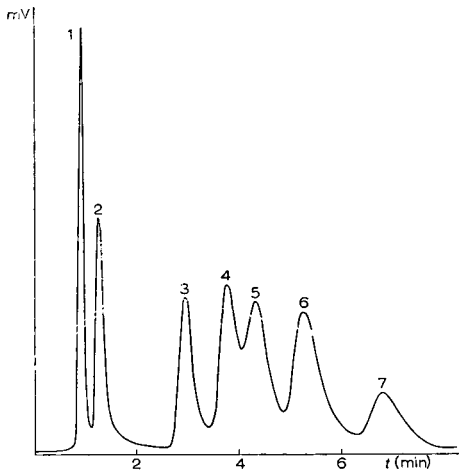
Gas–liquid chromatography of polar substances is generally complicated by the fact that the substance to be chromatographed interacts with the solid support, producing sharp non-symmetrical peaks on the chromatogram. Such interaction is particularly marked when acids or bases are chromatographed; the separation of pyridine bases entails just such complications. Acid–base interaction with the solid support is superimposed on the process of dissolution in the stationary phase. To eliminate such interaction, we used as a solid support either diatomite brick treated beforehand with 5 % alcoholic caustic soda with subsequent decantation[1], or sodium chloride for which acid–base interaction is practically precluded[2]. Either way has

TABLE I

RELATIVE RETENTION VOLUMES OF NITROGEN-CONTAINING HETEROCYCLIC COMPOUNDS

Item No.	*Compound*	*Mol. wt.*	*Boiling point (°C)*	$V_R^{rel.}$ *PEGA/diatomite brick*			*PEGA/NaCl*		
				150°	*180°*	*210°*	*150°*	*180°*	*210°*
1	Pyridine	79.1	115.3	1.0	1.0	1.0	1.0	1.0	1.0
2	α-Picoline	93.1	128.8	1.2	1.08	1.0	1.19	1.05	1.0
3	β-Picoline	93.1	143.8	1.72	1.43	1.16	1.75	1.4	1.2
4	γ-Picoline	93.1	145.4	1.85	1.42	1.16	1.9	1.4	1.2
5	2-Ethylpyridine	107.1	149.0	1.4	1.1	1.0	1.2	1.05	1.0
6	4-Ethylpyridine	107.1	165.0	3.3	2.1	1.2	3.2	2.0	1.1
7	4-Vinylpyridine	105.1	170.0	—	2.2	1.3	—	—	—
8	2,4-Lutidine	107.1	158.4	2.0	1.5	—	1.9	1.5	1.2
9	2,6-Lutidine	107.1	144.0	1.1	1.0	1.0	1.0	1.0	—
10	2,4,6-Trimethylpyridine	121.1	171.0	2.06	1.4	1.03	1.7	—	—
11	Ethyl nicotinate	151.2	223–224	—	3.9	2.6	3.14	2.4	1.63
12	Ethyl isonicotinate	151.2	216–217	—	3.44	2.38	2.82	2.2	1.55
13	3-Acetylpyridine	121.1	225	—	4.72	3.14	4.4	2.9	1.98
14	4-Acetylpyridine	121.1	215	—	4.0	2.6	4.08	2.8	1.8
15	3-Propionylpyridine	135.2	233	—	5.3	3.48	5.70	3.33	2.03
16	4-Propionylpyridine	135.2	225	—	4.98	—	—	—	—
17	3-Butyrylpyridine	149.2	240	—	6.35	3.8	7.3	3.94	2.33
18	4-Butyrylpyridine	149.2	230	—	5.66	3.4	6.7	3.7	2.2
19	3-Valerylpyridine	163	255	—	8.87	5.12	—	4.87	2.8
20	4-Valerylpyridine	163	240	—	7.7	—	—	—	—
21	α-Aminopyridine	94.1	210	—	4.26	3.74	—	—	3.0
22	Diethylamide of nicotinic acid	—	280	—	—	—	—	—	5.4
23	Piperidine	85.2	106	—	1.16	1.0	1.4	1.14	1.0
24	N-Acetylpiperidine	127.2	226–7	—	5.3	3.83	2.6	2.3	1.67
25	Quinoline	129.2	238	—	6.1	3.5	—	4.77	2.3
26	Isoquinoline	129.2	243	—	7.3	4.66	—	8.5	3.8
27	Quinaldine	143.1	247	—	6.35	3.6	—	5.7	2.46
28	Indole	117.1	223–4	—	14.8	8.9	—	7.65	4.1
	V_g (pyridine)						29.0	8.62	4.78

proved quite justifiable[3] (see Table I*). For example, an analysis was made in less than 10 min of a seven-component mixture of nitrogen-containing compounds on polyethyleneglycol adipate (PEGA) (Fig. 1). The latter, a stationary phase possessing high thermal stability and selectivity, was obtained by conventional methods[4]. A multicomponent mixture consisting, in the main, of pyridine derivatives could also be separated on a sodium chloride support (Fig. 2).



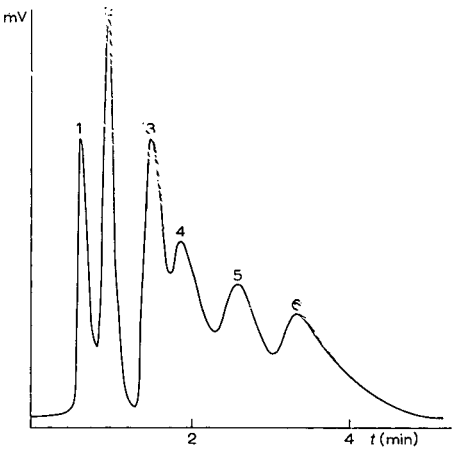


Fig. 1. Chromatogram of a mixture of nitrogen-containing compounds. Column: 20% of polyethyleneglycol adipate on diatomite brick treated with a 5% alcoholic solution of caustic soda; 2.6 m long; I.D., 6 mm; material, copper. Experimental conditions: temperature of the column and of the heat conductivity detector, 180°; helium flow rate, 67 ml/min. Notations of the peaks: 1 = pyridine; 2 = 2,4,6-trimethylpyridine; 3 = aniline; 4 = ethyl nicotinate; 5 = ethyl isonicotinate; 6 = 3-acetylpyridine; 7 = quinoline.

Fig. 2. Chromatogram of a mixture of nitrogen-containing compounds. Column: 1.0% polyethyleneglycol adipate on sodium chloride; 2.6 m long; I.D., 6 mm; material, copper. Experimental conditions: temperature of the column and of the heat conductivity detector, 180°; helium flow rate, 55 ml/min. Notations of the peaks: 1 = pyridine; 2 = aniline; 3 = ethyl nicotinate; 4 = 3-acetylpyridine; 5 = 3-butyrylpyridine; 6 = 3-valerylpyridine.

Polyethyleneglycol, when used on sodium chloride, possesses a lower selectivity than PEGA. Even at comparatively low temperatures (of the order of 150°), it is not possible to separate ethyl nicotinate and 3-acetylpyridine, which have similar boiling points, on it.

Pyridine, ethyl nicotinate and 3-acetylpyridine were chosen for the investigation of quantitative methods of analysis of compounds of the pyridine series. Aniline can be successfully used as an internal standard for analysis. The resultant method for the quantitative separation of various pyridine derivatives has made it possible to choose rapidly the optimal conditions in the catalytic synthesis of alkyl pyridyl ketones from aliphatic acids and esters of pyridinecarboxylic acids[5].

Reaction mixtures containing pyridine, a ketone of the aliphatic series, the products of its condensation, an ester of pyridinecarboxylic acid and alkyl pyridyl

* The boiling points given in the tables and figures were obtained by bringing the boiling point in a vacuum to normal conditions.

ketone were analysed in about 10 min (Fig. 3). The quantity of polymeric products formed in the course of the reaction, without leaving the column, was determined by adding aniline as internal standard. Information was obtained as to whether ketonization reaction takes place, and to what extent, without any preliminary treatment of the reaction mixture. Thus, there was no necessity to accumulate any catalysate, the stability of the catalyst was strictly and rapidly controlled, and all the components of the mixture were determined quantitatively.

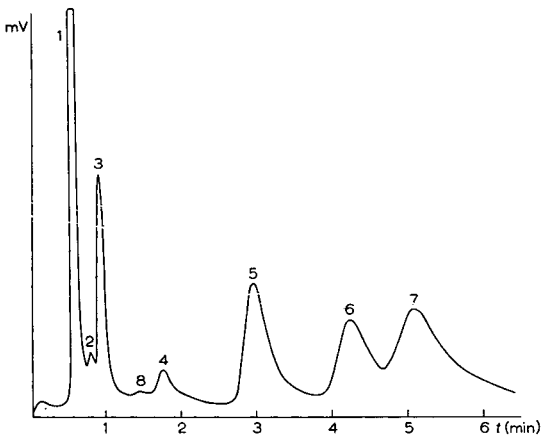


Fig. 3. Chromatogram of a catalysate in the ketonization reaction of ethyl nicotinate with acetic acid over an oxide alumozirconium catalyst. See legend of Fig. 1 for the column and the experimental conditions. Notations of the peaks: 1 = acetone; 2 = mesityl oxide; 3 = pyridine; 4 = non-identified peak; 5 = aniline; 6 = ethyl nicotinate; 7 = 3-acetylpyridine; 8 = diacetone alcohol.

Treatment of the solid support, diatomite brick, with an alkali leads to a reduction in the surface area of the brick from 20 m^2/g (before treatment) to 1 m^2/g (after treatment) and also apparently to partial suppression of the acid centres. Reduction of the surface area of the brick accounts for a shorter retention time, and suppression of the acid centres results in a decrease in "tailing". It was not possible, however, to completely eliminate "tailing". As can be seen from the above, a shorter retention time does not interfere with either qualitative or quantitative analysis. Complete symmetry of the peak was not observed even when sodium chloride was used as a solid support (Fig. 2). The existence of some interaction with the solid support (sodium chloride or diatomite brick after treatment with an alkali) is also corroborated by the relationship between the retention time (t_R) and the size of the sample (the greater the size of the sample, the smaller t_R).

We will now consider the possible modes of interaction of nitrogen-containing heterocyclic compounds (see Table I) with the stationary phase:

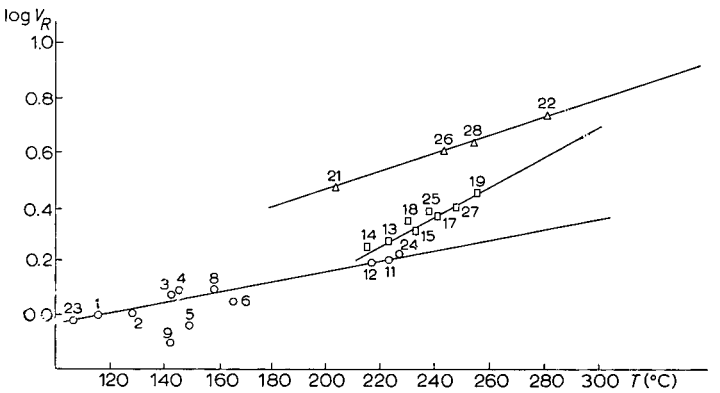
(a) acid–base (pyridine base: donor of electrons; stationary phase: acceptor of electrons);

(b) through the formation of a hydrogen bond.

Such interactions naturally depend on steric obstacles set up by the introduction of substituents into the molecule of the pyridine base.

The existence of a relationship between the retention volumes and the boiling points of nitrogen-containing heterocyclic compounds allows the establishment of a

number of rules (Fig. 4). By the nature of the interaction, the compounds under investigation may be classified into three groups: (1) alkyl derivatives of pyridine, (2) oxygen-containing compounds, and (3) derivatives with two nitrogen atoms in the molecule. Typical of the oxygen-containing pyridine bases is a larger angle of incline (0.01 Δ log $V_R^{rel.}$ per 1°) than that for alkylpyridines (0.005 per 1°). Hence, the coefficient of selectivity[6] for a pair of oxygen-containing alkyl derivatives on



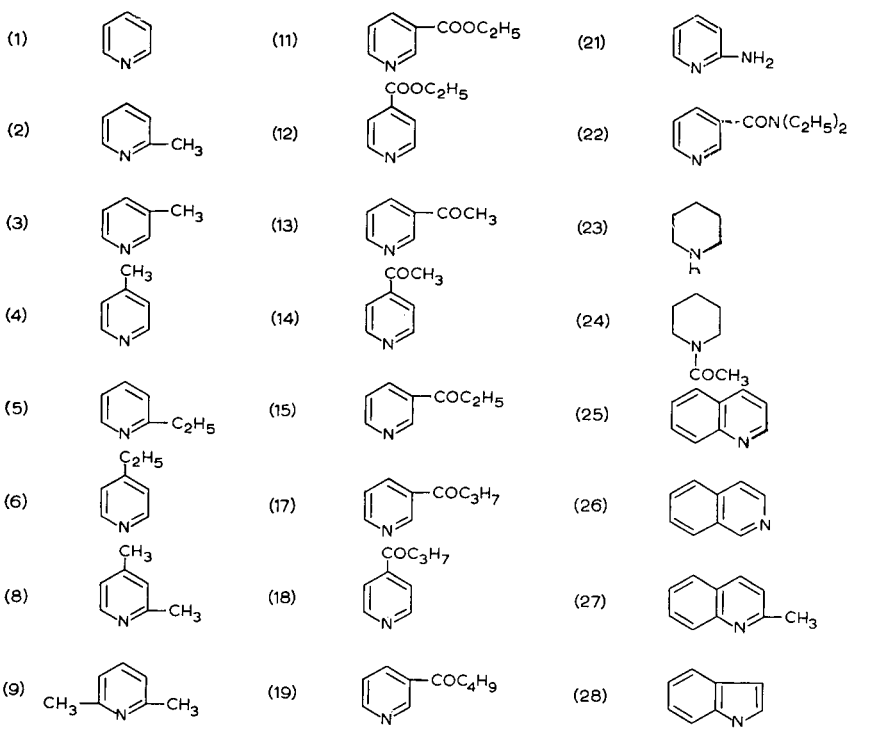


Fig. 4. Relationship between relative retention volumes (internal standard of pyridine $V_R^{rel.} = 1$) and boiling points. See legend of Fig. 2 for the column and the experimental conditions. Temperature: 210°. For compounds 7, 10, 16 and 20 no V_R data were determined at 210°.

polyethyleneglycol adipate equals 2. The addition of one more nitrogen atom to a pyridine molecule entails a sharp increase in the retention volume. The increase in $V_R^{rel.}$ for isoquinoline as compared with quinoline can be explained by an intensification of the basic properties in the case of isoquinoline. A larger $V_R^{rel.}$ for indole than for pyridine bases with approximately the same boiling points appears to be related to the formation of hydrogen bonding between the hydrogen of the indole NH and the oxygen of polyethyleneglycol adipate. Piperidine is a stronger base than pyridine, and, apart from this, is capable of forming a hydrogen bond with the stationary phase; for this reason its retention volume is greater than the one anticipated from its boiling point. Substitution of an acetyl group for hydrogen in piperidine precludes the possible formation of a hydrogen bond and sharply reduces the basicity; the retention volume begins to conform to the boiling point of an N-acylated derivative. The magnitude of the retention volume is also greatly influenced by steric effects in the molecule of the chromatographed substance.

As the nitrogen atom is the centre of basicity of pyridine bases, anything which impedes its interaction with the stationary phase will lead to a decrease in the retention volume. Such effects arise as a result of introducing substituents in the α-position of a pyridine ring. For instance, such steric hindrance is encountered in α-picoline only to a slight extent, is greater in 2-ethylpyridine owing to a more voluminous substituent, and increases still further with 2,6-dimethylpyridine, where both α-positions are occupied. As a result, the values of the retention volumes are below the curve log $V_R^{rel.}$–boiling point (Fig. 4). These differences cannot be attributed to a change in basicity since with an increase of the number of alkyl groups in substituted pyridines the basicity increases[7]. In the case of alkyl 3- or 4-pyridyl ketones, a linear relationship is observed between the retention volume and the number of carbon atoms in the side chain (Fig. 5); underlying this effect is the connection between the change in the logarithm of the retention volume and the increased work to transport a molecule of a substance from a solution to the gaseous phase on lengthening the carbon chain by one methylene group[8].

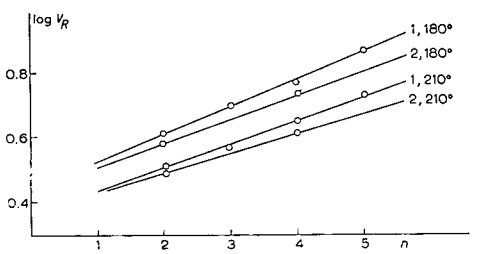


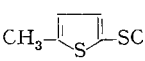
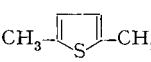
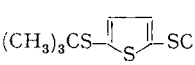
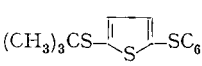
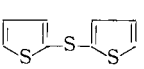
Fig. 5. Relationship between log $V_R^{rel.}$ (internal standard aniline) and the number of carbon atoms in the side chain of alkyl pyridyl ketones. 1 = Alkyl 3-pyridyl ketones; 2 = Alkyl 4-pyridyl ketones.

SULPHIDES OF THE THIOPHENE SERIES

Gas–liquid chromatography of high-boiling compounds represents a complex problem since the retention time at temperatures generally used in chromatography is so long that the substance does not even leave the column. At the same time a rise in temperature is undesirable and often impossible. The shorter retention time of

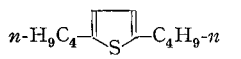
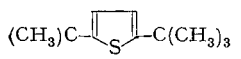
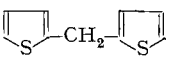
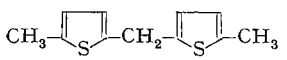
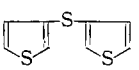
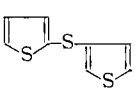
TABLE II

HEATS OF EVAPORATION AND SOLUTION OF THIOPHENE DERIVATIVES IN POLYETHYLENEGLYCOL ADIPATE AND SILICONE RUBBER SKTV

Thiophene derivatives	*Heat of evaporation at boiling point,* ΔH_V *(kcal/mole)*	*Heat of evaporation at 200°,* ΔH_V^{200} *(kcal/mole)*	*Polyethyleneglycol adipate on diatomite brick*		*Silicone rubber on sodium chloride*	
			Heat of solution, ΔH_S *(kcal/mole)*	*Excess heat of solution,* $\Delta \bar{H}_S^E$ *(kcal/mole)*	*Heat of solution,* ΔH_S *(kcal/mole)*	*Excess heat of solution,* $\Delta \bar{H}_S^E$ *(kcal/mole)*
	9.52	—	11.0	—2.40	9.60	—1.02
	9.85	—	11.4	—2.47	—	—
	10.16	10.3	12.0	—2.48	11.0	—1.64
	10.68	11.15	12.2	—1.83	12.6	—2.39
	9.93	9.93	11.7	—2.53	11.0	—1.83
	10.57	10.93	11.8	—1.30	12.9	—2.91
	10.91	11.41	13.2	—2.29	13.6	—3.13
	10.74	11.25	12.6	—2.46	13.5	—3.19
	11.87	13.08	—	—	13.4	—1.26
	11.49	12.48	14.05	—2.68	14.2	—2.66
	14.13	16.64	15.65	—0.19	19.5	—3.80
	14.84	17.77	—	—	16.0	+0.83
	15.07	18.11	—	—	17.6	—0.43
	12.01	13.17	13.95	—1.87	15.1	—2.87

(continued on p. 74)

TABLE II (*continued*)

Thiophene derivatives	*Heat of evaporation at boiling point, ΔH_V (kcal/mole*	*Heat of evaporation at 200°, ΔH_V^{200} (kcal/mole)*	*Polyethyleneglycol adipate on diatomite brick*		*Silicone rubber on sodium chloride*	
			Heat of solution, ΔH_S (kcal/mole)	*Excess heat of solution, $\Delta \bar{H}_S^E$ (kcal/mole)*	*Heat of solution, ΔH_S (kcal/mole)*	*Excess heat of solution, $\Delta \bar{H}_S^E$ (kcal/mole)*
	10.96	—	12.8	—1.98	19.4	—2.74
	10.46	10.78	11.0	—1.0	12.6	—2.76
	11.2	12.00	13.6	—2.71	13.2	—2.15
	11.87	13.08	15.6	—3.62	—	—
	12.36	13.84	14.95	—2.21	12.7	+0.20
	12.36	13.84	15.00	—2.26	14.0	—1.0
	7.52	—	6.83	—1.96	—	—

pyridine bases found after treatment of the solid support with an alkali (notably, as a result of its reduced surface) induced us to use it also for separating high-boiling sulphides of the thiophene series[3]* (Table II). At a temperature as low as 210° it was possible to elute sulphides of the thiophene series with a boiling point up to 460° on polyethyleneglycol adipate on diatomite brick treated with alkali (Fig. 6).

In this class of compounds, treatment of the solid carrier with alkali leads to a decrease in the retention volume by several magnitudes, to an extension of the band and the appearance of non-symmetry in the peak. The last two effects impair separation.

However, the temperature relationship of the logarithm of the corrected retention volume is the same for brick treated or untreated with alkali; in the case of methyl 2-thienyl sulphide, the heat of dissolution in polyethyleneglycol adipate is equal to 11 kcal/mole. Treatment of the solid support with an alkali does not tell either on the temperature relationship of other alkyl 2-thienyl sulphides and does not, consequently, affect the specific interaction of sulphides with the stationary phase. It was possible to separate a 12-component mixture of thiophene derivatives on untreated brick with polyethyleneglycol adipate as the stationary phase; the list

* Preparation of most sulphides of the thiophene series is described in refs. 9–12.

of the derivatives is given in the legend of Fig. 7. It is also possible to separate isomeric dithienyl sulphides, α-*tert.*-butylmercapto- and α-isobutylmercaptothiophenes and other compounds of a similar structure. The term θ (ref. 13) equals 0.7 or more. The method of separation developed has made it possible to demonstrate, notably, that *tert.*-butyl 2-thienyl sulphide obtained by addition of 2-mercaptothiophene to isobutylene does not contain an admixture of isobutyl 2-thienyl sulphide and that, likewise, α,β′- and β,β′-dithienyl sulphides obtained by reaction of the corresponding lithium thiophenes with dithienyl disulphides are free of any other isomers.

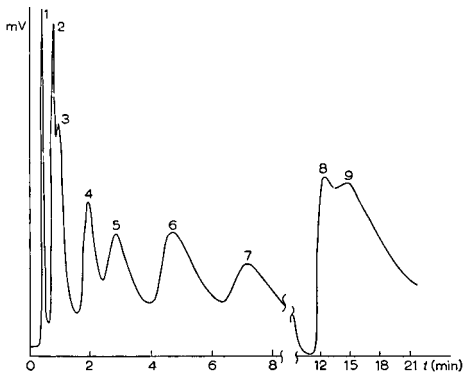
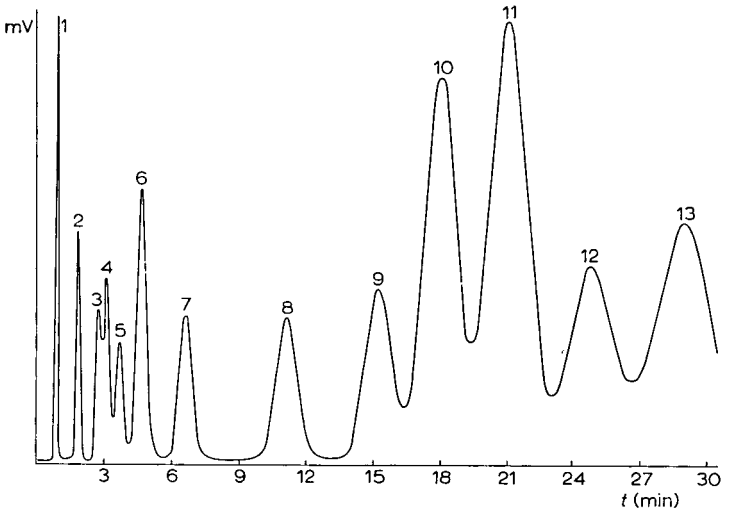


Fig. 6. Chromatogram of a mixture of the thiophene series sulphides. See legend of Fig. 1 for the column. Experimental conditions: temperature of the column and of the heat conductivity detector, 210°; helium flow rate, 60 ml/min. Notations of the peaks: 1 = solvent; 2 = methyl 2-thienyl sulphide; 3 = *tert.*-butyl (5-methyl-2-thienyl) sulphide; 4 = *n*-hexyl 2-thienyl sulphide; 5 = 2,5-bis-(*tert.*-butylmercapto)-thiophene; 6 = 2-*tert.*-butylmercapto-5-(isoamylmercapto)-thiophene; 7 = 2-*tert.*-butylmercapto-5-(*n*-hexylmercapto)-thiophene; 8 = 5-ethylmercapto-2,2′-dithienyl sulphide; 9 = 5-isobutylmercapto-2,2′-dithienyl sulphide.

In the case of some of the above-mentioned sulphides it was possible to observe a so-called "reaction chromatography", expressed by the fact that the compound underwent a transformation before entering the column; this occurred with a copper evaporator. The presence of a *tert.*-butyl group at the sulphide sulphur bound with the thiophene ring, or a bond of sulphide sulphur with this ring through a methylene group is common to all such compounds. If the *tert.*-butyl group is bound directly with the thiophene ring, the compound is not destroyed. This interesting fact may be used to confirm the structure of sulphides of the above-mentioned types.

There is a linear relationship between the retention volume and the boiling point of sulphides of the thiophene series by which it is possible to trace, as in the case of pyridine bases, the effect of the structure of the sulphide on the retention volume (Fig. 8). Typical of sulphides with two thiophene rings is the larger angle of incline than that for sulphides with one thiophene ring, and, consequently, a greater selectivity of the stationary phase. A comparison between the retention volumes of 2,2′-dithienylmethane and 2,2′-dithienyl sulphide indicates that substitution of a sulphur atom for a methylene group does not affect the interaction with the stationary phase, and both substances chromatograph in accordance with their boiling points (Fig. 8). In the case of 1,2-bis-(thienyl-2-mercapto)-ethane, *i.e.* when the thiophene rings are disrupted by two sulphur atoms and two methylene groups, interaction between the



(1) Solvent

(2) $(CH_3)_3C$–S–$C(CH_3)_3$

(3) S–SCH_3

(4) n-C_4H_9–S–C_4H_9-n

(5) S–SC_4H_9-iso

(6) S–SC_5H_{11}-iso

(7) S–SC_6H_{13}-n

(8) S–CH_2–S

(9) CH_3–S–CH_2–S–CH_3

(10) S–S–S

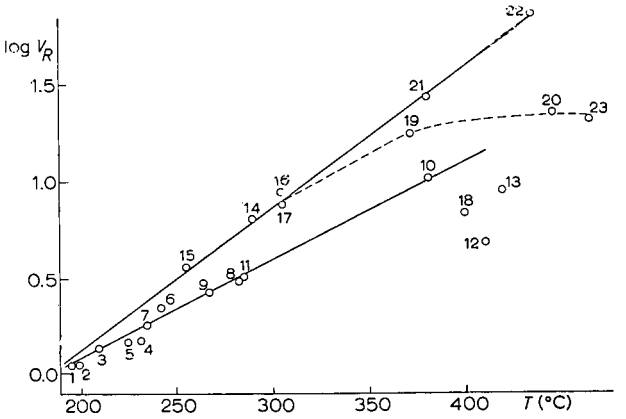
(11) S–S–S

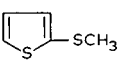
(12) S–S–S

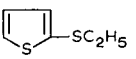
(13) S–$SC_{12}H_{25}$-n

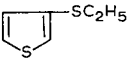
Fig. 7. Chromatogram of a mixture of sulphur-containing compounds (in the main, thiophene series sulphides). Column: 20% polyethyleneglycol adipate on diatomite brick; 2.6 m long; I.D., 6 mm; fraction of the solid support: 0.25–0.5 mm. Experimental conditions: temperature of the column and of the heat conductivity detector, 220°. Notations of the peaks: 1 = solvent; 2 = 2,5-di-*tert.*-butyl-thiophene; 3 = methyl 2-thienyl sulphide; 4 = 2,5-di-*n*-butyl thiophene; 5 = isobutyl 2-thienyl sulphide; 6 = isoamyl 2-thienyl sulphide; 7 = *n*-hexyl 2-thienyl sulphide; 8 = 2,2′-dithienylmethane; 9 = 5,5′-dimethyl-2,2′-dithienylmethane; 10 = 2,2′-dithienyl sulphide; 11 = 2,3′-dithienyl sulphide; 12 = 3,3′-dithienyl sulphide; 13 = *n*-dodecyl 2-thienyl sulphide.

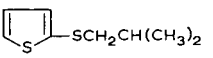
substance and the stationary phase is governed by the same rule. If an alkyl mercapto group is introduced into one of the α-positions of 2,2′-dithienyl sulphide, one thiophene ring is immediately substituted in the two α-positions and, as a result, shielded. The sharp decrease in the retention volume, recorded in this case, is, of course, a consequence of the difficulty of interaction with the stationary phase. Such an effect can be traced by the derangement of the relationship log $V_R^{rel.}$–boiling point (dotted line

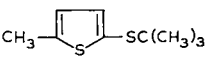


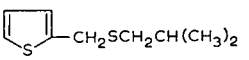
(1) 

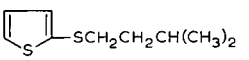
(2) 

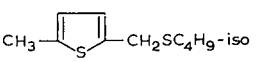
(3) 

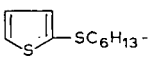
(4) 

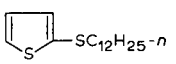
(5) 

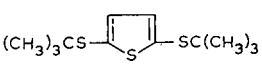
(6) 

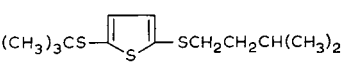
(7) 

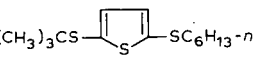
(8) 

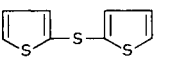
(9) 

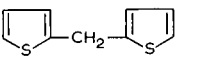
(10) 

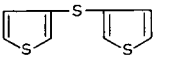
(11) 

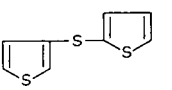
(12) 

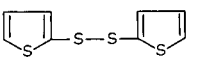
(13) 

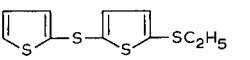
(14) 

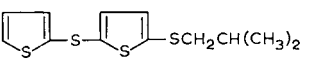
(15) 

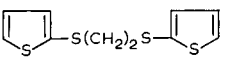
(16) 

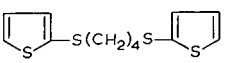
(17) 

(18) 

(19) 

(20) 

(21) 

(22) 

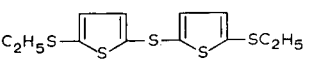
(23) 

Fig. 8. Relationship between the logarithm of relative retention volumes of thiophene sulphides (internal standard methyl 2-thienyl sulphide) and their boiling points. Column: polyethyleneglycol adipate applied to diatomite brick treated with an alkali. Temperature of the column and of the heat conductivity detector 210°.

in Fig. 8), which becomes greater as the size of the alkyl mercapto group increases. Similar effects are also observed in the series of sulphides with one thiophene ring (Fig. 8). In this case, it is necessary that two voluminous alkyl substituents should be present in both α-positions to reduce interaction with the stationary phase. The resultant relationships between the logarithm of the retention volume and the boiling point of the sulphide permit definite conclusions to be drawn regarding the structure of the compound under investigation.

Accurate and detailed information on interaction with the stationary phase and on the nature of the structural elements in the substances under investigation can be gained by considering the thermodynamics of solution. As is well known, the retention volume is linked with the free energy of solution and depends on the enthalpic and entropic terms[14,15]. The temperature relationship of the logarithm of the corrected retention volume allows the enthalpic component to be determined, *i.e.* the heat of solution (ΔH_S). The heat of solution determined experimentally includes the heat of evaporation (ΔH_V) and the excess heat of solution ($\Delta \bar{H}_S{}^E$)[14,15]. When solutions close to ideal ones are formed, the heat of solution equals that of evaporation and $\Delta \bar{H}_S{}^E = 0$. By determining the magnitude of $\Delta \bar{H}_S{}^E$, it is possible to assess the degree of interaction between the stationary phase and the substance under investigation. The relationship connected with the difference in the heats of evaporation is then precluded. At boiling point the former was calculated by Kistyakovsky's equation[16]:

$$\Delta H_V / T_{b.p.} = 8.75 + 4.576 \log T_{b.p.}$$

At a temperature differing from the boiling point (*e.g.* the temperature of the chromatogram), the heat of evaporation was calculated by Watson's equation[16]:

$$\frac{(\Delta H_V)_1}{(\Delta H_V)_2} = \left[\frac{1-(T_r)_1}{1-(T_r)_2}\right]^{0.37}$$

where $(\Delta H_V)_1$ and $(\Delta H_V)_2$ are the heats of evaporation at boiling point, T_1, and the temperature of the experiment, T_2; $(T_r)_1 = T_1/T_c$, where T_c is the critical temperature and $(T_r)_2 = T_2/T_c$. The critical temperature, T_c, for sulphur-containing compounds was determined according to the formula[12]:

$$T_c = 1.41 \cdot T_{b.p.} + 66$$

For thiophene, the value of ΔH_V calculated by Kistyakovsky's equation (7.29 kcal/mole) differs from the one calculated experimentally[17] (7.52 kcal/mole) by 3%. The values of ΔH_V, ΔH_S and $\Delta \bar{H}_S{}^E$ obtained for thiophene derivatives are summarized in Table II (within ± 0.05). In practically every case the term $\Delta \bar{H}_S{}^E$ is negative (exothermic effect in solution), which corresponds to the deviations in Raoult's law. Such deviations confirm the interaction between the compound to be chromatographed and the stationary phase.

Let us follow the change in the value of $\Delta \bar{H}_S{}^E$ when passing from one compound to another (Table II). The addition of an alkylmercapto group to a thiophene ring eads to an increase in the degree of interaction between the substance and the

stationary phase. Substitution of an ethylmercapto for a methylmercapto group does not markedly affect the magnitude of the excess heat of solution. An increase in the length of the alkyl chain or the presence of another α-substituent sharply reduces the interaction. Apparently there is not much difference when the alkylmercapto group is in either the α- or the β-position or when the sulphur atom is linked with the thiophene ring either directly or through the methylene group. The excess heat of solution is approximately the same for non-substituted thiophene and a compound with two thiophene rings bound by sulphur sulphide in an α-position. For alkyl-substituted thiophenes with an unbranched side chain the excess heat of solution is of the same magnitude as that for thiophene; it diminishes when the alkyl chain is branched (for 2,5-di-*tert.*-butylthiophene it amounts to —1.0) as a result of steric hindrance and the interaction between the substance and the stationary phase. The influence of steric hindrance on $\Delta\bar{H}_S{}^E$ should be distinguished from the reduction in the excess heat of solution upon an increase in the size of a molecule owing to the greater work required for drawing apart the molecules of the stationary phase[18]. The conclusion may be drawn that the thiophene ring and sulphur sulphide do not exercise such a reciprocal influence which would be expressed in a stronger interaction between sulphides of the thiophene series and the stationary phase.

A principal role in the interaction with the stationary phase is played by the thiophene ring whose shielding sharply reduces the interaction with the stationary phase. In a non-polar solvent (silicone rubber, Table II) the major part in the interaction with the stationary phase will be played by dispersion forces (and not a dipole–dipole interaction as in the case of the above-mentioned example with polyethyleneglycol adipate) which increase in proportion to the number of groups which come into direct contact with the stationary phase[19]. It is natural that there, too, steric hindrance will play a substantial part. But in general, as the hydrocarbon chain grows, interaction with the stationary phase increases. As can be seen from Table II, the nature of the change in $\Delta\bar{H}_S{}^E$ in the case of silicone rubber is opposite to that in $\Delta\bar{H}_S{}^E$ in the case of polyethyleneglycol adipate. The above leads to the assumption that comparative chromatographic characteristics permit the measurement of polarity of a substance and the peculiarities of its structure to be assessed with some reliability.

FURAN DERIVATIVES*

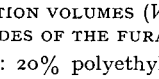
It was of considerable interest to compare the chromatographic features of similar thiophene and furan derivatives. A manifestation of the substitution of an oxygen atom for an atom of the sulphur in the ring, which leads to some increase in the dipole moment of the molecule, might be anticipated in such a comparison. Tables III and IV show the compounds under investigation.

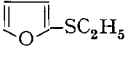
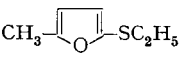
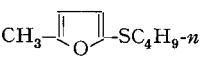
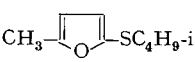
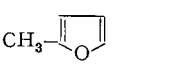
In the case of derivatives with one furan ring, a linear relationship is observed between log $V_R{}^{\text{rel.}}$ and the $T_{\text{b.p.}}$ of the substance (Fig. 9). A comparison of the chromatographic behaviour of furan and furylthienylmethane indicates that addition to the furan molecule of a thiophene ring linked through a methylene bridge with the furyl residue doubles the selectivity of separation. In this case both dithienylmethanes

* Synthesis of the substances is described in refs. 20–22.

TABLE III

RELATIVE RETENTION VOLUMES ($V_R^{rel.}$), HEATS OF EVAPORATION (ΔH_V) AND HEATS OF SOLUTION (ΔH_S) OF SULPHIDES OF THE FURAN SERIES

Stationary phase: 20% polyethyleneglycol adipate on diatomite brick treated with an alkali. For  , the retention time is 2.55 min at 137°, 1.05 min at 180°, and 0.6 min at 210°.

Item No.	*Compound*	*$T_{b.p.}$ at 760 mm Hg (°C)*	*$V_R^{rel.}$*			*ΔH_S (kcal/mole)*	*ΔH_V (kcal/mole)*
			137°	*180°*	*210°*		
1		158–160	0.78	0.88	—	11.0	9.0
2		179–180	1.0	1.0	1.0	12.2	9.5
3		218–219	1.96	1.46	1.41	13.6	10.4
4		209–210	1.53	1.28	—	—	—
5		65	0.22	—	0.71	—	—

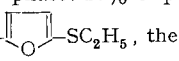
and furylthienylmethanes are subordinated to one linear relationship. Mobile π-electrons in the ring, and not the alkylmercapto group, seem to play a major part in the interaction with the stationary phase in this case. The degree of interaction between the stationary phase and the thiophene and furan ring is practically the same. Interaction between diphenylmethane and the stationary phase is considerably less than in the corresponding furan and thiophene derivatives. As in the case of alkyl 2-thienyl sulphides, the excess heat of solution of sulphides of the furan series in the stationary phase (in polyethyleneglycol adipate) is negative, *i.e.* there is an exothermic effect and negative deviations from Raoult's law during solution.

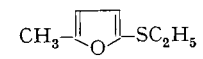
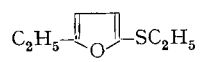
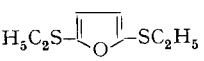
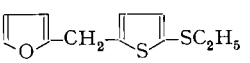
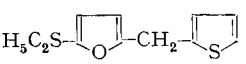
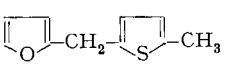
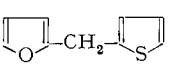
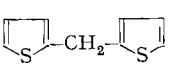
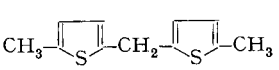
Low-boiling sulphides of the furan series with one furan ring are separated at 137° (Table III and Fig. 10); the last component has a boiling point of 220°. All separations of a five-component mixture take eight minutes. The retention time increases with the number of carbon atoms in a molecule. For compounds of the furan series which have branched alkyl substituents and lower boiling points, the retention time diminishes correspondingly. Fig. 11 shows a chromatogram of a seven-component mixture of higher-boiling furan and thiophene derivatives. The last component has a boiling point of 305°; the whole separation takes seven minutes at 210°. Introduction of methyl groups into the molecule of heterocyclic diarylmethane proves to be insufficient for its separation from non-substituted homologues; for this reason 2-methyl-5-(2-furfuryl)-thiophene appears on the chromatogram as a bend on the 2-furyl-2-thienylmethane peak (Fig. 11). In view of the fact that the boiling point of di-(2-thienyl)-methane is higher than of 2-thienyl-2-furylmethane, it is eluted later. The boiling points of 5-*n*-butylmercapto-2-methylfuran and 2-furyl-2-thienyl-

TABLE IV

RELATIVE RETENTION VOLUMES (V_R^{rel}) OF SULPHIDES OF THE FURAN SERIES AND OF DITHIENYL- AND FURYLTHIENYLMETHANE

Stationary phase: 20% of polyethyleneglycol adipate on diatomite brick treated with an alkali.

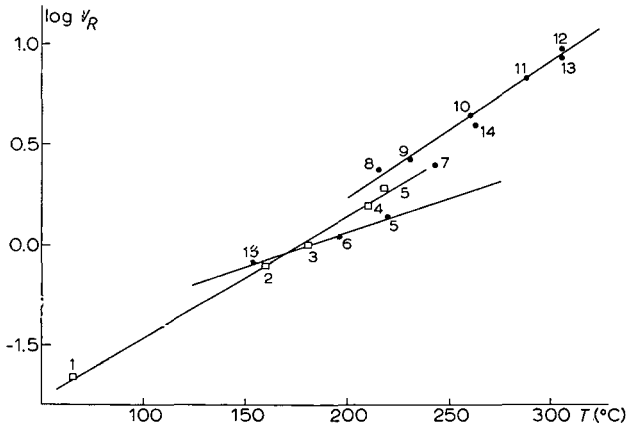
For  , the retention time is 0.6 min.

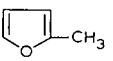
Item No.	*Compound*	*$T_{b.p.}$ at 760 mm Hg*	*$V_R^{rel.}$ at 210°*
1		179–180	1.0
2		195	1.05
3		240–245	2.42
4		305	8.7
5		305	8.3
6		230	2.67
7		215	2.50
8		260	4.42
9		285	7.0

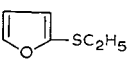
methane are nearly the same (215–218°). Nevertheless, the latter appears later, thereby corroborating the previous conclusion that the degree of interaction of the stationary phase with the thiophene of furan ring is higher than with the alkylmercapto group. A similar conclusion can be drawn by comparing the retention time of 2,5-bis-(ethylmercapto)-furan ($t_R^{210°}$, 1.45 min, $T_{b.p.}$ 240–245°) with the retention time of 2-methyl-5-(2-furfuryl)-thiophene ($t_R^{210°}$, 1.6 min, $T_{b.p.}$ 230°).

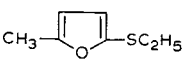
THIENOTHIOPHENES

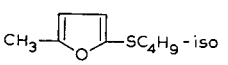
Investigation into the chromatographic behaviour of thienothiophene derivatives was of special interest. On the one hand, it was possible to explain the behaviour of condensed thiophene systems, and on the other, to resolve questions of identification of compounds since thienothiophenes can be obtained in different

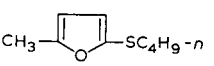


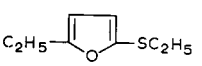
(1) 

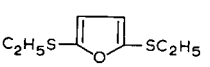
(2) 

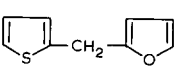
(3) 

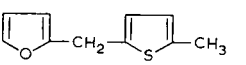
(4) 

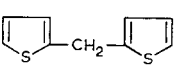
(5) 

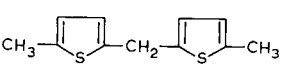
(6) 

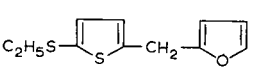
(7) 

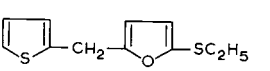
(8) 

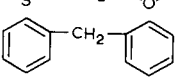
(9) 

(10) 

(11) 

(12) 

(13) 

(14) 

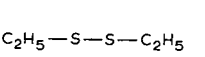
(15) 

Fig. 9. Relationship between relative retention volumes and boiling points. Column: 20% polyethyleneglycol adipate on diatomite brick treated with an alkali. Temperature: (□) 137°, (●) 210°. Notations of the points: 1 = sylvan; 2 = 2-ethylmercaptofuran; 3 = 5-ethylmercapto-2-methylfuran; 4 = 5-isobutylmercapto-2-methylfuran; 5 = 5-*n*-butylmercapto-2-methylfuran; 6 = 5-ethylmercapto-2-ethylfuran; 7 = 2,5-bis-(ethylmercapto)-furan; 8 = 2-furyl-2′-thienylmethane; 9 = 2-methyl-5-(2-furfuryl)-thiophene; 10 = di-(2-thienyl)-methane; 11 = bis-(5-methyl-2-thienyl)-methane; 12 = ethyl (5-furfuryl-thienyl-2) sulphide; 13 = ethyl (5-thenylfuryl-2) sulphide; 14 = diphenylmethane; 15 = diethyl disulphide.

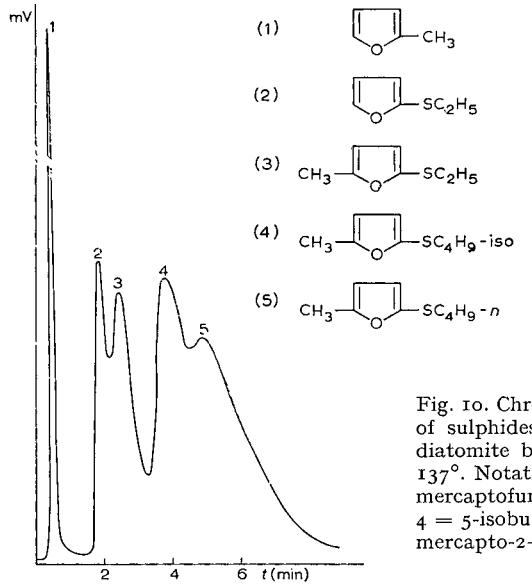


Fig. 10. Chromatogram of a mixture of the furan series of sulphides. Column: polyethyleneglycol adipate on diatomite brick treated with an alkali. Temperature: 137°. Notations of the peaks: 1 = sylvan; 2 = 2-ethylmercaptofuran; 3 = 5-ethylmercapto-2-methylfuran; 4 = 5-isobutylmercapto-2-methylfuran; 5 = 5-*n*-butylmercapto-2-methylfuran.

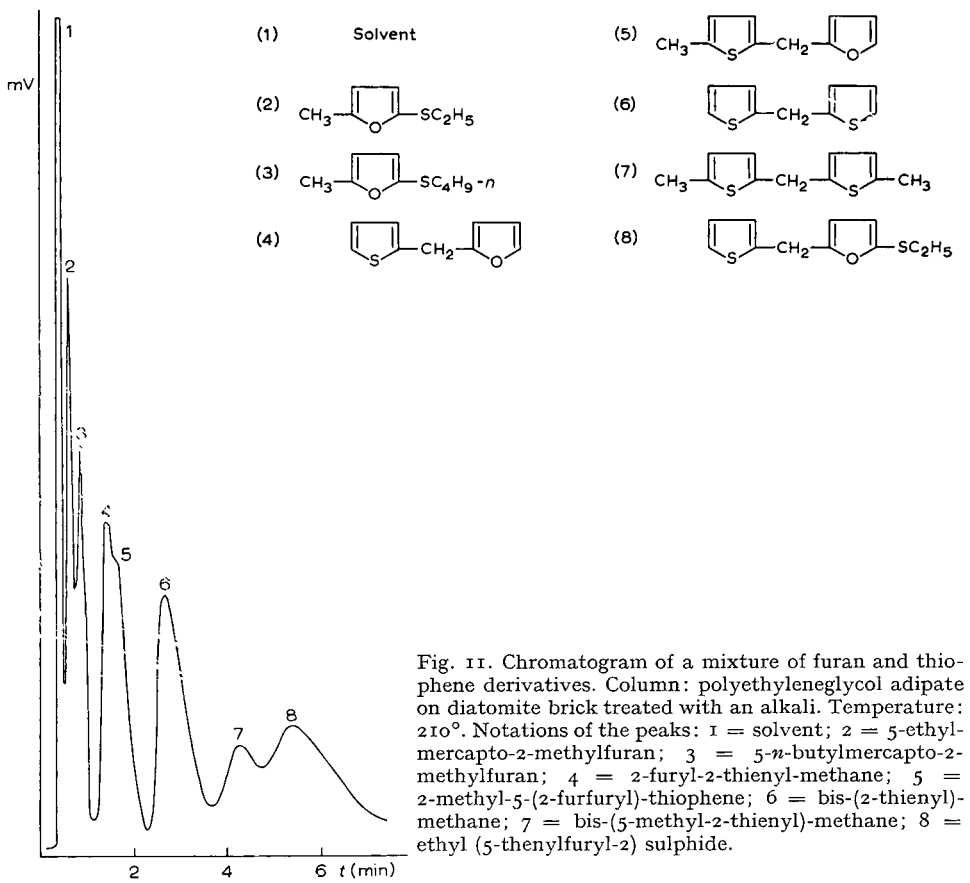


Fig. 11. Chromatogram of a mixture of furan and thiophene derivatives. Column: polyethyleneglycol adipate on diatomite brick treated with an alkali. Temperature: 210°. Notations of the peaks: 1 = solvent; 2 = 5-ethylmercapto-2-methylfuran; 3 = 5-*n*-butylmercapto-2-methylfuran; 4 = 2-furyl-2-thienyl-methane; 5 = 2-methyl-5-(2-furfuryl)-thiophene; 6 = bis-(2-thienyl)-methane; 7 = bis-(5-methyl-2-thienyl)-methane; 8 = ethyl (5-thenylfuryl-2) sulphide.

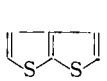
TABLE V

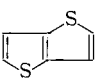
RELATIVE RETENTION VOLUMES AND COEFFICIENTS OF SEPARATION OF THIENOTHIOPHENE DERIVATIVES

Item No.	*Compound*	*Boiling point at 760 mm Hg (°C)*	*20% PEGA–diatomite brick + alkali, 2 m column*		
			150° $V_R^{rel.}$	*170°* $V_R^{rel.}$	*211°* $V_R^{rel.}$
1	3-Methyl-5-ethylthieno(3,2b)-thiophene	248	1.0 (V_R = 1565 ml) (t_R = 23.7 min)	1.0 (V_R = 660 ml) (t_R = 12 min)	1.0 (V_R = 263 ml) (t_R = 2.5 min)
2	3-Methyl-5-ethyl-thieno(2,3b)-thiophene	258	1.05	1.08	1.0
3	3-Methylthieno(3,2b)thiophene	230	0.47	0.647	0.647
4	3-Methylthieno(2,3b)thiophene	245		0.88	
5	3-Acetonylmercaptothiophene	257		0.556	
6	2-Acetonylmercaptothiophene	265		1.88	
7	Thieno(3,2b)thiophene	221–224		0.526	
8	2-Ethylthieno(3,2b)thiophene	250			
9	2-Ethylthieno(2,3b)thiophene	245			

* For the pair thieno(3,2b)thiophene and 3-methylthieno(2,3b)thiophene.
** θ is given for the following pairs: 3-methyl-5-ethylthieno(3,2b)thiophene and 3-methylthieno(3,2b)thiophene; 3-methyl-5-ethylthieno(2,3b)thiophene and 2-ethylthieno(2,3b)thiophene; 3-methylthieno(3,2b)thiophene and 2-ethylthieno(3,2b)thiophene.

ways. Thieno(2,3b)thiophene derivatives (I) are characterized by greater retention volumes than derivatives of thieno(3,2b)thiophene (II) (Table V, Fig. 12). This is


(I)


(II)

in agreement with the higher boiling points and the presence of a dipole moment in (I) (ref. 23). Introduction of an alkyl group raises the boiling point of the substance and, correspondingly, its retention time. Treatment of diatomite brick, the solid support, with an alkali results in a reduced retention time and in the appearance of "tailing". The latter effect is directly opposite to the one resulting from treat-

TABLE VI

QUANTITATIVE ANALYSIS OF A MIXTURE OF 3-METHYL-5-ETHYLTHIENO(2,3b)THIOPHENE (III) AND 3-METHYL-5-ETHYLTHIENO(3,2b)THIOPHENE (IV)

Standard mixture (%)		*Per cent determined*			
		*By the area of the peak**		*By the product height × retention time***	
III	*IV*	*III*	*IV*	*III*	*IV*
50	50	49.5	50.5	49	51
40	60	43	57	44	56
30	70	30	70	30	70

* On a column of polyethyleneglycol adipate applied to coated tile.
** On a column of polyethyleneglycol adipate applied to diatomite brick treated with alkali.

20 % PEGA–diatomite brick + alkali, 2.6 m column		*15 % PEGA–coated tile, 3 m column*		*1 % silicone rubber SKTV–NaCl, 2 m column*	
175° $V_R^{rel.}$	θ	*153°* $V_R^{rel.}$	θ	*200°* $V_R^{rel.}$	θ
1 (V_R = 2900 ml) (t_R = 44.5 min)	0.36	1.0 (V_R = 3560 ml) (t_R = 59.4 min)	0.42	1.0 (t_R = 5.1 min)	1.00
1.11	1.0	1.13		1.09	1.00**
0.60				0.59	1.0**
0.68					
0.405					
0.503	0.82*				
				0.79	1.0**
				0.79	1.0 **

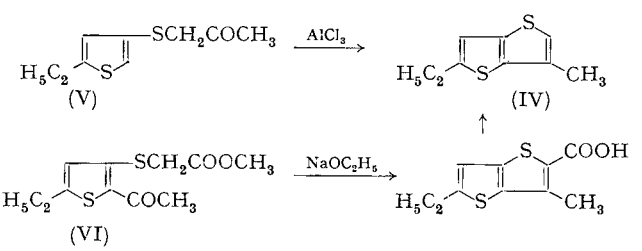
ment of the solid carrier with an alkali when pyridine bases are separated. This naturally impedes separation of isomeric thienothiophenes: instead of maxima, "shoulders" and bends appear on the chromatogram. Nevertheless, as shown in Table VI, this hardly interferes with the determination of the contents of isomers in the mixture.

After treatment of the support with an alkali, the retention time becomes even shorter than that on a column with 1 % of silicone rubber applied to sodium chloride.

The methods developed for the chromatographic separation of isomeric thienothiophenes have made it possible to resolve the following questions:

(a) identity of thienothiophene derivatives obtained in various ways;

(b) the route of the transformation upon cyclization of derivatives of acetonylmercaptothiophene. (IV), obtained by cyclization of 5-ethyl-3-acetonylmercaptothiophene (V) with aluminium chloride proved to be identical to the product obtained from methyl (5-ethyl-2-acetyl-3-thienylmercapto)-acetate (VI) (ref. 24).



No isomeric thienothiophene is formed in the reaction, *i.e.* there is no migration of the acetonylmercapto group from the 3- into the 2-position of the thiophene ring in the course of the reaction with aluminium chloride. 3-Methylthieno-(3,2 b)thio-

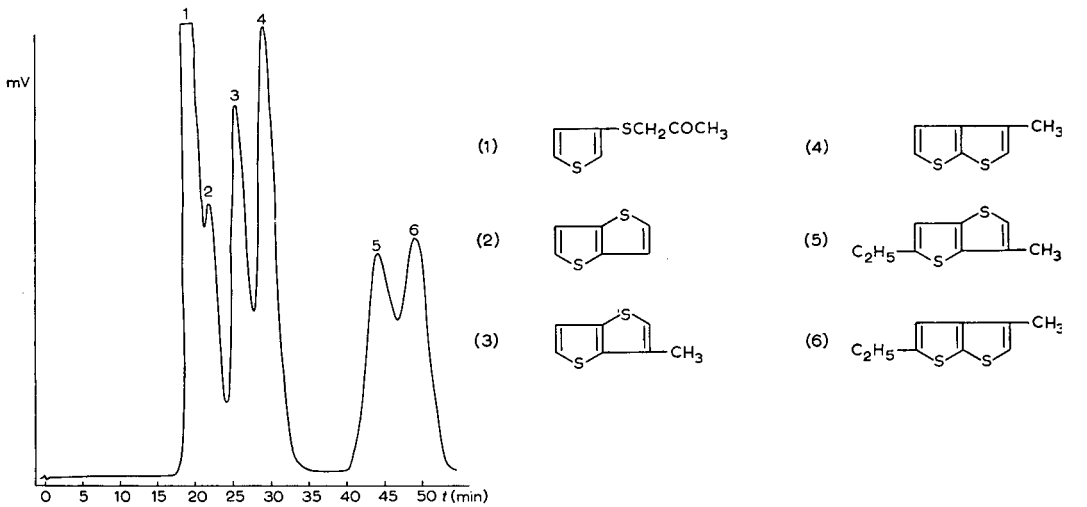
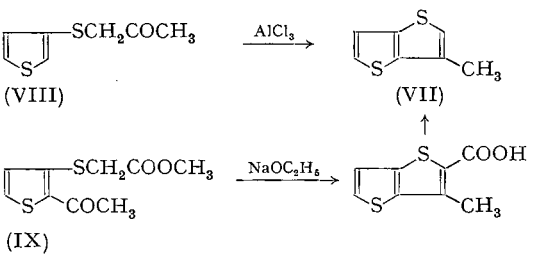


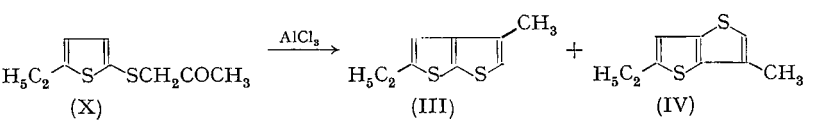
Fig. 12. Chromatogram of a mixture of isomeric thienothiophenes. Column: 20% polyethyleneglycol adipate on diatomite brick, 2.6 m long, I.D. 6 mm. Fraction of the solid support: 0.25–0.50 mm. Experimental conditions: temperature of the column and of the heat conductivity detector, 175°; helium flow rate, 66 ml/min; pressure at inlet, 0.45 kg/cm². Notations of the peaks: 1 = 3-acetonylmercaptothiophene; 2 = thieno(3,2b)thiophene; 3 = 3-methylthieno-(3,2b)thiophene; 4 = 3-methylthieno(2,3b)thiophene; 5 = 3-methyl-5-ethylthieno(3,2b)thiophene; 6 = 3-methyl-5-ethylthieno(2,3b)thiophene.

phene (VII) synthesized by cyclization of 3-acetonylmercaptothiophene (VIII) with aluminium chloride, is identical to the product obtained from methyl (2-acetyl-3-thienylmercapto)-acetate (IX) (ref. 24).



In this case, there is no migration of the acetonylmercapto group either.

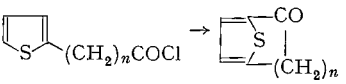
However, on cyclization of 5-ethyl-2-acetonylmercaptothiophene (X) in the presence of aluminium chloride, the acetonylmercapto group shifts to a 3-position, forming a mixture of isomeric dialkyl-substituted thienothiophenes (III) and (IV):



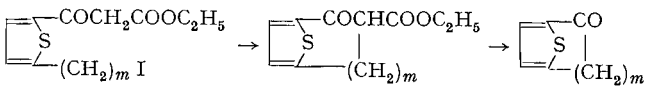
It was also shown by gas–liquid chromatography, that no migration of alkyl groups occurs in any of the above cases.

MACROCYCLIC COMPOUNDS INCLUDING A THIOPHENE RING

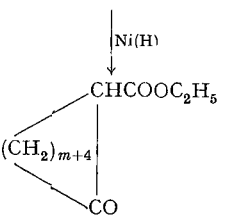
In recent years the Institute of Organic Chemistry developed a new method of synthesizing macrocyclic compounds based on thiophene[25], consisting in intramolecular acylation (Diagram 1) or alkylation (Diagram 2) of compounds of the thiophene series:



(1)

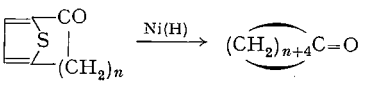


(2)



(2′)

The macrocyclic compounds so formed, which include a thiophene ring, can be easily transformed into macrocyclic ketones by reducing desulphurization on Raney nickel (Diagram 3).



(3)

Several highly interesting points arise from the viewpoint of chromatography:

(a) The possibility of analytically separating macrocyclic ketones which include a thiophene ring and alicyclic ketones. The complexity of separation is connected in this case with the high boiling point and the strong polarity of the substance to be separated, and apart from this, there is a difficulty here owing to the fact that the substances to be analysed must be prevented from contact with material such as copper, which is generally used in making the columns, the evaporator, and the heat conductivity detector.

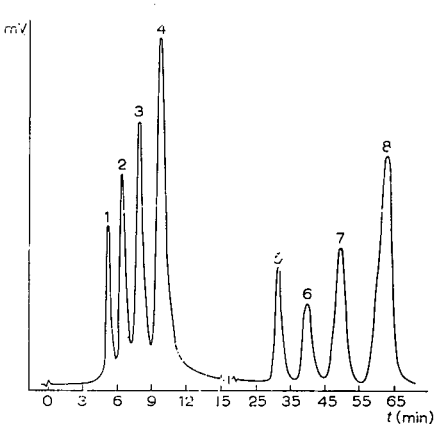
(b) The nature of interaction between ketones, which include a thiophene ring, and alicyclic ketones with the stationary phase, and the change in such interactions on a change in the size of the ring. As in previous cases, separations were carried out on a laboratory chromatograph made at the Institute of Organic Chemistry, U.S.S.R. Academy of Sciences, but in this case the heat conductivity detector was made of stainless steel with a tungsten filament; the evaporator and the U-shaped tubes were likewise made of stainless steel. Chromosorb W was used as solid support and coated with 15 % polyethyleneglycol succinate as the stationary phase. The data on the relative retention volumes are given in Table VII. Fig. 13 shows a chromatogram of an eight-component mixture of alicyclic C_{14}–C_{17} ketones and of corre-

TABLE VII

RELATIVE RETENTION VOLUMES AND COEFFICIENTS OF SEPARATION (θ) OF α-CYCLOTHIENONES AND ALICYCLIC KETONES

Compound	*Boiling point at 760 mm Hg (°C)*	*15% polyethyleneglycol succinate–Chromosorb W*						*PEGA–diatomite brick + alkali*		
		180°		*200°*		*217.5°*		*180°*	*200°*	*210°*
		$V_R^{rel.}$	θ	$V_R^{rel.}$	θ	$V_R^{rel.}$	θ	$V_R^{rel.}$	$V_R^{rel.}$	$V_R^{rel.}$
Cyclotetradecanone	288	0.778	0.90	0.807	0.86	0.826	0.8	0.746		0.71
Cyclopentadecanone	330	1.00 (V_R' = 30 ml) (t_R = 11.4 min)	0.85	1.00 (V_R' = 170 ml) (t_R = 6.5 min)	0.82	1.00 (V_R' = 98.2 ml) (t_R = 4.1 min)	0.75	1.00 (V_R' = 836 ml) (t_R = 15.2 min)	1.00 (V_R' = 445 ml) (t_R = 8.1 min)	1.00 (V_R' = 396 ml) (t_R = 7.2 min)
Cyclohexadecanone	345	1.26	0.90	1.23	0.87	1.20	0.83	1.38		1.19
Cycloheptadecanone	365	1.61	1.0	1.51	1.0	1.45	1.0	1.85	1.82	1.52
(10)-α-Cyclothienone-1	330	5.01	1.0	4.60	1.0	4.16	0.97		4.23	3.68
(11)-α-Cyclothienone-1	360	6.64	1.0	5.85	1.0	5.17	0.94			4.8
(12)-α-Cyclothienone-1	380	8.62	1.0	7.37	1.0	6.38	0.98			6.2
(13)-α-Cyclothienone-1	410	11.5		9.45		7.98				

sponding α-cyclothienones with boiling points ranging from 290 to 410°. A practically complete separation of the components of the mixture was recorded. With a rise in temperature from 180° to 220°, the time of analysis is shortened to 30 min, while separation does not deteriorate markedly. It is worth noting that the temperature at which separation was carried out was about 200° below the boiling point of the highest-boiling component. As can be seen from the graph of the relationship between logarithm of the retention volume and the number of carbon atoms in the ring (Fig. 14), the selectivity of the stationary phase for the ketones which include a thiophene ring is higher than that for the alicyclic ketones (the change in log $V_R^{rel.}$ for one carbon atom amounts to 0.125 in the first case, and to 0.1 in the second). This is possibly connected with the fact that the thiophene ring contributes additionally to the interaction with the stationary phase.

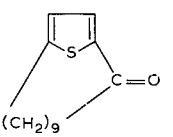


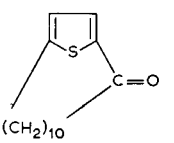
(1) $(CH_2)_{13}C{=}O$

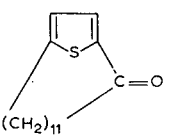
(2) $(CH_2)_{14}C{=}O$

(3) $(CH_2)_{15}C{=}O$

(4) $(CH_2)_{16}C{=}O$

(5) 

(6) 

(7) 

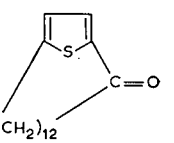
(8) 

Fig. 13. Chromatogram of an 8-component mixture of alicyclic ketones and α-cyclothienones. Column: 15% polyethyleneglycol succinate on Chromosorb W, U-shaped stainless steel, 2 m long; I.D., 4 mm. Fraction of the solid support: 60–80 mesh. Experimental conditions: temperature of the column and of the heat conductivity detector, 200.6°; helium flow rate, 50 ml/min; pressure at the inlet, 1.15 kg/cm². Notations of the peaks: 1 = cyclotetradecanone; 2 = cyclopentadecanone; 3 = cyclohexadecanone; 4 = cycloheptadecanone; 5 = (10)-α-cyclothienone-1; 6 = (11)-α-cyclothienone-1; 7 = (12)-α-cyclothienone-1; 8 = (13)-α-cyclothienone-1.

The heats of solution of alicyclic ketones and of α-cyclothienones in polyethyleneglycol succinate were determined by the temperature relationship of the corrected retention volumes within the range of 180–220°. ΔH_S is greater for α-

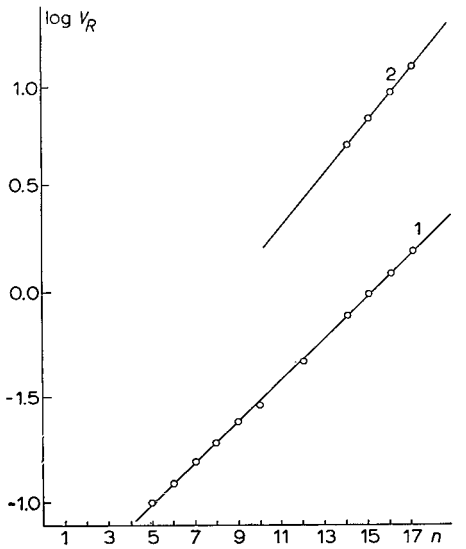


Fig. 14. Relationship between relative retention volumes ($V_R^{rel.}$ of cyclopentadecanone = 1) and the number of carbon atoms in a molecule at 180° on the polyethyleneglycol succinate–Chromosorb column. 1 = Alicyclic ketones; 2 = α-cyclothienones.

cyclothienones than for alicyclic ketones by 2.2 kcal/mole. In both series, ΔH_S grows linearly with the number of carbon atoms in a molecule. The change in ΔH_S for one methylene group amounts to 0.65 kcal/mole for both types of ketone. The resultant linear relationship between ΔH_S and the number of carbon atoms in a

TABLE VIII

HEAT OF EVAPORATION AND OF SOLUTION AND EXCESS HEAT OF SOLUTION OF ALICYCLIC KETONES AND

	C_4	C_5	C_6	C_7	C_8	C_9
Alicyclic ketones						
Heat of solution at 180–220°	5.91	6.56	7.21	7.86	8.51	9.16
Heat of evaporation at boiling point	7.63	8.33	8.90	9.47	9.84	10.16
Heat of evaporation at 200°	5.40	6.81	7.92	9.03	9.75	10.38
	(9.09*)	(10.17**)	(10.96***)			
Excess heat of solution at 200°	— 1.45	— 0.69	— 0.23	+ 0.23	+ 0.30	+ 0.28
α-Cyclothienones						
Heat of solution at 180–220°						
Heat of evaporation at boiling point						
Heat of evaporation at 200°						
Excess heat of solution at 200°						

* Heat of evaporation at 0°; according to published data, the heat of evaporation at 0° amounts to 9.18 kcal/mole (ref. 26).

** Heat of evaporation at 10°; according to published data, the heat of evaporation at 10° amounts to 10.41 kcal/mole (ref. 26).

*** Heat of evaporation at 30°; according to published data, the heat of evaporation at 30° amounts to 10.71 kcal/mole (ref. 26).

ring enables the determination of ΔH_S for any term of the series according to the formula:

$$\Delta H_S = 3.31 + 0.65\ n \text{ kcal/mole (acyclic ketones)},$$
$$\Delta H_S = 5.5\ + 0.65\ n \text{ kcal/mole (}\alpha\text{-cyclothienones)},$$

where n is the number of carbon atoms in the molecule.

Table VIII gives the heat of evaporation at the boiling point of ketone, calculated by the Kistyakovsky equation[16], the heat of evaporation at the average temperature of the chromatogram (200°), computed by Watson's equation[16], the experimentally determined heat of solution within a range of 180° to 220° for C_{14}–C_{17} ketones, and the heat of solution for alicyclic ketones C_4–C_{13}, found from the above linear relationship. Wherever possible, the calculated heat of evaporation was compared with published data. The maximum deviation did not exceed 2.5 %. From the excess heat of solution it was possible to appraise the nature of the interaction between the ketones and the stationary phase. In the case of alicyclic ketones, the nature of the interaction changes with an increase in the size of the ring. Typical of alicyclic C_4–C_6 ketones are the negative $\Delta\bar{H}_S{}^E$ values (Table VIII) (exothermic effect upon solution) and a negative deviation from the ideal state of a solution. In this case there is an interaction between the ketone molecules and the stationary phase molecules, the strength of the interaction dropping from the C_4-ketone to the C_6-ketone. Already with the C_7-ketone, $\Delta\bar{H}_S{}^E$ becomes positive (endothermic effect upon solution), which confirms the positive deviations from the ideal state of a solution. The ketone molecules are ejected, as it were, from the solution. However, when passing over to C_9–C_{11}-ketones, such a "negative" interaction between ketone and the stationary phase diminishes and $\Delta\bar{H}_S{}^E$ becomes again negative at C_{11}. Further on, up to C_{18},

α-CYCLOTHIENONES IN POLYETHYLENEGLYCOL SUCCINATE (kcal/mole)

C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}
9.81	10.46	11.11	11.76	12.46	13.14	13.95	14.36	15.01	15.66	16.31
10.39	10.62	11.08	11.43	11.97	12.95	13.30	13.77	14.24	—	—
10.83	11.28	12.20	12.90	13.98	16.0	16.72	17.70	18.69	—	—
+ 0.08	— 0.12	+ 0.15	+ 0.20	+ 0.58	+ 1.92	+ 1.83	+2.40	+ 2.74	—	—
		13.30†	13.95†	14.55	15.23	16.00	16.55	17.20	17.85	18.50
				12.95	13.65	14.13	14.84			
				14.78	15.89	16.65	17.77			
				— 0.71	— 0.28	— 0.29	+ 0.28			

† This value was obtained by extrapolation.

$\Delta\bar{H}_S{}^E$ is positive and rises continuously. Such a pattern of changes in the excess heat of solution with an increase in the size of the ring leads to the following conclusion. In homologues of a series with a small-sized ring, the work consumed in moving apart the molecules of the stationary phase is small, and the interaction between the dipole molecule of a ketone and the phase molecules is fairly large. As the size of the ring grows, the work required for moving apart the stationary phase molecules increases and the gain in energy diminishes. Somewhere in the region of C_{10} these two factors compensate each other and the solution approximates an ideal one. However, further on, up to C_{18}, the energy consumed by moving apart the stationary phase molecules becomes the fundamental magnitude, *i.e.* the energy required for a molecule of an alicyclic macroketone to find its place there. In the case of α-cyclothienones with the same size of ring, there appears an additional source of interaction: between the liquid phase and system of π-electrons of the thiophene ring. The work consumed for moving apart the stationary phase molecules is overlapped for C_{14}–C_{16} ketones by interaction with the ketone group and the thiophene ring. It is only at C_{17} that the size of the ring reaches such a magnitude that the interaction is accompanied by an endothermal, and not by an exothermal effect.

It appears that the relationships found should be regarded as preliminary since steric interactions in the molecule of a cyclic ketone were not taken into account in the discussion. This point merits special consideration, especially in view of the fact that anomalous values of $\Delta\bar{H}_S{}^E$ are to be found in the region of the middle-sized rings (C_9–C_{12}). This demonstrates once more the relationships existing between chromatographic behaviour of a substance and its structure.

SUMMARY

Gas–liquid chromatography, when used for heterocyclic compounds, opens up wide prospects for its analytical application. Highly polar and high-boiling substances can be chromatographed by using ether diatomite brick treated with an alkali (content of the stationary phase 15–20 %), or sodium chloride with a small amount of the stationary phase (about 1 %). Proceeding from the thermodynamics of the interaction between the investigated substances and the stationary phase, it is possible to reveal the fine effects related to the structure of a molecule.

REFERENCES

1 W. E. GOLDING AND C. A. TOWNSEND, *Chem. Ind.*, (1960) 1476;
Y. KOBASHI, *J. Chem. Soc. Japan, Pure Chem. Sect.*, 82 (1961) 1262, A79; and
A. L. BROWN AND K. B. BUCK, *Chem. Ind (London)*, (1961) 714;
E. D. SMITH AND R. D. RADFORD, *Anal. Chem.*, 33 (1961) 1160.
2 B. A. RUDENKO AND V. F. KUCHEROV, *Dokl. Akad. Nauk SSSR*, 145 (1962) 577.
3 V. I. YAKERSON, L. I. LAFER AND F. M. STOYANOVITCH, *Tezisy Dokl. Vses. Konf. Gaz. Khromatog.*, Moscow, 1964, p. 73.
4 V. V. KORSHAK, *Metody Vysokomolekulyarnoĭ Organicheskoĭ Khimii*. Vol. I. *Obshchei Metody Sinteza Vysokomolekulyarnykh Soedinenii*, Akad. Nauk, Moscow, 1953, p. 445.
5 A. M. RUBINSTHEIN, V. I. YAKERSON AND L. I. LAFER, *USSR Pat.*, 159,881 (1963), from *Byul. Izobret. i Tovarnykh Znakov*, No. 23 (1963) p. 16.
6 E. BAYER, *Gas-Chromatographie*, Springer-Verlag, Berlin, 1959.
7 A. ALBERT AND E. P. SERJEANT, *Ionization Constants of Acids and Bases*, Wiley, New York, 1962, pp. 141, 145 and 148.
8 N. A. FUKS, *Uzbeksk. Khim.*, 25 (1965) 845.
9 B. P. FEDOROV AND F. M. STOYANOVICH, *Zh. Obshch. Khim.*, 33 (1963) 2251.

10 E. PROFFT, *Chemiker-Ztg.*, 82 (1958) 298.
11 YA. L. GOLDFARB, M. B. IBRAGIMOVA AND O. A. KALINOVSKII, *Izv. Akad. Nauk SSSR, Otd. Khim. Nauk*, (1962) 1098.
12 B. P. FEDOROV AND F. M. STOYANOVICH, *Zh. Org. Khim.*, 1 (1965) 1282.
13 M. S. VIGDERGAUZ AND K. A. GOLBERT, *Neftekhimiya*, 2 (1962) 940.
14 D. H. DESTY AND W. T. SWANTON, *J. Phys. Chem.*, 67 (1961) 766.
15 P. E. PORTER, C. H. DEAL AND F. H. STROSS, *J. Am. Chem. Soc.*, 78 (1956) 2999.
16 E. HALA, J. PICK, V. FRID AND O. VILIM, *Vapour–Liquid Equilibrium*, Pergamon, Oxford, 1958.
17 G. WADDINGTON, G. W. KNOWLTON, D. W. SCOTT, G. D. OLIVER, S. S. TODD, W. H. HUBBERD, J. C. SMITH AND H. M. HUFFMAN, *J. Am. Chem. Soc.*, 71 (1949) 797.
18 B. I. ANVAER, A. A., ZHUKHOVITSKIĬ AND N. M. TURKELTAUB, *Gazovaya Khromatografiya*, Akad. Nauk, Moscow, 1962, p. 44.
19 G. SCHAY, *Theoretische Grundlagen der Gaschromatographie*, VEB Deutscher Verlag der Wissenschaften, Berlin, 1963.
20 YA. L. GOLDFARB, YA. L. DANYUSCHEVSKY AND M. A. VINOGRADOVA, *Dokl. Akad. Nauk SSSR*, 151 (1961) 332.
21 YA. L. GOLDFARB AND YA. L. DANYUSCHEVSKY, *Izv. Akad. Nauk SSSR, Otd. Khim. Nauk*, (1956) 1361.
22 YA. L. GOLDFARB AND YA. L. DANYUSCHEVSKY, *Zh. Obshch. Khim.*, 31 (1961) 3654.
23 V. P. LITVINOV AND YA. L. GOLDFARB, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1963) 2183.
24 YA. L. GOLDFARB, V. P. LITVINOV AND S. A. OZOLIN, *Izv. Akad. Nauk SSSR, Otd. Khim Nauk*, (1965) 510.
25 YA. L. GOLDFARB, S. Z. TAITS AND L. I. BELENKII, *Tetrahedron*, 19 (1963) 1851.
26 S. W. BENSON AND G. D. KISTIAKOVSKY, *J. Am. Chem. Soc.*, 64 (1942) 80.

THE QUANTITATIVE ESTIMATION OF SUBSTANCES ON PAPER CHROMATOGRAMS

I. A MACHINE FOR THE TREATMENT OF PAPER STRIPS WITH CHEMICAL REAGENTS

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INTRODUCTION

Almost as soon as partition chromatography on paper had been introduced by CONSDEN, GORDON AND MARTIN[1] attempts were made to determine substances on chromatograms by the direct measurement of the color density of the zones that could be produced with suitable color reagents. These early attempts have been reviewed in detail by BLOCK, DURRUM AND ZWEIG[2]. In summary, it would be fair to say that reasonable estimations (approx. errors $\pm$ 10 %) could be achieved by rigorous attention to details of technique but that by 1958 this method of approach had not been adopted to any great extent for practical problems in biochemical estimation (see also CHIBNALL[3]). Instead, quantitative estimation in conjunction with chromatography was carried out by the application of more or less conventional colorimetric methods to the effluents from columns, or to the eluates from segments of paper chromatograms cut out by hand. Continuous mixing of the effluent of a column with reagent, and monitoring the optical density of the mixture with a photometer and potentiometric chart recorder was developed for amino acids by SPACKMAN, MOORE AND STEIN[4] and has become widely used as a result of the commercial manufacture of their apparatus.

The author re-examined the question of the direct estimation ("scanning") of colored zones on paper chromatograms in 1953 and devised an apparatus for mechanizing the procedure in 1955. It was found that certain general rules seemed to hold for the successful quantitative scanning of paper chromatograms whatever class of substances were being measured. Knowing these rules, it was relatively easy to establish quantitative methods of low error, and to devise reproducible techniques without a prolonged empirical search for the right conditions with each new colorimetric method. The course of this work has been described in two review articles (BUSH[5,6]) and a book (BUSH[7], Chap. 4) but many details remain unpublished or are scattered through papers published since 1957 (*e.g.* BUSH AND WILLOUGHBY[8]). In view of numerous private enquiries about the apparatus that has been devised it is hoped to collect this unpublished material in the series of papers of which this is the first. Schematic descriptions of the original apparatus and a discussion of the rationale

of the technique in general have been given previously and will only be discussed briefly in this series of papers (BUSH[6,7]).

The first paper deals with the design and construction of a machine capable of treating paper chromatograms reproducibly with chemical reagents.

DESIGN AND CONSTRUCTION OF THE MACHINE

Early attempts and difficulties

The first machine was made in late 1955 and was similar in layout to the fourth model described in this paper. Crude as it was, this prototype sufficed to demonstrate the considerable reproducibility with which the reagent roller unit (BUSH[7], Chap. 4) was capable of applying a film of liquid reagent to successive paper strips, and the efficiency with which color reactions could be carried out by using a zone in which hot air was forced at high speed over the paper.

The second model (Fig. 1) was designed to provide a much longer path in the heat-treatment zone and employed a zig-zag path of the belts to secure reasonable compactness. Many successful runs were made in 1959–1960 but the tension required

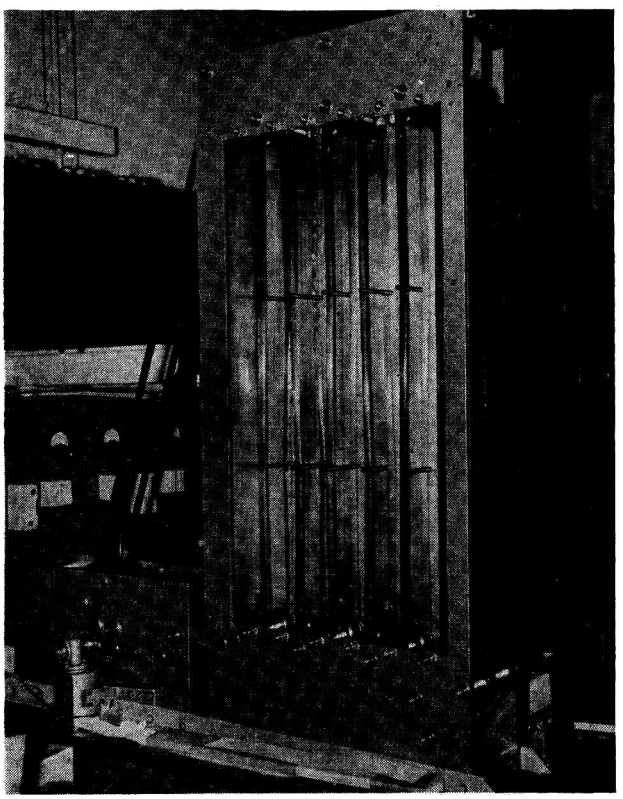


Fig. 1. The second model of the machine. The reagent applicator is at the left and the reaction-drying chamber at the right. The front panels of the latter are removed to show the zig-zag path of the belts. Heating units and blowers were attached to the front panels which also carried partitions dividing the chamber into six partly separated compartments.

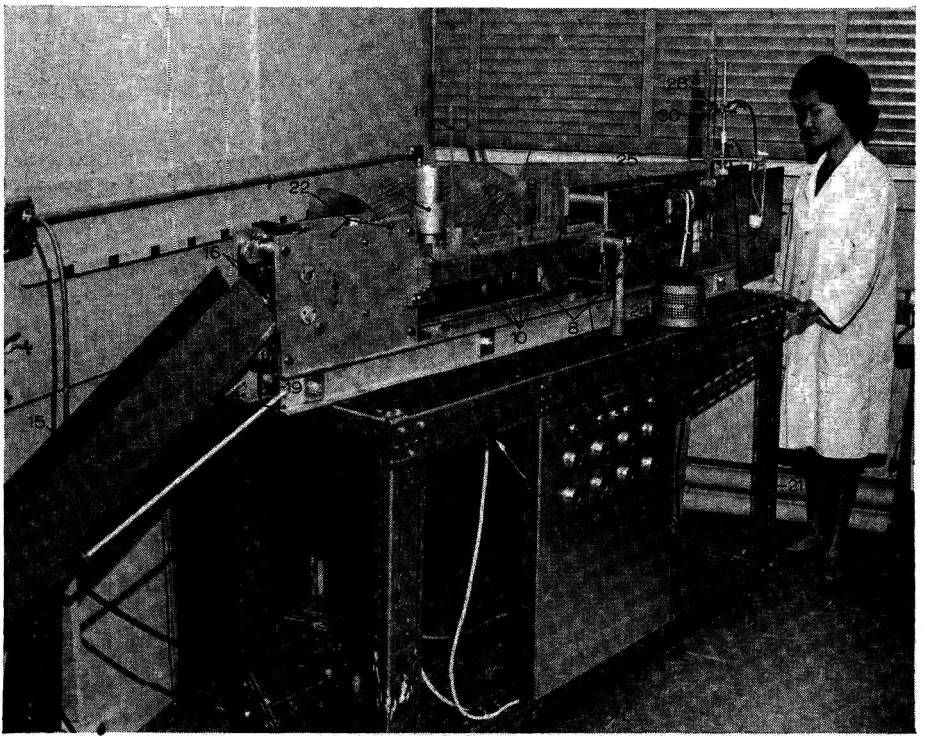


Fig. 2a. The fourth model described in the paper. The reference numbers are used throughout the paper. 6 = Variac controlling reagent-roller motor; 7 = heater-blower, beneath and behind top of carriage; 8 = hot-air conduits to reaction chamber; 9 = reaction chamber; 10 = slide valves to entry ports of reaction-chamber; 11 = thermometer; 12 = exhaust tube; 13 = variable transformer for heater; 15 = collecting tray for ejected strips; 16 = main driving pulleys; 19 = crossbars of main supporting rails; 20 = main supporting rails; 21 = dexion frame on casters; 22 = tensioning auxiliary pulleys for belts; 24 = driving chain for reagent-rollers; 25 = sprockets on shafts to the two reagent-rollers; 26 = PTFE reagent feed tube to reagent-roller; 27 = constant-level chamber of reagent-feed; 28 = upper chamber of reagent-feed; 29 = lower chamber of reagent-feed; 30 = clamps supporting reagent feed; 31 = control panel.

to keep the belts running true on the one-flanged pulleys (approx. 15 kg) led to fairly rapid distortion of the stainless-steel belts by cold-flow, and their frequent replacement was uneconomical and time-consuming.

The third model reverted to a simple straight-through reaction chamber. An inadequately rigid base failed to maintain proper lateral stability of the belts. The fourth model employed the same overall design, corrected the remaining errors of the third, and has given excellent and reliable service since it was completed nearly two years ago. The belts have been replaced twice, and the machine has treated several thousand chromatograms.

Description of the fourth model

The machine is shown in Figs. 2 a, b and c and a schematic elevation in Fig. 3. It consists of three main sections. The first is the reagent-applicator unit (extreme right of Fig. 2). An aluminum guide-rail (1, Figs. 3 and 7 a) receives the strips of paper (5.1 cm

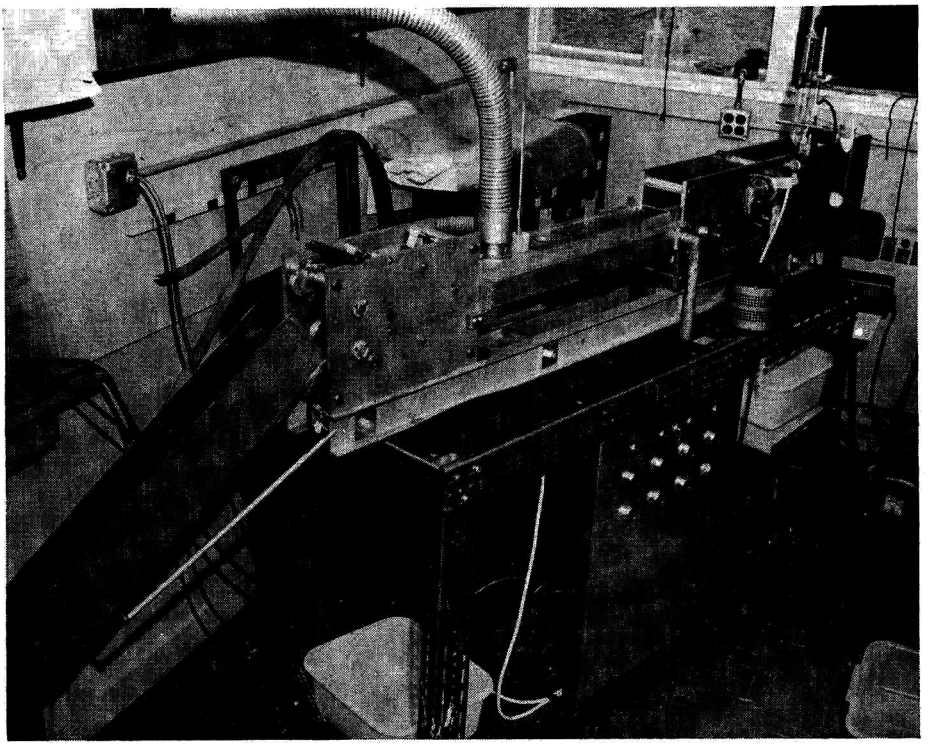


Fig. 2b. The fourth model equipped with an auxiliary exhaust system. The flexible metal hose is connected to an exhaust duct discharging through a window-board at the right. To the left, an opening to the room (not shown) is controlled as a bypass with a butterfly valve.

wide) which are pushed along until the edges of the leading part of the strip are gripped by the upper and lower belts on each side at their meeting point (2, Fig. 3). The paper is then gently flattened, if convex upwards, by the idling roller (3, Fig. 2c) which is made of polytetrafluoroethylene (PTFE). The paper then passes over the stainless-steel reagent-roller (4, Figs. 3 and 7a) in light contact with it and takes up the reagent from the film on the roller by capillary action. There is provision for a second reagent-roller (5, Fig. 3) but it has not been necessary to use it up to the present.

The speed of movement of the paper is fixed at 1 cm/sec which is determined by the motor speed, gearing, and diameter of the driving pulleys in the take-off unit described later. This speed is between 1.5 and 2.0 times the minimum speed required to ensure that the longitudinal capillary movement of typical liquid reagents in the paper is slower than the paper movement itself. This is necessary to ensure that the reagent does not move or distort zones of material on the chromatograms.

The speed of the reagent-roller is adjusted with a variable transformer (Variac, Duratrak, Type V5HM, Zenith Electric Co., Birmingham, England) and is usually in the range 40–120 r.p.m. with reagents of ordinary viscosity (6, Fig. 2a).

The paper, soaked with reagent, is now carried through the reaction-chamber or middle zone of the apparatus. This is a steel and aluminum box of rectangular cross-section and 65.5 cm long. The side-walls are double, the two outer compartments

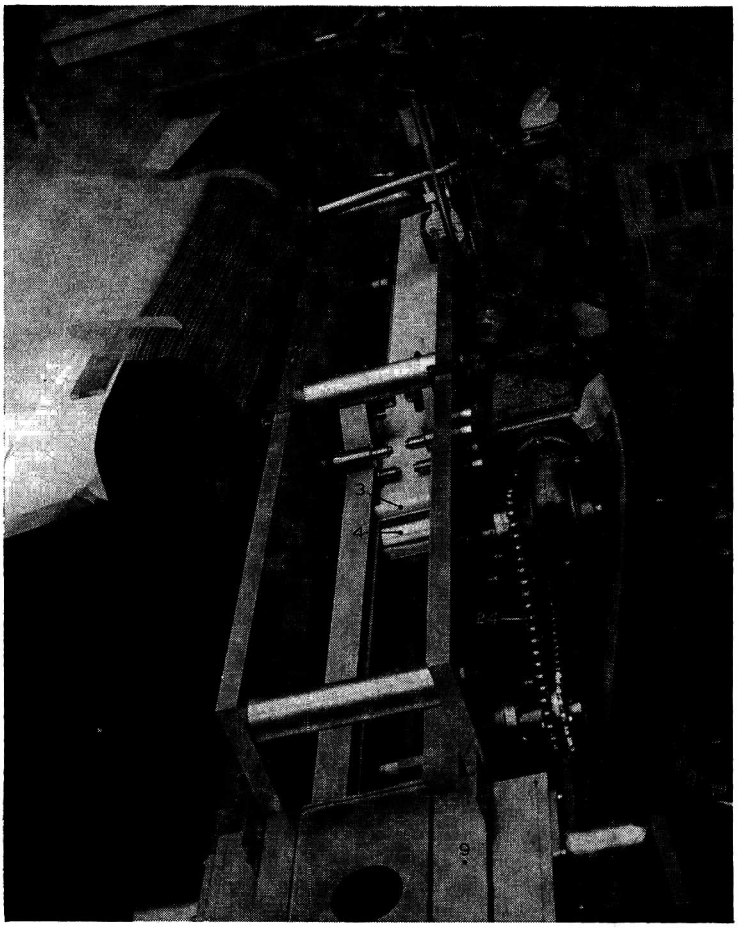


Fig. 2c. A view showing the reagent applicator unit with a chromatogram just about to make contact with the reagent roller; 3 = idling roller. Other reference numbers as in Figs. 2a and 3.

(cross-section, inside dimensions 2.54 × 3.81 cm) acting as conduits for hot-air supplied by the heater-blower (7, Figs. 2a and 3) via the Y-tube and connectors (8, Figs. 2a and 3). These conduits (9, Figs. 2a and 3) communicate with the reaction-chamber itself (inside cross-section, 11.5 × 5.0 cm) by entry ports 3.2 cm in diameter which can be opened or closed by simple slide valves (10, Figs. 2a and 3). The lid of the reaction chamber is perforated with inspection holes which are covered with a sheet of plate glass. Similar holes are used to hold a mercury-bulb thermometer (11, Fig. 2a) and the exhaust-tube (12, Figs. 2a and 3). The latter is a simple venturi tube driven by a centrifugal fan mounted behind the machine. In practice it has been found necessary to supplement this fan by connecting the exhaust-tube with flexible metal hose to a conventional exhaust fan discharging to the outside air (not shown in Fig. 2). The reaction chamber contains a strip heater (1.5 kW) in its floor for ballast heat but this has not been used frequently. A variety of patterns of heating and drying can be achieved by varying the setting of the variable transformer controlling the heater-

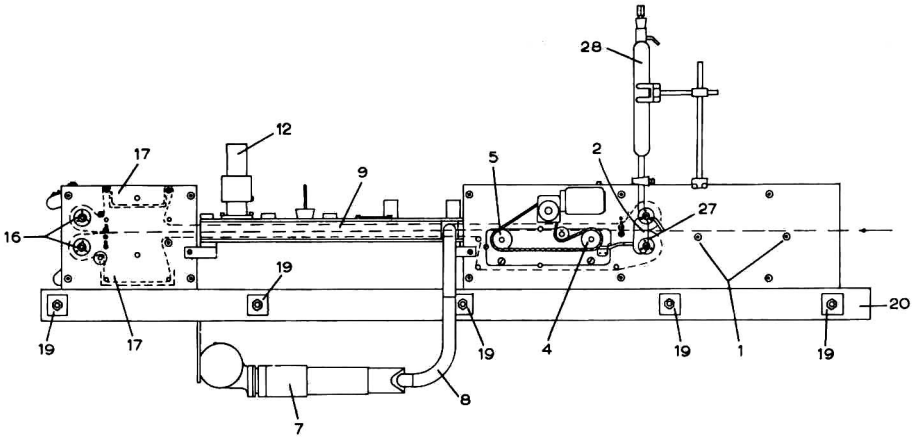


Fig. 3. Side-elevation of the fourth model excluding the supporting carriage. 1 = Supports for entry guide-rails; 2 = meeting point of upper and lower belts at which paper strips are gripped; 4 = sprocket and axle of first reagent-roller; 5 = sprocket and axle of second reagent-roller; 17 = washing baths for upper and lower pairs of belts. Other reference numbers as in Fig. 2a.

Fig. 4. The reaction-chamber with lid removed to show arrangement of the belting system. A strip of paper is entering from the right. The central guideposts (14) are visible: the first pair are not visible but their position is indicated by the reference numbers. (Other reference numbers as in Fig. 2a.) The upper and lower belts are seen on their return passage through the reaction chamber.

blower (13, Fig. 2a) and by using different settings of the 4 entry-ports (10, Figs. 2a and 3).

To ensure that the belts are pressed together and grip the edges of the paper strips in at least two positions on each strip (approx. 45 cm long) at all times, three guide posts (14, Fig. 4) are fixed to the sides of the reaction chamber. These are ridged to assist the maintenance of correct lateral alignment of the belts through the chamber, and are placed so as to produce a slight up and down deviation of the belts from a straight horizontal path. The third, middle, pair of guide-posts was only added to the machine recently. Although good performance was achieved with a straight, horizontal, path of the belts through the machine, strips were occasionally dislodged and lost in the reaction chamber due to an inadequate gripping action by the PTFE-coated fiber-glass belts which have an extremely low coefficient of friction. To minimize this problem the belts had to be run at rather high tension, thus increasing wear and reducing their useful life. Foolproof performance, with lower tension on the belts, has been achieved by the addition of the middle pair of guide-posts which deflect the belts from a straight path. The distances between the three pairs of posts are 27 cm and 28 cm.

The paper is passed out of the reaction chamber and ejected into the collecting tray (15, Fig. 2a) by the third section of the machine called the take-off unit. The two belts on each side of the paper disengage on passing through the main driving pulleys (16, Figs. 3 and 5) and are taken by means of small doubly-flanged auxiliary pulleys through two washing-baths (17, Figs. 3 and 6b) one for the upper, the second for the lower, belts. A stream of hot tap water is fed to these baths by tubes and sucked off

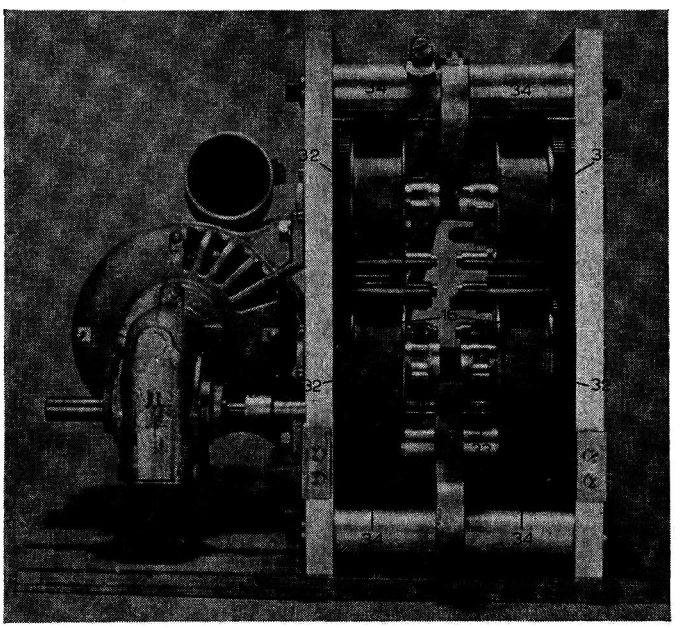


Fig. 5. End-view of main drive-pulleys of the take-off unit. Key as in Fig. 2a. The belts are removed for clarity. 32 = Spur gears on main drive-pulleys; 33 = common spur driving 32; 34 = rubber pressure rollers.

by a water-pump via drain tubes which maintain a constant level in the baths. Any drops of water are wiped from the belts as they emerge from the baths by small plastic-sponge wipers mounted on metal brackets (18, Fig. 6b). The belts then return to the reagent-applicator unit *through* the reaction-chamber so that they are completely dried before re-engaging and taking in fresh strips of paper from the loading guide-rail.

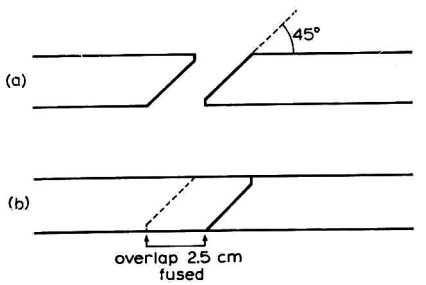


Fig. 6a. Diagram of method of joining the ends of the belts. The fusion was carried out by the manufacturer.

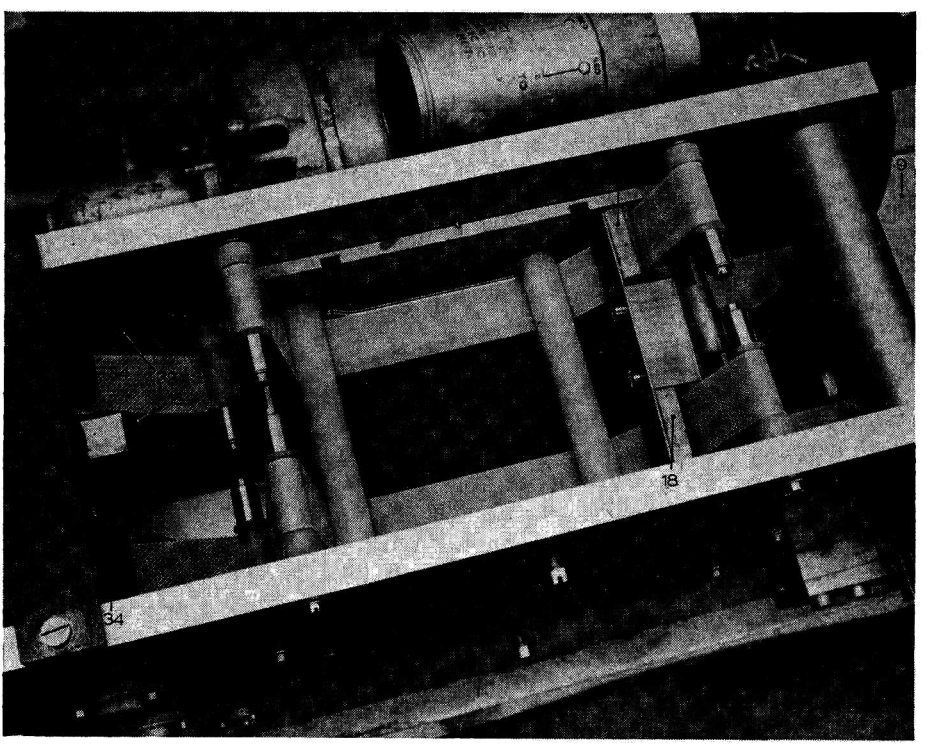


Fig. 6b. The washing-trough for the upper pair of belts. The lower washing trough is similar. The PTFE rollers in the trough are inserted by the vertical sections of the two grooves seen on the back wall of the trough, and similar grooves (not seen) on the front wall. The belts return to the right via the reaction-chamber (see Fig. 4). 18 = Plastic sponge wipers. Other numbers as in Figs. 2a and 5.

The heater-blower is a standard commercial unit rated at 2.0 kW on the heater, and moving approximately 325 l/min. The centrifugal fan is run at constant speed. The supply voltage to the heater is controlled by a variable transformer (Variac Type 100 RM, Claude Lyons & Co., Liverpool, England).

The reagent-applicator and take-off units are made on the watch-plate principle, the main side plates being of hard aluminum alloy 1.25 cm thick, joined together by 2.54 cm diameter mild-steel spacing rods to which they are fixed by counterbored screws. The pulley shafts are of steel (diam. 0.9 cm), and all bearings are of presoaked spongy phosphor-bronze mounted in steel bushes. The main driving pulleys are contained in the take-off units and their arrangement is shown in Fig. 5. Equal velocities of the two sides and of the upper and lower belts are obtained by the steel spur gears (32, Fig. 5) the lower pair of which is driven by a common long spur (33, Fig. 5). The motor is a synchronous motor of 46 kg·cm torque with built-in gear box and an axle speed of 36 r.p.m. (Parvalux Motors, Poole, Dorset, England).

The reaction-chamber is slung between the reagent-applicator unit and the take-off unit by means of brackets which allow the reaction-chamber to expand at high temperatures by a horizontal sliding movement.

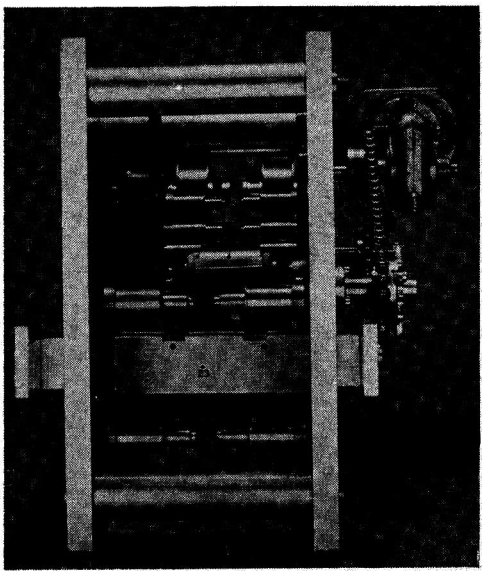
Because of the low coefficient of friction of the PTFE-coated belts, rubber pressure rollers (34, Fig. 5) have been inserted to ensure that the belts do not slip on the steel driving pulleys.

The first and third units of the machine are rigidly fixed with cross-bars and steel-wire ties (0.63 cm diam., 19, Fig. 2a) around the bottom spacing rods, to a heavy rail (20, Figs. 2a and 3) made of two I-section steel girders (7.5 × 3.8 cm) bolted together against steel spacing rods (5.1 cm diam.). The whole apparatus can then be mounted on a fairly light frame (21, Fig. 2a) which moves on rubber casters.

The electrical controls are mounted on the front panel (31, Fig. 2). The order of switching is controlled by relays so that switches 1, 2, 3, and 4 (from left to right) can only be switched on in that order, and switching off any one switch automatically opens all switches to its right. The switches 1–4 control in order—the fan of the heater-blower, the heater of the heater-blower, the motor driving the reagent-roller, and the motor driving the belts.

The belts and their mountings are very important parts of the machine and need a detailed description. They are endless and arranged in two pairs. The upper belts are 324.1 cm long and the lower belts are 321.4 cm long. They are 2.23 cm wide and 0.023 cm thick. They are made of woven glass fiber coated and impregnated with PTFE. They are joined by overlapping and fusing, the area of overlap being an oblique parallelogram (Fig. 6a) to present minimum resistance to their passing through or over pressure-points on their paths through the machine. Two firms have been able to supply us with adequate belts after consultation with them. The first set were 0.030 cm thick (0.012 in.) and made by Tygadure Co. Ltd., Nottingham, England. The second pair were supplied by A.A.A. Plastics Ltd., Cambridge, Mass., U.S.A. and are 0.023 cm thick (0.009 in.). The operating tension of these belts has usually been in the range 1–3 kg as measured indirectly by displacement with a weight of 100 g.

All pulleys driving, supporting, or deflecting the belts are mounted on stub shafts which leave a clear central gap of 1 cm right down the length of the machine. This is a very considerable convenience in the servicing and replacement of belts since, after loosening the tensioning pulleys (22, Figs. 2a and 3), the belts can be lifted



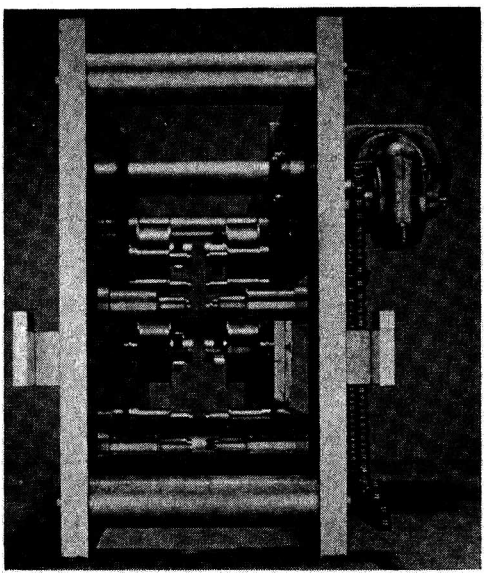


Fig. 7a. The reagent-applicator unit seen from the end normally attached to the entry of the reaction-chamber. The belts are removed for clarity. 23 = Cross-bar supporting reagent-trough and roller No. 2. The support for reagent-trough No. 1 is seen immediately behind 23. Other numbers as in Figs. 2a and 3.

Fig. 7b. Reagent-applicator unit with reagent-trough-roller unit removed.

off the pulleys and out of the machine via the gaps between the stub-shafts. This arrangement is clearly seen in Figs. 2c, 5 and 7.

In Fig. 7 the arrangement of the reagent-roller is seen. The loading guide-rail is seen at the end of the unit distant from the reader in Fig. 7a. The belts have been removed for clarity. The reagent-trough is made of PTFE end-plates with a near-circumferential groove into which is pressed an approx. 2/3-cylinder of PTFE radius 2.54 cm, length 5.1 cm, and thickness 0.18 cm. The trough is held together by 0.161 cm diameter steel-tie bars secured with nuts. The trough is mounted on a rectangular aluminum cross-bar (23, Fig. 7a) mounted in turn on a face-plate which is let into a rectangular hole in the front main plate of the reagent-applicator unit. The whole trough-roller section is demountable from the front main plate after disengaging the driving chain (24, Figs. 2a and 7a) from the sprockets (25, Fig. 7a) (see Fig. 7b). A PTFE tube (I.D. 0.30 cm, O.D. 0.50 cm; 26, Figs. 2a and 8) leads from the lower part of the front edge of the trough out through the subsidiary face-plate to the constant-level chamber of the reagent feed (27, Figs. 2a and 8).

The reagent-feed is a simple constant-level device with a capacity of approximately 180 ml. With the tap closed, the upper chamber (28, Fig. 2a) is filled with reagent and the stopper is inserted, well-lubricated with excess reagent or an inert solvent. The tap is then opened and reagent flows into the constant-level chamber and thence to the trough. Further fluid will only pass from the upper to the lower chamber when the level drops below the orifice of the control tube (29, Fig. 8), thus allowing a bubble of air to pass up into the upper chamber and displace the fluid downwards. The level is set by sliding the unit vertically in the laboratory clamps

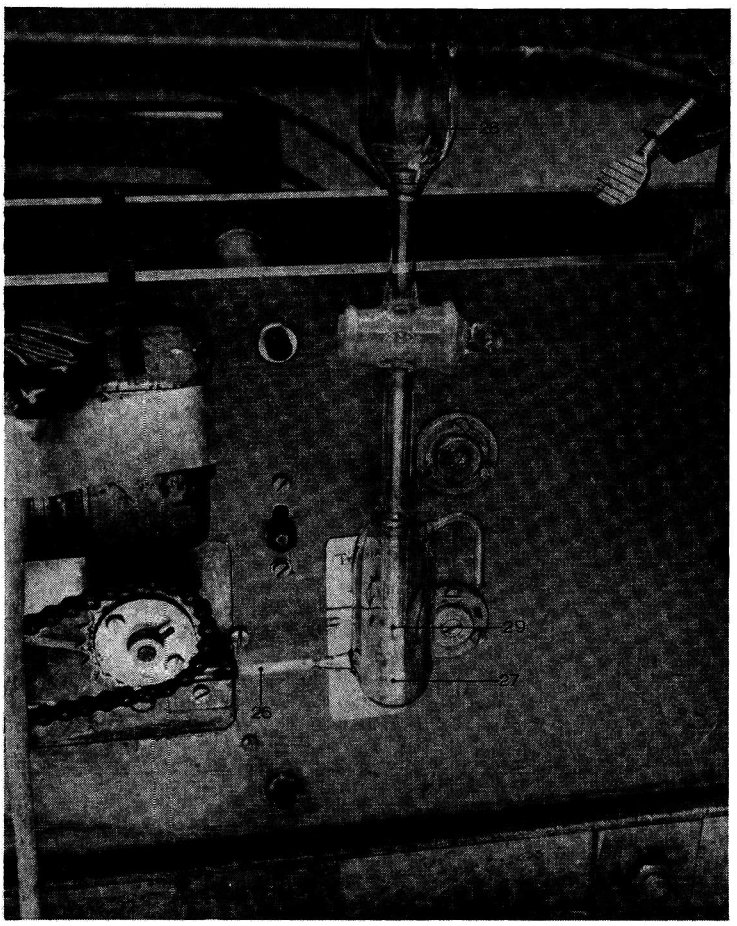


Fig. 8. Constant-level chamber and inlet tube to reagent-trough and roller. 26 = PTFE connecting tube to reagent-trough and roller; 27 = reagent feed constant-level chamber; 28 = reagent reservoir-feed, upper chamber; 29 = orifice of control inlet tube from upper chamber.

(30, Fig. 2a) until the orifice of the control tube is level with the mark indicating the desirable fluid level in the reagent trough.

OPERATION AND USE OF THE MACHINE

Method of operation

The machine was first subjected to calibration of the variable transformer (Variac) controlling the heater of the heater-blower unit. The room temperature (26°) was noted and the Variac turned to give maximum voltage. Ports 1 and 2 were opened and the exhaust fan switched on. After 15–20 min at an ambient temperature of 26° the thermometer in the reaction-chamber of the present machine reached a steady reading of 150° (uncorr.) which was checked four times at intervals of 5 min and showed a range of variation of approx. $\pm$ 0.2°.

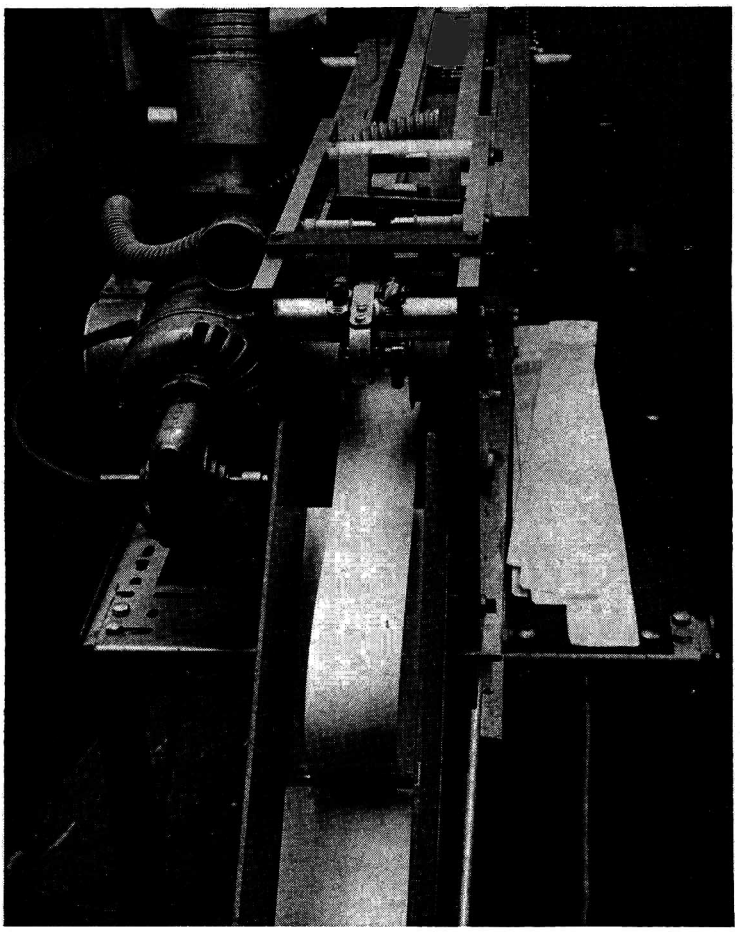


Fig. 9. View of ejection of a strip from the take-off unit showing the action of the ridge in the collecting trough. The strip is about to roll over and fall "tail-first" into the collecting tray.

The belt drive was then switched on and the temperature of the reaction-chamber fell in 10 min to a steady value of 148°. The belt-drive was kept running throughout the subsequent calibration. The Variac was turned back to 80% of full-scale and a new steady temperature was reached in approx. 10 min. The process was repeated in approx. 10% steps on the Variac dial until a reading of 42° was obtained. A typical calibration is shown in Fig. 10.

This calibration curve is used in day-to-day operation of the machine to set the Variac to achieve the desired temperature. If necessary, calibration curves for a range of ambient temperatures can be made. After a 15–20 minute period allowed for warming up, a final adjustment of the Variac is made until the thermometer indicates the exact temperature that is desired for the reaction to be carried out. Usually the belt drive is switched on 5 min before using the machine to avoid unnecessary wear and tear. Final adjustment of the starting temperature *must* be made with the belts running.

Without servo-control of the temperature, however, this *starting* temperature will *not* be maintained when strips soaked with reagent start passing through the reaction-chamber. The proper starting temperature depends both on the thickness of the paper strips to be treated and the nature of the reagent, and is determined by trial and error when investigating the optimal conditions for each color or fluorescence

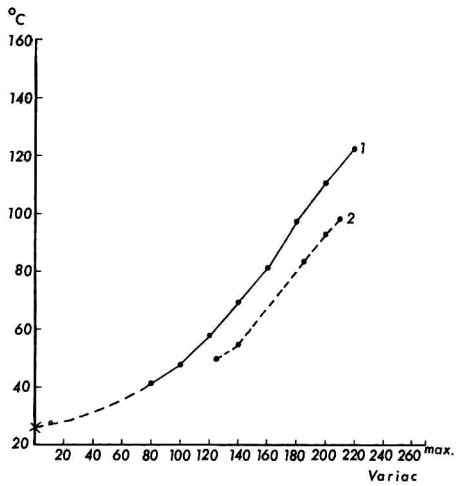


Fig. 10. Calibration curve for temperature of reaction chamber as a function of the setting of the Variac controlling the heater of the heater-blower unit. 1 = Room temperature 26°; 2 = room temperature 22°.

reaction. With the present machine a fall of 2–10° is seen with Whatman No. 2 paper when using reagents dissolved in common alcohols or solvents. Since control to within $\pm$ 0.5° is essential for adequately reproducible results a simple technique has been devised to achieve a steady-state before feeding strips into the machine.

Having achieved the appropriate starting temperature, the reagent reservoir is filled with reagent, the stopper inserted, and the tap opened. The constant-level chamber and reservoir are usually kept at the appropriate level that fills but does not cause overflowing of the reagent-roller trough. One or two clean strips of filter paper, 56 cm long, of the type used for the chromatograms are now fed into the machine via the entry guide rails, and the temperature is observed by an assistant. If the temperature fails below the desired operating temperature for the reaction, the Variac is readjusted and further clean strips passed into the machine until the correct temperature has been achieved. Usually, one or at most two "leader" strips of this sort are needed, and the operating temperature is achieved without readjustment of the Variac.

The "leader" strip is also used to adjust the speed of the reagent-roller. The speed of rotation of the roller is adjusted, while watching the strip at its point of contact with the roller, until the trailing edge of the wetted zone holds a steady position several mm *behind* the actual point of contact with the roller itself, and is convex backwards in the area between the inner edges of the belts and the outer edges of the roller (see Fig. 11). This ensures maximal loading of the paper with reagent, without feeding excess reagent on to the edges of the paper and between the belts.

The chromatograms to be treated are now fed into the machine by hand, making sure that the gaps between the ends of successive strips do not exceed 1–2 cm. It is also possible and eminently satisfactory to link the strips head-to-tail in a zig-zag pack with clean cotton thread, or by slots cut with scissors. The first of the train is fed in manually after which the entire operation is automatic.

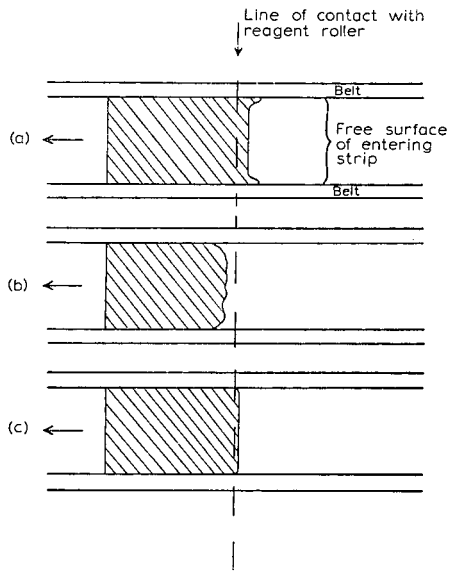


Fig. 11. Diagram of the pattern of wetting of strips with reagent when the speed of rotation of the reagent roller is (a) too large, (b) too small, (c) optimal.

As soon as the last chromatogram has entered the machine, a clean, 56 cm long strip of filter paper is fed in as a "trailer" strip to maintain a steady-state in the reaction-chamber while the last chromatogram is passing through. Without this precaution the temperature will rise and the last chromatogram will be overheated over at least its trailing half. With most reagents this will result in a rising background optical density along its length when the chromatogram is scanned.

If the exit of the machine is not directly coupled with a scanner (see later papers in this series) the treated chromatograms are now piled in order in the collecting tray and are ready for scanning or other procedures.

The speed of operation is determined by the length of the machine and the fixed velocity of the belts of 1 cm/sec. In the present model the entry and ejection points are 127 cm apart so that a 50 cm long chromatogram takes 177 sec to pass right through the machine. This, however, is the "dead time" for the first strip: after this time successive 50 cm strips emerge at one every 50 sec, *i.e.* at 72 strips/h. The "productivity" of such a machine is therefore very large. Allowing 30 min for warming up and the initial adjustment, and 30 min for cleaning up at the end of the day, it would be possible to treat 500 chromatograms 50 cm long in an 8-hour working day. A team of one skilled worker and a trained assistant could conveniently maintain such a full-time schedule, and in addition pass the treated strips through a suitable scanner if the device of linking the strips with scissor-cuts or cotton was employed.

If a series of different color reactions are to be used, approximately 20 min must be allowed with the present machine for the readjustment of temperature and cleaning of the reagent-roller, trough, and constant-level reservoir, for each change of color-reaction.

DISCUSSION

The need for rigorous control of conditions in the treatment of paper chromatograms with chemical reagents

The filter paper used for chromatography is an incompletely described form of hydrated or solvated cellulose. Any chemical reactions carried out *in situ* may be complicated in unknown ways by this circumstance, as well as by the fact of their being performed in a heterogeneous rather than in a homogeneous system. Extensive work on this problem has been carried out with amino acids[3,10,18–20]. Many examples in the steroid field have been described by the author (BUSH[5,6,7]) and will be described in more detail in later papers of this series. Briefly, it is well-known that different types of filter paper vary in properties that affect the running of chromatograms. In addition, its previous exposure to solvents and the atmosphere is also a source of considerable variation in properties (HANES *et al.*[10], and earlier papers; TOMISEK AND ALLAN[11]). This variation can be eliminated by appropriate pre-treatment of the chromatograms.

As with other aspects of the scanning method for quantitative estimation of substances separated on paper chromatograms, there are a number of relatively unexpected features which are of critical importance to the success or failure of the method as a whole. It must be re-emphasized (BUSH[7,8,9]) that these are relatively easy to *control* once they have been discovered, and that most of them are common in greater or lesser degree to *all* colorimetric reactions which we have examined by this method. There is no doubt that mechanization of the treatment of paper strips with chemical reagents improves very greatly the degree of control achieved over those critical factors.

Assuming that removable impurities in the original filter paper and chromatographic solvents have been brought to a satisfactory and minimal level, there are two main factors affecting the design of a machine for treating chromatograms with chemical reagents. The first is that the most common deficiency of the conventional color reagents used in paper chromatography is that they are *too dilute* (BUSH[5,6]). A calculation has been given previously demonstrating just how serious this can be (BUSH[7], Chap. 4). In all cases the use of a more concentrated reagent gives rise to a more or less serious increase in background color. In some cases this is so severe that the concentration of the reagent can only be increased to an extent which provides a marginal excess per unit area of the paper.

These two reasons alone make it necessary to ensure that any reagent is applied as evenly as possible over the *whole* length of *all* the strips of paper to be scanned.

The second main factor to be controlled is the reproducible heating and drying of the strips once the reagent has been applied. The even and reproducible heating of sheets or strips impregnated with color reagents presents many problems (BLOCK[12], OUGH *et al.*[13]). The complications are easily envisaged and will not be discussed in detail.

In order to solve these problems the machine to be described is designed to move strips of filter paper at a steady speed through a zone in which the reagent is applied to it by a roller and trough, followed by a zone in which they are exposed to heat and a moving air stream. This method has two sets of advantages. In the first place, it is easier to maintain temperature and other relevant conditions constant with time in small volumes of space, rather than attempt to control large volumes of space. In the second, a continuous, sequential movement of strips through the machine is more easily made automatic than the stepwise or batchwise treatment of strips. This approach is justified by the accumulated experience of industrial processes for the production of uniform materials in the form of sheets and strips, and for dyeing and processing paper, cloth, and plastics.

Mechanical requirements

The mechanical specifications of a satisfactory machine of the type under consideration are determined by a combination of mechanical and chemical problems which cannot always be treated separately. Thus, smooth and precise mechanical operation is required to ensure that the spatial relationships of the paper to the treatment zones of the machine are held constant. The working parts of the machine must also be chemically inert and resistant to corrosion. The paper must be supported in its passage through the machine in such a way that all parts are treated with reagent, heated, and dried in identical fashion. Preferably, heating and drying should take place simultaneously on both sides of the paper.

These requirements were met by a pulley and belt system which gripped the paper strips by their edges (BUSH[14]). This meant that the main bearing pulleys in the region where the belts gripped the paper could not have internal flanges. Auxiliary double-flanged pulleys and precise machining are essential to secure trouble-free running of the belts. A more expensive method, but suitable for a commercially produced model, would be the use of chain-belts made of die-cast or moulded units, or a perforated belt, running on grooved pulleys or on sprockets or pin wheels to provide lateral fixation of the belts.

To avoid twisting or off-axis movement of the paper strip, the pairs of belts gripping each edge of the paper must have exactly equal velocities. To avoid displacement of the paper strip by uneven vibration in the air-stream used for drying, the belts must be pressed together at several points in their passage so that the paper is held tightly in position and cannot move laterally by slipping between the pairs of belts.

General features of the design

The most important and possibly questionable features of the present machine are:

(a) The linear, sequential arrangement of the machine and thus of the process it carries out;

(b) The belting-system supporting and transporting the paper strips by their extreme edges; and

(c) The use of a solid roller and trough for applying the liquid reagent.

The main reasons for the linear arrangement of the machine, and of the continuous sequential treatment of strips have been described above. Furthermore,

gradients of important variables along the axis of the controlled space are not only tolerable but can be produced and used if this is desirable. A further advantage is that the machine can be directly linked with the scanner, thus achieving the desirable property of the system as a whole that each zone along the length of each strip is scanned at exactly the same time interval after completion of the reaction in that zone. This enables the use of relatively evanescent color or fluorescence reactions for quantitative work.

The belting system was designed with three main aims in mind. The first was to minimize or eliminate as far as possible all preliminary maneuvers with the paper strips such as mounting them on frames or cutting or folding them into complicated forms. This both saves a lot of time when many strips have to be processed and reduces the risk of getting the strips contaminated with dirt and foreign chemicals. Secondly, it was not considered feasible to provide reproducible treatment of the strips with reagent by sprays or feeds from above, without considerable expense on the development of such equipment. The paper must be fed with a film of reagent from below, and its central region must therefore be freely accessible. Finally, both optical and chemical requirements of the method as a whole make it desirable, if possible, to heat and dry the reagent-soaked strips from both sides at equal rates at all stages of the process. This also requires that the working area of the strips be fully exposed throughout their passage through the machine.

The use of a solid roller and trough rather than a porous roller with a sponge-action (*e.g.* HRUBANT[15]) was preferred for a variety of reasons. In the first place, the release of fluid by a porous roller depends on the capillary properties of *both* the paper and the roller and is very sensitive to variations in the pressure between the two. Second, the dead-space of such a system includes both the porous volume of the roller as well as the volume of fluid between the roller and its trough. Such a dead-space, and particularly its porous nature, increases the risk of slowly exchanged reagent deteriorating during the course of a run and of decomposition products being applied in greater quantity to the later than the earlier strips in a train. Finally, the production of a really reliably inert and durable porous material in roller form capable of resisting attack by a wide range of reagents is expensive and not too easy.

A criticism has been made fairly frequently that the author's apparatus cannot handle two-dimensional paper chromatograms. This criticism seems to have relatively little weight for the following reasons. My experience with the problems afforded by one-dimensional chromatograms suggested very early on that a machine capable of really good performance with two-dimensional chromatograms would be excessively expensive to develop. Moreover, the zones on two-dimensional chromatograms are more diffuse and often much more irregular than those on one-dimensional strips, which makes the already more complex problem of scanning a two-dimensional array of zones subject to additional sources of error which would have to be overcome. Finally, the use of sequences of one-dimensional chromatograms (column or paper) to obtain sub-fractions of an extract prior to final one-dimensional chromatography has numerous advantages over two-dimensional methods when quantitative work is in question.

Important details of design and construction

Many features of the present machine are not critical, and a detailed description

has not been given of those dimensions and features which can readily be ascertained approximately from the figures, or are obvious to a good machinist. Indeed, certain features are not optimal, having been forced upon me by financial and other circumstances, and will be discussed below. Here I shall simply emphasize those features which are of crucial importance to the achievement of a successful machine.

There is a great advantage in using simple, unpatterned, belts for transporting the paper strips. The manufacture of patterned belts (*i.e.* belts with some form of ridges or holes to give lateral fixation) from corrosion-resistant material of low specific heat is extremely expensive unless large-scale production is contemplated. In order to use simple unpatterned belts successfully, however, one must obtain considerable geometrical accuracy and rigidity over the whole length of the machine, and ensure that thermal expansion of the reaction-chamber does not disturb this. This is obtained by the following features:

(*a*) very accurate machining of the main plates of the first and last sections, their spacing rods, and the stub shafts mounting the pulleys;

(*b*) the provision of a very rigid, heavy base or rail to which the first and last units are secured;

(*c*) the suspension of the reaction chamber on brackets which allow sliding to take up thermal expansion;

(*d*) the provision of lateral guide-posts or rails for the belts in the reaction chamber, and a small deflection of the belts from a straight horizontal path to provide pressure-points gripping the edges of the paper strips.

If the defects mentioned below can be overcome, PTFE-coated woven fiber glass belts seem ideal from a number of points of view. They are relatively inelastic and show little stretching or distortion when used under reasonable tensions. They are chemically inert and resistant to chemical destruction. Even more important is their virtue of being unwettable and of low thermal conductivity and specific heat. The former property allows a very simple washing tray to maintain adequate cleanliness with endless belts, and prevents overloading of the edges of the strips with reagent. The latter property reduces the difficulty of maintaining a constant temperature of the reaction-chamber, and minimizes underheating or overheating of the edges of the strips of paper, which can otherwise be a nuisance under certain conditions.

A disadvantage of PTFE-coated belts is their low coefficient of friction. Contact over a large fraction of the circumferences of the main driving pulleys must be obtained by using auxiliary pulleys or rollers. Other alternatives are considered below.

The entry and exit of air to and from the reaction-chamber must be regulated by using fans or blowers at both points. Although the diaphragms and deflecting baffles are of use in confining hot air to the chamber itself, and in securing a uniform heating and drying action, they are not adequate to the task on their own. In the absence of an exhaust fan capable of matching the inlet blower, hot air blows out of the ends of the chamber. This can heat up the take-off unit to an undesirable extent and lead to distortion and excessive wear due to uneven thermal expansion. Even worse, the reagent trough in the applicator unit can be gradually heated up. This causes a slow change in the conditions to which successive paper strips are subjected, and in many cases causes evaporation or deterioration of the reagent in the trough itself.

A small detail of the collecting tray is important (see Fig. 9). The tray is con-

structed with a floor of Formica (melamine-plywood laminate with a low coefficient of friction on the hard upper surface) and hardboard sides. At any angle which ensures that the strips stay in the tray after being ejected from the machine, successive strips tend to stick on, and between, one another and pile up as an irregular many-layered sandwich, the "tail" of which stays close to the ejection point and blocks or hinders the ejection of succeeding strips unless the strips are continually pushed down the tray by hand. This is overcome by the ridge (Fig. 9) placed (for 50 cm strips) approximately 28 cm horizontally beyond and 14 cm vertically below the ejection point (*i.e.* the point at which the upper and lower belts disengage from the paper). The leading edge of the paper strip catches on the ridge, thus forcing the emerging strip to roll over as it is pushed out from the rear (see Fig. 8). If the ridge is positioned properly by trial and error, the center of gravity of the strip is beyond the ridge by the time the end of the strip is released at the ejection point and the strip rolls over on itself. This ensures that it drops smoothly and sharply down to the end of the tray, ending up with the leading edge at the *upper* end of the tray. The operator can thus devote his whole attention to loading the strips into the machine and checking the controls.

Plenty of scope must be available in the tensioning pulleys to obtain the proper tension in all four belts. The manufacture of continuous belts of the correct length is subject to error, and even the exact measurement of the long and tortuous path taken by the belts is not easy. Their specification must always allow a safe margin of slack, and the tensioning pulleys must have sufficient movement to take up the maximum amount of slack likely to be encountered. With adequate accuracy of the machining and alignment of the main components, and with the use of guideposts in the reaction-chamber, the adjustment of belt tension is not difficult and not too critical a factor in the reliable running of the belts (see also below).

Undesirable features of the present machine

While the fourth machine described above is a serviceable and apparently effective piece of apparatus, as will appear in later papers of this series, it contains a number of defects which should be corrected in future models.

First, it is not possible to use the present machine for the treatment of strip chromatograms with predominantly aqueous reagents. The method of supporting and transporting the strips fails to allow for the swelling of paper that takes place when reagents containing a large fraction of water are used, and also gives inadequate support to the central area of the paper. It is also difficult to secure complete drying of the strips in the short path-length of the reaction-chamber if the reagent contains more than 50 % of water by volume.

The short, straight path, in the reaction-chamber is traversed in 65 sec. This, again, means that certain reactions which need prolonged exposure to the color reagent before finally drying the strips, cannot be completed in the machine. In this respect, the long zig-zag path used in the second model (Fig. 1) was greatly superior as it allowed a ten-minute exposure to the reagent.

These two defects are, however, not too serious for the following reasons. The majority of useful color and fluorescence reactions are carried out with reagents dissolved in organic solvents containing little or no water. Such reagents do not swell or weaken the paper strips sufficiently to prevent their efficient carriage by the belt system. Second, while many color reactions for paper chromatograms are normally

carried out by procedures which involve exposure to the reagent at room temperature, or with moderate heating, for several minutes or hours, the excellent control of conditions which is obtained by mechanizing the process allows one to increase the velocity of such reactions by using elevated temperatures. In addition, the application of a blast of hot air may in itself increase the speed and efficiency of color reactions on paper chromatograms by mechanisms which are not fully understood (see *e.g.* KOCHAKIAN AND STIDWORTHY[16]; SWALE[17]). Thus, for instance, better control of the ninhydrin reaction is usually achieved in conventional methods for paper chromatograms of amino acids by prolonged exposure (2–24 h) to the reagent at low or moderate temperatures, rather than by brief exposure to higher temperatures (*e.g.*, BARROLLIER[18,19] and PATTON AND CHISM[20]). However, completely satisfactory results have been achieved with ninhydrin in the third and fourth machines described here by using a working temperature of 115°.

Another undesirable feature of the present machine is the absence, for reasons of economy, of any servo-control on air-flow and temperature in the reaction-chamber. The difficulties arising from this can be overcome to a large extent by the general technique described above, but reproducible operation of the machine tends to be difficult, or to require much preliminary adjustment, if the room temperature or main electricity supply varies appreciably.

It will be seen in subsequent papers that these defects, while making it essential to have reasonably skilled workers operating the present machine, by no means prevent it from being a serviceable instrument of considerable and practical usefulness.

Problems encountered in using the fourth machine

Most of the problems that have been encountered have been due either to the defects mentioned earlier or to difficulties with materials or the environmental conditions.

The belting system is designed to grip 5.08 cm wide strips by an overlap of their edges of 0.318 cm. Care must be taken to see that a batch of strips from the supplier is not used for chromatograms if they are more than 1.5–2.0 mm undersize in width. Oversized strips can be managed quite adequately as long as the guide-rails at the loading point are adjustable in width, which is easily arranged (Fig. 12).

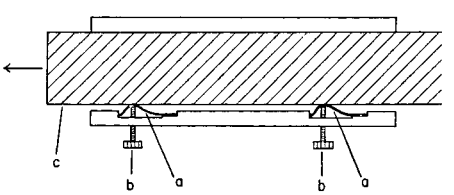


Fig. 12. Arrangement for providing an adjustable width to the entry guide-rails. a = Leaf spring inset on side face of guide rail; b = screw control; c = paper strip in guide rails.

Another problem is common to all methods of carrying out reactions on paper chromatograms, namely atmospheric contamination with agents affecting the reaction being carried out. This is particularly dangerous with this machine because of the relatively large air-flow through the reaction-chamber, and the necessary use of an exhaust fan leading to the outside air. Care must be taken to see that the outflow

through the exhaust system is balanced by an inflow of air to the room from an *uncontaminated* region of the atmosphere, and that other exhaust systems in the laboratory (*e.g.* hoods and air-conditioning systems) are either similarly balanced or are switched off during operation of the machine. A dust-filter on the intake to the heater-blower is desirable unless a dust-free atmosphere is obtained in the room by air-conditioning apparatus.

In the presence of rapid or extreme variations in ambient temperature, humidity, or mains voltage supply, it is difficult to achieve reproducible results with the present machine because of its lack of servo-controls. Under reasonable laboratory conditions in England and the U.S.A. such difficulties have not often been sufficiently severe to cause serious trouble. At worst a slightly lengthened period for adjustment of working temperature has been required.

Care is also needed in arriving at the optimum arrangement of the entry ports to the reaction chamber. The present machine with its single rather short reaction-chamber is not as flexible as is desirable. It is important that the exhaust system be adjustable so that inflow from the blower-heater is as nearly as possible exactly balanced by the exhaust outflow. If, in order to lengthen the time before each chromatogram is completely dried, one attempts to reduce the air-flow by reducing the total area of open entry ports *without* reducing the flow to the exhaust fan, colder room air will be sucked into the open ends of the reaction-chamber. This both lowers the temperature, and, more serious, tends to produce a laminar air-flow in the reaction-chamber with a central cold air stream, flanked by peripheral hot air streams from the entry ports (see Fig. 13). In the present machine, balance is achieved manually with a butterfly-valve which controls a bypass to the exhaust system. The pressure difference at the opening of the reaction-chamber is observed by means of a thin strip of tissue paper hanging in the opening which is brought to a vertical undisturbed position when the pressure difference is zero.

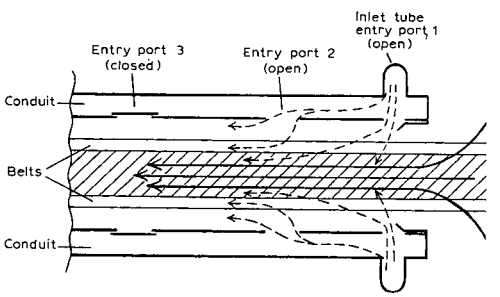


Fig. 13. Streamlining of cool air in the reaction chamber when inlet air-flow is less than exhaust outlet air-flow. Hot air as dashed lines, room air as solid lines.

The belt-washing baths have worked well, but the PTFE coating of the fiber glass belts is often not as continuous or as impervious as could be desired. The best way that I have found of testing such belts for selection is to soak them in 1.0 *N* KOH in 50 % ethanol for 24 h at 23–28° and then rinse them well in cold tap-water for 5–10 min. The washed belts are now immersed in phenolphthalein (1 % w/v in ethanol) which rapidly reveals as pink spots all serious defects through which KOH has penetrated. Although quite feasible technically, it is not usually economical to

obtain narrow belts of this sort which are covered with PTFE at their edges. Instead, they are cut from much wider sheets of PTFE-fiber glass and the cut edges can only be "sealed" with PTFE at great expense if success is to be guaranteed. Again the joints of the belts are a potential source of weakness. Mechanical strain is increased by the extra thickness and irregularity, and the fusion process sometimes removes some of the original PTFE coating and exposes the fiber glass to corrosive action.

The only economical solution to this problem, and to the similar one of preserving the stainless-steel reagent roller in good condition, is rapid and complete cleaning immediately after each use of the machine. The belts are most seriously endangered by flooding with strongly alkaline reagents, while the only reagent that has threatened to corrode the reagent roller in our experience has been 20 % w/v phosphomolybdic acid in alcohols. The stainless-steel roller has, however, lasted well and has not had to be replaced in eighteen months of frequent use.

Performance of the machine

The performance of the machine must be assessed in terms of two separate functions:

(a) The evenness of the application of reagents to different parts of each strip of paper and to different strips in a train of them.

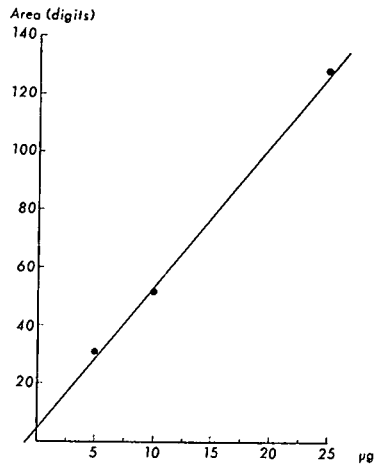
(b) The evenness of heating and drying the strips.

The first function has been assessed largely independently of the other by substituting a colored solution for the reagent, and setting the temperature of the reaction chamber at the minimum required to achieve almost, but not quite, complete drying of the strips. After treatment in the machine, they were hung horizontally (BUSH AND WILLOUGHBY[8]) in a hood to complete their drying. The strips were then scanned at the wavelength of maximum absorption of the dye that had been used. The first model was tested in this way using Sudan red in ethanol; later models were tested using the dye 4-amino-1-methylaminoanthraquinone (NEHER[21]). Such strips were compared with similar ones which were dipped by hand over the dye solution in a watchglass (BUSH AND WILLOUGHBY[8]) and dried horizontally in a hood. The scanner was used at a higher sensitivity than usual in order to accentuate variations in the artificial background color that had been produced.

The maximum variation in optical density over the length of any one strip treated by the machine (*i.e.* the "worst" strip) was invariably much less (5–10 fold) than the variation on the "best" strip of the series treated by hand. In view of the clearcut superiority of the mechanized process no statistical analysis of these records was carried out.

The evenness of heating and drying of the strips is best determined by using a color-reagent which requires a moderate temperature for completion in the 65 sec period in the reaction chamber, and which gives a very high background on overheating. An even more critical test is provided by running the machine at a temperature *below* the optimum for such a reaction so that the reaction is incomplete. In these circumstances the calibration curve for a set of standards in the usual working range of concentrations is nonlinear and highly susceptible to any variation in the heating and drying conditions that were used. The best test of this sort is provided by the phosphomolybdic acid reagent, used for a wide range of hydroxylic steroids (KRITCHEVSKY AND KIRK[22]) and other alcohols of high-molecular weight. Under the usual

conditions of heating the paper in an oven at 90° the reaction usually gives little color in 1.0 min, an optimum color contrast (*i.e.* zone/background = blue/yellow-green) in approx. 1.5 min, and a uselessly high background, in which the whole paper becomes deep blue-black in color, in approx. 2.2 min. It has been found almost impossible to control the conditions sufficiently by manual methods to obtain reliable quantitative results with this reagent by scanning, unless the much slower, low-temperature method of MARTIN[23] is used. As will be shown in paper 3 of this series the machine enables one



(a)

(b)

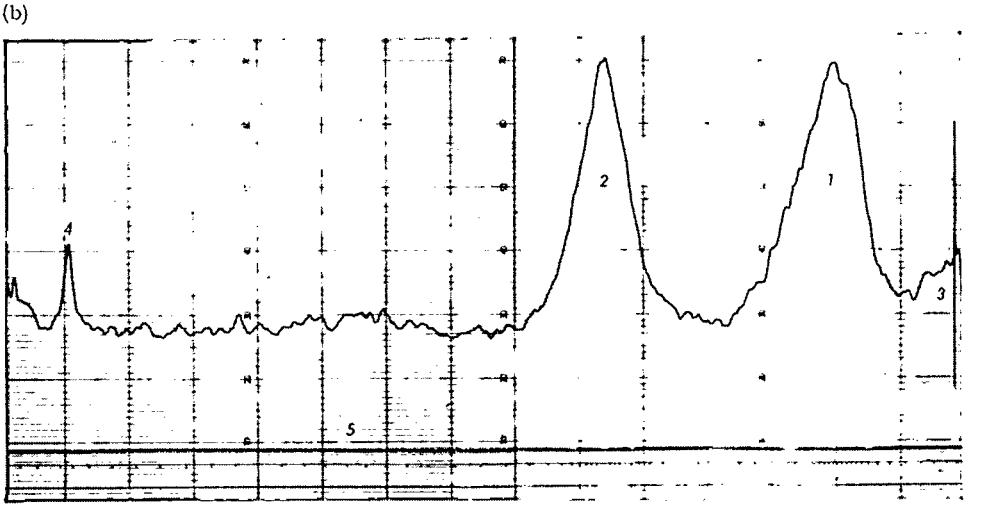


Fig. 14. Calibration curve (a) and typical scanning record (b) for dehydroepiandrosterone determined by the phosphomolybdic acid reagent carried out by the machine. Starting temperature: 95.5°. Steady-state operating temperature, 94.5°. Chromatograms on Whatman No. 2 paper, run 3.5 h with the system light petroleum–toluene–methanol–water (67:33:85:15, by vol.) at 25°. 1 = Androsterone; 2 = dehydroepiandrosterone; 3 = impurities at front; 4 = pencil line at origin; 5 = digital integral record (units, tens, hundreds, thousands from top to bottom). The recorder was run with minimal damping during this experiment in order to emphasize background fluctuations. Androsterone was too close to the front material for precise estimation.

to obtain quantitative estimation of hydroxylic steroids which is almost as precise as any other colorimetric method carried out by the scanning method.

A typical run is shown in Fig. 14. The starting temperature was 95.5° and the operating temperature during the run was 94.7° ± 0.7°. The calibration curve is linear up to 25 μg and the background optical density, while showing the usual short-length (1–3 mm) irregularity typical with dry filter paper (BUSH[7]), did not vary by more than ± 2 small divisions of the chart record from the mean over a 45 cm long strip. In another similar run with a train of 12 strips, each 50 cm long, the difference in the backgrounds of the strip with the highest and the strip with the lowest background optical density was 3 small divisions on the chart record. The scanner was run at moderate sensitivity and minimum damping (see paper 2 of this series) to accentuate small and rapid fluctuations in optical density, and a 25 μg zone of dehydroepiandrosterone (5 cm wide) gave a peak height of 34 small divisions of the chart. During this period the drift of the scanner and light source produced a change in optical density of 2–3 small divisions on the chart recorder over periods of 50 sec (the time needed to scan one strip) and a ripple of ± 0.5 small divisions at approximately 2 c.p.s.

Strips treated manually over the same period (dipping over a watchglass and heating before an open electric heater (BUSH[7])) showed a minimum background variation (with the same sensitivity of the scanner) on any one strip (*i.e.* the "worst" strip) of ± 5 small divisions, and the differences between the background optical density of the strips with highest and lowest mean backgrounds was 20 small divisions on the chart.

Similar but less extreme results have been obtained with the Zimmermann reaction for 17-ketosteroids (alkaline *m*-dinitrobenzene, BUSH AND MAHESH[9]). Manually treated strips usually require constant readjustment of the background-setting potentiometer during scanning to cope with the wide variation (± 8 small divisions) in backgrounds of successive strips. With machine-treated strips little or no readjustment is required during the scanning of trains of 20–30 strips (see paper 2).

Other uses of the machine

Apart from its main use, which is the rapid and reproducible achievement of color and fluorescence reactions on paper chromatograms, the machine can be used for many other treatments of filter paper strips which are useful in paper chromatography. Thus, it can be used to pretreat filter paper with solvents or buffer solutions necessary for some types of paper chromatography (see *e.g.* LEVY AND CHUNG[24]). The reproducibility afforded by the continuous, mechanized, process is greatly superior to spraying or dipping by hand, and is especially so when drying or heating to a precisely controlled degree is desired. Similarly, the pretreatment of chromatograms after they have been run is often a valuable preliminary before certain color reactions are carried out. Thus, the final elimination of traces of the solvent system is often only achieved by "steaming" (*e.g.* ISHERWOOD[25]) or other similar procedures (*e.g.* BUSH[7], Chaps. 3 and 4 and later papers in this series). In such cases the reproducible application of a liquid solvent or reagent and its controlled removal by evaporation is crucial to the achievement of satisfactory results, and can be done by the machine much more reliably than with manual methods, ovens, hair-driers and the like.

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SUMMARY

A machine is described for the reproducible treatment of paper chromatograms with liquid chemical reagents. Strips of filter paper 5 cm wide are passed through the machine by two pairs of belts which grip the edges of the strip leaving the central area exposed on both sides. A film of liquid reagent is fed on to the undersurface of the paper strip by means of a roller and trough which is fed from a simple constant level device. The reagent-soaked strips pass immediately into a straight tunnel into which a stream of hot air is introduced by ports opening from manifolds running along the sides of the tunnel. The air stream is passed out of the top of the tunnel by means of an exhaust tube and fan connected to its distal end. The strips are passed out of the machine by disengagement of the belts which are returned to the starting point of the machine after being washed, and dried on their return passage through the hot air tunnel. Operating temperatures up to 150° can be achieved and are adequate for producing quantitative and complete reactions with the majority of color reagents that are useful for the quantitative estimation of substances by direct scanning of paper chromatograms (*e.g.* Zimmerman reagent, ninhydrin, phosphomolybdic acid). The mechanization of the treatment of paper chromatograms with color reagents improves greatly the reproducibility of the results, the evenness and reproducibility of background, and enables the successful use of color reactions which cannot be controlled sufficiently by manual methods to allow their quantitative estimation. It also enables the treatment of very large numbers of chromatograms: the present apparatus is capable of treating 40 cm long chromatograms at a rate of up to 88 per h or over 500 per day. The development, important features of design, and method of operation of the machine are described.

REFERENCES

1 R. CONSDEN, A. H. GORDON AND A. J. P. MARTIN, *Biochem. J.*, 38 (1944) 224.
2 R. J. BLOCK, E. L. DURRUM AND G. ZWEIG, *A Manual of Paper Chromatography and Paper Electrophoresis*, Academic Press, New York, 1958.
3 A. C. CHIBNALL, *Brit. Med. Bull.*, 10 (1954) 183.
4 D. H. SPACKMAN, W. H. STEIN AND S. MOORE, *Anal. Chem.*, 30 (1958) 1190.
5 I. E. BUSH, *Mem. Soc. Endocrinol.*, 8 (1960) 24.
6 I. E. BUSH, in D. GLICK (Editor), *Methods of Biochemical Analysis*, Vol. 11, Interscience, New York, 1964, p. 149.
7 I. E. BUSH, *The Chromatography of Steroids*, Pergamon, Oxford, 1961.
8 I. E. BUSH AND M. L. N. WILLOUGHBY, *Biochem. J.*, 67 (1957) 689.
9 I. E. BUSH AND V. B. MAHESH, *J. Endocrinol.*, 18 (1959) 1.
10 C. S. HANES, C. K. HARRIS, M. A. MOSCARELLO AND E. TIGANE, *Can. J. Biochem. Physiol.*, 39 (1961) 163.
11 A. J. TOMISEK AND P. W. ALLAN, *J. Chromatog.*, 14 (1964) 232.
12 R. J. BLOCK, *Anal. Chem.*, 22 (1950) 1327.
13 L. D. OUGH, A. JEANES AND J. E. PITTSLEY, *J. Chromatog.*, 6 (1961) 80.
14 I. E. BUSH, *British Pat.*, 893,648; filed Oct. 17th, 1958, published April 11th, 1962.
15 H. E. HRUBANT, *J. Chromatog.*, 6 (1961) 94.
16 C. D. KOCHAKIAN AND G. STIDWORTHY, *J. Biol. Chem.*, 199 (1952) 607.
17 J. SWALE, personal communication, 1954.
18 J. BARROLLIER, *Naturwiss.*, 42 (1955) 416.
19 J. BARROLLIER, *Z. Physik. Chem.*, 309 (1957) 219.
20 A. R. PATTON AND P. CHISM, *Anal. Chem.*, 23 (1951) 1683.
21 R. NEHER, in M. LEDERER (Editor), *Chromatographic Reviews*, Vol. 1, Elsevier, Amsterdam, 1959, p. 99.
22 D. KRITCHEVSKY AND M. KIRK, *Arch. Biochem. Biophys.*, 35 (1952) 346.
23 R. P. MARTIN, *Biochim. Biophys. Acta*, 25 (1957) 408.
24 A. L. LEVY AND D. CHUNG, *Anal. Chem.*, 25 (1953) 396.
25 F. A. ISHERWOOD, *Brit. Med. Bull.*, 10 (1954) 202.

STUDIES IN THE RELATIONSHIP BETWEEN MOLECULAR STRUCTURE AND CHROMATOGRAPHIC BEHAVIOUR

V. THE BEHAVIOUR OF SOME ALKYL ETC. PHENOLS CHROMATOGRAPHED ON ALUMINA-IMPREGNATED PAPERS AND ON THIN LAYERS OF ALUMINA

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INTRODUCTION

In the thin-layer chromatographic separation of alkylphenols the following substrates have been used: silica gel[1–4], polyamide[5,6], unbound alumina[7,8] and ion-exchange resins[9].

For isomeric cresols[1], it was found that the R_F values of the *m*- and *p*-compounds showed little difference from those of the parent phenol, but that the R_F value of *o*-cresol was higher.

PETROWITZ[2] showed that the R_F values of the isomeric xylenols were similarly dependent upon the presence, and the number of *o*-methyl groups. These findings for the behaviour of these compounds on silica gel have been confirmed by other workers[3,4].

Attempts to substantiate the MARTIN additivity[10] principle for an homologous series, have been made by HALMEKOSKI AND HANNIKAINEN[5]. These workers chromatographed phenol and the first three members of the alkylphenols (methyl, ethyl, *n*-propyl) both on silica gel and polyamide layers, using a series of solvent systems.

On polyamide layers, there is evidence that the MARTIN[10] additivity principle is valid for these phenols in each solvent system. The separations using silica gel are such that no convincing evidence is available to substantiate the validity of the principle.

The results obtained by WANG[6], using polyamide layers, confirm the effect of *ortho* substituents and using his results, it is possible to show for phenols substituted in the *meta* or *para* positions that the MARTIN relation is approximately correct.

Using loose layers of alumina, HERMANEK and co-workers[7] showed that the R_F values of phenol, and 3-methyl- and 4-methylphenol, were approximately the same, but the values obtained for 2-methylphenol were all significantly higher, showing an increased mobility of the phenol, over the polar substrate.

KHEIFITS *et al.*[8] also confirmed this *ortho* effect of substituents, when some other alkylphenols were chromatographed on alumina. They also found that alkyl

groups in the 3- or 4-position had little effect on the R_F values relative to those of phenol, even when the alkyl group was a bulky one, *e.g.* a *tert.*-butyl group.

In an earlier work dealing with the separation of nitrophenols on alumina-impregnated papers[12], we stated that the mechanism of the chromatographic process was a result of hydrogen bonding between:

(1) the oxygen atom of the phenolic group, and

(2) any other proton acceptor group within the molecule, and the hydrogen atoms of the hydroxyl groups on the alumina surface.

In this present work, we have chromatographed a large number of alkylphenols, a small number of arylphenols, and some alkoxyphenols on the same grades of alumina-impregnated papers previously used[12], and also on thin layers of unbound alumina. The results obtained, under carefully standardised conditions, are considered in terms of the chromatographic process outlined above. The validity of the MARTIN[10] relation, and reasons for deviations from it are also considered.

The choice of a suitable solvent system in all forms of chromatography cannot be isolated from a consideration of the nature of the molecule to be separated and the nature of the stationary phase to be used. Suitable guides to the suitability of any eluent systems are the so-called elutiotropic series, which arrange organic liquids in order of their increasing polarities either qualitatively[11,13,14] or semi-quantitatively[15]. SNYDER[15] reported that the calculated eluent strengths agreed with the experimental strengths for the same solvent mixtures. It is considered that these series are only of value when it is not possible for solute–eluent interactions[16] to occur. When studying the behaviour of nitrophenols on alumina-impregnated papers, with special reference to hydrogen bonding between the alumina and the solute, we[12] chose anhydrous cyclohexane as the solvent, in order to minimise interactions (*viz.* hydrogen bonding) between the phenols and the development solvent. In this work, where hydrogen bonding between the alkylphenols and the solvent is probably reduced because of the generally lower polarity of the alkylphenols, compared with the nitrophenols, other solvent systems were used. These were chosen to give a variation in the relative polarities of the solute–solvent system.

EXPERIMENTAL

Chromatography on impregnated paper

The four grades of alumina-impregnated papers were:

(a) cellulose paper (Whatman No. 1) impregnated with 2 % of alumina;

(b) cellulose paper (Whatman No. 1) impregnated with 7.5 % of alumina;

(c) glass fibre "paper" (Whatman) impregnated with 7.5 % of alumina;

(d) cellulose paper (Schleicher and Schüll No. 288) impregnated with 25 % of alumina.

The pretreatment of the papers, the application of the phenols and the development conditions were as previously described[12].

Thin-layer chromatography

Alumina (Hopkin and Williams M.F.C. (Camag) grade, neutral, Brockmann activity I–II, 100–200 mesh) was used as the substrate.

Preliminary experiments showed that this grade was too coarse to given satis-

factory layers when slurried with water. The alumina was therefore crushed and sieved, and the various sieve fractions tested, that passing a 200-mesh sieve but retained by a 230-mesh sieve gave a material suitable for the preparation of the thin layers.

Alumina (40 g) was slurried with water (40 ml) and applied to clean, grease-free glass plates using a Shandon thin-layer applicator*. The quantities given were sufficient to coat 5 glass plates (20 × 20 cm), layer thickness 0.25 mm.

When the surplus water had evaporated, the coated plates were stored in racks and air dried for 24 h at a constant temperature of 25° ± 0.5°.

In order to equate the results obtained from the thin layers with those obtained from the impregnated papers, the activation of the layers was the same as for the papers[12], namely, heating in an air oven for 15 min at 110°, followed by cooling in an evacuated desiccator over molecular sieve type 4 A (British Drug Houses).

The application of the phenols and development conditions

The phenols (1 μl of 0.25 % v/v solutions in suitable solvents) were applied to the activated plates with the multiple-spotting device previously described[17].

The prepared plates were placed in a double saturation chamber[17] and eluted by an ascending technique at a constant temperature of 25° ± 0.5°. The length of run was standardised as being $14^1/_2 \pm {}^1/_2$ cm. The time taken for the solvent front to travel this distance was 90 min.

Eluent systems

The following eluents were used:

(I) Cyclohexane (see below)
(II) Dioxane (see below)
(III) Cyclohexane–dioxane (75:25, v/v)
(IV) Cyclohexane–dioxane (1:1, v/v)
(V) Benzene–methanol (95:5, v/v)
(VI) Benzene–ethanol (95:5, v/v)
(VII) Benzene–ethyl acetate (3:7, v/v).

Purification of the solvents

Cyclohexane. This was purified as previously described[12].

Dioxane. DASLER AND BAUER[18] recommended the removal of explosive peroxides and water from dioxane by standing the solvent (Analar grade) over alumina. This was done here. The solvent was then distilled from sodium wire and the fraction boiling at 101° under 759 mm pressure was collected.

Benzene. Benzene (Hopkin and Williams, M.F.C. grade) was dried over sodium wire. The dried solvent was distilled, and the fraction boiling at 80° under 760 mm pressure was collected.

Methyl alcohol. Methyl alcohol (Hopkin and Williams, Analar grade) was dried over alumina. The solvent was redistilled and the fraction boiling at 65° under 760 mm pressure was collected.

Ethyl alcohol. Super dry alcohol was prepared as previously described[19].

* Available from Shandon Scientific Co., Pound Lane, London.

Ethyl acetate. This was purified by heating ethyl acetate (Hopkin and Williams, M.F.C. grade), acetic anhydride and a little concentrated sulphuric acid under reflux for 4 h. The mixture was distilled, and the distillate was neutralised by shaking it with anhydrous potassium carbonate. After removal of the excess solid the solvent was redistilled; the fraction boiling at 77° under 760 mm pressure was used.

Preparation of the mixed eluents

Eluent mixtures were prepared by mixing together the appropriate volumes of the components.

All eluents were allowed to come to the temperature at which the chromatograms were to be eluted.

Detection of the phenols

After elution, the chromatograms were dried and then sprayed with an alkaline potassium permanganate solution (0.515 g anhydrous sodium carbonate and 0.5 g potassium permanganate in 100 ml of aqueous solution). The phenols appeared as yellow spots on a pale purple background. The spots were stable long enough to permit the marking of the spots.

RESULTS

The results are shown in Table I. Each result is the average of at least four runs on plates or papers carrying an internal standard. For the results obtained to be considered, the results for the standard on each plate/paper had to agree within $\pm$ 0.01 R_F units with the pre-determined mean for that standard when run under the same conditions as the subsequent chromatograms. No chromatograms had to be discarded. Further, the R_F values for each individual phenol did not vary more than $\pm$ 0.01 R_F unit from the mean values quoted.

DISCUSSION OF RESULTS

Solvent effect

SNYDER[15,20] when discussing the role of the solvent in linear elution column chromatography attempted to quantitise the strength of the solvents discussed, and quoted the values for cyclohexane as 0.04 units, dioxane as 0.63 units and benzene as 0.32 units. The strengths of the mixed eluents used were calculated, assuming the additivity of strengths, thus the relative strengths of the eluents used are:

Eluent	*Strength*
Cyclohexane	0.04
Cyclohexane–dioxane (75:25, v/v)	0.19
Cyclohexane–dioxane (50:50, v/v)	0.34
Dioxane	0.63
Benzene–methanol (95:5, v/v)	0.35
Benzene–ethylacetate (30:70, v/v)	0.19
Benzene–ethanol (95:5, v/v)	0.35 (approx.)

TABLE I

R_F VALUES OF PHENOLS ON VARIOUS SOLVENT/SUPPORT SYSTEMS

Key	Phenol	R_F values for solvent/support system No.													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Phenol	0.10	0.05	0.06	0.00	1.00	1.00	1.00	0.25	0.00	0.48	0.95	0.26	0.29	0.50
2	2-Methyl	0.24	0.12	0.13	0.00	1.00	1.00	1.00	0.49	0.00	0.65	1.00	0.40	0.38	0.67
3	3-Methyl	0.14	0.07	0.12	0.00	1.00	1.00	1.00	0.29	0.00	0.53	1.00	0.34	0.32	0.56
4	4-Methyl	0.12	0.06	0.10	0.00	1.00	1.00	1.00	0.27	0.00	0.52	1.00	0.33	0.32	0.55
5	2,3-Dimethyl	0.26	0.12	0.18	0.00	1.00	1.00	1.00	0.55	0.00	0.75	1.00	0.43	0.41	0.70
6	2,4-Dimethyl	0.25	0.11	0.19	0.00	1.00	1.00	1.00	0.53	0.00	0.74	1.00	0.41	0.40	0.69
7	2,5-Dimethyl	0.28	0.12	0.19	0.00	1.00	1.00	1.00	0.55	0.00	0.75	1.00	0.43	0.41	0.70
8	2,6-Dimethyl	0.56	0.36	0.43	0.00	1.00	1.00	1.00	0.76	0.00	0.87	1.00	0.52	0.54	0.80
9	3,4-Dimethyl	0.16	0.08	0.14	0.00	1.00	1.00	1.00	0.32	0.00	0.53	1.00	0.32	0.33	0.58
10	3,5-Dimethyl	0.19	0.18	0.14	0.00	1.00	1.00	1.00	0.35	0.00	0.53	1.00	0.32	0.32	0.60
11	2,3,4-Trimethyl	0.26	0.14	0.20	0.00	1.00	1.00	1.00	0.59	0.00	0.73	1.00	0.39	0.42	0.70
12	2,3,5-Trimethyl	0.27	0.16	0.21	0.00	1.00	1.00	1.00	0.62	0.00	0.76	1.00	0.39	0.44	0.72
13	2,3,6-Trimethyl	0.54	0.34	0.37	0.05	1.00	1.00	1.00	0.84	0.03	0.85	1.00	0.52	0.56	0.83
14	2,4,5-Trimethyl	0.26	0.16	0.19	0.00	1.00	1.00	1.00	0.61	0.00	0.75	1.00	0.39	0.44	0.72
15	2,4,6-Trimethyl	0.54	0.34	0.36	0.05	1.00	1.00	1.00	0.84	0.03	0.85	1.00	0.50	0.55	0.83
16	3,4,5-Trimethyl	0.12	0.08	0.05	0.00	1.00	1.00	1.00	0.32	0.00	0.53	1.00	0.30	0.33	0.62
17	2,3,4,5-Tetramethyl	0.30	0.16	0.20	0.00	1.00	1.00	1.00	0.62	0.00	0.73	1.00	0.39	0.44	0.70
18	2,3,4,6-Tetramethyl	0.55	0.34	0.38	0.04	1.00	1.00	1.00	0.85	0.03	0.89	1.00	0.52	0.54	0.86
19	2,3,5,6-Tetramethyl	0.55	0.36	0.39	0.04	1.00	1.00	1.00	0.86	0.03	0.90	1.00	0.54	0.55	0.86
20	2-Ethyl	0.28	0.15	0.16	0.00	1.00	1.00	1.00	0.67	0.00	0.73	1.00	0.45	0.46	0.75
21	3-Ethyl	0.14	0.07	0.08	0.00	1.00	1.00	1.00	0.37	0.00	0.53	1.00	0.36	0.36	0.59
22	4-Ethyl	0.12	0.06	0.07	0.00	1.00	1.00	1.00	0.35	0.00	0.54	1.00	0.35	0.36	0.58
23	2-*n*-Propyl	0.32	0.18	0.19	0.02	1.00	1.00	1.00	0.76	0.00	0.81	1.00	0.49	0.49	0.84
24	4-*n*-Propyl	0.18	0.07	0.07	0.00	1.00	1.00	1.00	0.37	0.00	0.53	1.00	0.35	0.35	0.62
25	2-Isopropyl	0.18	0.07	0.08	0.00	1.00	1.00	1.00	0.40	0.00	0.55	1.00	0.41	0.38	0.62
26	4-Isopropyl	0.19	0.07	0.07	0.00	1.00	1.00	1.00	0.37	0.00	0.53	1.00	0.35	0.35	0.62
27	4-*n*-Butyl	0.19	0.07	0.07	0.00	1.00	1.00	1.00	0.37	0.00	0.53	1.00	0.35	0.35	0.63
28	2-*sec.*-Butyl	0.41	0.26	0.26	0.02	1.00	1.00	1.00	0.79	0.00	0.91	1.00	0.56	0.51	0.85
29	4-*sec.*-Butyl	0.25	0.08	0.19	0.00	1.00	1.00	1.00	0.40	0.00	0.63	1.00	0.38	0.40	0.65
30	2-*tert.*-Butyl	0.59	0.29	0.27	0.02	1.00	1.00	1.00	0.84	0.02	1.00	1.00	0.61	0.59	0.92
31	3-*tert.*-Butyl	0.27	0.10	0.10	0.00	1.00	1.00	1.00	0.51	0.00	0.63	1.00	0.45	0.40	0.65
32	4-*tert.*-Butyl	0.28	0.10	0.13	0.00	1.00	1.00	1.00	0.51	0.00	0.64	1.00	0.45	0.43	0.65
33	4-*n*-Amyl	0.24	0.10	0.11	0.00	1.00	1.00	1.00	0.46	0.00	0.58	1.00	0.35	0.35	0.65
34	4-*sec.*-Amyl	0.31	0.10	0.10	0.00	1.00	1.00	1.00	0.53	0.00	0.68	1.00	0.38	0.39	0.67
35	4-*tert.*-Amyl	0.28	0.11	0.09	0.00	1.00	1.00	1.00	0.49	0.00	0.67	1.00	0.44	0.43	0.67
36	4-(3-Methylbutyl)	0.13	0.07	0.06	0.00	1.00	1.00	1.00	0.40	0.00	0.56	1.00	0.35	0.35	0.64
37	4-*tert.*-Octyl	0.14	0.08	0.08	0.00	1.00	1.00	1.00	0.53	0.00	0.76	1.00	0.44	0.45	0.78
38	2-*n*-Octyl	0.60	0.40	0.39	0.03	1.00	1.00	1.00	0.90	0.03	0.98	1.00	0.63	0.62	0.92
39	4-*n*-Nonyl	0.14	0.06	0.09	0.00	1.00	1.00	1.00	0.53	0.00	0.68	1.00	0.42	0.42	0.74
40	2-Allyl	0.24	0.09	0.10	0.00	1.00	1.00	1.00	0.63	0.00	0.68	1.00	0.47	0.48	0.70
41	4-Allyl	0.10	0.04	0.06	0.00	1.00	1.00	1.00	0.34	0.00	0.46	1.00	0.35	0.35	0.53
	[illegible]	0.11	0.06	0.06	0.00	1.00	1.00	1.00	0.35	0.00	0.50	1.00	0.36	0.36	0.56

43	2-Phenyl	0.32	0.14	0.16	0.03	1.00	1.00	1.00	0.66	0.00	0.75	1.00	0.51	0.53	0.76
44	4-Phenyl	0.07	0.03	0.03	0.00	1.00	1.00	1.00	0.15	0.00	0.33	1.00	0.30	0.30	0.42
45	4-Benzyl	0.08	0.03	0.03	0.00	1.00	1.00	1.00	0.16	0.00	0.44	1.00	0.40	0.42	0.69
46	4-Cumyl	0.08	0.03	0.03	0.00	1.00	1.00	1.00	0.34	0.00	0.50	1.00	0.42	0.44	0.71
47	4-Cyclopentyl	0.14	0.07	0.06	0.00	1.00	1.00	1.00	0.39	0.00	0.55	1.00	0.36	0.37	0.66
48	4-Cyclopent-2-enyl	0.12	0.06	0.05	0.00	1.00	1.00	1.00	0.32	0.00	0.53	1.00	0.37	0.37	0.64
49	2-Cyclohexyl	0.33	0.11	0.11	0.03	1.00	1.00	1.00	0.84	0.00	0.81	1.00	0.54	0.54	0.81
50	4-Cyclohexyl	0.17	0.08	0.08	0.00	1.00	1.00	1.00	0.38	0.00	0.60	1.00	0.38	0.39	0.69
51	2-Methyl-4-*tert.*-butyl	0.28	0.17	0.16	0.02	1.00	1.00	1.00	0.55	0.00	0.76	1.00	0.42	0.44	0.81
52	3-Methyl-5-ethyl	0.16	0.06	0.07	0.01	1.00	1.00	1.00	0.42	0.00	0.58	1.00	0.35	0.35	0.62
53	3-Methyl-4-isopropyl	0.16	0.06	0.09	0.00	1.00	1.00	1.00	0.46	0.00	0.61	1.00	0.35	0.35	0.68
54	3-Methyl-5-isopropyl	0.19	0.06	0.09	0.02	1.00	1.00	1.00	0.47	0.00	0.60	1.00	0.35	0.35	0.66
55	3-Methyl-5-*sec.*-butyl	0.24	0.07	0.13	0.01	1.00	1.00	1.00	0.54	0.00	0.66	1.00	0.40	0.42	0.69
56	3,5-Di-*tert.*-butyl	0.33	0.12	0.20	0.00	1.00	1.00	1.00	0.86	0.00	0.70	1.00	0.48	0.49	0.86
57	2-Methyl-4-octyl	0.30	0.11	0.11	0.02	1.00	1.00	1.00	0.58	0.00	0.80	1.00	0.46	0.46	0.84
58	2-*tert.*-Butyl-3-methyl	0.59	0.28	0.28	0.05	1.00	1.00	1.00	0.93	0.03	1.00	1.00	0.63	0.64	1.00
59	2-*tert.*-Butyl-4-methyl	0.59	0.28	0.26	0.04	1.00	1.00	1.00	0.96	0.03	1.00	1.00	0.63	0.64	1.00
60	2-Octyl-4-methyl	0.70	0.40	0.40	0.11	1.00	1.00	1.00	0.97	0.04	1.00	1.00	0.63	0.63	1.00
61	2,6-Dimethyl-4-*n*-propyl	0.60	0.45	0.51	0.09	1.00	1.00	1.00	0.91	0.04	0.97	1.00	0.60	0.56	0.89
62	2,6-Dimethyl-4-allyl	0.59	0.42	0.47	0.08	1.00	1.00	1.00	0.90	0.06	0.93	1.00	0.60	0.58	0.89
63	2,6-Di-*tert.*-butyl	0.97	0.93	0.91	0.83	1.00	1.00	1.00	1.00	0.57	1.00	1.00	1.00	0.97	1.00
64	2-Methyl-4,6-di-*tert.*-butyl	0.90	0.84	0.83	0.75	1.00	1.00	1.00	0.96	0.10	0.96	1.00	0.77	0.77	1.00
65	2,6-Di-*tert.*-butyl-4-methyl	0.98	0.91	0.89	0.81	1.00	1.00	1.00	1.00	0.54	1.00	1.00	1.00	0.97	1.00
66	2-Methoxyl	0.25	0.13	0.13	0.00	1.00	1.00	1.00	0.33	0.00	0.55	1.00	0.30	0.41	0.68
67	3-Methoxyl	0.03	0.00	0.04	0.00	1.00	1.00	1.00	0.23	0.00	0.38	0.76	0.20	0.39	0.65
68	4-Methoxyl	0.04	0.00	0.04	0.00	1.00	1.00	1.00	0.24	0.00	0.40	0.78	0.20	0.38	0.66
69	3,5-Dimethoxyl	0.00	0.00	0.00	0.00	1.00	0.92	0.93	0.07	0.00	0.25	0.70	0.15	0.28	0.39
70	4-Ethoxyl	0.05	0.00	0.00	0.00	1.00	1.00	1.00	0.26	0.00	0.50	0.82	0.22	0.37	0.65
71	4-Cyclopentyloxyl	0.11	0.00	0.00	0.00	1.00	1.00	1.00	0.26	0.00	0.60	0.90	0.22	0.37	0.67
72	4-Heptoxyl	0.07	0.00	0.00	0.00	1.00	1.00	1.00	0.31	0.00	0.72	0.90	0.25	0.44	0.70
73	4-Dodecyloxyl	0.07	0.00	0.00	0.00	1.00	1.00	1.00	0.35	0.00	0.80	0.90	0.26	0.43	0.70
74	4-Tetradecyloxyl	0.06	0.00	0.00	0.00	1.00	1.00	1.00	0.39	0.00	0.80	0.90	0.32	0.43	0.70
75	4-Hexadecyloxyl	0.07	0.00	0.00	0.00	1.00	1.00	1.00	0.40	0.00	0.82	0.90	0.32	0.43	0.71
76	4-Phenoxyl	0.05	0.00	0.00	0.00	1.00	1.00	1.00	0.14	0.00	0.45	0.65	0.21	0.39	0.58
77	2,5-Dicarbamethoxyl	0.00	0.00	0.00	0.00	1.00	1.00	1.00	0.02	0.00	0.08	0.31	0.12	0.11	0.22

* Key to solvent/support system Nos.:

1 = Cyclohexane/cellulose paper and 2 % of alumina
2 = Cyclohexane/cellulose paper and 7.5 % of alumina
3 = Cyclohexane/glass fibre paper and 7.5 % of alumina
4 = Cyclohexane/cellulose paper and 25 % of alumina
5 = Cyclohexane–dioxane (75:25, v/v)/cellulose paper and 2 % of alumina
6 = Cyclohexane–dioxane (75:25, v/v)/cellulose paper and 7.5 % of alumina
7 = Cyclohexane–dioxane (75:25, v/v)/glass fibre paper and 7.5 % of alumina
8 = Cyclohexane–dioxane (75:25, v/v)/cellulose paper and 25 % of alumina
9 = Cyclohexane/alumina thin layers
10 = Cyclohexane–dioxane (1:1, v/v)/alumina thin layers
11 = Dioxane/alumina thin layers
12 = Benzene–methanol (95:5, v/v)/alumina thin layers
13 = Benzene–ethanol (95:5, v/v)/alumina thin layers
14 = Benzene–ethyl acetate (3:7, v/v)/alumina thin layers.

The strength of the benzene–ethanol system could not be calculated accurately because SNYDER has not determined a value for ethanol. However, for the purposes of the discussion, it was assumed that the value for ethanol will lie between that for isopropanol (0.82) and methanol (0.95) and is probably nearer to the latter value. Hence the strength of the benzene–ethanol system will be close to that of benzene–methanol.

From a comparison of the eluent strengths it would be expected that the R_F values obtained from thin-layer chromatograms would be in the order:

Cyclohexane < cyclohexane–dioxane (50:50, v/v) < benzene–ethyl acetate (30:70, v/v) < benzene–ethanol (95:5, v/v) < benzene–methanol (95:5, v/v) < dioxane.

The results show that this is not so and would in our opinion indicate some solute/eluent interaction in the systems containing esters or alcohols.

To simplify the overall picture, the phenols are subdivided by an arbitrary classification, and eluent systems in which no separation is achieved are omitted. In these systems the phenols either stayed at the point of application or moved with the solvent front.

(*a*) *Methylated phenols*

Here, the effect of the addition of successive methyl groups to the phenyl nucleus has to be considered. Electronically, it would be expected that because of the inductive effect of the methyl group, electrons would be displaced into the ring and hence towards the phenolic oxygen atom. This, in turn, would increase the strength of the hydrogen bond between this atom and the hydroxylated alumina surface. In addition, the effect of the eluent on this hydrogen bonding, and also steric effects have to be considered.

The results in Table II show that the effects of the addition of one or more methyl groups to the phenyl nucleus are dependent on the position of the substituent group or groups relative to the phenolic group. This means that these phenols can be classified into three groups.

Group 1: *no ortho substituent.* In all eluents, the addition of a single methyl group to either the 3- or 4-position causes a slight increase in R_F values relative to that of the unsubstituted phenol, the 4-compound having lower R_F values than the 3-compound. Further additions of methyl groups to these positions to give the 3,4-, 3,5-, and 3,4,5-compound result in little or no change in the R_F values, except in the system benzene–ethyl acetate, where a progressive increase in R_F values with increased methylation results. These results suggest that the fine electronic effects of the methyl group on the strong bond formed between the phenolic oxygen atom and the hydroxylated alumina are offset by the increase in the solubility of the non-polar part of the molecule in the organic mobile phases.

Group 2: *one ortho substituent.* The addition of a methyl group *ortho* to the parent phenolic group results in a substantial increase in the R_F value of the 2-methylphenol relative to that of the parent compound. Addition of further methyl groups to the 3-, and 4-positions of the molecule has little effect on the R_F values relative to that of the 2-methylphenol. Evidence of the effects of the position of substituents relative to each other is seen in so far as the R_F values of the 2,4-dimethylphenol are slightly lower than the 2,3-, and 2,5-dimethylphenols in all systems. The 2,3,4-trimethylphenol is generally lower than the 2,3,5-, or the 2,4,5-isomers.

TABLE II

R_F VALUES (× 100) OF METHYLATED PHENOLS

Phenol	*Solvent/support system No.*							
	1	*2*	*3*	*8*	*10*	*12*	*13*	*14*
Phenol	10	5	6	25	48	26	29	50
2-Methyl	24	12	13	49	65	40	38	67
3-Methyl	14	7	12	29	53	34	32	56
4-Methyl	12	6	10	27	52	33	32	55
2,3-Dimethyl	26	12	18	55	75	43	41	70
2,4-Dimethyl	25	11	19	53	74	41	40	69
2,5-Dimethyl	28	12	19	55	75	43	41	70
2,6-Dimethyl	56	36	43	76	87	52	54	80
3,4-Dimethyl	16	8	14	32	53	32	33	58
3,5-Dimethyl	19	18	14	35	53	32	32	60
2,3,4-Trimethyl	26	14	20	59	73	39	42	70
2,3,5-Trimethyl	27	16	21	62	76	39	44	72
2,3,6-Trimethyl	54	34	37	84	85	52	56	83
2,4,5-Trimethyl	26	16	19	61	75	39	44	72
2,4,6-Trimethyl	54	34	36	84	85	50	55	83
3,4,5-Trimethyl	12	8	5	32	53	30	33	62
2,3,4,5-Tetramethyl	30	16	20	62	73	39	44	70
2,3,4,6-Tetramethyl	55	34	38	85	89	52	54	86
2,3,5,6-Tetramethyl	55	36	39	86	90	54	55	86

Group 3: two ortho substituents. Table III shows the R_F values of 2,6-dimethyl-substituted phenols relative to those of 2-methylphenol and phenol. It can be seen that the presence of a second methyl group in an *ortho* position greatly increases the R_F-values. Once again, however, addition of methyl groups to the 3- or 4-position of the molecule has little effect on the R_F values, though results indicate that the 2,3,4,6-tetramethylphenol is slightly more strongly adsorbed than its 2,3,5,6-isomer.

From the results, it would appear that the major constitutive effect modifying the strength of the hydrogen bond between the phenolic group and the substrate is one of complete or partial steric hindrance, and that electronic interactions, if they occur, are small.

TABLE III

R_F VALUES (× 100) OF PHENOLS WITH TWO *ortho* SUBSTITUENTS

Phenols	*Solvent/support system No.*							
	1	*2*	*3*	*8*	*10*	*12*	*13*	*14*
Phenol	10	5	6	25	48	26	29	50
2-Methyl	24	12	13	49	65	40	38	67
2,6-Dimethyl	56	36	43	76	87	52	54	80
2,3,6-Trimethyl	54	34	37	84	85	52	56	83
2,4,6-Trimethyl	54	34	36	84	85	50	55	83
2,3,4,6-Tetramethyl	55	36	39	86	90	54	55	86
2,3,5,6-Tetramethyl	55	36	39	86	90	54	55	86

(*b*) *Other alkyl, etc., phenols*

Once again these may be classified into 3 groups according to the number of positions *ortho* to the phenolic group which are substituted.

Group 1: *no ortho substituents.* Talbe IV shows that the addition of hydrocarbon groups of increasing chain length to either the 3- or, more particularly, the 4-position has little or no effect on the strength of the hydrogen bond between the phenolic group and the substrate when a straight-chain hydrocarbon is considered. This is in accord with the findings of KHEIFITS *et al.*[8].

TABLE IV

R_F VALUES (× 100) OF ALKYLATED PHENOLS

Phenol	*Solvent/support system No.*							
	1	*2*	*3*	*8*	*10*	*12*	*13*	*14*
Phenol	10	5	6	25	48	26	29	50
3-Methyl	14	7	12	29	53	34	32	56
4-Methyl	12	6	10	27	52	33	32	55
3-Ethyl	12	6	8	37	53	36	36	59
4-Ethyl	12	6	7	35	54	35	36	58
4-*n*-Propyl	18	7	7	37	53	35	35	62
4-*n*-Butyl	19	7	7	37	53	35	35	63
4-*n*-Amyl	24	10	11	46	58	35	35	65
4-*n*-Nonyl	14	6	9	53	68	42	42	74

This is particularly so for the alcoholic eluents (KHEIFITS *et al.*[8] used benzene–methanol (9:1, v/v) in their investigation). There are, however, some non-regular deviations from this generalisation. These are probably caused by differences in the solubilities of individual phenols in a given eluent system. The R_F values in the benzene–ethyl acetate system show a slight but fairly regular increase with increasing chain length. This behaviour parallels that of the methylated phenols in this system.

TABLE V

EFFECT OF A DOUBLE BOND IN THE SIDE CHAIN ON R_F VALUES (× 100)

Phenol	*Solvent/support system No.*							
	1	*2*	*3*	*8*	*10*	*12*	*13*	*14*
Phenol	10	5	6	25	48	26	29	50
4-*n*-Propyl	18	7	7	37	53	35	35	62
4-Allyl	10	4	6	34	46	35	35	53
4-*n*-Butyl	19	7	7	37	53	35	35	63
4-Crotyl	11	6	6	35	50	36	36	56
4-Cyclopentyl	14	7	6	39	55	36	37	66
4-Cyclopent-2-enyl	12	6	5	32	53	37	37	64
4-Cyclohexyl	17	8	8	38	60	38	39	69

The effect of the presence of a double bond in the side chain is shown in Table V. With the exceptions of the two systems which contain an alcohol, the presence of a double bond slightly lowers the R_F values, probably as a result of the interaction between the π-electrons of the double bond and the hydroxyl groups of the substrate.

In the case of the alcoholic systems, it is probable that such interaction is nullified by a competitive mechanism involving the double bond and the hydroxyl group of the alcohol.

TABLE VI

R_F VALUES (× 100) OF ARYLPHENOLS

Phenol	*Solvent/support system No.*							
	1	*2*	*3*	*8*	*10*	*12*	*13*	*14*
Phenol	10	5	6	25	48	26	29	50
4-Phenyl	7	3	3	15	33	30	30	42
4-Benzyl	8	3	3	26	44	40	42	69
4-Cumyl	8	3	3	34	50	42	44	71

The effect of the addition of an aromatic substituent to the 4-position is shown in Table VI. In the eluent systems other than the alcoholic ones, the addition of a phenyl group reduces the R_F value relative to that of phenol. That this cannot be caused simply by the presence of 3 additional double bonds to the molecule can be seen from the results for *p*-benzyl- and *p*-cumylphenol. These contain the same number of double bonds as the phenyl derivative but have higher R_F values. In these last two, however, the conjugated double bond system is destroyed by the presence of the essentially aliphatic group separating the phenyl rings. It would therefore appear that the presence of a conjugated double bond system has a greater effect on R_F values than a non-conjugated double bond system. This is in accord with the views of LISBOA[21], for the separation of steroids. The results for the alcoholic benzene systems may be caused in part by the increased solubilities of these aromatic systems in the aromatic fraction of the eluent, and in part to the interaction of the π-electrons with the alcoholic hydroxyl groups.

The effect of a bulky group in the 4-position is shown in Table VII. From the results it can be seen that such a group can have some effect on the R_F values. It is suggested that these groups prevent the adsorbed molecule from lying in the plane of the substrate, forcing the non-polar part of the molecule into the mobile phase, thus slightly increasing the solubility in the non-polar phase and hence the R_F values. The value for *tert.*-octylphenol in system 1 is considered to be anomalous.

The results for the poly-alkylated phenols (Table VIII) are, qualitatively, the expected ones. The additional alkyl group having little effect on the R_F values, except where bulky groups are involved.

Group 2: one ortho substituent. From the results in Table IX, it can be seen that the addition of a straight-chain hydrocarbon to the 2-position results in a fairly regular increase in R_F values. It has already been shown that the addition of a straight-chain hydrocarbon to the phenyl nucleus has little polar effect, hence it must be concluded that the increases in R_F values are a result of a steric effect, the *o*-alkyl

TABLE VII

EFFECT OF THE SIZE OF THE SUBSTITUENT ON R_F VALUES (× 100)

Phenol	*Solvent/support system No.*							
	1	*2*	*3*	*8*	*10*	*12*	*13*	*14*
4-*n*-Propyl	18	5	7	36	53	35	35	62
4-Isopropyl	19	7	7	37	53	35	35	62
4-*n*-Butyl	19	7	7	37	53	35	35	63
4-*sec.*-Butyl	25	8	19	40	63	38	40	65
3-*tert.*-Butyl	27	10	10	51	63	45	40	65
4-*tert.*-Butyl	28	10	13	51	64	45	43	65
4-*n*-Amyl	24	10	11	46	58	35	35	65
4-*sec.*-Amyl	31	10	10	53	68	38	39	67
4-*tert.*-Amyl	28	11	9	49	67	44	43	67
4-(3-Methyl butyl)	13	7	6	40	56	35	35	64
4-*tert.*-Octyl	14	8	8	53	76	44	45	78

TABLE VIII

R_F VALUES (× 100) OF POLY-ALKYLATED PHENOLS

Phenol	*Solvent/support system No.*							
	1	*2*	*3*	*8*	*10*	*12*	*13*	*14*
4-*n*-Propyl	18	7	7	36	53	35	35	62
3-Methyl-5-ethyl	16	6	7	42	58	35	35	62
3-Methyl-4-isopropyl	16	6	9	46	61	36	35	68
3-Methyl-5-isopropyl	19	6	9	47	60	35	35	66
3-Methyl-5-*sec.*-butyl	24	7	13	54	66	40	42	69
3,5-Di-*tert.*-butyl	33	12	20	86	70	48	49	86

TABLE IX

EFFECT OF AN *ortho* GROUP ON THE R_F VALUES (× 100) OF ALKYLATED PHENOLS

Phenol	*Solvent/support system No.*							
	1	*2*	*3*	*8*	*10*	*12*	*13*	*14*
Phenol	10	5	6	25	48	26	29	50
2-Methyl	24	12	13	49	65	40	38	67
2-Ethyl	28	15	16	67	73	45	46	75
2-*n*-Propyl	32	18	19	76	81	49	49	84
2-*iso*-Propyl	18	7	8	40	55	41	38	62
2-*sec.*-Butyl	41	26	26	79	91	56	51	85
2-*tert.*-Butyl	59	29	27	84	100	61	59	92
2-*n*-Octyl	60	40	39	90	98	63	62	92
2-Allyl	24	9	10	63	68	47	48	70
2-Phenyl	32	14	16	66	75	51	53	76
2-Cyclohexyl	33	11	11	84	81	54	54	81
2-Methyl-4-*tert.*-butyl	28	17	16	55	76	42	44	81
2-Methyl-4-octyl	30	11	11	58	80	46	46	89
2-*tert.*-Butyl-3-methyl	59	28	28	93	100	63	64	100
2-*tert.*-Butyl-4-methyl	59	28	26	96	100	63	64	100
2-Octyl-4-methyl	70	40	40	79	100	63	63	100

substituent reducing the availability of the phenolic group for hydrogen bonding with the alumina surface. This view is further substantiated by a consideration of the results for 2-*sec.*-butyl- and 4-*tert.*-butylphenols, where branching of the hydrocarbon chain results in an increase in the size of the *ortho* substituent, giving an increased contribution to the steric effect, and hence higher R_F values. The values for 2-isopropylphenol are anomalous. The results for 2-*tert.*-butyl-3-methyl-, 2-*tert.*-butyl-4-methyl- and 2-octyl-4-methylphenol are the expected ones, the addition of the methyl group to the 3-, or 4-positions having no effect on the R_F values. The addition of the 4-*tert.*-butyl group to 2-methylphenol has the expected effect of slightly increasing the R_F value relative to 2-methylphenol. Spectroscopic evidence has shown that hydrogen bonding between the phenolic hydrogen atom and the π-electrons of the double bond in the 2-allyl group is possible[22]. It would therefore be expected that the R_F value of 2-allylphenol should be higher than that of 2-*n*-propylphenol. This is not so, probably because of the formation of a competing, and stronger, hydrogen bond between the double bond electrons and the hydroxyl groups of the substrate. In contrast to the behaviour of the 4-phenyl substituent, which has a lower R_F value than phenol, the same substituent in the 2-position greatly increases the R_F value relative to phenol. This again suggests that for substituents in the 2-position, steric effects are of greater significance than polar ones. That the R_F value of 2-phenyl- is lower than that of 2-cyclohexylphenol, an alicyclic derivative containing the same number of carbon atoms, may be taken as evidence that polar effects are not entirely absent. It is therefore suggested that where the gross effect of hydrogen bonding between the phenolic group and the substrate is weakened, the MARTIN[10] relation is valid, modified, however, by constitutive effects of chain branching, double bonds, and ring systems. The relation cannot be extended to include substituents in the 3-, or 4-positions of *ortho*-substituted phenols.

TABLE X

EFFECT OF DI-*ortho* SUBSTITUTION ON R_F VALUES (× 100) OF ALKYLATED PHENOLS

Phenol	*Solvent/support system No.*								
	1	*2*	*3*	*8*	*9*	*10*	*12*	*13*	*14*
Phenol	10	5	6	25	0	48	26	29	50
2,6-Dimethyl	56	36	43	76	0	87	52	54	80
2,6-Dimethyl-4-*n*-propyl	60	45	51	91	4	97	60	56	89
2,6-Dimethyl-4-allyl	59	42	47	90	6	93	60	58	89
2,6-Di-*tert.*-butyl	97	93	91	100	57	100	100	97	100
2-Methyl-4,6-di-*tert.*-butyl	90	84	83	96	10	96	77	77	100
2,6-Di-*tert.*-butyl-4-methyl	98	91	89	100	54	100	100	97	100

Group 3: *two ortho substituents*. The results for these are as expected (Table X). Di-*ortho* substitution with 2 bulky *tert.*-butyl groups greatly increase the R_F value of this compound relative to that of 2,6-dimethylphenol. The additivity of steric effects, proposed for mono-*ortho*-substituted phenols, is supported by the results for 2-methyl-4,6-di-*tert.*-butyl- and 2,6-di-*tert.*-butylphenols. Increase in the R_F values of the 2,6-dimethyl-, 4-substituted phenols (4-*n*-propyl- and 4-allyl) are probably caused by solubility effects.

(c) *Alkoxyphenols*

The R_F values for these compounds are given in Table XI.

The presence of the methoxyl group *ortho* to the phenolic group increases the R_F value relative to phenol and relative to the 3- and 4-methoxyl isomers. This is probably a steric effect rather than a polar one. The lower R_F values of the 3- and 4-methoxyl and also the 4-phenoxyphenol compared with that of phenol are probably the result of hydrogen bonding between the ether oxygen atom and the hydroxyl

TABLE XI

R_F VALUES (× 100) OF ALKOXYPHENOLS

Phenol	*Solvent/support system No.*						
	1	*8*	*10*	*11*	*12*	*13*	*14*
Phenol	10	25	48	95	26	29	50
2-Methoxyl	25	33	55	100	30	41	68
3-Methoxyl	3	23	38	76	20	39	65
4-Methoxyl	4	24	40	78	20	38	66
3,5-Dimethoxyl	0	7	25	70	15	28	39
4-Ethoxyl	5	26	50	82	22	37	65
4-Cyclopentoxyl	11	26	60	90	22	37	67
4-Heptoxyl	7	31	72	90	25	44	70
4-Dodecyloxyl	7	35	80	90	26	43	70
4-Tetradecyloxyl	6	39	80	90	32	43	70
4-Hexadecyloxyl	7	40	82	90	32	43	71
4-Phenoxyl	5	14	45	65	21	39	58
3,5-Carbamethoxyl	0	2	8	31	12	11	22

groups of the substrate. That the phenoxyl derivative has a lower R_F value than the 3- or 4-methoxyl derivatives is probably caused by the greater inductive effect of the phenyl nucleus, compared with the methyl radical building up the electron density at the ether oxygen atom to form a stronger intramolecular hydrogen bond with the surface. This is in accord with the view expressed by GRAHAM AND STONE[23]. The presence of additional oxygen centres for the formation of more such intramolecular hydrogen bonds probably accounts for the R_F values of 3,5-dimethoxyl and 3,5-dicarbamethoxyphenols. These results are also in agreement with the above suggestions and with the mechanism postulated earlier[12]. With increasing chain length of the alkyl group, attached to the ether oxygen atom, the R_F values show corresponding increases.

CONCLUSION

In the systems studied, the gross effect of the intramolecular hydrogen bonding between the phenolic group and the hydroxylated alumina is so strong that in the simple alkylphenols, with no *ortho* substituents, the substituent effect on the chromatographic behaviour is negligible, and hence the MARTIN relation is not valid for these nuclear substituted compounds. Where these substituents are present in one or both *ortho* positions, the R_F values increases with an increase in chain length and the number of methylene groups added. This is attributed to steric effects rather than to

electronic effects. It is suggested that there is steric hindrance of the approach of the phenolic group to the surface. The long-chain substituents cause some hindrance, but the same number of methylene groups as a bulky unit causes a greater effect. The bulkier is the substituent group, then the greater is this effect.

ACKNOWLEDGEMENTS

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SUMMARY

Seventy-seven alkyl-, aryl- and alkoxyphenols were chromatographed using 14 different stationary phase/mobile phase systems, each containing alumina in the stationary phase. From a consideration of the R_F values, mechanisms for the separations obtained are suggested. The effect of substituents *ortho* to the phenolic group is pronounced and is discussed in detail. It is suggested that variations in the behaviour of such compounds is mainly attributable to steric hindrance of the approach of the phenolic group to the surface, and hence to the variations in the strength and amount of hydrogen bonding possible between the phenol and the alumina.

REFERENCES

1 G. WAGNER, *Pharmazie*, 10 (1955) 302.
2 H.-J. PETROWITZ, *Erdöl Kohle*, 14 (1961) 923.
3 G. PASTUSKA AND H.-J. PETROWITZ, *Chemiker Ztg.*, 86 (1962) 311.
4 D. MCHALE, *Lab. Pract.*, 13 (1964) 512.
5 J. HALMEKOSKI AND H. HANNIKAINEN, *Suomen Kemistilehti*, B36 (1963) 24.
6 K.-T. WANG, *J. Chinese Chem. Soc. (Taiwan)*, 8 (1961) 241.
7 S. HERMANEK, V. SCHWARZ AND Z. CEKAN, *Pharmazie*, 6 (1961) 556.
8 L. A. KHEIFITS, G. I. MOLDOVANSKAY AND L. M. SHALOV, *Zh. Analit. Khim.*, 18 (1963) 267.
9 J. SHERMA AND L. V. S. HOOD, *J. Chromatog.*, 17 (1965) 307.
10 A. J. P. MARTIN, *Biochem. Soc. Symp. (Cambridge, Engl.)*, 3 (1950) 4.
11 W. TRAPPE, *Biochem. Z.*, 305 (1940) 150.
12 L. S. BARK AND R. J. T. GRAHAM, *Talanta*, 11 (1964) 839.
13 W. TRAPPE, *Biochem. Z.*, 306 (1940) 316.
14 H. STRAIN, *Chromatographic Adsorption Analysis*, Interscience, New York, 1942.
15 L. SNYDER, *J. Chromatog.*, 8 (1962) 178.
16 S. HERMANEK, V. SCHWARZ AND Z. CEKAN, *Collection Czech. Chem. Commun.*, 28 (1963) 2031.
17 L. S. BARK, R. J. T. GRAHAM AND D. MCCORMICK, *Talanta*, 12 (1965) 122.
18 W. DASLER AND C. D. BAUER, *Ind. Eng. Chem., Anal. Edition.*, 18 (1946) 52.
19 A. I. VOGEL, *A Text Book of Practical Organic Chemistry*, 3rd Ed., Longmans Green, London, 1956, p. 168.
20 L. SNYDER, *J. Chromatog.*, 16 (1964) 55.
21 B. P. LISBOA, *J. Chromatog.*, 14 (1964) 319.
22 R. WEST, *J. Am. Chem. Soc.*, 81 (1959) 1614.
23 R. J. T. GRAHAM AND C. W. STONE, *Talanta*, 11 (1964) 947.

THE PAPER CHROMATOGRAPHY OF SOME ISOMERIC MONOSUBSTITUTED PHENOLS. II.

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INTRODUCTION

The paper chromatography of phenols has been the subject of a number of recent studies[1–4]. The first part of this paper reported the use of water, toluene saturated with water, and mineral spirits saturated with water as mobile solvent systems. The use of ceric ammonium nitrate as a spot-locating agent for phenols was also reported[4]. The effect of the orientation of substituent groups on the R_F' value in the various solvent systems was also studied. The types of phenols considered were limited to monosubstituted isomers. For purposes of general information, however, a few single phenols were considered.

The work presented in this paper is a continuation of the previous work. A number of additional isomeric phenols are considered in the same three solvent systems and ceric ammonium nitrate is used as the spot-locating agent whenever possible. The effect of orientation on the R_F' value is considered along with some generalizations correlating R_F' value and structure.

EXPERIMENTAL

Reagents

The mobile solvents used were water, toluene saturated by shaking with distilled water and mineral spirits (Kauri-butanol value 37–38, aniline point 56–59°) saturated with distilled water. The spot-locating reagents used were: (1) ceric ammonium nitrate reagent[4], (2) *p*-nitrobenzenediazonium fluoborate solution in acetone and 0.1 *N* alcoholic potasssium hydroxide[5], and (3) alkaline potassium permanganate prepared by mixing equal volumes of 1 % potassium permanganate and 2 % sodium carbonate[6].

Procedure

The toluene and mineral spirits were kept saturated with water by the presence of a lower water layer in the tanks. The tanks were lined with paper sheets extending into the water layer. All chromatograms were run at 25° with Whatman No. 1 paper using the ascending development method. The papers were allowed to equilibrate 20 min in the tank before immersion. The solvents were allowed to travel 10 cm requiring 30–45 min. In a few cases in which the phenols were relatively volatile no equilibration was used and solvent travel was limited to 5 cm.

The paper sheets were sprayed with the reagent as soon as they were removed from the tank and then washed thoroughly with water to remove excess reagent. In cases where the ceric ammonium nitrate reagent was ineffective, *p*-nitrobenzenediazonium fluoborate was used followed by 0.1 *N* alcoholic potassium hydroxide. The papers were not washed when using this method. In any cases where *p*-nitrobenzenediazonium fluoborate was ineffective, alkaline potassium permanganate was used. All papers were air dried. The R_F' values reported are the distance traveled by the spot front divided by the distance traveled by the solvent front[7].

RESULTS AND DISCUSSION

Examination of the data obtained confirms the earlier findings that water, mineral spirits and toluene are quite suitable as mobile solvents for mono-substituted phenols. In general, water and mineral spirits are more satisfactory than toluene since toluene tends to carry too many phenols along with the solvent front. However, all three solvents are of value in separations involving mixtures of phenols where two dimensional paper chromatography is required[8]. It is interesting to note that of the seventy-eight phenols examined in both parts of this paper only one (*p,p'*-diphenol) had an R_F' value of zero in all three solvents.

The use of ceric ammonium nitrate as a spot locating agent was continued in this study. Some limitations were found in its use especially with *ortho* substituted phenols. Many of the *ortho* substituted phenols gave colors so light that the spots could not be detected. Of the fifteen *ortho* substituted phenols only seven gave spots having enough color to be determined. The only group of isomeric phenols that were completely unresponsive to ceric ammonium nitrate were the methoxy phenols. When ceric ammonium nitrate gave colors too weak to determine, *p*-nitrobenzenediazonium fluoborate was used as the spot-locating agent. In a few cases, this reagent gave poor results and an alkaline solution of potassium permanganate was used.

Of the three solvents considered, toluene normally gives the highest R_F' values within an isomeric group of phenols. There are some exceptions to this depending on the type of substituent. The exceptions are *o*-fluorophenol, *p*-hydroxyacetophenone, all isomers of hydroxybenzaldehydes, hydroxybenzylalcohols and hydroxyacetanilides. All of these exceptions have greater R_F' values in water. A similar effect was noted in the first part of this paper with the aminophenols, the hydroxyphenols and the hydroxybenzoic acids. None of the phenols examined had greater R_F' values in mineral spirits than in toluene, although some gave R_F' values of 1.0 with both solvents.

Previously it was found that the phenylphenols, the iodophenols, the *tert.*-butylphenols and the naphthols have greater R_F' values in mineral spirits than in water. To this list can now be added *o*-hydroxyacetophenone, *o*-hydroxypropiophenone, *o*-hydroxy-*n*-butyrophenone, *o*-methoxyphenol, *o*-ethoxyphenol, *m-tert.*-butylphenol, *ortho*- and *para* benzylphenols, *ortho* and *para n*-propylphenols, *ortho* and *para sec.*-butylphenols, *ortho* and *para* cyclohexylphenols, and all isomeric ethylphenols. These phenols appear to be the least readily ionized, thus enhancing their organic structure and minimizing their ionic nature. This tends to affect the partition of these phenols in favor of the organic solvent. This is supported by the fact that these phenols without exception, have greater R_F' values in toluene than in water. The large number of *ortho* substituted phenols in this group containing

TABLE I

R_F' VALUES OF ISOMERIC MONOSUBSTITUTED PHENOLS

Phenol	R_F' *value at 25°*			*Spot color*	*Reagent*
	Water	*Mineral spirits*	*Toluene*		
o-Hydroxyacetophenone[a]	0.82	1.00	1.00	Brown	Alkaline $KMnO_4$
m-Hydroxyacetophenone[a]	0.73	0.07[b]	0.73	Tan-yellow	Ceric ammonium nitrate
p-Hydroxyacetophenone[a]	0.73	0.00	0.39	Gray-green	Ceric ammonium nitrate
o-Hydroxypropiophenone	0.86	1.00	1.00	Brown	Alkaline $KMnO_4$
m-Hydroxypropiophenone	0.66	0.22[b]	1.00	Very light tan	Ceric ammonium nitrate
p-Hydroxypropiophenone	0.64[b]	0.00	0.66[b]	Very light gray	Ceric ammonium nitrate
o-Hydroxy-*n*-butyrophenone	0.00	1.00	1.00	Brown	Alkaline $KMnO_4$
p-Hydroxy-*n*-butyrophenone	0.66[b]	0.17	1.00	Tan-yellow	Ceric ammonium nitrate
o-Hydroxybenzaldehyde	0.87	0.12	0.76	Red to purple	*p*-Nitrobenzenediazonium fluoborate
m-Hydroxybenzaldehyde	0.74	0.07	0.61	Tan	Ceric ammonium nitrate
p-Hydroxybenzaldehyde	0.73	0.00	0.39	Light gray-green	Ceric ammonium nitrate
o-Hydroxybenzyl alcohol	0.85	0.07	0.41	Dark gray-green	Ceric ammonium nitrate
m-Hydroxybenzyl alcohol	0.81	0.00	0.07[b]	Light tan	Ceric ammonium nitrate
p-Hydroxybenzyl alcohol	0.84	0.00	0.06[b]	Light tan	Ceric ammonium nitrate
o-Benzylphenol	0.64[b]	0.97	1.00	Light brown	Ceric ammonium nitrate
p-Benzylphenol	0.00	0.86	1.00	Tan	Ceric ammonium nitrate
o-Methoxyphenol	0.69	0.74	1.00	Tan to gray	*p*-Nitrobenzenediazonium fluoborate
m-Methoxyphenol	0.74	0.26	0.96	Maroon	*p*-Nitrobenzenediazonium fluoborate
p-Methoxyphenol	0.79	0.22	0.92	Purple	*p*-Nitrobenzenediazonium fluoborate
o-Ethoxyphenol	0.20	0.55	1.00	Tan-gray	*p*-Nitrobenzenediazonium fluoborate
m-Ethoxyphenol	0.73	0.45	1.00	Brown	Ceric ammonium nitrate
p-Ethoxyphenol	0.67[c]	0.34[c]	1.00	Tan	Ceric ammonium nitrate
o-Ethylphenol	0.76	0.81	1.00	Purple	*p*-Nitrobenzenediazonium fluoborate
m-Ethylphenol	0.67[b]	0.74	1.00	Light tan	Ceric ammonium nitrate
p-Ethylphenol	0.58[b]	0.74	1.00	Very light yellow	Ceric ammonium nitrate
o-*n*-Propylphenol	0.64	0.93	1.0	Tan	Ceric ammonium nitrate
p-*n*-Propylphenol	0.00	0.85	1.0	Bleaches spot white	Ceric ammonium nitrate
o-*sec*.-Butylphenol[a]	0.00	0.92	1.00	Light yellow	Ceric ammonium nitrate
p-*sec*.-Butylphenol[a]	0.00	0.92	1.00	Gray to tan	Ceric ammonium nitrate
m-*tert*.-Butylphenol	0.00	0.96	1.00	Maroon	*p*-Nitrobenzenediazonium fluoborate
o-Cyclohexylphenol	0.48[b]	0.99	1.00	Yellow-green	Ceric ammonium nitrate
p-Cyclohexylphenol	0.00	0.92[b]	1.00	Light gray	Ceric ammonium nitrate
o-Fluorophenol[a]	0.89	0.00	0.87	Light yellow	Ceric ammonium nitrate
m-Fluorophenol[a]	0.81	0.43	1.00	Light brown	Ceric ammonium nitrate
p-Fluorophenol[a]	0.79	0.33	0.96	Very light tan	Ceric ammonium nitrate
o-Hydroxyacetanilide	0.80[c]	0.00	0.00	Purple	Ceric ammonium nitrate
m-Hydroxyacetanilide	0.77	0.00	0.00	Tan	Ceric ammonium nitrate
p-Hydroxyacetanilide	0.83	0.00	0.00	Pink to purple	Ceric ammonium nitrate
p,*p*′-Isopropylidine diphenol (Bisphenol-A)	0.61[b]	0.10	0.89	Light yellow	Ceric ammonium nitrate

[a] Vaporizes rapidly. [b] Spot streaks. [c] Spot bleached.

either methylene or oxygen in the side chain indicates that these products are less ionic in nature than the corresponding *meta* and *para* isomers. This is probably caused by intramolecular hydrogen bonding with the resulting effect of reducing the overall ionic nature of the molecule. The effect of this hydrogen bonding would not only reduce the ionic nature but would also explain the fact that many of these phenols did not give any detectable spots with ceric ammonium nitrate. The phenones were apparently bonded so strongly that they did not even react with the *p*-nitrobenzenediazonium fluoborate.

Phenols such as the cresols, hydroxyphenols, hydroxybenzoic acids, aminophenols, and diphenols were previously found to have greater R_F' values in water than in mineral spirits. To this group can be added, *p*-hydroxy-*n*-butyrophenone, *p,p'*-isopropylidene diphenol (Bisphenol-A), *meta* and *para* hydroxyacetophenone, *meta* and *para* hydroxypropiophenone, *meta* and *para* methoxyphenol, *meta* and *para* ethoxyphenol, and all isomers of hydroxybenzaldehyde, hydroxybenzyl alcohol, fluorophenol and hydroxyacetanilide. These phenols can be subdivided into two groups relative to their R_F' values in toluene. Those that contain ionizable substituents have higher R_F' values with water than with toluene. This group includes substituents such as benzyl alcohol, aldehyde, carboxyl and amino. The second group contains less ionizable substituents and consequently the phenols in this group have greater R_F' values with toluene than with water. The substituents in this group include methyl, fluoro, *meta* and *para* methoxy and *meta* and *para* ethoxy.

The so called "*ortho* effect" appears consistently throughout the data observed. Very minor deviations in which an *ortho* isomer has a smaller R_F' value than the *meta* or *para* isomer may be due only to experimental error. However, a few significant exceptions to the "*ortho* effect" are resorcinol with toluene, *p*-hydroxy-*n*-butyrophenone with water, all isomeric methoxyphenols with water, all isomeric ethoxyphenols with water and all isomeric fluorophenols with toluene and mineral spirits. The R_F' values in the three solvents, the spot-locating reagents and the spot colors are all shown in Table I.

The effect of the orientation of the substituent groups on the R_F' value of the phenols with water as the mobile solvent is shown in Fig. 1. When water is used as the mobile solvent, the separation mechanism involved is adsorption. When toluene and mineral spirits are the mobile solvents, the mechanism is partition.

For phenols in which all three isomers were available, Fig. 1 shows that the substituents of the *meta* and *para* isomers are practically all in the same order as the R_F' values decrease. The exceptions are all isomers of fluoro- and ethylphenols. The order of decreasing R_F' values is altered considerably in the *ortho* substituted phenols where other factors such as intramolecular hydrogen bonding influence the R_F' value. These factors are minimized with the *meta* and *para* isomers resulting in more uniformity in the order.

Figs. 2 and 3 show the effect of orientation when the mobile solvents are mineral spirits and toluene. With both solvents the decreasing order of R_F' values is the same for both *meta* and *para* substitution. In addition, the decreasing order of R_F' values is very nearly the same in either toluene or mineral spirits, although the order in water is very different and tends to be in a reverse order. This shows the similarity of the two partition systems and their difference from an adsorption system. The same effect was observed in the first part of this paper.

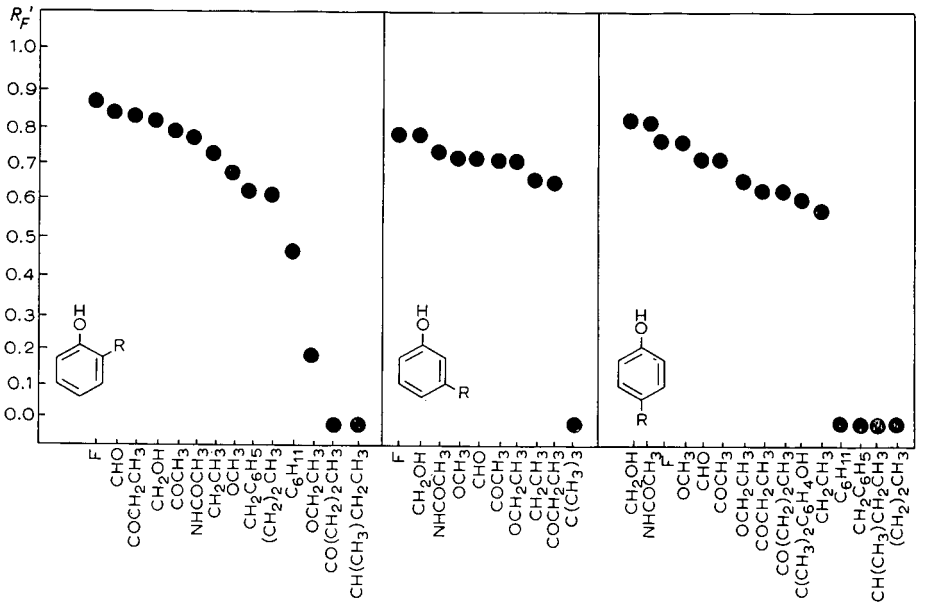

Fig. 1. Effect of the orientation of substituent groups on the R_F' value of phenols with water.

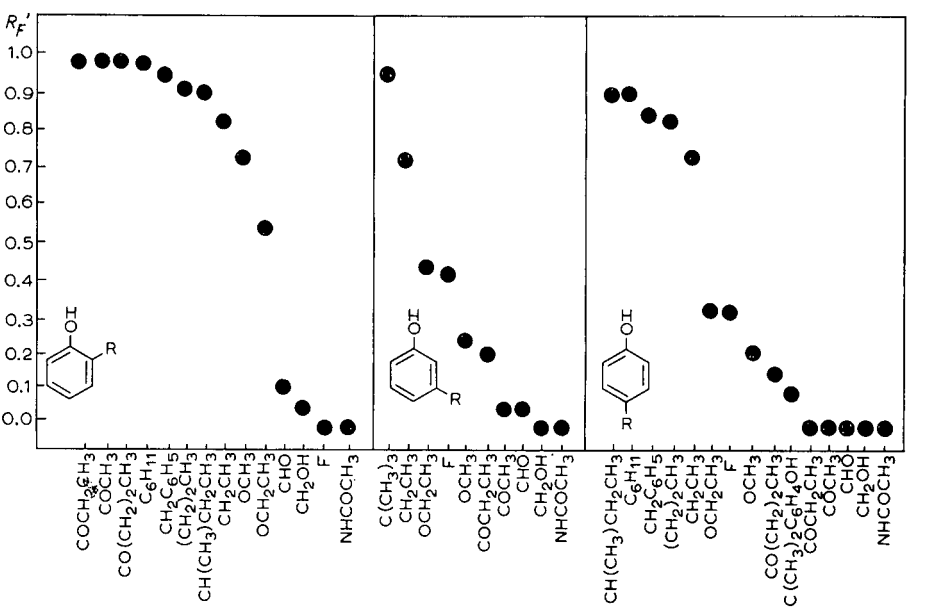

Fig. 2. Effect of the orientation of substituent groups on the R_F' value of phenols with mineral spirits.

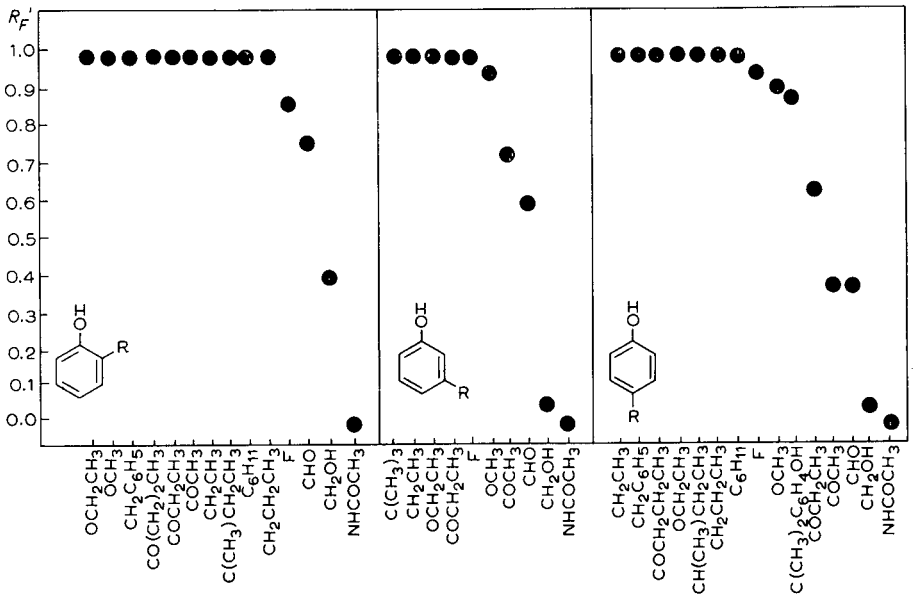

Fig. 3. Effect of the orientation of substituent groups on the R_F' value of phenols with toluene.

A comparison of the R_F' values of the halogenated phenols in all three solvents has been made. The results indicate that as the molecular weight of the side chain increases the R_F' values vary depending on the solvent. Fig. 4 shows that with water as the mobile solvent a slight decrease is noted in the R_F' values as the molecular weight increases. Fig. 5 shows that with mineral spirits a slight increase in R_F' values is noted for the *meta* and *para* isomers as the molecular weight increases. The *ortho* isomers do not follow this pattern, particularly in the case of *o*-fluorophenol. When toluene is used as the mobile solvent, the same type of curves are obtained as when mineral spirits is the solvent although the differences are less pronounced.

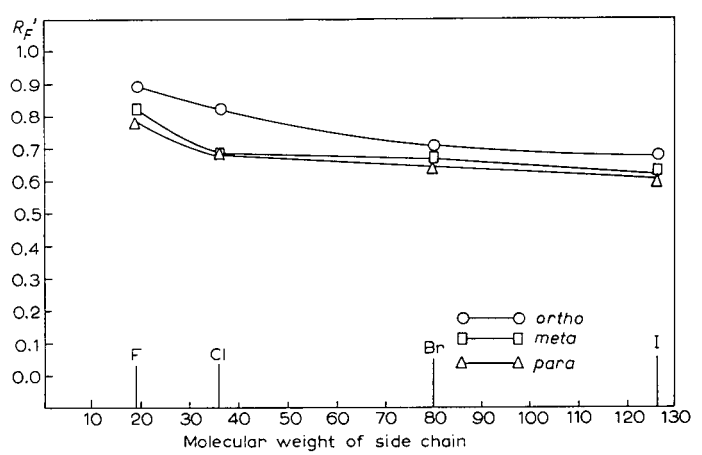

Fig. 4. R_F' value in water *vs.* mol.wt. of the halogen substituent.

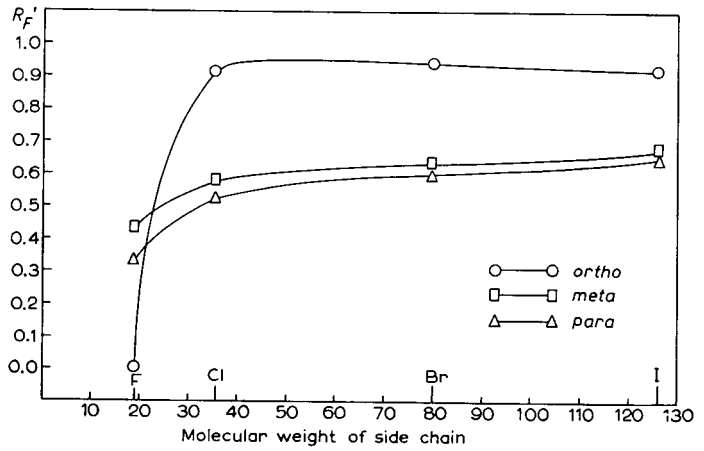

Fig. 5. R_F' value in mineral spirits *vs.* mol.wt. of the halogen substituent.

This demonstrates again the similarity of the two partition systems and their difference to the adsorption system. The figure for the data with toluene is not included since all the R_F' values are above 0.95 with the exception of *o*-fluorophenol.

The R_F' values for the homologous series of substituted phenols from the cresols to the *n*-propyl phenols have been compared. Fig. 6 shows the variation in R_F' values of the *ortho* and *para* isomers as the molecular weight of the substituent increases when water is the mobile solvent. In this case, the R_F' values show a decrease as molecular weight increases. This should be expected since the compounds tend to become less ionic in nature as the molecular weight of the side chain increases. Fig. 7 shows the increase in R_F' values as the molecular weight increases when the mobile solvent is mineral spirits. The *meta* isomers have not been included in these figures since not all of them were available. However, the values for *meta*-cresol and *meta*-ethylphenol are intermediate between the two curves obtained and it should be

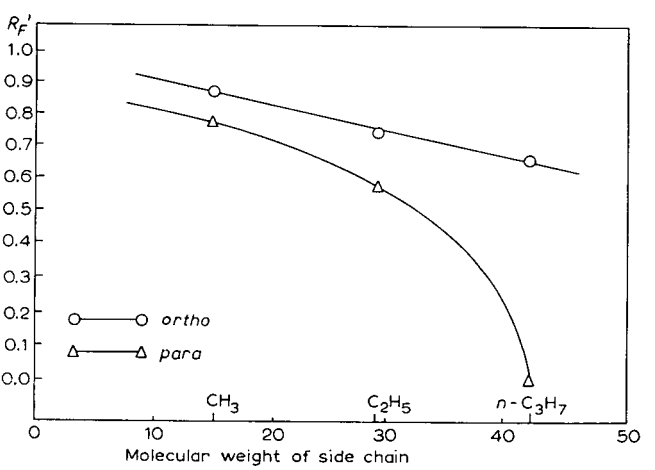

Fig. 6. R_F' value in water *vs.* mol.wt. of non-branched hydrocarbon.

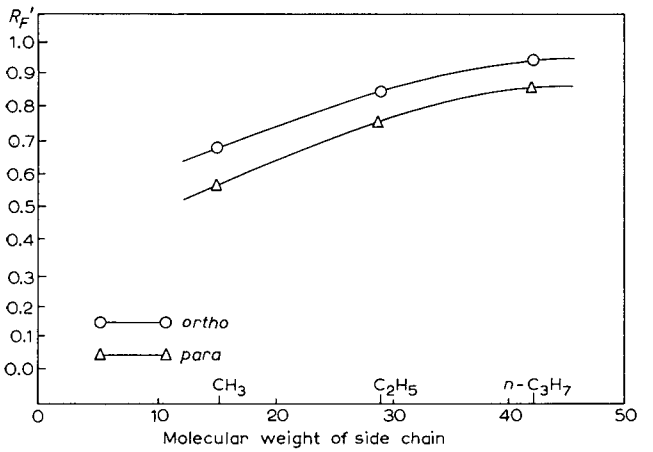

Fig. 7. R_F' value in mineral spirits *vs.* mol.wt. of non-branched hydrocarbon.

safe to assume that the values for *meta-n*-propylphenol would also be intermediate.

An attempt to correlate the R_F' values and the molecular weight of the substituents of the series from acetophenone to *n*-butyrophenone with the various solvents did not give conclusive results. Apparently, the nature of the substituent has a varying effect on the degree of change of the R_F' value in a given solvent.

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SUMMARY

The R_F' values of a number of isomeric monosubstituted phenols have been determined for three solvent systems. This is a continuation of our previous data. The results confirm the earlier work showing that most of the phenols have significant variations in R_F' value between these three solvents. Some limited correlations between R_F' values and the molecular weight of the side chain were found for members of homologous series. The use of ceric ammonium nitrate as a spot locating agent for phenols was found to have certain limitations, particularly for *ortho* substituted phenols. In general, however, this reagent is quite suitable for most phenols.

REFERENCES

1 I. T. CLARK, *J. Chromatog.*, 15 (1964) 65.
2 E. SUNDT, *J. Chromatog.*, 6 (1961) 475.
3 P. COLOMBO, D. CORBETTA, A. PIROTTA AND G. RUFFINI, *J. Chromatog.*, 6 (1961) 467.
4 D. L. GUMPRECHT, *J. Chromatog.*, 18 (1965) 336.
5 W. M. ANDERSON, G. B. CARTER AND A. J. LANDUA, *Anal. Chem.*, 31 (1959) 1214.
6 I. M. HAIS AND K. MACEK, *Paper Chromatography*, 3rd Ed., Academic Press, New York, 1963.
7 R. A. EVANS, W. H. PARR AND W. C. EVANS, *Nature*, 164 (1949) 674.
8 D. L. GUMPRECHT, *J. Chromatog.*, 20 (1965) 546.

THE VARIOUS QUENCHING EFFECTS IN THIN-LAYER CHROMATOGRAPHY

APPLICATION TO AIR POLLUTION

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INTRODUCTION

Quenching effects on phosphorescent[1,2] or fluorescent[3] reagents have been useful in locating non-fluorescing ultraviolet-absorbing chemicals on thin-layer chromatograms.

Recently a few selective quenching reagents used in the developer or after development were introduced for the location and preliminary characterization of fluorescent compounds containing various types of functional groups[4]. These techniques are useful primarily in the analysis of fluorescent compounds. We wish to emphasize that the procedure could be applied also to the direct analysis of non-fluorescent compounds by chemically changing these latter compounds on the plate into fluorescent derivatives. The synthesis of fluorescent derivatives on the plate followed by direct fluorometric examination is readily performed, as shown in recent work[5,6].

In this paper we discuss the ramifications and possibilities of fluorescence quenching in thin-layer chromatographic analysis. Examples are given to show the potentiality of this technique.

EXPERIMENTAL*

Chemicals and equipment

All solvents were distilled before use. All polynuclear compounds were obtained pure and were recrystallized to a constant melting point when chromatography showed that they were impure. An Aminco-Bowman Spectrophotofluorometer with a solid-state attachment was used in the direct fluorometric examination of thin-layer chromatograms.

Chromatography

A mixture of 20 g alumina and 10 g 2,4-dinitrophenoxy starch (Anheuser-Busch, Inc., St. Louis, Mo.) in 96 ml of absolute methanol was blended for $^1/_2$ min. Five

* Mention of commercial products does not constitute endorsement by the Public Health Service.

plates were coated to a thickness of 500 μ. They were then air dried and were ready for use in an hour. In the separation the solvent front reached 5 cm in 10 min, 10 cm in 30 min, and 15 cm in 60 min. Separation on alumina took twice as long.

DISCUSSION AND RESULTS

Eight main types of quenching techniques have been used to some extent in thin-layer chromatographic analysis. The most common one involves chromatography on an adsorbent in which a fluorescent or phosphorescent compound is evenly dispersed[7]. Rhodamine B, fluorescein, and fluoranthene have been used as the fluorescent compound; inorganic phosphors of various types have been used as the phosphorescent compound. Under ultraviolet light ultraviolet-absorbing compounds are seen as dull to black spots against the fluorescent background.

In the second technique the developed chromatogram is sprayed (or dipped) with a solution of the fluorescent or phosphorescent material[8]. The ultraviolet-absorbing materials are then readily located under the ultraviolet light as dark non-fluorescing spots.

A third quenching technique involves the use of a fluorescent screen to locate an ultraviolet-absorbing spot[9].

A fourth type of quenching effect that has proved valuable in thin-layer chromatography is obtained with the use of a volatile quencher in the developing solvent[4,10]. Volatile quenchers that can be used are diacetyl, carbon disulfide, isopropylamine, 2-butanone, trifluoroacetic acid, trifluoroacetic anhydride, nitrogen dioxide, nitromethane, and pyrrole. The boiling points of the volatile quenchers range from about 30 to 100°.

With pentane plus 2-nitropropane as a developing solvent fluoranthenic hydrocarbons can be distinguished from other types of polynuclear aromatic hydrocarbons. While the plate is wet with the nitroalkane, the non-fluoranthenic hydrocarbons do not fluoresce while the fluoranthenic ones do. After the plate has dried, all the hydrocarbons fluoresce.

A fifth type of quenching effect involves the use of a nonvolatile quencher in the developer[4]. Pentane–nitrobenzene (9:1) has been used. Such a compound quenches the fluorescence of the aromatic hydrocarbons while other types of compounds fluoresce. The concentration of nitrobenzene in the developer can be adjusted so as to quench almost all fluorescent compounds. Another type of quenching agent could be used so that the polynuclear aromatic hydrocarbons would fluoresce while the fluorescences of other types would be quenched; *e.g.* with N,N-dimethylaniline, the fluorescence of the aza heterocyclic hydrocarbons would be quenched while the fluorescence of many of the aromatic hydrocarbons would be unaffected.

A sixth quenching effect is obtained when a developed chromatogram is sprayed, fumed, or treated with a volatile quencher[4]. By use of appropriate quenching gases a plate can be exposed to fumes in a closed container or each separate spot can be treated individually. Some of the volatile quenchers used in our laboratory are carbon disulfide, nitrogen dioxide, nitromethane, trifluoroacetic acid, alkyl nitrites, and alkyl nitrates. Nitrogen dioxide–trifluoroacetic acid (7:3, v/v) has been used successfully to differentiate aromatic hydrocarbons from aza heterocyclic hydrocarbons and to test for an aromatic hydrocarbon through its loss of fluorescence on treatment[4].

TABLE I

EFFECT OF QUENCHING AGENTS ON VARIOUS COMPOUNDS*

*Quencher***	*8-Amino-fluoranthene*	*Benz(c)-acridine*	*7H-Benz-(d,e)-anthra-cen-7-one*	*Morin*	*Pyrene*	*Tryptophan*
	Quencher in Al_2O_3 adsorbent					
1-Aminopyrene	Q ⟶ Q	Q ⟶ B	Q ⟶ Q	Q ⟶ B	Q ⟶ Q	Q ⟶ Q
Aniline	G ⟶ G	Q ⟶ B	Q ⟶ YO	G ⟶ G	B ⟶ B	B ⟶ B
o-Cresol	G ⟶ lG	Q ⟶ Q	Q ⟶ Q	G ⟶ G	B ⟶ Q	B ⟶ B
Nitrobenzene	Q ⟶ Q	Q ⟶ G	lB ⟶ Y	G ⟶ G	Q ⟶ Q	B ⟶ B
1-Nitropyrene	Q ⟶ Q	Q ⟶ Q	Q ⟶ Q	G ⟶ G	Q ⟶ Q	Q ⟶ Q
Phenylhydrazine	B ⟶ G	Q ⟶ B	Q ⟶ YO	G ⟶ G	Q ⟶ B	B ⟶ B
Phloroglucinol	G ⟶ BG	Q ⟶ BG	Q ⟶ YO	G ⟶ G	B ⟶ B	lB ⟶ B
Picric acid	Q ⟶ Q	Q ⟶ B	Q ⟶ Y	Q ⟶ G	Q ⟶ Q	lB ⟶ B
2,4-Dinitrophenoxy starch	Q ⟶ Q	Q ⟶ G	Q ⟶ Y	G ⟶ G	Q ⟶ Q	B ⟶ B
	Volatile quencher after development					
N,N-Dimethylhydrazine	G	Q	Q	G	B	fB
NO_2–TFA (1:1)	Q	B	Y	Q	Q	Q
	Non-volatile quencher after development					
Aniline	G	Q	Q	G	Q	Q
o-Cresol	G	Q	Q	G	lB	fB
Nitrobenzene	Q	Q	Q	Q	Q	Q
Pyrrole	G	Q	Q	G	Q	fB

* B = blue, f = faint, G = green, l = light, O = orange, Q = quenched, Y = yellow, and ⟶ = fluorescence colors before and after trifluoroacetic acid treatment.

** Liquids: 0.2 g per g alumina; solids: 0.1 g per g alumina; dinitrophenoxy starch–alumina (1:1).

The diverse results obtained with two types of volatile quenchers are shown in Table I for six fluorescent compounds, each containing a distinctive functional group. With this type of temporary quenching, compounds containing the functional group of interest could be located in a quick preliminary, nondestructive procedure.

The seventh type of quenching effect is obtained when the developed plate is treated with a nonvolatile quencher. Some useful quenchers are phenols, anilines, nitrobenzenes, nitrosobenzenes, hydrazines, benzaldehydes, quinones, polycyano compounds, and potassium permanganate. Much work needs to be done to investigate the ramifications and possibilities of this new type of functional group analysis.

If necessary, a strong quencher could be diluted to allow maximum use of its quenching ability. Such a quencher could be used at the highest concentration at which it would have a maximal effect on the interferences while the test substances remained fluorescent.

Dependent on the functional group of the test substance, a quencher can be used in alkaline or acidic solution to allow maximal use of its selective property. For example, the nonvolatile quenchers in Table I are best used for the analysis of the 8-aminofluoranthene and morin types of compounds. With the addition of acid to the quencher the fluorescence of most aromatic amines would be quenched while many of the polynuclear aza heterocyclic compounds would become fluorescent in acidic aniline and nitrobenzene.

The usefulness of the seventh technique is indicated by comparison of Figs. 1 and 2. The red visible spot, a natural internal marker, is valuable in comparing these

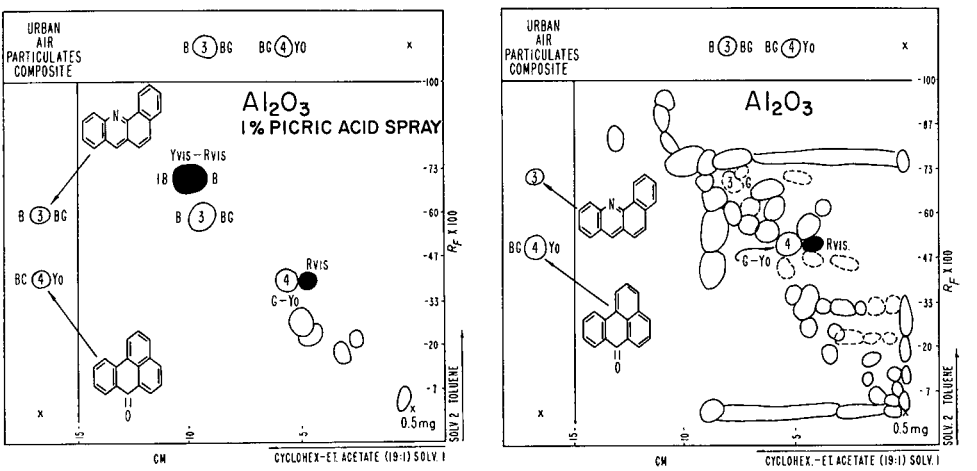

Fig. 1. Two-dimensional alumina thin-layer chromatogram of 0.5 mg of composite airborne particulate sample separated as in Fig. 2. Following development the plate is sprayed with 1 % aqueous picric acid. Benz(*c*)acridine and 7H-benz(*d*,*e*)anthracen-7-one standards run one-dimensionally. Definitions as in Fig. 2.

Fig. 2. Two-dimensional alumina thin-layer chromatograms of 0.5 mg of the benzene extract of a composite of airborne particulate samples collected from about 100 American cities. Standards run one-dimensionally: (3) benz(*c*)acridine and (4) 7H-benz(*d*,*e*)anthracen-7-one. B = blue, G = green, l = light, O = orange, Y = yellow. Letters at left of spot represent fluorescence color on the wet chromatograms; letters at right, fluorescence colors after fuming with trifluoroacetic acid. Spots encircled by dashed line appeared only after fuming. Rvis = spot with red visible color.

figures (and also Fig. 3) since its location clarifies the relative positions of the various spots. Of all the spots originally present on this plate (similar to Fig. 2, a different run, which shows plate before treatment with quencher), only nine fluoresce after treatment of the plate with 1 % picric acid spray (Fig. 1). Benz(*c*)acridine and 7H-benz(*d*,*e*)-anthracen-7-one were readily characterized on the plate. The quenching treatment simplified the location and characterization of these compounds.

An eighth type of quenching effect is obtained through the use of an insoluble inorganic or organic compound as a quenching material in the adsorbent. Examples of some organic quenchers would be polymers or large insoluble compounds containing nitro, acetyl, phenolic hydroxyl, aromatic amino, hydrazino, polycyano, quinonic, or other appropriate groups. These types of quenchers produce a selective quenching effect with various families of fluorescent compounds and this provides a type of functional group analysis that can be performed directly on the thin-layer plate. This technique should allow the development of many new kinds of characterization and assay methods. The highly selective quenchofluorometric technique of analyzing for polynuclear hydrocarbons containing the fluoranthenic ring system in the presence of all other types of polynuclear aromatic hydrocarbons[10] could be developed for direct fluorometric assay on a thin-layer chromatographic plate.

The usefulness of this technique is shown in a comparison of Figs. 2 and 3. Both figures represent two-dimensional separation of the same benzene extract of a composite sample of urban airborne particulate (collected from approximately

100 large American cities). In the separation on alumina, Fig. 2, a large number of fluorescent spots were obtained. Also a large, vague fluorescent area was seen along both solvent fronts, but especially in the area diagonally opposite the origin. Benz(*c*)-acridine and 7H-benz(*d*,*e*)anthracen-7-one were characterized by direct fluorometric examination. In addition a red visible spot of unknown composition was readily noticeable.

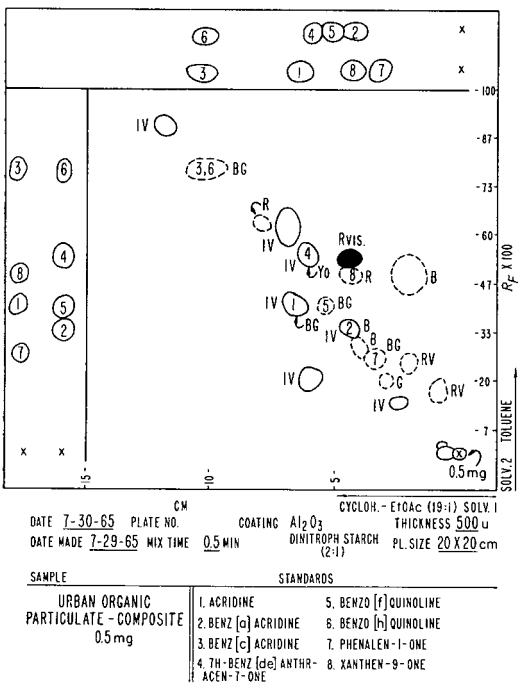

Fig. 3. Two-dimensional thin-layer chromatogram on alumina–2,4-dinitrophenoxy starch (2:1) of the composite airborne particulate sample. Definitions as in Fig. 2; in addition R = red, V = violet.

Separation of the same mixture on alumina–dinitrophenoxy starch (2:1), Fig. 3, gave fewer fluorescent spots, of which approximately half were readily characterized. Apparently the fluorescent spots containing polynuclear aromatic hydrocarbons and some polynuclear heterocyclic compounds were quenched. Treatment of this plate with trifluoroacetic fumes brought out the fluorescence of many of the polynuclear aza and carbonyl compounds.

Although the quencher used in this procedure definitely proved the usefulness of this technique, it presented one serious drawback. When treated with trifluoroacetic acid fumes, the plate acquired a blue fluorescent background color. This fluorescence interfered with direct fluorometric examination of the plate. We overcame this difficulty by eluting the appropriate spot, placing the solution of the spot on glass-fiber paper, treating with trifluoroacetic acid fumes, and examining the area fluorometrically. In this fashion spots containing acridine, benz(*a*)acridine, benz(*c*)acridine, 7H-benz(*d*,*e*)anthracen-7-one, benzo(*f*)quinoline, benzo(*h*)-quinoline,

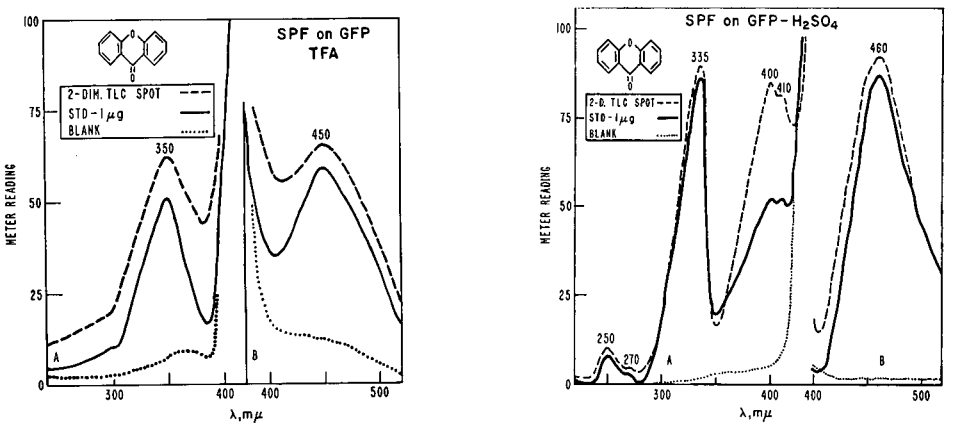

Fig. 4. Fluorescence excitation (A) and emission (B) spectra on glass-fiber paper treated with trifluoroacetic acid of a two-dimensional alumina thin-layer chromatographic spot (see Fig. 3, No. 8) (— — —), 1 μg of xanthen-9-one (———), and the blank (· · ·).

Fig. 5. Fluorescence excitation (A) and emission (B) spectra on glass-fiber paper treated with sulfuric acid of spot no. 8 in Fig. 2: Unknown (– – –) and standard xanthen-9-one (———). Blank (· · ·).

phenalen-1-one, and xanthen-9-one were readily characterized (Fig. 3). Examples of the characterization are given in Figs. 4 and 5. The fluorescence spectra of the spot were closely similar to that of xanthen-9-one whether the spot was treated with trifluoroacetic acid (Fig. 4) or sulfuric acid (Fig. 5). Xanthone has never been found in the air previously.

An aromatic fraction obtained from an organic airborne particulate sample from Detroit was separated on alumina two-dimensionally with pentane followed by cyclohexane. More than 50 spots were found on the plate. When this same fraction was separated on an alumina–dinitrophenoxy starch (2:1) plate in the same way, only eight spots were obtained. Treatment with trifluoroacetic acid fumes did not bring out any other fluorescent spots. This result indicates the absence of weakly basic polynuclear ring-carbonyl and the somewhat more basic aza heterocyclic compounds.

The two-dimensional separation on alumina of a coal-tar-pitch basic fraction with cyclohexane–ethyl acetate (9:1) followed by toluene showed the presence of more than 50 fluorescent spots. The basic fraction is extremely complicated and contains thousands of compounds[11]. When the same sample was separated on alumina–dinitrophenoxy starch plate, only eight fluorescent spots could be seen. Treatment with trifluoroacetic acid brought out about eight more spots, five of which contained the aza compounds characterized in Fig. 3. Obviously, the use of an appropriate polymeric quencher in the adsorbent on a thin-layer plate should facilitate the characterization and estimation of fluorescent compounds. Many such polymers should be prepared and tested for such applications.

The effect of various types of quenching agents in the adsorbent is shown in Table I. These quenchers could not be used with any developer that would dissolve them. At most they could be slightly soluble in the developer, or else they could be incorporated into an insoluble polymer before use. Their selective quenching efficiency depends to a large extent on the hydroxy, amino, hydrazino, and nitro functional

groups. Thus, morin would be the only compound of the six to fluoresce when the adsorbent contains 1-nitropyrene, and 8-aminofluoranthene would fluoresce in the presence of aniline, *o*-cresol, phenylhydrazine or phloroglucinol while benz(*c*)acridine would not. Table I indicates other possibilities.

SUMMARY

Eight fluorescence quenching techniques of value in the direct analysis of spots on thin-layer chromatograms are discussed.

Use of an insoluble quencher in the adsorbent is of benefit in the selective analysis of fluorescent compounds. It is predicted that with the help of polymeric materials containing nitro, phenolic, amino, anilino, thiocarbonyl, ketonic carbonyl, hydrazine, azo, and nitroso groups quick highly selective methods of direct quenchofluorometric analysis of spots on a plate will be possible for compounds containing various types of functional groups. This type of functional group analysis should approach the simplicity of colorimetry.

Examples are given of the application of some of these techniques to the analysis of urban air pollutants. The following compounds have been readily and quickly characterized by the fluorescence quenching techniques: acridine, benz(*a*)acridine, benz(*c*)acridine, 7H-benz(*d*,*e*)anthracen-7-one, benzo(*f*)quinoline, benzo(*h*)quinoline, phenalen-1-one and xanthen-9-one. All of these compounds have been found in the examined polluted urban atmospheres.

REFERENCES

1 J. W. SEASE, *J. Am. Chem. Soc.*, 69 (1947) 2242 and 70 (1948) 3630.
2 J. G. KIRCHNER, J. M. MILLER AND G. J. KELLER, *Anal. Chem.*, 23 (1951) 420.
3 E. STAHL, *Chemiker Ztg.*, 82 (1958) 323.
4 E. SAWICKI, W. C. ELBERT AND T. W. STANLEY, *J. Chromatog.*, 17 (1965) 120.
5 N. SEILER AND M. WIECHMANN, *Z. Physiol. Chem.*, 337 (1964) 229.
6 T. W. STANLEY AND E. SAWICKI, *Anal. Chem.*, 37 (1965) 938.
7 E. STAHL, *Thin-Layer Chromatography*, Academic Press, New York, 1965.
8 J. M. BOBBITT, *Thin-Layer Chromatography*, Reinhold, New York, 1963, p. 87.
9 Z. STRANSKY, *Chem. Listy*, 55 (1961) 1084.
10 E. SAWICKI, T. W. STANLEY AND W. C. ELBERT, *Talanta*, 11 (1964) 1433.
11 E. SAWICKI, T. W. STANLEY AND W. C. ELBERT, *Occupational Health Rev.*, 16 (1964) 8.

CHROMATOGRAPHIE UND ELEKTROCHROMATOGRAPHIE ANORGANISCHER SALZE MIT HYDROGENSULFAT- UND PYROSULFAT-SCHMELZEN

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Bei der Chromatographie anorganischer Ionen bietet die Verwendung geschmolzener Salze als Laufmittel die Möglichkeit, die Adsorption an der festen Phase in vielseitiger Weise zu variieren. Als Adsorbens wurden hierfür verschiedene Glas-, Al_2O_3-, SiO_2-Pulver oder Glasfaserpapier eingesetzt. Umfangreiche Untersuchungen liegen vor allem für niedrig schmelzende Nitratgemische vor. Erste Arbeiten dieser Art wurden von GRUEN und Mitarb.[1–3] sowie etwas später von BENARIE[4] veröffentlicht, wobei durch Zusatz von Chloriden die adsorbierten Ionen wieder eluiert werden konnten.

Aus Bestimmungen der Adsorptionsgleichgewichte von Co^{2+}- und Cd^{2+}-Ionen zwischen Al_2O_3 und Nitratschmelzen mit bestimmtem Chloridzusatz errechneten LILJENZIN und Mitarb.[5,6] die Konstanten für die stufenweise Bildung von Chlorokomplexen in der Schmelze. LINDNER UND JOHNSSON[7] gelang die Trennung des La von Nd und des Tb von Tm durch Adsorption in Säulen mit Al_2O_3 oder Pyrexglas-Pulver. Die Salze der Seltenerdmetalle waren dabei in einer $LiNO_3$-Schmelze gelöst und liessen sich anschliessend durch Zusatz von $ZnCl_2$ zur Nitratschmelze eluieren.

DRUDING[8] benutzte die Dünnschichtchromatographie-Technik, um an Silicagel Ag, Pb, Tl(I) und Hg(I) mit $LiNO_3$–KNO_3-Schmelze zu trennen. Aus geschmolzenen Alkalichloriden findet wegen der Bildung von Chlorokomplexen keine Adsorption der Metallionen statt, doch konnten ROACH UND HIMMELBLAU[9] zeigen, dass aus KCl–$ZnCl_2$-Schmelzen Ca und Sr an Al_2O_3 adsorbiert werden.

Zusätzliche Trenneffekte sind durch die Methoden der Elektrochromatographie zu erwarten, da sich hier noch die unterschiedlichen Wanderungsgeschwindigkeiten der verschiedenen Ionen auswirken und nicht mehr allein eine gewisse Adsorption an der festen Phase vorausgesetzt werden muss. Auf dementsprechende Arbeiten soll im zweiten Abschnitt näher eingegangen werden.

In der vorliegenden Arbeit werden die Anwendungsmöglichkeiten von Hydrogen- und Pyro-sulfatschmelzen untersucht.

I. CHROMATOGRAPHIE

Die Chromatographieversuche wurden in einer elektrisch beheizten, mit der gepulverten, festen Phase gefüllten Glassäule ausgeführt. Durch eine geeignete Vor-

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richtung wurde ein bestimmter Gasdruck auf das Laufmittel ausgeübt, um eine ausreichende Durchflussgeschwindigkeit der Schmelze zu erhalten. Die Salzschmelze tropfte aus der Säule auf eine darunter langsam rotierende Glasscheibe, wo sie zu einem Ring erstarrte, in dem die transportierten Salze analytisch nachgewiesen wurden.

Schmelze, Adsorbens und Metallsalze

Alkalihydrogensulfatschmelzen stehen in dem für die Chromatographieversuche bevorzugten Temperaturbereich um 250° nach der Gleichung:

$$2\ HSO_4^- \rightleftarrows S_2O_7^{2-} + H_2O$$

mit einem bestimmten Wasserdampfdruck und einer Pyrosulfatkonzentration im Gleichgewicht[10]. Da diese thermische Zersetzung bei den Sulfaten mit kleinen Kationen begünstigt ist, wurde bevorzugt $KHSO_4$ eingesetzt. Dieses schmilzt bei 207° und die Pyrosulfatbildung verläuft auch bei 250° noch so langsam, dass weder die Entwicklung von Dampfblasen noch eine merkliche Veränderung der Eigenschaften der Schmelze durch Pyrosulfat eintritt; dabei ist die Viskosität bereits so niedrig, dass sich bei der später beschriebenen Versuchstechnik ausreichende Laufgeschwindigkeiten ergeben. Höhere Temperaturen führen dagegen zu Störungen. So berichten THILO UND VAN LAMPE[11], dass eine $KHSO_4$-Schmelze, die 15 Std. bei 310° gehalten wird, sich zu 90–95 % zum $K_2S_2O_7$ umsetzt. Nach Abspaltung des gesamten Wassers enthält die Schmelze *ca.* 5 % SO_4^{2-}, das durch die Abgabe von SO_3 aus dem Pyrosulfat entstand. Diese Ergebnisse stehen mit Raman-spektroskopischen Messungen von WALRAFEN, IRISH UND YOUNG[12] im Einklang, die zeigten, dass bei 500° ein Maximum des $S_2O_7^{2-}$-Gehaltes erreicht wird, der bei höheren Temperaturen durch Zersetzung zu SO_3 und SO_4^{2-} wieder abnimmt.

Kaliumpyrosulfat, das bei 350–400° schmilzt, wurde im Temperaturbereich um 430° eingesetzt, um adsorbierte Ionen zu eluieren.

Natriumhydrogensulfat (FP 178°) zersetzt sich bereits bei 210° recht erheblich[11] und führte schon bei 230° zu Störungen, da sich in der Füllung der Chromatographiersäule Dampfblasen entwickelten.

Bei der Wahl der *festen Phase* war die Beständigkeit gegen die Schmelzen Voraussetzung. Insbesondere wurden Silicatgläser herangezogen, für die eine gewisse Ionenaustauscherwirkung zu erwarten war. Sie wurden in einer einheitlichen Korngrössenfraktion von 0.075–0.12 mm eingesetzt, die eine günstige Durchlaufgeschwindigkeit ermöglichte.

Reine *Na- und K-Silicate* wurden durch Zusammenschmelzen von Alkalicarbonat und Quarz in Mischungsverhältnissen hergestellt, die annähernd den Formeln $K_2Si_2O_5$, $Na_2Si_2O_5$ und $K_2Si_4O_9$ entsprachen. Sie setzten sich mit der Hydrogensulfat-Schmelze zumindest an der Oberfläche zu Kieselsäuren um, wobei sich durch Quellung und K_2SO_4-Abscheidung die Säulen verstopften. Nach einer Vorbehandlung mit Schmelze oder konz. Schwefelsäure zeigten die Silicatkörner bei der Verwendung keine weitere Veränderung. Da aber gegenüber den später beschriebenen technischen Gläsern keine besonderen Adsorptionseffekte auftraten, wurde auf ihre weitere Untersuchung verzichtet.

Bei Silicaten der Zusammensetzung 32.5 % SiO_2, 55.3 % B_2O_3, 12.2 % Na_2O

und 39.5 % SiO_2, 16.5 % Al_2O_3, 44.0 % Na_2O (Gew.-%) wirkte sich der Kontakt mit der Schmelze dahingehend aus, dass die zunächst einheitlichen Kornfraktionen undurchsichtig wurden und sich in wesentlich kleinere Partikel zerteilten. Ein kieselsäurereicheres Borosilicatglas der Zusammensetzung 48 % SiO_2, 28 % B_2O_3, 24 % Na_2O zeigte nach dem Erhitzen mit der Schmelze zwar eine Trübung der Körner, wurde aber nicht weiter angegriffen. Es verhielt sich bei Trennversuchen praktisch ebenso wie Alkalicalciumsilicat-Gläser.

Für die meisten Versuche wurde Jenaer Glas G20 eingesetzt, das lediglich bei der Elution mit Pyrosulfat über 400° spurenweise Aluminium abgab.

Um die Adsorption des Al^{3+} und die Trennung anderer Ionen davon zu untersuchen, wurde ein Al-freies Spezialglas der "Deutschen Spiegelglas A.G. Grünenplan" eingesetzt, das im folgenden als DSG-Glas bezeichnet wird*. Tabelle I gibt die Analysen dieser Gläser zusammen mit den Vergleichswerten für normales Fensterglas.

Die zu untersuchenden Ionen wurden in Form ihrer z. T. wasserhaltigen Sulfate in $KHSO_4$-Schmelze gelöst, die nach dem Erstarren pulverisiert wurde. Für die Versuche wurde jeweils soviel Substanz eingesetzt, dass 0.2–0.4 mg der Metallionen auf den Startpunkt der Säule kamen.

Die Löslichkeit von $NiSO_4$ und $MnSO_4$ wurde in $KHSO_4$ bei 250° durch eine einfache Filtermethode quantitativ bestimmt. Die gelöste Menge blieb nach 60 Min. praktisch konstant und betrug im Falle des $NiSO_4$ 0.54, beim $MnSO_4$ 0.46 Gew.-%.

Bei Al-, Ce(III)- und Fe(III)-Salzen ergaben sich keine klaren Lösungen. Da die milchig trüben Mischungen aber auch noch die feinsten Glasfritten passierten, war die später beobachtete Adsorption des Eisens und Aluminiums sicher nicht auf einen Filtriereffekt zurückzuführen.

Im Falle des Vanadyl(IV)sulfates erreichte die verhältnismässig grosse Löslichkeit nach 70 Min. mit 4.5 Gew.-% ein Maximum, um dann wieder langsam abzusinken, so dass in der Schmelze, die nach 3 Std. auch schon einen höheren Pyrosulfatgehalt aufwies, nur mehr 3 % Vanadin vorlagen. Die Farbe hatte sich dabei von grünblau nach gelbgrün verändert. Dieses Verhalten der Vanadinsalze führte auch bei den späteren Versuchen zu besonderen Effekten.

Apparatur

Die Chromatographiersäule bestand aus einem 23 cm langen Glasrohr von 6 mm Innendurchmesser, das sich oben zu einem 16 cm langen Teil von 18 mm Durchm. erweiterte, der die als Laufmittel dienende Schmelze aufnahm. Am oberen Ende des Rohres befand sich ein NS 29-Schliffkern. Der enge Teil der Säule, der beim Versuch das Glaspulver enthielt, lief unten zu einer Spitze mit einer 0.1–0.2 mm weiten Öffnung aus. Oben wurde die Säule mit einer durch Stahlfedern gesicherten Schliffkappe verschlossen, die mit der Druckgasleitung verbunden war.

Zum Beheizen der Säule erwies sich ein kleiner Elektroofen als zweckmässig, dessen Kern ein 35 cm langer, zylindrischer Aluminiumblock von 35 mm Durchm. bildete. Er war in der Längsachse mit einer Bohrung von 8 mm Durchm. versehen, die sich oben so erweiterte, dass man den Säulenkopf mit dem Schmelzevorrat noch *ca.* 10 cm tief in den Heizblock senken konnte. Auch am unteren Ende des Ofens

* Für die kostenlose Herstellung der Glasproben danken wir der obengenannten Firma, insbesondere Herrn Dr. O. GOTT.

TABELLE I

ZUSAMMENSETZUNG DER TECHNISCHEN GLÄSER

Nr.	*Glas*	SiO_2	B_2O_3	Na_2O	K_2O	*Cao*	Al_2O_3	*BaO*	*MgO*	As_2O_3	Sb_2O_3
		Gew.-%									
1	Jena G 20	75.3	7.5	5.7	0.8	0.8	6.0	3.5	—	0.1	—
2	DSG	71.0	—	17.3	—	11.0	—	—	0.5	—	0.2
3	Normalglas	71.5	—	14.0	—	13.0	1.5	—	—	—	—
		Mol.-%									
1	Jena G 20	80.45	6.91	5.91	0.55	0.92	3.77	1.46	—	0.03	
2	DSG	70.77	—	16.71	—	11.74	—	—	0.74	—	0.04
3	Normalglas	66.3	—	12.59	—	12.92	8.19	—	—	—	—

war diese Bohrung erweitert, so dass sich die Spitze der Säule zwar innerhalb des Heizraumes befand, jedoch die austretenden Schmelzetropfen nicht mit dem Metall in Berührung kamen. Der Aluminiumblock trug eine entsprechend isolierte Heizwicklung. Mit Hilfe eines Temperaturreglers, der durch ein in der Mitte der Heizwicklung angebrachtes Thermoelement gesteuert wurde, liessen sich konstante Temperaturen bis 600° einstellen.

Den Aufbau eines Quecksilberventils, das zum Festlegen des auf die Schmelze wirkenden Gasüberdruckes diente, zeigt Fig. 1.

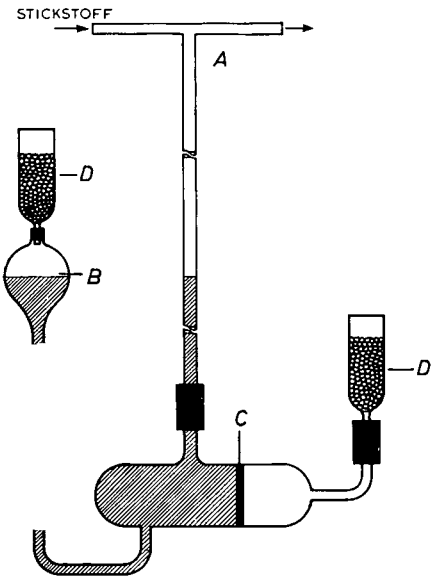


Fig. 1. Quecksilberventil zur Herstellung konstanter Gasdrucke. A–D, siehe Text.

Es war durch ein T-Stück (A) in die Rohrleitung geschaltet, die einen sehr langsamen Stickstoffstrom von der Druckgasflasche zur Schliffkappe der Chromatographiersäule führte. Mit Hilfe der Niveaubirne (B) wurde ein bestimmter Druck eingestellt, nach dessen Erreichen der Gasüberschuss über eine G-3-Glasfritte (C) im unteren Teil des Gerätes nach aussen abströmte. Um Hg-Dämpfe zu absorbieren, trugen Austrittsöffnung und Niveaubirne mit Jodkohle gefüllte Glasröhrchen (D). Das Gerät gestattete, Drucke bis zu 700 mm Hg-Säule einzustellen, ohne dass das Quecksilber durch die Glasfritte drang.

Zum Auffangen der Schmelze eigneten sich Duranglasscheiben von 30 cm Durchm., die durch einen Uhrwerksantrieb mit 1 Umdrehung in 8 oder 12 Stunden rotierten.

Versuchsablauf

Auf einen kleinen Glaswollepfropf in der unteren Spitze der Säule wurde so viel Glas der Korngrösse 0.075–0.12 mm in den engen Rohrteil gefüllt, dass oben noch 2 cm frei blieben. Dann wurden 5–10 g $KHSO_4$ zugegeben und nach dem Einsetzen der Säule in den Ofen aufgeschmolzen. Unter Gasdruck wanderte die Schmelze

langsam durch die Glasfüllung bis ihr Meniskus die obere Grenze des Glaspulvers erreicht hatte. Dann wurde die Schliffkappe vorsichtig entfernt und die Mischung der zu transportierenden Salze mit der Trägerschmelze (*ca.* 100 mg) durch einen langhalsigen Trichter in die Säule gebracht. Nachdem die Mischung geschmolzen war, wurde sie durch Gasdruck unter die Obergrenze des Glaspulvers bewegt und noch soviel Glaspulver nachgefüllt, bis dieses noch 5 mm hoch im erweiterten Teil stand. Danach wurde eine ausreichende Menge Trägersalz zugegeben. Nachdem dieses geschmolzen war, wurde die Säule endgültig geschlossen und ein Druck von *ca.* 400 Torr. auf die Schmelze gegeben. Diese tropfte aus der Säule auf die rotierende Scheibe, wo sie zu einem gleichmässigen Ring erstarrte. Die Durchflussgeschwindigkeit war so bemessen, dass die Tropfen im Abstand von 60 Sek. abfielen. Ihr Gewicht betrug *ca.* 40 mg, so dass in einer Stunde etwa 2.5 g Salz austraten. Eine langsamere Tropfenfolge von 120 Sek. hatte keinen Einfluss auf den Grad der Trennung. Glaspulver wesentlich geringerer Korngrösse als 0.07 mm konnte nicht verwendet werden, da dann selbst bei höherem Druck die Schmelze nicht mehr durchfloss.

Der Schmelzering wurde am Ende des Versuches in Sektoren geeigneter Grösse aufgeteilt, die ausgewogen und auf ihren Gehalt an transportierten Ionen hin analysiert wurden. Zum Nachweis dienten die üblichen photometrischen Bestimmungsmethoden, wie sie in den Vorschriften von ZIMMERMANN[13] und SANDELL[14] vorliegen. Die Konzentrationen werden im folgenden in mg Metallion/g Schmelze angegeben.

Versuchsergebnisse

Die Salze der Alkalimetalle, des Li, Mn(II), Co(II), Ni, Cu(II), U(VI) und Ce(III) wurden von keinem der verwendeten Gläser adsorbiert und traten folglich mit der Front der Schmelze in einer sehr engen Zone quantitativ aus der Säule aus. Das durch seine Färbung gut erkennbare Kobalt- oder Nickelsulfat wurde deshalb benutzt, um die Lage der Front der Schmelze zu kennzeichnen. Um die Verteilung besonders der stärker adsorbierten Ionen im Schmelzering zu erfassen, wurden ihre Konzentrationen in Abhängigkeit von der nach Erscheinen der Front aus der Säule getropften Salzmenge in Gramm aufgetragen.

Aluminium wurde aus $KHSO_4$-Schmelze bei 250° von allen Glassorten vollkommen adsorbiert. Ebenso wurde Fe(III) von Jenaer- und DSG-Glas stark adsorbiert und trat auch nach längerer Laufzeit des Versuches nur in Spuren unter 5 γ/g in der Schmelze auf. Dabei war jedoch zu beobachten, dass mit der Front innerhalb der ersten 3 g der Schmelze etwas grössere Konzentrationen zwischen 5 und 15 γ Fe erschienen. Es dürfte sich dabei um Spuren zweiwertigen Eisens aus Verunreinigungen der Glasmasse handeln. Eisen(II)-Ionen befanden sich bei Verwendung von Jenaer Glas an der Front der Schmelze, doch verteilte sich ein Rest über eine lange Strecke des Schmelzeringes. Bei Verwendung anderer Glasarten war sogar manchmal ein zweites Maximum zu beobachten. Dies beruht auf einer teilweisen Oxydation zum Fe^{3+} durch das Laufmedium.

Bei einer Korngrösse von 0.075–0.12 mm besassen die Gläser eine Oberfläche von etwa 150 cm^2/g. Es wurde nicht untersucht, inwieweit bei Al- und Fe(III)-Salzen auch dann noch einwandfreie Trennungen zu erzielen sind, wenn bei gleicher Dimensionierung der Säulen wesentlich grössere Mengen eingesetzt werden. Die Adsorption des Eisens und Aluminiums beruht vermutlich auf der hohen Affinität zu den Oxidionen in der Glasoberfläche. In diesem Zusammenhang ist auch die

Feststellung von GRJOTHEIM und Mitarb.[15] wichtig, dass Al^{3+} in Sulfatschmelzen eine so hohe Acidität besitzt, dass es bei 800° unter Ausfällung als Al_2O_3 Schwefeltrioxid freisetzt.

Ein komplizierteres Verhalten zeigten Cr(III), Ti(IV) und Vanadin(IV), besonders bei den späteren Versuchen zur Trennung von Fe(III). Beim Titan befand sich der Hauptanteil an der Front der Schmelze, während ein kleiner Rest unter Schwanzbildung erst durch grössere Mengen des Hydrogensulfates desorbiert wurde. Beim Chrom lief etwa ein Drittel der eingesetzten Menge mit der Front und der Rest verteilte sich über mehr als 40 g des Laufmittels. Auch beim Vanadin wanderte nur etwa die Hälfte als grünblaue Zone mit der Front. Der andere Teil blieb mit grüngelber Farbe im oberen Teil der Glasfüllung adsorbiert und konnte erst mit Pyrosulfat bei 400° eluiert werden.

Eine Steigerung der Temperatur oder eine Erhöhung der Pyrosulfatkonzentration bewirkten eine zunehmende Elution des adsorbierten Eisens bzw. Aluminiums. Ab 400° wurden dieses und alle anderen adsorbierten Ionen durch $K_2S_2O_7$-Schmelze quantitativ eluiert.

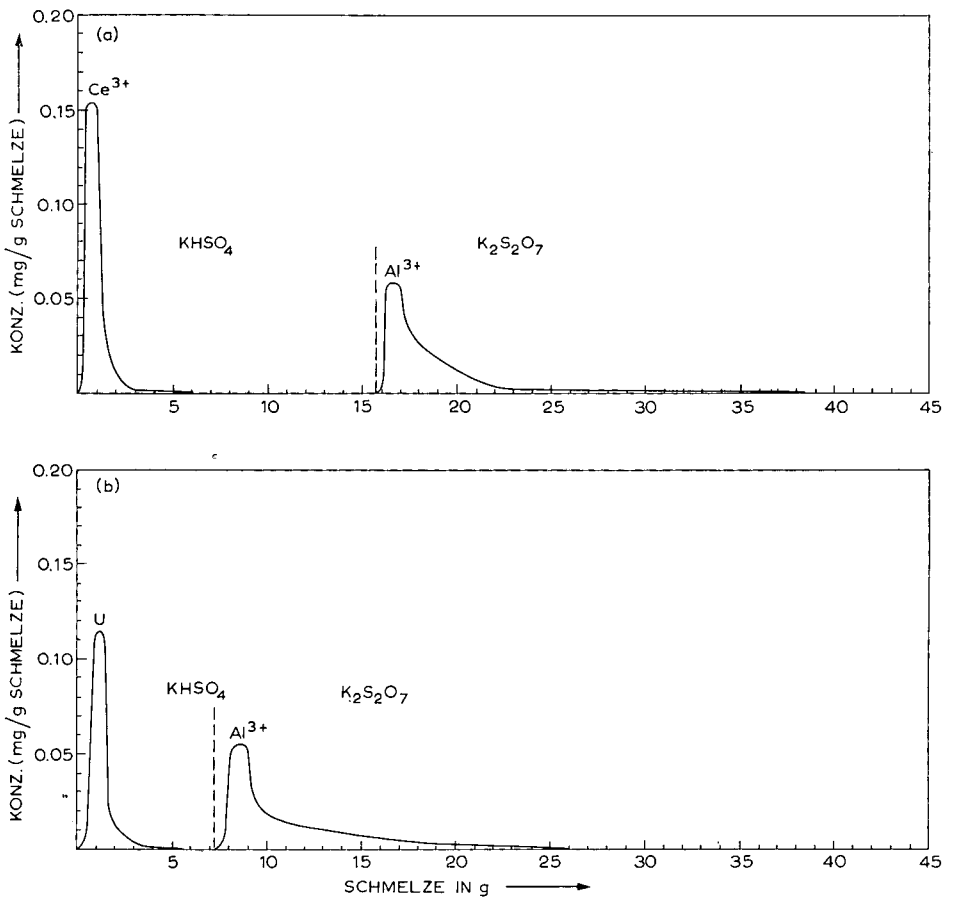


Fig. 2. Trennungen durch Chromatographie an DSG-Glas der Korngrösse 0.075–0.12 mm mit $KHSO_4$ bei 250° bzw. $K_2S_2O_7$ bei 420°. (a) Ce(III)–Al; (b) U(VI)–Al.

Damit konnten Trennungen erreicht werden, indem man zunächst die aus $KHSO_4$-Schmelze nicht adsorbierbaren Salze mit der Front der Schmelze durchlaufen liess und anschliessend die adsorbierten Ionen bei 400–430° mit Pyrosulfat eluierte. So wurde mit DSG-Glas das Al vom Co(II), Ni(II), Ce(III) und U(VI) getrennt, wie es Fig. 2 für die Beispiele einer Ce(III)–Al- und einer U(VI)–Al-Mischung aus etwa gleichen Mengen von 0.3 mg zeigt. Ganz analoge Kurven ergaben sich für die anderen Salze. Auch eine Ti(IV)–Al-Trennung ist möglich, wenn die Elution nicht zu früh stattfindet.

Trennungen vom dreiwertigen Eisen wurden an Jenaer Glas durchgeführt. Sie verliefen glatt mit Co(II), Ni, Cu(II), Ce(III) und U(VI). Titanylsulfat verursachte ein teilweises Mitlaufen des Fe(III), wie das Verteilungsdiagramm der Fig. 3a zeigt. Dieser merkwürdige Effekt war unabhängig davon, ob Eisenammoniumalaun oder $Fe_2(SO_4)_3 \cdot xH_2O$ eingesetzt wurde. Es kann sich auch nicht um eine Verdrängung des Eisens durch das Titan handeln, denn wenn an Stelle des reinen $KHSO_4$ eine Lösung mit 1 mg Ti/g Schmelze als Laufmittel benutzt wurde, verhielt sich das Fe^{3+} wie bei der reinen Hydrogensulfatschmelze. Beim Versuch einer Trennung des Cr^{3+} vom Titan lief auch hier fast das gesamte Chrom zusammen mit dem Titan, während in dessen Abwesenheit der grössere Anteil in der Säule verblieb.

Ein ähnlicher Mitführeffekt war auch beim Vanadyl(IV)sulfat, wenn auch nicht so ausgeprägt, festzustellen (siehe Fig. 3b) und auch $UO_2SO_4 \cdot 7\ H_2O$ bewirkt eine gerade noch erkennbare, erhöhte Elution des Eisens durch $KHSO_4$. Eine Erklärung dieser Erscheinung würde weitgehend spekulativen Charakter tragen und bedarf noch weiterer experimenteller Untersuchung.

II. ELEKTROCHROMATOGRAPHIE

Um den elektrolytischen Transport verschiedener Ionen in Salzschmelzen zu untersuchen, wurden bisher, wie auch im Falle der gewöhnlichen Chromatographie, die Nitrate bevorzugt. So bestimmten z.B. ARNIKAR, BONIN UND CHEMLA durch Markierung mit radioaktiven Isotopen die relativen Wanderungsgeschwindigkeiten von Li^+ und Na^+[16,17] sowie anderer Alkali- und der Halogenidionen[18–20]. Die Trennung der verschiedensten anorganischen Ionen haben ALBERTI und Mitarb.[21,22] sowie BAILEY UND STEGER[23] durch Elektrophorese in KNO_3–$LiNO_3$- oder LiCl–KCl-Eutektika ausgeführt.

Für die eigenen Arbeiten wurde als Elektrolyt das bei 203.5° schmelzende Eutektikum aus 10 Mol-% $KHSO_4$ und 90 Mol-% $K_2S_2O_7$ verwendet[24] und eine geschlossene Trennsäule aus Pyrexglas entwickelt, um Veränderungen des HSO_4^-–$S_2O_7^{2-}$-Verhältnisses durch das Abdampfen von Wasser zu vermeiden. Ihr Aufbau geht aus Fig. 4 hervor.

Die beiden Elektrodenkammern waren zwischen Schliff und Glasfritte 45 cm lang, bei einem Durchm. von 22 mm. Die Gesamtlänge der eigentlichen Trennsäule betrug *ca.* 54 cm und ihr Lumen 5 mm. Die Schliffkerne trugen während der Elektrolyse Verschlusskappen mit den Elektroden. Die gesamte Elektrolysesäule stand mit ihrem unteren Teil 40 cm tief in einem Luftofen, der aus einem Asbestkasten bestand, der mit Elektroheizung, einem Ventilator zur Luftumwälzung und Schaugläsern ausgestattert war. Darin konnten konstante Temperaturen bis 500° erreicht werden.

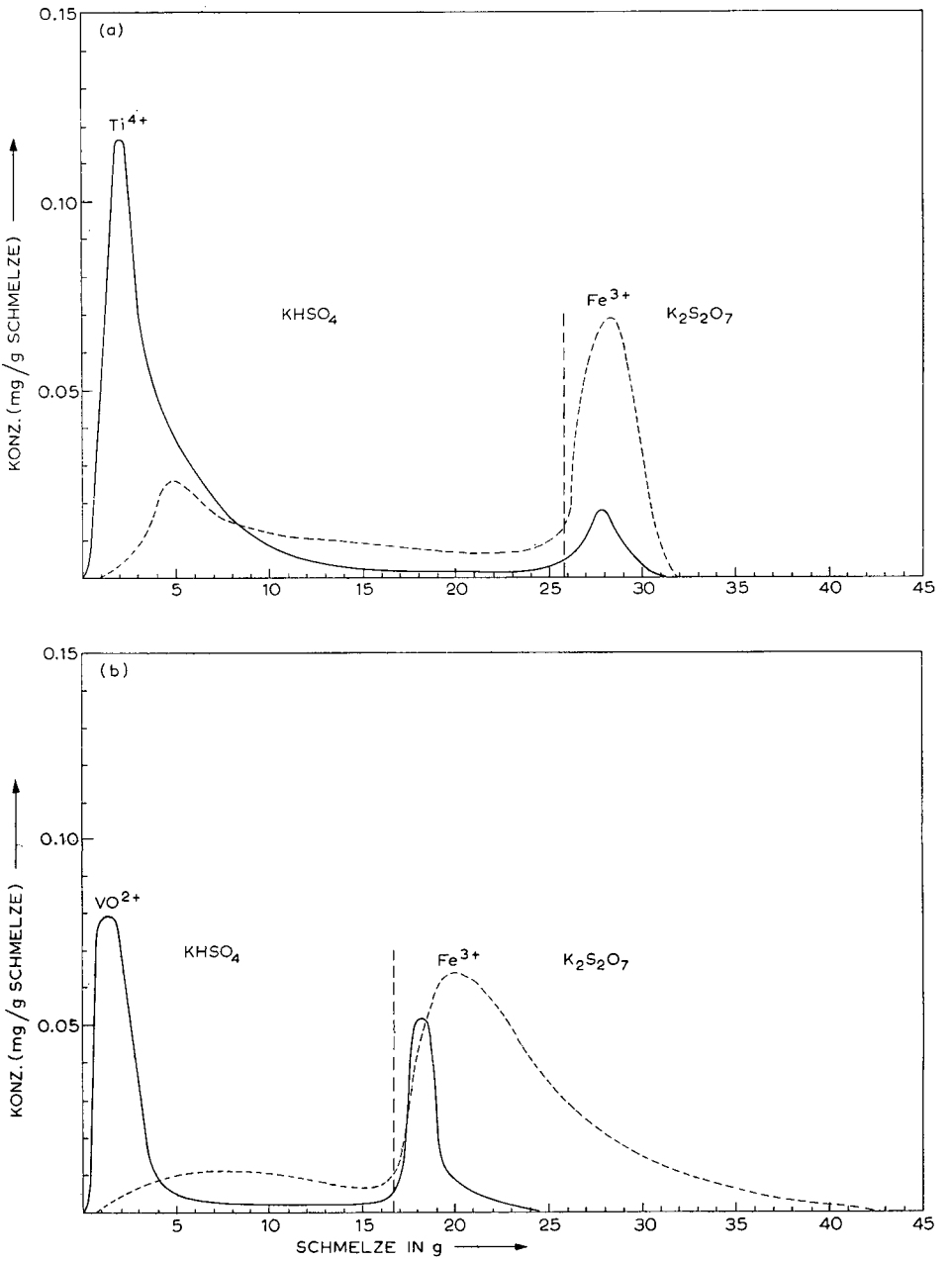
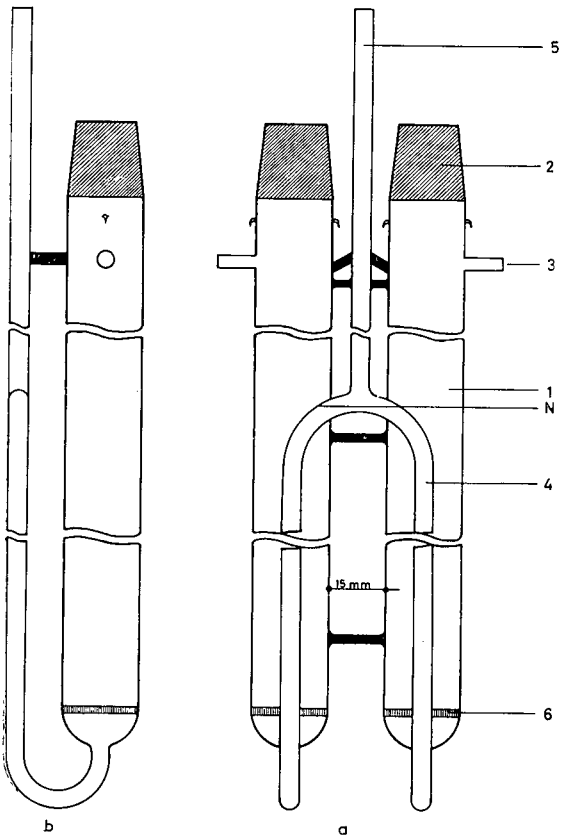


Fig. 3. Chromatographieversuche an Jenaer Glas der Korngrösse 0.075–0.12 mm mit $KHSO_4$ bei 250° bzw. $K_2S_2O_7$ bei 420°. (a) Ti(IV)–Fe(III); (b) V(IV)–Fe(III).

Die Elektrode bildete ein starker Draht aus der Heizleiterlegierung Kanthal A*. Diese Legierung wurde als Anode zwar auch von der Schmelze angegriffen, doch war sie im Vergleich zu anderem Material noch am beständigsten. Elektroden aus Substanzen wie Graphit, W, Mo, Ti, Au, Pt usw. wurden schnell zerstört. Siliciumcarbid erwies sich zwar als beständig, überzog sich aber mit einer isolierenden Deckschicht.



a. VORDERANSICHT
b. SEITENANSICHT

Fig. 4. Säule für Elektrochromatographie. 1 = Elektrodenraum; 2 = Schliff NS 29; 3 = Stutzen für Gasableitung; 4 = Trennrohr; 5 = Einfüllrohr für zu trennende Salze; 6 = Glasfritte Go.

Versuchsablauf

In die Elektrodenräume (1) wurde, jeweils 10 cm hoch, reines geglühtes Quarzpulver der Korngrösse 0.6–0.75 mm gefüllt. Durch das Rohr am U-förmigen Mittelteil (4) der Transportsäule wurde ebenfalls Quarz der Kornfraktion 0.5–0.6 mm soweit eingefüllt, dass ein kleiner Teil an der Einmündung des Zugaberohres leer blieb. In diesen Raum kam später das zu trennende Salzgemisch. Wählte man die Körnung des Quarzes zu grob, so entstanden beim Elektrolysieren in der Trennsäule

* Fe–Cr–Al–Co-Legierung der Firma Aktiebolaget Kanthal, Hallstahammar.

Gasblasen, dadurch Engstellen und oft unter Funkenbildung örtliche Überhitzungen, die zur Zerstörung des Gerätes führten. Bei zu geringer Korngrösse ergab sich ein zu hoher Widerstand der Zelle. Die Quarzfüllung der Elektrodenräume wurde mit einem Plättchen aus grober Glasfritte abgedeckt und mit einem Glasstab belastet, um ein Hochsteigen der Quarzkörner in der Schmelze zu vermeiden. Nach dem Aufheizen im Luftofen wurden in beide Elektrodenkammern gleichzeitig gleiche Mengen des vorher aufgeschmolzenen Eutektikums gegossen. Dadurch stieg die Schmelze durch die Fritten und das Quarzpulver von beiden Seiten gleichmässig in das Trennrohr, um sich am Ansatzpunkt des Einfüllrohres (5) zu vereinigen. Die Menge wurde so bemessen (*ca.* 200 g), dass das Niveau in Höhe der in Fig. 4 mit N gekennzeichneten Linie stand. Nach dem in 2 bis 3 Std. abgeschlossenen Niveauausgleich wurden 10–20 mg der zu transportierenden Salze, die ganz analog wie bei der Säulenchromatographie mit 200 mg des Eutektikums verdünnt waren, durch das Füllrohr (5) in die Mitte der Trennröhre gebracht, so dass eine 1 cm lange Zone der gelösten Salze entstand. Im Kathodenraum wurden noch 3 ml konz. H_2SO_4 zugefügt und nach Einsetzen der Elektroden bei 320–350° mit einer Gleichspannung von 180–270 V bei einem Strom von 60 mA elektrolysiert.

Für die Trennungsversuche wurden die gleichen Sulfate wie bei der Säulenchromatographie benutzt; vorwiegend mit Kationen, die durch ihre Farbe das Wandern der Zone direkt erkennen liessen. Im Falle des Ce(III) und Ti(IV) wurde die Säule nach Beendigung des Versuchs in kleine Stücke zertrennt und der Inhalt sektorenweise analysiert. Uran liess sich sehr empfindlich durch seine Fluoreszenz unter der UV-Lampe nachweisen.

Ergebnisse der Versuche

Der Gesamtwiederstand der Trennsäulen betrug 2900–3800 Ω. Der spezifische Widerstand im Trennrohr mit Quarz der Kornfraktion 0.5–0.6 mm fiel mit steigender Temperatur von 595 $\Omega \cdot$cm bei 325° auf 450 $\Omega \cdot$cm bei 350°. An der Anode entwickelte sich nach den Reaktionen:

$$4\ SO_4^{2-} - 4\ e^- = 2\ S_2O_7^{2-} + O_2$$

bzw.:

$$2\ S_2O_7^{2-} - 4\ e = 4\ SO_3 + O_2.$$

Sauerstoff und etwas Schwefeltrioxid, das in den Abzug geleitet wurde. An der Kathode wurde Wasserstoff freigesetzt. Da sich hierbei nach der Gleichung:

$$2\ HSO_4^- + 2\ e^- = H_2 + 2\ SO_4^{2-}.$$

Sulfat anreicherte, war der Zusatz von konz. H_2SO_4 nötig, um Störungen durch die Ausscheidung von K_2SO_4 zu vermeiden.

Die Ergebnisse und Versuchsdaten sind in Tabelle II zusammengefasst. Als zurückgelegter Weg wurde dabei die Lage des Intensitätsmaximums der Farbzone, (bzw. bei analytischer Untersuchung, des Punktes maximaler Konzentration) vom Startpunkt bezeichnet. Eine spezifische Geschwindigkeit V wurde dabei berechnet aus der Beziehung:

$$V = \frac{s}{t} \cdot \frac{q}{I}$$

TABELLE II

VERSUCHSERGEBNISSE DER ELEKTROCHROMATOGRAPHIE

Nr.	*Getrennte Ionen*	*Eingesetzte Mengen der Salze (mg) als Metallion*	*Versuchs-temperatur (°C)*	*Elektrolyse-dauer (Std.)*	*Wanderungs-richtung Kathode (—) Anode (+)*	*Strommenge (A·Std.)*	*Zurückgelegter Weg (mm)*	*Spezifische Geschwindigkeit (cm^3/A·Std.)*
1	Co^{2+}–VO^{2+}	20 + 10 als Salze	325	26	Co (—) V (+)	2.22	Co 56 V 60	0.83 0.90
2	Co^{2+}–UO_2^{2+}	1 + 1	330–340	38	Co (—) U (+)	2.28	Co 55 U 65	0.80 0.94
3	Ni^{2+}–VO^{2+}	2.5 + 1.5	330–340	44	Ni (—) V (+)	2.11	Ni 56 V 60	0.88 0.94
4	Cu^{2+}–VO^{2+}	5 + 5	350	22	Cu (—) V (+)	1.8	Cu 55 V 57	1.01 1.05
5	Ni^{2+}–UO_2^{2+}	5 + 2.5	350	22	Ni (—) U (+)	1.8	Ni 54 U 50	0.996 0.92
6	Cu^{2+}–UO_2^{2+}	5 + 2.5	350	24	Cu (—) U (+)	1.63	Cu 40 U 52	0.81 1.05
7	Co^{2+}–TiO^{2+}	2 + 2	345–350	24	Co (—) Ti (+)	1.78	Co 45 Ti 50	0.84 0.93
8	Ce^{3+}–UO_2^{2+}	0.6 + 0.6	350	38	Ce (—) U (+)	2.7	Ce 75 U 80	0.92 0.98
9	Ce^{3+}–VO^{2+}	0.6 + 0.6	350–355	38	Ce (—) V (+)	2.55	Ce 55 V 65	0.72 0.85
10	Co^{2+}–Cr^{3+}	—	300	—	Co (—) Cr (—)	—	— —	—

Hierin bedeuten

s = Weg der Zone in der Zeit t (cm),
I = Stromstärke (A),
t = Elektrolysedauer (Std.),
q = Querschnitt des Trennrohres (0.332 cm^2).

Diese Grösse gilt jedoch nur für eine Quarzpackung bestimmter Korngrössenfraktion. Sie stellt auch nur ein relatives Mass für die Bewegung der Ionen dar, da die Schmelze durch Elektrophorese selbst eine langsame Verschiebung in Richtung zur Anode erfährt. Absolute Messungen der Ionenbeweglichkeit wären nur mit Hilfe einer radioaktiven Indizierung sowohl der Sulfat als auch der Kaliumionen zu erreichen.

Das Diagramm der Fig. 5 gibt einen Überblick über die Konzentrationsverteilung der Ionen am Ende der Versuche. Die relativ breiten Zonen, die sich in der Mitte noch überlappen, sind hier auf die im Vergleich zu den Säulenchromatographie-Versuchen wesentlich grösseren Salzmengen zurückzuführen, welche zwischen 2 und 5 mg Metallion lagen. Lediglich beim leicht lokalisierbaren U(VI) und dem analytisch untersuchten Ce^{3+} wurden kleinere Mengen eingesetzt, die dann auch vollkommen voneinander zu trennen waren.

Es wandern demnach Uran-, Titan- und Vanadinsalze nach der Anode, Nickel, Kobalt(II), Kupfer(II) und Cer(III) nach der Kathode, und man kann schliessen, dass die erstgenannten Salze komplexe Anionen bilden während die anderen in kationischer Form gelöst sind.

Beim Versuch, Chrom(III) von Kobalt zu trennen, blieb in dem pyrosulfatreichen Eutektikum die Hauptmenge des Chromsulfates ungelöst. Es verschob sich als trüb olivgrüne Zone sehr langsam in Richtung zur Kathode, wogegen das Kobalt wesentlich schneller abwanderte, so dass auch hier zumindest qualitativ eine Trennung stattfand.

Vor dem Einbringen der Kobalt–Uranyl- bzw. Kobalt–Titanylsulfatgemische mussten diesen ein paar Tropfen konz. H_2SO_4 zugesetzt werden, damit das Kobaltsalz in Lösung blieb.

Im Falle der Trennungen vom Vanadin, das in Form des $VO(SO_4)_2 \cdot 5\ H_2O$ verwendet worden war, ist zu bemerken, dass nur der Teil ins Diagramm aufgenommen wurde, der als grünblaue Zone zur Anode wanderte. Ein erheblicher Anteil war mit bräunlich gelber Farbe am Startpunkt verblieben. Das ganz ähnliche Verhalten bei der Säulenchromatographie zeigt, dass dieses in zwei verschiedenen Formen gelöst ist. Wahrscheinlich handelt es sich bei der blauen Zone um noch teilweise hydratisierte Ionen und bei der gelben Form um wasserfreie Komplexe. Diese Annahme wird auch durch die Farbänderung bei der stufenweisen Entwässerung des $VOSO_4 \cdot 5\ H_2O$ nahegelegt[25]. Dass ein Teil zum Vanadin (V) oxydiert wurde, ist weniger wahrscheinlich.

Um das Verhalten der gelösten Ionen unter einem gemeinsamen Gesichtspunkt betrachten zu können, wird zweckmässigerweise das Kationenpotential, der Quotient aus Kationen-Ladungszahl und -Radius, herangezogen. Die Werte für die bei der Säulenchromatographie stark adsorbierten Ionen des Fe(III) und Al liegen dann bei 5.0 und 6.0 $Å^{-1}$, während Cr^{3+} mit dem Ionenpotential 4.7 $Å^{-1}$ nur noch teilweise zurückgehalten wird. Cer(III) mit 2.9 $Å^{-1}$ fliesst bereits mit der Front der Schmelze, so dass wohl auch für die anderen Seltenerdelemente (Ionenpotential des Lu^{3+} = 3.2 $Å^{-1}$) an den bisher untersuchten Adsorbentien mit $KHSO_4$-Schmelze

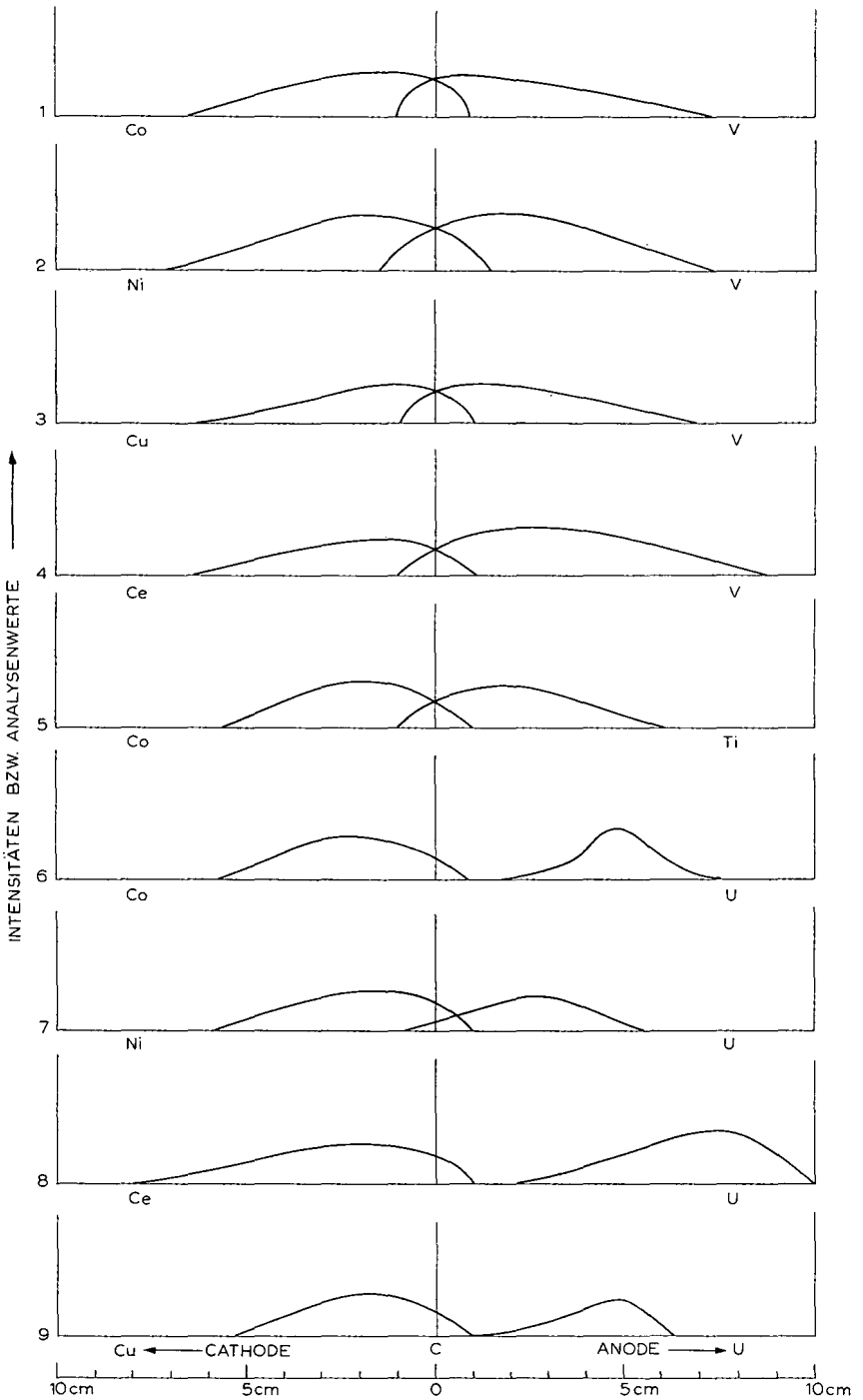


Fig. 5. Die geschätzten relativen Farb- und Fluoreszenzintensitäten bzw. Analysenwerte nach der elektrochromatographischen Trennung von Ionen in $KHSO_4$–$K_2S_2O_7$-Eutektikum.

keine Trenneffekte zu erwarten sind. Auch alle zweiwertigen untersuchten Ionen besitzen Ionenpotentiale unter 3.0 $Å^{-1}$ und werden somit nicht mehr adsorbiert. Ionen mit sehr hohem Potential, wie U(VI) bilden anionische Komplexe. Bei dem besonderen Verhalten des Titans und Vanadins dürfte die Neigung zur Bildung der Titanyl- und Vanadylionen eine Rolle spielen, denn mit 5.9 bzw. 6.0 $Å^{-1}$ liegt das Kationenpotential in einem Bereich, in dem man eine Adsorption wie beim Aluminium erwarten könnte. Tatsächlich war ja im Falle der Säulenchromatographie des Titans für einen kleinen Anteil eine stärkere Adsorption festzustellen und noch ausgeprägter war dies beim Vanadin für denjenigen Anteil gültig, der bei der Elektrochromatographie nicht zur Anode wanderte.

DANK

Herrn Prof. Dr. P. EHRLICH möchten wir unseren Dank für sein lebhaftes Interesse aussprechen. Ferner danken wir der Deutschen Forschungsgemeinschaft sowie dem Fonds der Chemischen Industrie für Geräte und finanzielle Unterstützung. Zu besonderem Dank sind wir der Friedrich-Ebert-Stiftung für die Gewährung eines Stipendiums verpflichtet.

ZUSAMMENFASSUNG

Es wurden Untersuchungen zur Trennung verschiedener anorganischer Ionen durch Säulen- und Elektrochromatographie an Silicat-Glaspulvern unter Verwendung von Alkalihydrogensulfat- und Pyrosulfat-Schmelzen als Laufmittel durchgeführt.

Aus $KHSO_4$-Schmelze adsorbierten bei 250° Jenaer Glas und aluminiumoxidfreie Gläser Al^{3+} und Fe^{3+}. Alkalimetallionen, zweiwertiges Mn, Co, Ni und Cu, ferner Ce(III), Ti(IV) und U(VI) wurden nicht adsorbiert und konnten dadurch von Eisen und Aluminium getrennt werden. Letztere liessen sich mit $K_2S_2O_7$ bei 420° vollständig eluieren.

Bei der Elektrochromatographie wandern in eutektischer $KHSO_4$–$K_2S_2O_7$-Schmelze bei 325–350°, U(VI), Ti(IV) und zum Teil Vanadin(IV) zur Anode, zweiwertige Ionen sowie das Ce(III) zur Kathode,

SUMMARY

The separation of different inorganic ions by means of column- and electrochromatography has been studied, using powdered silicate glasses as adsorbing medium and molten $KHSO_4$ or $K_2S_2O_7$ as liquid phase.

Al^{3+} and Fe^{3+} were adsorbed from a $KHSO_4$ melt by "Jenaer" glass and Al_2O_3-free glasses. The alkali metal ions and divalent Mn, Co, Ni, Cu as well as Ce(III), Ti(IV) and U(VI) were not retained and could be separated from iron and aluminium. Complete elution of all adsorbed ions was achieved by molten $K_2S_2O_7$ at 420°.

During electrochromatographic experiments with eutectic $K_2S_2O_7$–$KHSO_4$ melts at 325–350° migration towards the anode was observed in the case of U(VI), Ti(IV) and partially V(V). The divalent cations and Ce(III) moved in the direction of the cathode.

LITERATUR

1 D. M. GRUEN, *Nature*, 178 (1956) 1181.
2 D. M. GRUEN, *Proc. 16th Intern. Congr. Pure Appl. Chem., Paris, 1957.*
3 D. M. GRUEN, S. FRIED, P. GRAF UND R. L. MCBETH, *Proc. U.N. Conf. Peaceful Uses At. Energy, 2nd, Geneva*, 28 (1958) 112.
4 M. M. BENARIE, *Science*, 128 (1958) 1008.
5 J. O. LILJENZIN, R. LINDNER, H. REINHARDT UND H. WIRRIES, *Radiochim. Acta*, 1 (1963) 161.
6 J. O. LILJENZIN, H. REINHARDT, H. WIRRIES UND R. LINDNER, *Z. Naturforsch.*, 18a (1963) 840.
7 R. LINDNER UND S. JOHNSSON, *Z. Elektrochem.*, 64 (1960) 1063.
8 L. F. DRUDING, *Anal. Chem.*, 35 (1963) 1744.
9 T. L. ROACH UND D. M. HIMMELBLAU, *J. Inorg. Nucl. Chem.*, 17 (1961) 373.
10 H. HAGISAWA UND T. TAKAI, *Bull. Inst. Phys.-Chem. Research*, 16 (1937) 29.
11 E. THILO UND F. VAN LAMPE, *Z. Anorg. Allgem. Chem.*, 319 (1963) 387.
12 G. E. WALRAFEN, D. E. IRISH UND T. F. YOUNG, *J. Chem. Phys.*, 37 (1962) 662.
13 M. ZIMMERMANN, *Photometrische Metall- und Wasseranalysen*, Wissenschaftl. Verlagsges. mbH, Stuttgart, 1954.
14 E. B. SANDELL, *Colorimetric Determination of Traces of Metals*, Interscience Publishers, London, 1959.
15 K. GRJOTHEIM, T. HALVORSEN UND S. URNES, *Can. J. Chem.*, 37 (1959) 1170.
16 M. CHEMLA UND A. BONIN, *Compt. Rend.*, 241 (1955) 1288.
17 M. CHEMLA, *Compt. Rend.*, 242 (1956) 1450.
18 H. J. ARNIKAR, *Compt. Rend.*, 244 (1957) 2241.
19 H. J. ARNIKAR UND M. CHEMLA, *Radioisotopes Sci.Res.,Proc. Intern. Conf., Paris*, 2 (1957) 421.
20 H. J. ARNIKAR, *Ann. Phys.*, 4 (1959) 1291.
21 G. ALBERTI, G. GRASSINI UND R. TRUCCO, *J. Electroanal. Chem.*, 3 (1962) 283.
22 G. ALBERTI UND S. ALULLI, *J. Chromatog.*, 15 (1964) 420.
23 R. A. BAILEY UND A. STEGER, *J. Chromatog.*, 11 (1963) 122.
24 LANDOLT-BÖRNSTEIN, II. Band, 3. Teil, *Schmelzgleichgewichte*, Springer, Berlin, 1956.
25 J. ROCH, *Compt. Rend.*, 248 (1959) 3549.

Notes

Gas chromatography of acetonitrile and its chlorinated derivatives

In the course of investigations carried out in another section of this laboratory, a method was required for the analysis of mixtures containing acetonitrile and its chlorinated derivatives, *i.e.* monochloro-, dichloro- and trichloroacetonitrile.

Although some work has been reported on the gas chromatography of acetonitrile[1] and some other unsubstituted aliphatic nitriles[2], there does not appear to be any reference to the analysis by gas chromatography of mixtures of acetonitrile and its chlorinated derivatives. In this paper the retention times of these compounds relative to chloroform are reported for four different liquid phases. Area correction factors for conversion of peak areas into weight per cent results are also given.

An attempt has been made to explain the order of elution of these compounds on the various liquid phases.

Experimental

Apparatus. The gas chromatograph used was a Perkin Elmer Vapor Fractometer model 154-D equipped with a thermistor thermal detector. The columns used were 5/32 in. I.D. and 6 ft. in length.

Helium was used as the carrier gas and its flow rate was adjusted so that the chloroform peak was eluted approximately 7 min after the air peak. This corresponded to flow rates of about 50 ml/min.

The columns contained 20 % by weight of liquid phase. The solid support was "Gas Chrom Z" for the Apiezon L and "Embaphase" Silicone Oil; and "Gas Chrom P" for the QF-1 and XF-1150 liquid phases. Particle size range was 80–100 mesh. Elution times were determined at 40° except for Apiezon L. In this case an oven temperature of 70° was used to avoid the peak broadening found at lower temperatures with this phase.

Reagents. Acetonitrile: Spectro grade Eastman Organic Chemicals D.P.I., Rochester, N.Y.

Monochloroacetonitrile: Benzol Products Company, Newark, N.J.

Dichloroacetonitrile: K. & K. Laboratories, Inc., Plainview, N.Y.

Trichloroacetonitrile was prepared in these laboratories by the dehydration of trichloroacetamide[4].

All reagents used gave one peak when examined by gas chromatography.

Area correction factors. Area correction factors for conversion of peak areas to weight percentages were determined at 40° using either the Embaphase silicone or QF-1 columns. These factors were determined relative to trichloroacetonitrile as this was the major component in the samples submitted for analysis

Peak areas were determined by triangulation, the area being calculated as the product of the peak height and the peak base[3].

TABLE I

AREA CORRECTION FACTORS AND RELATIVE RETENTION TIMES OF CHLOROACETONITRILES

Compound	*B.p.* (°C)	*A.C.F.* (40°)	*Relative retention time* ($CHCl_3$ = 1.00)			
			Apiezon L (70°)	*Embaphase silicone* (40°)	*QF-1* (40°)	*XF-1150* (40°)
CH_3CN	82	0.56	0.20	0.27	2.88	1.63
$ClCH_2CN$	124*	0.74	1.12	1.50	7.72	15.0
Cl_2CHCN	112*	0.90	2.10	2.14	5.77	11.2
Cl_3CCN	83*	1.00	1.46	1.74	2.62	0.86
$CHCl_3$	61	0.85	1.00	1.00	1.00	1.00
n-C_6H_{14}	68	0.89	0.82	1.00	1.04	0.085

* See ref. 4.

Results

Boiling points, area correction factors, and retention times relative to chloroform are shown in Table I. Chloroform was present as a minor impurity in most mixtures of chlorinated acetonitriles and hence made a useful reference point. The retention data are also given in terms of the Kovats Retention Index in Table II.

The most useful stationary phase for the analysis of the acetonitrile mixtures appears to be the non-polar Embaphase Silicone Oil. Retention times on the cyanoethyl silicone XF-1150, were very long and the acetonitrile peaks were well separated but chloroform and trichloroacetonitrile overlap. A short column of this phase would be ideal for the analysis of acetonitrile mixtures if chloroform is known to be absent.

TABLE II

KOVATS RETENTION INDICES OF CHLOROACETONITRILES

Compound	*Retention index*			
	Apiezon L (70°)	*Embaphase silicone* (40°)	*QF-1* (40°)	*XF-1150* (40°)
CH_3CN	457	460	828	959
$ClCH_2CN$	675	643	948	1434
Cl_2CHCN	717	681	911	1200
Cl_3CCN	630	659	817	877
$CHCl_3$	621	600	696	896

Discussion

The boiling points of the chloroacetonitriles decrease as their molecular weights increase. Trichloroacetonitrile with the highest molecular weight in the series has a boiling point close to that of acetonitrile. Monochloroacetonitrile with the lowest molecular weight has the highest boiling point.

The presence of the electron attracting nitrile group and chlorine atoms in the molecule tends to deplete the α-carbon position of electrons causing hydrogen atoms attached to that site to become protonic. These hydrogen atoms can then bond with electron rich positions in other molecules. It is suggested that this hydrogen bonding

is a controlling factor in determining the boiling points of these compounds. Trichloroacetonitrile with no hydrogen atoms in the molecule is presumably the least associated of these compounds in the liquid state and therefore has a low boiling point. Monochloroacetonitrile with two hydrogen atoms has the highest boiling point.

The number of hydrogen atoms in the acetonitrile molecules is also of importance in determining their elution volumes (and hence elution times) during gas chromatography. The factors influencing the magnitude of solute retention on a stationary phase have been discussed by BROWN[5].

The relation of the retention volume V to the activity coefficient γ_1, of the solute in solution in the stationary phase and the vapour pressure p°_1, of the pure solute is:

$$V \propto \frac{1}{\gamma_1 p^\circ_1}$$

γ_1 is controlled by the balance of intermolecular forces between solute 1 and stationary phase 2. The number of hydrogen atoms in the acetonitrile molecule is important in controlling p°_1 as shown above and also the (1,2) interaction between solute and polar stationary phases such as QF-1 and XF-1150. On these phases the acetonitriles (including acetonitrile itself) tend to elute in boiling point order. Retention times are large due to a large (1,2) interaction (hydrogen bonding or dipole–dipole interaction) causing γ_1 to be small and hence V large. The retention times of trichloroacetonitrile on these phases appears to be smaller than expected. In this case the strong association of the stationary phase with itself [(2,2) interaction] would tend to "squeeze" out the solute resulting in a high value of γ_1 and hence a low retention volume.

In the non-polar stationary liquids the retentions are still mainly controlled by p°_1. However retention volumes are small as the associated solutes give large values of γ_1 [(1,1) interaction predominates]. The highly associated monochloroacetonitrile has a lower retention time than expected from its boiling point as the effect of the high value of γ_1 apparently overshadows the effect of the low value of p°_1.

Further evidence of these interactions has been obtained by BROWN[6] using phases of higher polarity than those investigated in this report.

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1 J. K. CAMPBEL, J. W. RHOADES AND A. L. CROSS, *Nature*, 199 (1963) 991.
2 Y. ARAD-TALMI, H. LEVY AND D. VOFSI, *J. Chromatog.*, 10 (1963) 417.
3 BRITISH STANDARDS INSTITUTION, BS 3282 (1963)
4 BEILSTEIN, *Handbuch der organische Chemie*, Band 2, pp. 212, 205, 201.
5 I. BROWN, *J. Chromatog.*, 10 (1963) 284.
6 I. BROWN, private communication.

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A simple device for making a concave concentration gradient for general use in column chromatography

ALM, WILLIAMS AND TISELIUS[1] pioneered the use of concentration gradients for the separation of solutes on chromatographic columns. They deduced on the basis of adsorption isotherms that sharpest peaks and optimal separation would generally be obtained by the use of a gradient in which the concentration of the de-sorbing solvent increases in more than linear proportion, *i.e.* one whose plot of concentration ordinate against volume is concave. Such concave gradients have been widely used, and may be made by a variety of methods[2–9], some simple and some requiring complex apparatus. As WREN[9] points out, many of these methods assume equality of density between the dilute and concentrated solvents; he alone takes account of the more usual circumstance where one solvent is denser than the other, and he has improved the theory to take this into account. The device here to be described is simple to assemble, and has characteristics which do not alter for a given pair of vessels whatever solvents may be used. General accounts of gradients elution systems have often been given[3,4,6,10], but the special features of this system made it seem worthwhile to describe it.

The device consists of a conical reservoir standing on its base, filled with concentrated solvent, and feeding through a floating siphon[11] a stirred cylindrical mixing

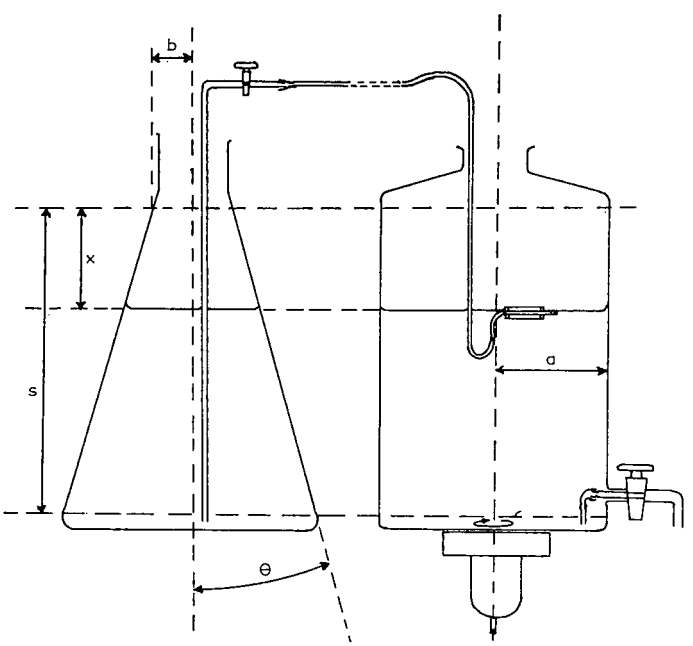

Fig. 1. Apparatus for constructing a concave elution gradient. The conical flask contains concentrated eluent which siphons over into the stirred cylindrical aspirator as outflow proceeds through the tap. The siphon floats at the surface of the mixed solvent, thus compensating for density differences between the liquids; a measured maximum difference in levels of about 2 mm occurred in practice with flow rates of 250 ml per h with a siphon of 3 mm bore. Only a very small difference in levels is required for the siphon to flow. The dimensions are those referred to in the theoretical discussion.

vessel, which is of similar height to the cone, and stands beside it (Fig. 1). The chromatography column is fed from the base of the cylinder. Standard "Pyrex" glassware was used (J. A. Jobling, Ltd., Sunderland, England), conical flasks and aspirator bottles being available with dimensions suitable for a wide range of assembly sizes and volumes. The siphon consists of a flexible tube about 1–3 mm bore depending upon the scale of the assembly. One end of the tube may conveniently be attached to a glass tube which dips to the base of the conical reservoir; the other end is made to float at the surface of the liquid in the mixing vessel. Suitable floats may be made simply in several ways (Fig. 2). Narrow tubing of sufficient flexibility is obtainable from Portland Plastics Ltd., Hythe, Kent, England (type 4) or from Precision Rubbers Ltd., Bagworth, Leicester, England (grade B. 502).

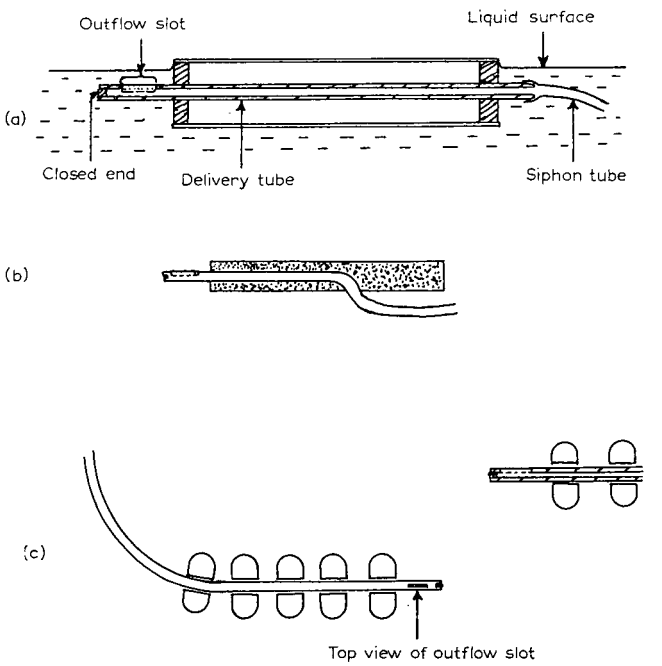

Fig. 2. Construction of floating siphon. The delivery tube may be made from polythene tubing, approximately 2.5 mm bore, 3.5 mm O.D. The flexible siphon tube is attached to the proximal end. The distal end, through which the concentrated eluent is delivered, has proved to function best if the eluent flows from a slot about 3 mm long, 0.5 mm wide cut along the top of the tube. The open bore is then plugged. This device results in an upward outflow which prevents blockage by air bubbles. Floats: (a) The delivery tube is surrounded by about 6 cm of thin-walled polythene tubing of 10 mm I.D., its ends sealed with rubber rings cut from tubing of 10 mm O.D., 3 mm bore. (b) A thin disc of expanded polymer or cork, about 5 × 0.5 cm through the thickness of which the delivery tube is threaded. (c) Hollow glass beads, advantageous for organic solvents.

Automatic adjustment of the levels of the two liquids is now ensured, however disparate their respective densities. Providing the rate of efflux to the column does not exceed the capabilities of the siphon tube, the system will reproduce the same gradient with any two liquids. The characteristics of the gradient may be determined by a trial run using water in the cylinder and either acid or a coloured substance in the cone, sampling at several stages of the measured effluent.

Details of technique

Vessels of such a volume are chosen that the total volume of the system is used for the chromatogram. The narrowest part of the cone is mounted level with the top of the cylinder, and the vessels are filled to this level, which is then marked on each vessel for future reference. In a similar way, the lowest convenient level of the system is marked on each vessel using a spirit level. The gradient will comprise the combined volume in both vessels between the initial and final levels.

It is an advantage to de-gas the concentrated solvent at a suction pump to avoid the blockage by air bubbles of the siphon tube. The siphon tube is then filled, the stopcock permitting easy handling of the filled siphon, and the float inserted into the mixing vessel whose liquid vessel is adjusted, and the elution may commence. With 2 mm bore siphon tubing a flow rate of 300 ml per h has been attained without overloading the siphon delivery. If desired the apparatus may be operated at increased pressure by equal application to both vessels of the required amount. The system has proved successful in chromatography of nucleotides and crude protein extracts, giving sharp peaks without tailing. By varying the initial and final levels subtle variations of the gradient are produced, while inverting the cone, or varying the shape of either vessel allows an astonishing variety of gradient forms to be produced. A full account of these with their complete theoretical analysis is being prepared for submission to a mathematical journal.

Theory

This type of system is complicated to analyse, since as outflow proceeds a varying proportion of concentrated eluent is drawn into the mixing vessel, whose contents are progressively diminishing in volume. An elementary analysis by the "mass-conservation" principle has been made. The function which defines effluent concentration C in terms of the concentration B of the eluent in the reservoir is:

$$\frac{C}{B} = 1 - \exp\left[\frac{\tan^2\theta}{a^2}\left\{\left(\frac{b}{\tan\theta} + s\right)^2 \log\frac{s-x}{s} + \frac{x^2}{2} + xs + \frac{2bx}{\tan\theta}\right\}\right] \qquad (1)$$

where

C = concentration of effluent when the surface is x cm below the initial level, defining the initial contents of the mixing vessel as having concentration zero;

s = total depth from initial to final liquid levels;

θ = cone angle;

a = radius of cylinder;

b = radius of cone at initial solvent level (see Fig. 1).

Expression (1) gives the relation between effluent concentration and the distance x travelled by the liquid level; there is no simple relationship between the volume of flow and concentration. Volume of flow V is related to x by the expression:

$$V = \frac{\pi x}{3}\{3a^2 + 3b^2 + 3bx\tan\theta + x^2\tan^2\theta\} \qquad (2)$$

Values of C/B from expression (1) were computed for 150 values of x in a system of measured dimensions. The curve of x against V was constructed manually from

expression (2) and hence the volumes of flow corresponding to each value of x could be read off, and the concentration gradient for the particular system was drawn.

The experimental gradient was determined by titration in a system of hydrochloric acid and water. Good agreement between theory and experiment was obtained Fig. 3).

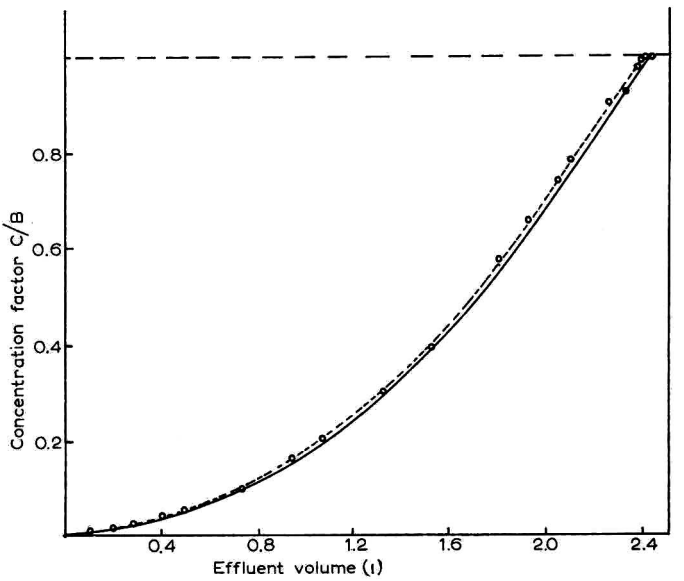

Fig. 3. Theoretical and experimental gradients. Solid line: curve computed using the exponential function (1). Broken line with points: curve as determined by titration of acid appearing in effluenf during gradient formation in the following system: conical reservoir, 1.027 *N* HCl, nominal size ot cone 1 l; mixing chamber, nominal size 2 l, initially filled with water. Total volume of gradient (*i.e.* volume between initial and final liquid levels): 2454 ml.

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1 R. S. ALM, R. J. P. WILLIAMS AND A. TISELIUS, *Acta Chem. Scand.*, 6 (1952) 826.
2 V. DESREUX, *Rec. Trav. Chim.*, 68 (1949) 789.
3 T. K. LAKSHMANAN AND S. LIEBERMAN, *Arch. Biochem. Biophys.*, 53 (1954) 258.

4 R. M. BOCK AND N. S. LING, *Anal. Chem.*, 26 (1954) 1543.
5 C. W. PARR, *Biochem. J.*, 56 (1954) xxvii.
6 B. DRAKE, *Arkiv. Kemi*, 8 (1955) 1.
7 E. A. PETERSON AND H. A. SOBER, *Anal. Chem.*, 31 (1959) 857.
8 E. A. PETERSON AND J. ROWLAND, *J. Chromatog.*, 5 (1961) 330.
9 J. J. WREN, *J. Chromatog.*, 12 (1963) 32.
10 P. LEBRETON, *Bull. Soc. Chim.*, (1960) 2188.
11 *Pat. Appl.*, 17030 (1966).

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An improved spray reagent for detecting lipids on thin-layer chromatograms

During the separations of lipids on thin-layer plates using silica gel a number of reagent sprays have been examined, both destructive (sulphuric acid, chromic acid, phosphoric acid, iodine) and non-destructive (Rhodamine B, 2′,7′-dichlorofluorescein, 2′,7′-dibromofluorescein, bromthymol blue, water). As the analysis of fatty acids of

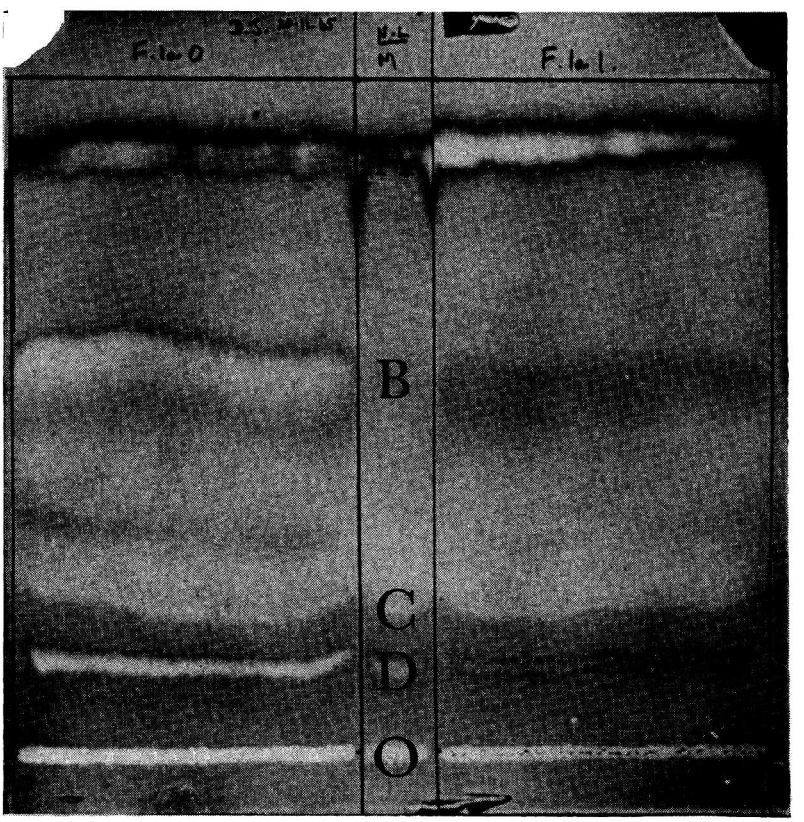

Fig. 1. Neutral lipids of chicken liver. A = Cholesterol esters; B = triglycerides; C = free fatty acids; D = cholesterol; O = origin (containing phospholipids).

the eluted lipids by gas chromatography was required[1], destructive reagents could not be used; many of the non-destructive reagents did not localise lipids sufficiently accurately, or cause sufficient contrast with background, for accurate photographic records. For this reason a mixed spray of a non-destructive nature has been developed which gives excellent contrast for both colour and black-and-white photography. This spray has proved superior to any so far described. Its composition is as follows:

Rhodamine B	100 mg
2′,7′-dichlorofluorescein	35 mg
diethyl ether	150 ml
95% ethanol	70 ml
water	16 ml

Chromatograms are sprayed and allowed to dry before examination under ultra-violet light (366Å). Lipids fluoresce orange or orange-purple on a green background (Figs. 1–3).

These colours appear as the chromatogram dries. A high proportion of ether is therefore added to the reagent to assist rapid evaporation of the solvent. It is essential to have water in the spray reagent otherwise colours are not intense; colours can be further intensified by heavy spraying. Plates to separate phospholipids

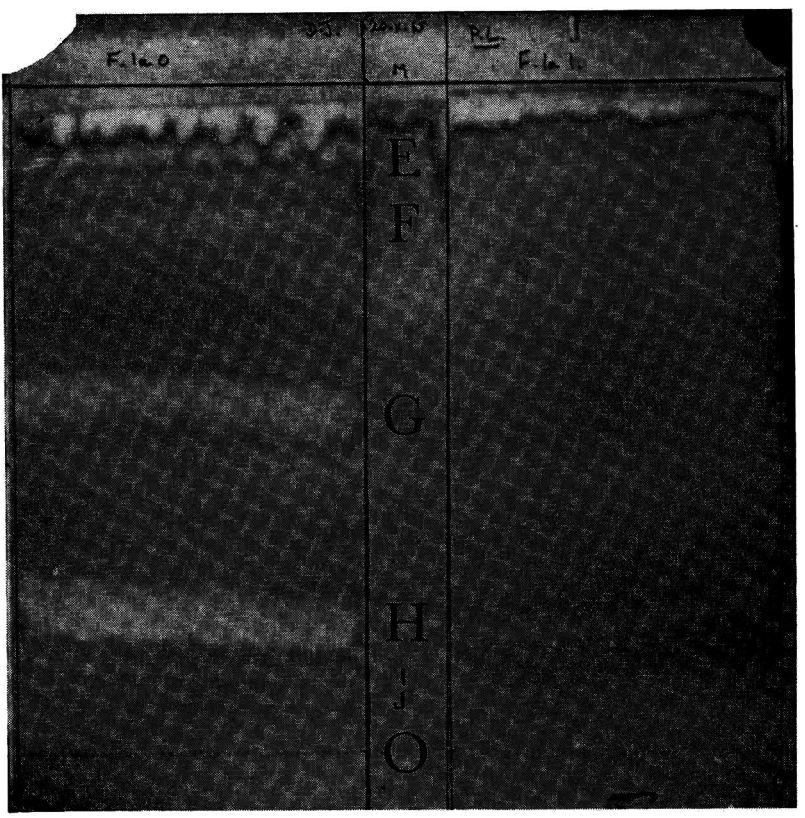

Fig. 2. Phospholipids of chicken liver. E = Neutral lipids; F = free fatty acids; G = phosphatidyl ethanolamine; H = phosphatidyl choline; I = sphingomyelin; J = lysolecithins; O = origin.

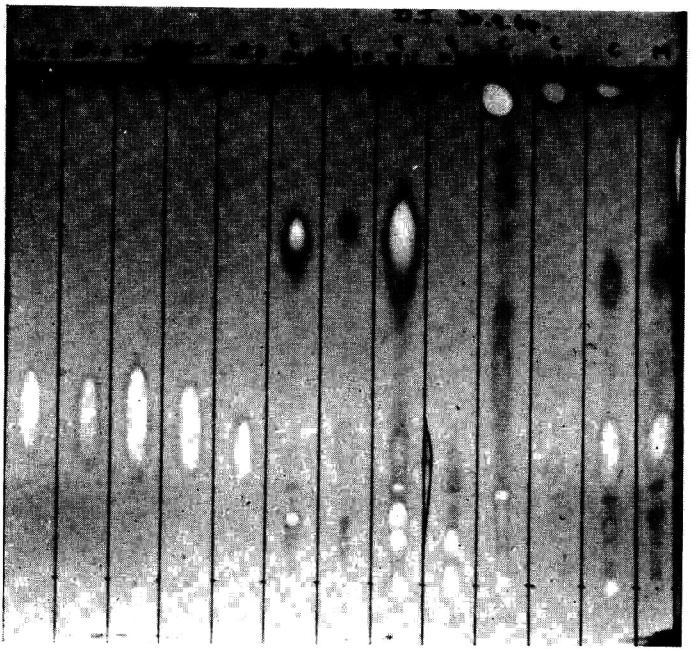

Fig. 3. Various neutral lipids (ex British Drug Houses). Columns 1–5: free fatty acids; columns 6–8: triglycerides; column 9: glyceryl monoricinoleate; columns 10–11; cholesterol esters; column 12: cholesterol; column 13: marker. These lipids were as received.

developed in chloroform–methanol–water (60:25:4) show colours within one or two minutes. Plates to separate neutral lipids developed in petrol–ether–acetic acid (85:15:1) are somewhat slower to show colour, for the acetic acid, which evaporates slowly, suppresses colour formation.

This work was supported in part by a grant (H. 6300) from the National Institute of Health, Bethesda, Md., U.S.A., The British Heart Foundation and a personal grant to one of us (D.J.) from the British Egg Marketing Board.

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1 D. E. BOWYER, W. F. M. LEAT, A. N. HOWARD AND G. A. GRESHAM, *Biochim. Biophys. Acta*, 70 (1963) 423.

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Gas chromatography of N-chloroethyl and N,N-bis-(chloroethyl) carbamates

The N-chloroethyl urethanes represent an interesting class of compounds in their intimate structural relationship to nitrogen mustard derivatives. Such compounds have been assayed colorimetrically via γ-(4-nitrobenzyl)-pyridine (NBP)[1–7]. This reagent has also been employed in the study of comparative chemical and biological activities of such alkylating agents[8]. The colored product formed by the NBP-alkylating agent couple has been described by BARDOS and his coworkers[8]. Alkylating agents have alternately been studied by a cytotoxic metabolic inhibition test[9,10] and by fluorimetry[11]. The analysis of β-chloroethyl carbamate included in a group of simple carbamates, and a diverse selection of N-substituted urethanes as analyzed by gas chromatographic means has been reported earlier[12,13].

The object of this note is to report the gas chromatographic elution pattern of the N-chloroethyl urethanes on several chromatographic liquid phases.

Experimental

The N-chloroethyl carbamate standards employed in this study were obtained from BASF, Ludwigshafen, Germany. Gas chromatographic analysis was carried out on (1) 15 % Versilube F-50 copper and (2) 10 % SE-30 stainless steel columns housed in an F&M model 1502 dual column oven containing a hot-wire detector and operated from an F&M model 500 gas chromatograph; on a (3) 4 % QF-1 glass column in an F&M model 1609 flame ionization gas chromatograph; and on a (4) 5 % Dow-11 glass column in an Aerograph model 600-B containing an electron capture detector. Specific analytical operating conditions are given in the footnotes to Table I.

Results and discussion

The results of chromatographic analysis of the chloroethyl carbamates on the various liquid partitioning phases are given in Table I. The relative elution behavior followed a similar pattern on all of the columns employed with no reversals being noted. The best resolution appeared to be obtained on Dow-11. The effective number of plates for the elution of N,N-bis-(2-chloroethyl) ethyl carbamate, however, indicated Versilube F-50 (66 plates/ft.) was slightly more efficient than Dow-11 (60 plates/ft.). The greatest number of total effective plates was obtained on the QF-1 column (300 plates), while the lowest efficiency was observed on SE-30 (22 plates/ft.). As would be expected, the order of elution on such relatively non-polar phases was found to be a function of molecular weight. It should be mentioned that several peaks were obtained on the electron capture detector for all of the carbamates analyzed. The elution results reported by electron capture refer to the principal component detected on the recorder chart. Gas chromatographic analysis on a selective phase (Carbowax 20 M) was also attempted initially. The alkyl carbamates eluted successfully, but the aryl derivatives, and the 2,4,6-trichlorophenyl carbamate derivative in particular, were extremely retained on this substrate. Even on a non-selective phase such as QF-1, elution of the samples required a column operating temperature in the vicinity of 200°.

This study of the chromatographic behavior of chloroethyl carbamates is being extended to other classes of chlorinated carbamates.

TABLE I

GAS CHROMATOGRAPHY OF CHLOROETHYL CARBAMATES

$(R_1)(R_2)N-\overset{\overset{O}{\|}}{C}-O-R_3$

R_1	R_2	R_3	*Relative retention*[a]			
			Dow-11[b]	*QF-1*[c]	*Versilube F-50*[d]	*SE-30*[e]
Methyl	2-chloroethyl	methyl	0.020	0.036	0.031	0.036
Methyl	2-chloroethyl	ethyl	0.028	0.045	0.038	0.043
2-Chloroethyl	2-chloroethyl	ethyl	0.12	0.12	0.10	0.12
2-Chloroethyl	2-chloroethyl	*p*-tolyl	1.0	1.0	1.0	1.0
2-Chloroethyl	2-chloroethyl	*p*-chlorophenyl	1.5	1.3	1.4	1.2
2-Chloroethyl	2-chloroethyl	2,4,6-trichlorophenyl	3.1	1.9	2.6	2.3

[a] Relative to N,N-bis-(2-chloroethyl) *p*-tolyl carbamate as 1.0. Retention of this derivative was 5.6 min on QF-1, 5.9 min on SE-30, 6.3 min on Dow-11, and 23.3 min on Versilube F-50.

[b] 5% w/w on 60–80 mesh Chromosorb W, 3 ft. by 0.125 in. O. D. glass column. Operating conditions: column 150°; injection port 70 V; detector 150°; output sensitivity 1 ×; input impedance 10^7; detector titanium tritide 250 mc; nitrogen carrier 35 ml/min.

[c] 4% w/w on 80–100 mesh Chromosorb W (HMDS pretreated), 6 ft. by 0.25 in. glass column. Operating conditions: column 198°; injection port 70 V; detector 210°; range 1000; flame ionization detector; hydrogen 74 ml/min; air 400 ml/min; nitrogen carrier 60 ml/min.

[d] 15% w/w on 60–80 mesh Chromosorb W (HMDS pretreated), 4 ft. by 0.125 in. copper column. Operating conditions: column 195°; injection port 235°; detector 250°; filament current 150 mA; hot-wire detector; helium carrier 26 ml/min.

[e] 10% w/w on 60–80 mesh Chromosorb W, 8 ft. by 0.125 in. stainless steel column. Operating conditions: column 195°; injection port 235°; detector 250°; filament current 150 mA; hot-wire detector; helium carrier 33 ml/min.

Acknowledgements

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1 B. K. AUSMAN, S. J. GEMMILL AND J. B. AUST, *Proc. Am. Assoc. Cancer Res.*, 3 (1959) 4.
2 B. K. AUSMAN, G. E. CRESAR, H. HAGEDORN, T. J. BARDOS AND J. L. AMBRUS, *J. Am. Med. Assoc.*, 178 (1961) 143, 735.
3 J. EPSTEIN, R. W. ROSENTHAL AND R. J. ESS, *Anal. Chem.*, 27 (1955) 1835.
4 O. KLATT, A. C. GRIFFIN AND J. S. STEHLIN, *Proc. Soc. Exptl. Biol. Med.*, 104 (1960) 629.
5 O. M. FRIEDMAN AND E. BOGER, *Anal. Chem.*, 33 (1961) 906.
6 Y. L. TAN AND D. R. COLE, *Clin. Chem.*, 11 (1965) 58.
7 H. G. PETERING AND G. J. VAN GIESSEN, *J. Pharm. Sci.*, 52 (1963) 1159.
8 T. D. BARDOS, N. DATTA-GUPTA, P. HEBBORN AND D. J. TRIGGLE, *J. Med. Chem.*, 8 (1965) 167.
9 R. P. SANDERSON, J. M. MCKENNA AND W. S. BLAKEMORE, *Nature*, 205 (1965) 479.
10 R. M. MCALLISTER, P. W. GRUNMEIER, L. L. CORIELL AND W. S. BLAKEMORE, *Cancer*, 12 (1959) 938.
11 L. B. MELLETT AND L. A. WOODS, *Cancer Res.*, 20 (1960) 518, 524.
12 W. L. ZIELINSKI, JR. AND L. FISHBEIN, *J. Gas Chromatog.*, 3 (1965) 192.
13 W. L. ZIELINSKI, JR. AND L. FISHBEIN, *J. Gas Chromatog.*, 3 (1965) 333.

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Nachweis von Enzymen nach elektrophoretischer Trennung an Polyacrylamid-Säulchen

Seit der Entwicklung der disc-Elektrophorese an Polyacrylamid-Gelen durch ORNSTEIN[1] und DAVIS[2] hat diese Technik vielfältige Anwendung zur Trennung von Protein-Gemischen gefunden. Umso überraschender ist es, dass der Nachweis von Enzymen nur gelegentlich beschrieben ist. So gelang es z.B. ALLEN[3] und ALLEN UND GOCKERMANN[4] Phosphatase nachzuweisen.

Die Reihe der nachzuweisenden Enzyme an Polyacrylamid-Gel konnte von uns unter Verwendung bekannter Farbstoffe um Peroxydase, LDH und MDH erweitert werden.

Trennmethode

Der enzymatisch aktive Extrakt wird an 10%igem Acrylamid nach der Vorschrift von ORNSTEIN[1] und DAVIS[2] unter Verwendung von "spacer" und "sample" Gel getrennt. Die Trennung dauert bei 4° und einer Stromstärke von 2.5 mA/per Säulchen *ca.* 45 min. Der Trennvorgang wird beendet, sobald der als Markierung mitlaufende Farbstoffe Bromphenolblau das Gel verlässt. Sodann wird das Säulchen mittels eines scharfen Wasserstrahles durch eine Injektionskanüle von der Innenwand des Röhrchens gelöst und in ein weitlumigeres Glasröhrchen übertragen, das an der Innenseite an zahlreichen Stellen nadelförmig ausgezogen ist (Fig. 1). In diesem "blotched"

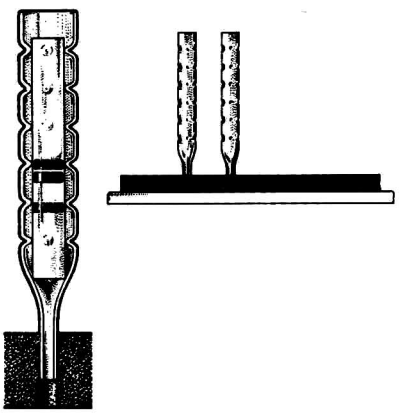


Fig. 1. Skizze eines "Pickel"-Röhrchen. Links: Längsschnitt, mit Polyacrylamid-Säulchen. Rechts: Aufstellung in einer Gummiplatte von 1 cm Dicke mit Bohrungen.

Röhrchen wird das hinderliche Ankleben an die Glaswand vermieden und eine allseitige Benetzung während der folgenden Operationen sichergestellt. Das "Pickel-Röhrchen" wird mit destilliertem Wasser gefüllt, in dem die Gel-Säulchen bei 4° bei einmaligem Wechsel eine Stunde verbleiben. Durch diese Wässerung wird erreicht, dass die nachfolgende Farbreaktion schneller eintritt und eine Untergrundfärbung unterbleibt.

Enzymnachweise

(*a*) *Peroxydase.* Der Nachweis erfolgt mit Benzidin–Guajakol[5] bei Zimmer-Temperatur in 15–45 min. Es ist darauf zu achten, dass Tageslicht ferngehalten wird;

nur dann ist eine Untergrundfärbung zu verhindern. Das gewässerte Gel wird in eine stets frisch bereitete Lösung folgender Zusammensetzung gelegt:

2 ml Natriumacetat 0.2 *M*,
0.25 ml Benzidin–Guajakol-Lösung,
0.10 ml Mangansulphat 0.005 *M*,
0.10 ml Wasserstoffperoxyd-Lösung 0.12 %.

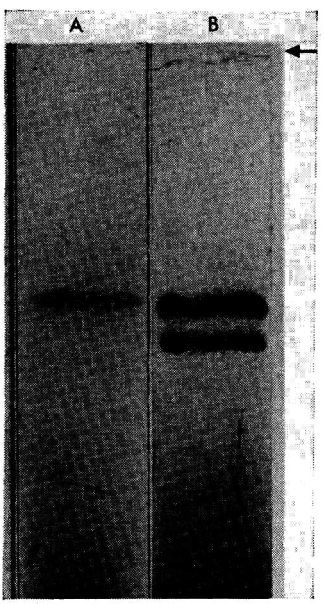
Die Benzidin–Guajakol-Lösung hat folgende Zusammensetzung:

50 mg Benzidin,
135 mg Guajakol,
25 ml Essigsäure p.a. 10 %ig.

Das Gemisch ergibt bei Erwärmen auf *ca.* 40° eine klare Lösung.

An der Oberfläche des Gel-Säulchens entsteht nach *ca.* 10 min an den Stellen von Peroxydase-Aktivität eine braunrote Färbung, welche nach *ca.* 30 min ihre maximale Farbtiefe erreicht (Fig. 2). Das Gel wird sodann aus der Substrat-Lösung herausgenommen, in destilliertem Wasser gespült, und in 2 %iger Essigsäure bewahrt. Wird das Gel im Dunkeln aufbewahrt, so verändert sich die Farbintensität der Bänder innerhalb einer Woche nur geringfügig.

(*b*) *Milchsäure-Dehydrogenase* (*LDH*). Das in dest. Wasser gespülte Gel wird bei einer Temperatur von 37° in die Inkubationsflüssigkeit gebracht. Diese hat fol-



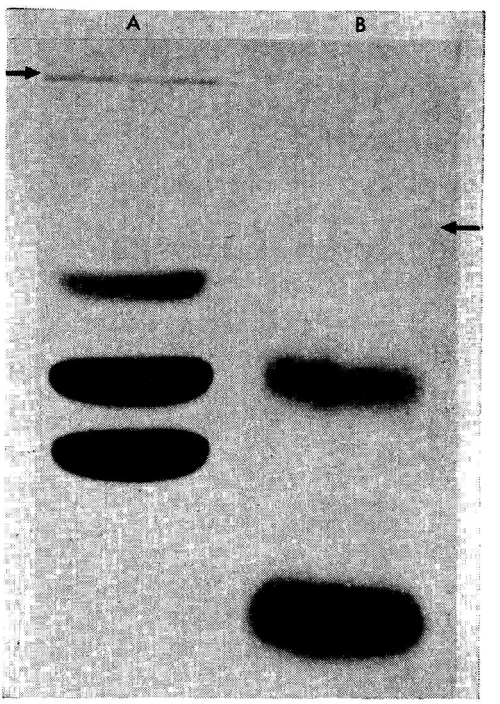


Fig. 2. Nachweis von Peroxydase (aus Meerrettich, Boehringer) an 7.5 % Acrylamid. (A) 2 γ; (B) 12 γ.

Fig. 3. Trennung eines identischen Blutserums und anschliessender LDH-Nachweis. (A) An 10 % Acrylamid (6 μl Serum); (B) an Agar (ausgeführt nach der Methode von VAN DER HELM *et al.* 1962 im Biochemischen Laboratorium der Klinik für Innere Medizin, Leiter: Dr. A. P. JANSEN).

gende Zusammensetzung (siehe VAN DER HELM *et al.*[6]): 7.2 ml Lösung I; 0.2 ml Lösung II; und 8 mg Nikotinamidadenindinukleotid (NAD); (pH 7.6).

Das Gemisch muss täglich frisch aus den folgenden Stamm-Lösungen bereitet werden:

Lösung I: 1 ml Natriumlaktat (70–72 %); 50 mg Natriumcyanid; 1.8690 g Dinatriumhydrogenphosphat ($Na_2HPO_4 \cdot 2H_2O$); 0.2722 g Kaliumdihydrogenphosphat (KH_2PO_4); und 25 mg Tetrazolium-Nitroblau (Nitro-BT). Diese Substanzen werden in dest. Wasser gelöst. Sodann wird auf 90 ml aufgefüllt und filtriert, wobei möglichst alles in Lösung gehen soll.

Lösung II: 10 mg Phenazinmethosulphat (PMS), gelöst in 10 ml dest. Wasser.

Die beiden Lösungen I und II sind mindestens 14 Tage haltbar, sofern sie vollständig dunkel bewahrt werden.

Die Farbentwicklung auf LDH ist bereits nach *ca.* 15 min zu sehen (Fig. 3). Inkubation bei 37° länger als 2 Stunden ist nachteilig. Gleiche Farbentwicklung wird bei Inkubation während der Nacht bei Zimmertemperatur erreicht, sofern diese im Dunkeln erfolgt.

Das Gel wird nach der Färbung aus der Inkubationsflüssigkeit genommen, in destilliertem Wasser gespült und in 2 %iger Essigsäure bewahrt. Innerhalb einer Woche tritt keine Farbintensitätsänderung der Bänder auf.

Es muss darauf hingewiesen werden, dass die Trennung am Polyacrylamid nicht in allen Fällen das gleiche Verteilungsmuster liefert, wie eine Auftrennung mittels Agar-Elektrophorese (Fig. 3).

(*c*) *Apfelsäuredehydrogenase* (*MDH*). Der Nachweis von MDH erfolgt analog der

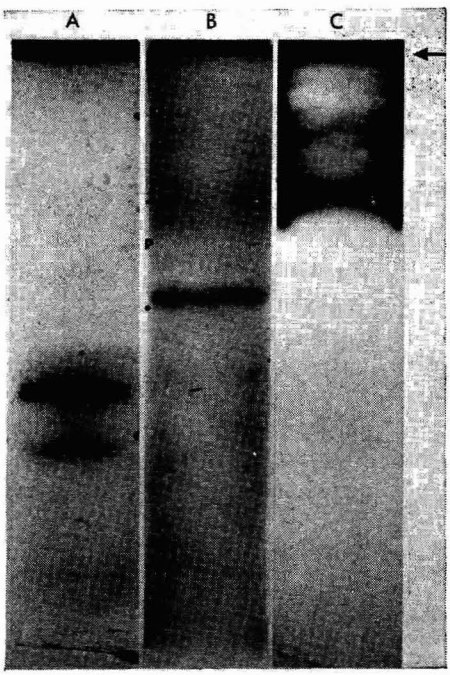


Fig. 4. Enzym-Trennung an 10 % Acrylamid. Total-Extrakt des Tapetums von *Lilium henryi*, Tetraden-Stadium der Pollenmeiose. A = Peroxydase; B = LDH; C = MDH.

Vorschrift für LDH; an Stelle von 1 ml Natriumlaktat in der Lösung I wird 0.580 mg Apfelsäure, gelöst in 5 ml dest. Wasser und neutralisiert mit 0.1 *N* Natronlauge, zugefügt (Fig. 4).

Die Anwendung der genannten Farbreaktionen ergab bei Pflanzenextrakten (Fig. 4), die durch disc-Elektrophorese getrennt worden waren, scharfe und eindeutige Banden (LINSKENS[7]).

Dank

Für die Überlassung von LDH-Substrat danke ich Herrn Dr. A. P. JANSEN sehr.

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1 L. ORNSTEIN, *Ann. N.Y. Acad. Sci.*, 121 (1964) 321.
2 B. J. DAVIS, *Ann. N.Y. Acad. Sci.*, 121 (1964) 404.
3 J. M. ALLEN, *J. Histochem. Cytochem.*, 11 (1963) 542.
4 J. ALLEN UND J. GOCKERMANN, *Ann. N.Y. Acad. Sci.*, 121 (1964) 616.
5 H. F. LINSKENS, in H. F. LINSKENS (Herausgeber), *Papierchromatographie in der Botanik*, 2. Aufl., Springer-Verlag, Berlin-Göttingen-Heidelberg, 1959, S. 183–184.
6 H. J. VAN DER HELM, H. A. ZONDAG, H. A. PH. HARTOG UND M. W. VAN DER KOOIJ, *Clin. Chim. Acta*, 7 (1962) 540.
7 H. F. LINSKENS, *Planta*, 69 (1966) 79.

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J. Chromatog., 23 (1966) 177–180

Separation of alcohol mixtures by thin-layer chromatography

Thin-layer chromatography (TLC) was applied by LAWSON AND GETZ[1] to the purification of selachyl alcohol and later by SUBBARAO *et al.*[2] to mixtures of alcohols, especially hydroxy-acids, employing petroleum ether–diethyl ether solvent systems. In the present study, ternary, quaternary and quinary artificial mixtures of alcohols were submitted to TLC resolution and the sequences of separation compared with the boiling points of the individual components.

Glass plates measuring 20 × 20 × 0.4 cm were cleaned with chromic acid and washed successively with tap and distilled water. The slurry of silica gel G was applied uniformily at a thickness of 0.25 mm. The coated plates were air-dried at 25° for 16 h, heated in an oven for 30 min at 110°, cooled and stored over silica gel. The alcohols of high purity originated from Union Carbide and Carbon Co., Stephan Chemical Co., Eastman Kodak Co., Armour and Co. and Air Reduction Chemical Co. The last source supplied 2-methyl-3-butyn-2-ol and 3-methyl-1-pentyn-3-ol, boiling at 103–104° and 120–121°, respectively.

A series of alcohol mixtures was prepared containing equivalent concentrations of each component and the total volume of the solutions was kept constant. The samples in amount of 5 μl were applied with a microsyringe 2.0 cm from the lower edge of the plate and dried by air blower. Ascending development was affected at

TABLE I

SEPARATION OF ALCOHOL MIXTURES BY TLC

Mixture	*Solvent system*	*Sequence of resolution*[a]				
		A	*B*	*C*	*D*	*E*
I	95% Ethanol[b]	1-Tetradecanol (0.93)	1-Octadecanol (0.84)	1-Heptadecanol[c] (0.84)		
II	1-Butanol saturated with water	2-Methyl-3-butyn-2-ol (0.88)	3-Methyl-1-pentyn-3-ol (0.76)	1-Hexadecanol (0.24)		
III	1-Butanol saturated with water	4,9,12-Trimethyl-7-pentadecanol (0.81)	Oleyl alcohol (0.70)	Ricinolyl alcohol (0.64)	1-Hexadecanol (0.25)	
IV	1-Butanol saturated with water	2-Ethylhexanol (0.89)	1-Decanol (0.74)	1-Tetradecanol (0.67)	1-Hexadecanol (0.25)	1-Octadecanol[d] (0.00)
V	1-Butanol saturated with water	2-Methyl-3-butyn-2-ol (0.87)	3-Methyl-1-pentyn-3-ol (0.75)	1-Dodecanol (0.64)	1-Heptadecanol (0.57)	
VI	1-Butanol saturated with water	Tetrahydrofurfuryl alcohol (0.93)	1-Dodecanol (0.64)	1-Octadecanol (0.00)		
VII	1-Butanol + 2% NH_4OH[b]	3-Methyl-1-pentyn-3-ol (0.90)	D-Borneol (0.78)	1-Hexadecanol (0.09)		
VIII	Water[b,e]	Tetrahydrofurfuryl alcohol (0.53)	Phenylethyl alcohol (0.34)			

[a] Position of the spot or zone in relation to the point of application, the furthermost one being designated as A. Average R_F values appear in parentheses and the standard deviation was $\pm$ 0.02 in each case.

[b] No satisfactory separation occurred with 1-butanol saturated with water as solvent.

[c] Sequence as indicated or virtually of the same R_F as 1-octadecanol; trailing was very prominent with the latter.

[d] This component did not migrate.

[e] Although resolution was rather poor, the above sequence is indicated.

24° in a glass chamber equilibrated at least 8 h previously with the solvent. The plates were then dried and the spots located by spraying with concentrated sulfuric acid containing 0.5 % potassium dichromate. The spots were identified by use of reference standards. The respective findings are presented in Table I. Generally sharp separations resulted, except for mixtures I and VIII. Of great interest is the observation that the sequence of separation of components appeared to parallel the respective boiling points of the alcohols.

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1 D. D. LAWSON AND H. R. GETZ, *Chem. Ind. (London)*, (1961) 1404.
2 R. SUBBARAO, M. W. ROOMI, R. R. SUBBARAM AND K. T. ACHAYA, *J. Chromatog.*, 9 (1962) 295.

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Electrophoresis and detection of proteins on thin layers of alumina

The usefulness of electrophoretic techniques for separation of proteins is well-established. In recent years chromatography and electrophoresis on thin layers have been used to advantage in separations of a great variety of organic and inorganic compounds. Thin-layer techniques are frequently favored over chromatography or electrophoresis on, for example, paper because they are far faster and permit a wide variation in composition of supporting medium. Thin-layer chromatography has been used to some extent on proteins, but the only solid supports that have been used are hydroxyl-apatite[1] and various forms of Sephadex[2–7] (Pharmacia, Uppsala, Sweden), a gel filtration medium composed of cross-linked dextran. We are not aware of any published reports of electrophoresis of proteins on thin layers.

A major difficulty in the application of thin-layer techniques to proteins has been the locating of the proteins on the finished plates[6]. Conventional methods for staining proteins on paper strips[8] include steps involving washings, and are therefore inapplicable to thin-layer plates. MORRIS[6] has succeeded in circumventing this problem by overlaying the developed plates with filter paper, but a simpler method is clearly desirable. One method is outlined in this report.

Experimental

Glass plates (20 × 20 cm) were coated with alumina (aluminum oxide G, Stahl) thin layers by the usual methods. These were equilibrated with 0.1 *M* phosphate buffer (pH 7.7), and spotted with 2–10 μg of trypsin, α-chymotrypsin, or bovine

serum albumin. Electrophoresis was conducted on an RSCo. Model E-800-2B Electrophoresis Migration Chamber (Research Specialties Co., Richmond, California) at 400 V, with water cooling. The contact between the thin-layer plate and buffer trough was via filter paper wicks. Current varied from 44 to 56 mA. At the conclusion of the electrophoresis, the plates were dried, then sprayed with 2,7-dichlorofluorescein (0.2% in methanol) and viewed under ultraviolet light.

Results and discussion

Under the conditions described, trypsin, chymotrypsin and serum albumin migrated at the rate of 2.5 to 3.0 cm/h. This would suggest that electrophoresis on thin layers is potentially in the category of "rapid-electrophoresis" techniques. The migration of the applied spots was accompanied by very little if any increase in spot size, and no tailing was observed. The detection method used is comparable to that described by MORRIS[6] in sensitivity. MORRIS reports the lower limit to be about 1 μg of protein; using 2,7-dichlorofluorescein we find 2 μg of protein as a detectable amount, with 10 μg giving very obvious, well-defined spots. A possible drawback to the method described here is that prolonged exposure to the ultraviolet lamp resulted in gradual disappearance of the spots.

Although we have not conducted extensive studies, it seems reasonable to expect that the advantages of thin-layer electrophoresis, as opposed to paper, found for low molecular weight compounds apply to proteins as well.

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1 A. F. HOFMANN, *Biochim. Biophys. Acta*, 60 (1962) 458.
2 B. G. JOHNSON AND L. RYMO, *Acta Chem. Scand.*, 16 (1962) 2067.
3 T. WIELAND AND H. DETERMANN, *Experientia*, 18 (1962) 431.
4 H. DETERMANN, *Experientia*, 18 (1962) 431.
5 B. G. JOHNSON AND L. RYMO, *Acta Chem. Scand.*, 18 (1964) 217.
6 C. J. O. R. MORRIS, *J. Chromatog.*, 16 (1964) 167.
7 P. ANDREWS, *Biochem. J.*, 91 (1964) 222.
8 R. J. BLOCK, E. L. DURRUM AND G. ZWEIG, *Paper Chromatography and Paper Electrophoresis*, Academic Press, New York, 1958.

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Book Reviews

Chromatographic Separation of the Lanthanide and Actinide Elements, Bibliographical Series No. 11, International Atomic Energy Agency, Vienna, 1964, 117 pp. price $ 2.50.

The bibliography which contains 554 references was compiled by Mr. O. VOJTECH of the documentation section of the AIEA and covers work up to 1962. As pointed out in the introduction, work on lanthanide and actinide separation reached a maximum in the years 1960–1962 and is decreasing in 1963, thus a comprehensive bibliography seems really justified at this moment.

The compilation, it is stated, was carried out by the use of abstracting journals and is in alphabetical order of the name of the first author, each reference having also a number and being followed by an adequate abstract of about 10 lines. A subject and an author index permit easy orientation.

MICHAEL LEDERER (Rome)

Chromatography, by L. SAVIDAN, edited by D. I. DAVIES, Ph.D., Iliffe Books Ltd., London, 1965, 112 pp., price 35s net.

The original French edition of SAVIDAN's book appeared in 1958 and there was, it seems, a further edition in 1963. In French it served, at the time, quite a useful purpose as an introduction to chromatography even if rather incomplete.

The reviewer entirely fails to understand why this book has been translated now, when already much better introductions to all fields of chromatography are available in the English language. It is an equal mystery why the translators, the editor and the publishers invented a new chemical terminology in this translation. Thin-layer chromatography is called "plate chromatography", inorganic chemistry becomes "mineral chemistry". On further reading it becomes evident that the new terminology is not intentional but simply bad translation.

There are also sentences such as "Much of this chromatography is carried out on ion exchange columns so that by disregarding them we see only a part of the story" or "The zones are disclosed by a mixture of silver nitrate and fluorescein". The chemical nomenclature is equally careless *e.g.* "tryptophane" (p. 46) and "adenin" (p. 39).

The book cannot be recommended as an introduction and there is no need for such in the English language.

Paper and Thin-Layer Chromatography and Electrophoresis: a Teaching Level Manual, by IVOR SMITH AND J. G. FEINBERG, for use with the Shandon "Unikit" Apparatus, 2nd edition, Shandon Scientific Company Ltd., London, 1965, 241 pp.

This already well-known manual has been enlarged by the addition of a long chapter on inorganic paper chromatography written by (the late) Dr. F. H. POLLARD and Dr. G. NICKLESS, and by a third section on thin-layer chromatography. There are excellent colour photographs of some striking separations which will undoubtedly encourage students.

The new edition like its predecessor may be recommended for class experiments but has two shortcomings, it is linked to only one kind of apparatus (the Shandon "Unikit") and is confined to teaching techniques only, instead of combining the new techniques with some pertinent organic, physico-chemical or inorganic chemistry so as to show the possibilities in the various fields.

Gas Chromatography of Metal Chelates, by ROSS W. MOSHIER AND ROBERT E. SIEVERS, Pergamon Press, Oxford, 1965, 163 pages, price 35 s.

Application of gas chromatography to metal analysis is a very attractive field and the authors of this book should be praised for making available, in one volume, the work which has been carried out on this subject. It is a thorough review of various approaches which have been followed to extend the potentialities of gas chromatography to metals and to show the various applications of metal chelates in qualitative and quantitative analysis, in the study of reactions and equilibria.

The book is well written and nicely presented. It is a good source of references for all complexes which may be used in gas chromatography.

There is no doubt that a large number of people engaged in metal chemistry will appreciate this volume.

A. LIBERTI (Naples)

Das Destillat, Unsere Verlagsarbeit in Proben und Berichten, Verlag Chemie, GmbH, Weinheim/Bergstr.

During 1965 Verlag Chemie started this small newspaper which keeps its readers informed about the various activities of the publishing house, such as the appearance of new books, the participation in expositions and congresses, etc.

The first two numbers contained 16 pages each and also featured sample pages of books due to appear.

Two very welcome features: the inside of the back cover contains some really good jokes about chemists and chemistry and the newspaper is distributed free of charge.

News

Forthcoming Events

TECHNICON INSTRUMENTS COMPANY LTD.

European Symposium 1966

We feel that we can confidently claim that the Scientific Symposia on Automation in Analytical Chemistry which we have sponsored throughout Europe during the past five years, have proved to be of value not only to users of our equipment, but to scientific workers generally.

We believe that the time has now come when we should concentrate our efforts into one main European Symposium to be alternated annually between the principal cities in Europe.

We are very much aware of the demands made on the time of scientific workers who have papers to present and we feel that by concentrating our efforts into one main meeting, it will be more convenient for scientists not only from Europe, but from all parts of the world to attend.

In 1966 the European Symposium will be held in Paris on the 2nd, 3rd and 4th November 1966. The venue will be the Salon de la Chemie, 27 bis, Rue Saint Dominique, Paris VIIe.

We are planning a rather more comprehensive meeting than has been possible on previous occasions and in due course we shall be publishing a copy of the programme showing the papers from all over the world which have been accepted for presentation by the Scientific Committee.

Adequate multilingual simultaneous translation facilities will be provided and the whole of the Technicon European Technical Staff will be in attendance.

We are making arrangements for cheap travelling facilities and charter flights from the United Kingdom to Paris for the 1966 Symposium and details will be announced later. Additionally we can tell you that arrangements for hotel accommodation in Paris are being put into the hands of a reliable and internationally known travel agency.

We sincerely hope that this first announcement of our plans for the 1966 Technicon European Symposium will find favour with you and that you will be able to attend.

As on previous occasions, the Symposium will be supported by a large exhibition of the latest Autoanalyzer systems and devices and adequate time will be allowed in the programme for free discussions between scientists with common interests.

Conference on Molecular Sieves

The Society of Chemical Industry is organizing a conference on molecular sieves in London on April 4th–6th, 1967.

Provisional Programme

A. Mineralogy and structure

Dr. K. S. Deffeyes (U.S.A.). Area: Zeolite deposits and occurrence.
Prof. W. M. Meier (Switzerland). Area: Zeolite structure (survey).
Prof. J. V. Smith (U.S.A.). Area: Structure of sorption complexes in zeolites.

B. Synthesis

Air Liquide (France). Area: Mordenite synthesis.
Prof. R. M. Barrer (U.K.). Area: Synthesis of molecular sieve zeolites.
Dr. D. W. Breck (U.S.A.). Area: Synthesis and properties of Linde Co. zeolites.
Dr. L. B. Sand (U.S.A.). Title: Synthesis of large port and small port mordenites.
Prof. H. S. Zhdanov (U.S.S.R.). Area: Zeolite synthesis.

C. Catalysis

Prof. R. M. Barrer and Dr. S. Kravitz (U.K.). Title: Reactions of some alkyl halides, catalysed by molecular sieves.
Dr. H. W. Habgood and Dr. D. W. Bassett (Canada). Area: Cyclopropane isomerization.
Dr. R. L. Mays (U.S.A.). Area: Molecular sieve catalysis.
Dr. P. B. Weisz (U.S.A.). Area: Catalysis—making use of specific molecular shape properties.

D. Zeolite modifications and regeneration

Prof. R. M. Barrer and Dr. B. Coughlan (U.K.). Title: Molecular sieves derived from clinoptilolite by progressive removal of framework charge: characterization by sorption of CO_2 and Kr.
Dr. V. Bosacek (Czechoslovakia). Title: The role of cations in chromatographic separations of gases on X type zeolites.
Dr. G. T. Kerr (U.S.A.). Area: Decationized zeolites.
Prof. F. Wolf (Germany). Area: Modification of molecular sieves for chromatographic separation.

E. Applications

Air Liquide (France). Area: Separation with molecular sieves.
Dr. L. L. Ames, Jr. (U.S.A.). Area: Cation exchange on a natural or synthetic zeolite related to waste disposal.
Dr. J. M. Milton (U.S.A.). Area: Survey of applications.

F. Sorption and diffusion phenomena

Prof. R. M. Barrer and Dr. B. Coughlan (U.K.). Title: The influence of crystal structures upon zeolite carbon dioxide. Part I: Isotherms and selectivity. Part II: Heats and entropies.
Prof. A. V. Kiselev (U.S.S.R.). Title: The energy of adsorption by zeolites of molecules of different geometrical and electronic structure. The adsorption by zeolites from liquid solutions.
Dr. D. L. Peterson (U.S.A.). Area: Sorption characteristics of zeolites.
Prof. R. W. H. Sargent (U.K.). Title: Transient behaviour of beds of molecular sieve pellets.
Prof. W. Schirmer (Germany). Title: Thermodynamics and kinetics of adsorption of the *n*-alkanes C_{10}–C_{20} on zeolites. The adsorption of mixtures of hydrocarbons and polar molecules on zeolites.

G. General properties

Dr. P. A. Egelstaff (U.K.) Area: The motion of defect molecules in zeolite crystals.
Dr. L. V. C. Rees (U.K.). Area: Radiation damage.
Dr. D. N. Stamires (U.S.A.). Area: E.S.R. studies in zeolites.
Dr. D. J. C. Yates (U.S.A.). Area: Review of infrared studies on zeolites.

The registration fee for the conference, which entitles a member to one copy of preprints of the papers which will be despatched before the meeting, is 200s (501s to members of the Society of Chemical Industry).

Those wishing to receive the final programme and registration form should contact the Honorary Secretary, Conference on Molecular Sieves, Society of Chemical Industry, 14 Belgrave Square, London, S.W.1.

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EUCHEM-CONFERENCES IN 1966

September: *Far Infrared Spectroscopy*
In Great Britain.
Organised by:
Prof. H. W. THOMPSON,
St. John's College,
Oxford,
Great Britain.

September 12th–17th: *Chemistry of Insects*
Villa Monastero, Varenna, Italy.
Organised by:
Prof. A. QUILICO,
Politechnico di Milano,
Istituto di Chimica Milano,
Milano,
Italy.

October 24th–28th: *Synthesis and Characterisation of Organic Radicals*
Schloss Elmau b. Mittenwald, Germany.
Organised by:
Prof. K. DIMROTH,
Chemical Institute of the University,
355 Marburg, Bahnhofstr. 7,
Germany.

General enquiries and suggestions for future conferences should be sent to Prof. H. W. THOMPSON, St. John's College, Oxford, Great Britain.

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ARBEITSTAGUNG "ANGEWANDTE RADIOAKTIVITÄT"

In der Zeit vom 11. bis 14. Oktober 1966 veranstaltet das Institut für angewandte Radioaktivität der Deutschen Akademie der Wissenschaften zu Berlin eine internationale Arbeitstagung über angewandte Radioaktivität in Leipzig.

Für diese Tagung sind folgende Themenkreise vorgesehen:

Radiochemische Analytik
Physikalische Analysenverfahren mit Radionukliden
Traceruntersuchungen in der chemischen Kinetik
Metrologie radioaktiver Substanzen
Markierungssynthesen und Präparationen
Spezielle Anwendungen von Radionukliden.

Nähere Auskünft erteilt das Organisationskomitee im Institut für angewandte Radioaktivität der DAW, 705 Leipzig, Permoserstr. 15, D.D.R.

Recent Events

A TWO-DAY RESEARCH CONFERENCE ON PROGRESS IN GAS CHROMATOGRAPHY

The University of California, Los Angeles (UCLA), Department of Chemistry in cooperation with University of California Extension, Los Angeles, Calif., presented the following programme by a group of experts who discussed recent developments in gas chromatography, with emphasis on their own work.

Monday, January 31st, 1966

1. Study of chemical equilibria by gas chromatography. J. H. PURNELL, University College, Swansea, Wales.
2. Theoretical background and experimental tests of preparative scale gas chromatography. D. T. SAWYER, University of California, Riverside, Calif.
3. Preparative gas chromatography. K. P. DIMICK, Wilkens Instrument and Research, Inc.
4. Preparative gas chromatography. A. J. MARTIN, F & M Scientific Division, Hewlett-Packard Corporation.

Tuesday, February 1st, 1966

5. Reaction gas chromatography. M. BEROZA, Pesticide Chemicals Research Branch, U.S.D.A.
6. Pyrolysis gas chromatography by electric discharge. J. C. STERNBERG, Beckman Instruments, Inc.
7. Interpretation and processing of data in gas chromatography. H. W. JOHNSON, Jr., Shell Development Co.

Bibliography Section

Paper Chromatography

2. FUNDAMENTALS, THEORY AND GENERAL

KLEIN, P. D. AND TYLER, S. A.: Accuracy in determination of chromatographic mobility and its significance in identification of compounds. *Anal. Chem.*, 37 (1965) 1280–1281 — PC and TLC.

WAKSMUNDZKI, A. AND RATAJEWICZ, D.: (Influence of the organic phase type on the course of the dependence R_F = f(pH) of some acridine derivatives). *Chem. Anal. (Warsaw)*, 10 (1965) 1129–1131.

3. TECHNIQUES I

AMATO, H.: A time-saving method of paper chromatography applied to cardiotonic glycosides containing the aglycone digoxigenin. *J. Chromatog.*, 20 (1965) 629–631.

HARRIS, E. R., REITH, W. S. AND SMITH, F. R. G.: Streaking apparatus for paper chromatography. *Biochem. J.*, 97 (1965) 45P–46P — end of a polytetrafluoroethylene capillary faces gas nozzle on the other surface of the paper.

HUNTER, L. D.: Improved technique for detecting fluorescent compounds on chromatograms. *J. Chromatog.*, 20 (1965) 595.

IRVINE, D. G. AND ANDERSON, M. E.: Autotransfer chromatography: facile two-dimensional chromatography in which the stationary phase is changed. I. Autotransfer from thin-layer to paper. *J. Chromatog.*, 20 (1965) 541–545.

MORI, I. AND CHIKUI, S.: An apparatus for the rapid quantitative determination of inorganic substances by paper chromatography, combined with conductometric and polarographic methods. *J. Chromatog.*, 20 (1965) 627–629.

5. HYDROCARBONS AND HALOGEN DERIVATIVES

BOYLAND, E. AND SIMS, P.: The metabolism of benz[*a*]anthracene and dibenz[*a,h*]anthracene and their 5,6-epoxy-5,6-dihydro derivatives by rat-liver homogenates. *Biochem. J.*, 97 (1965) 7–16.

MAŠEK, V.: (Chromatographic determination of 3,4-benzopyrene). *Chem. Průmysl*, 15 (1965) 47–50; *C.A.*, 63 (1965) 10639d.

6. ALCOHOLS

CASSELMAN, A. A. AND BANNARD, R. A. B.: Chromatographic separation of the 1-methoxy-2(3)-hydroxy-3(2)-bromocyclohexanes and their derivatives. *J. Chromatog.*, 20 (1965) 424–428 — PC and TLC of 30 compounds.

RŮŽIČKA, B.: (Separation and quantitative determination by paper chromatography of C_1–C_5 alcohols in water from the Fischer Tropsch synthesis). *Chem. Anal. (Warsaw)*, 10 (1965) 1165–1169 — xanthates of alcohols.

7. PHENOLS

GUMPRECHT, D. L.: Paper chromatographic analysis of some by-products from alkaline fusion of benzenesulfonic acid. *J. Chromatog.*, 20 (1965) 546–555.

WRIGHT, A. S., AKINTONWA, D. A. A., CROWNE, R. S. AND HATHWAY, D. E.: The metabolism of 2,6-di-*tert.*-butyl-4-hydroxymethylphenol (Ionox 100) in the dog and rat. *Biochem. J.*, 97 (1965) 303–310.

8. SUBSTANCES CONTAINING HETEROCYCLIC OXYGEN

KARIMDZHANOV, A. K., ISMAILOV, A. I. AND SADYKOV, A. S.: (The formation of phenolic compounds in the cotton shoots). *Khim. Prirodn. Soedin., Akad. Nauk Uz. SSR*, (1965) 350–352.

NORTJÉ, B. K. AND KOEPPEN, B. H.: The flavonol glycosides in the fruit of *Pyrus communis* L. cultivar Bon Chrétien. *Biochem. J.*, 97 (1965) 209–213.

PETRENKO, V. V.: (Quinqueloside — a new flavonoid glycoside from *Leonuris quinquelobatus* Gilib.). *Khim. Prirodn. Soedin., Akad. Nauk Uz. SSR*, (1965) 414–419.

SAAYMAN, H. M. AND ROUX, D. G.: The origins of tannins and flavonoids in black-wattle barks and heartwoods, and their associated "non-tannin" components. *Biochem. J.*, 97 (1965) 794–801.

9. OXO COMPOUNDS

SHINOHARA, T.: Simultaneous paper chromatography of carbonyl compounds. *Kagaku Keisatsu Kenkyusho Hokoku*, 18 (1965) 10–15; *C.A.*, 63 (1965) 11256f.

10. CARBOHYDRATES

BARBIROLI, G.: (Separation and determination of sugar mixtures by circular paper chromatography). *Rass. Chim.*, 17, No. 2 (1965) 62–64; *C.A.*, 63 (1965) 11843d.

BRUNDISH, E. D., SHAW, N. AND BADDILEY, J.: The glycolipids from the non-capsulated strain of *Pneumococcus* I-192R, A.T.C.C. 12213. *Biochem. J.*, 97 (1965) 158–165.

GERHARDT, W.: (Certain sucrose derivatives. I. Preparation and properties of oxy-ethylated sucroses). *J. Prakt. Chem.*, 39 (1965) 300–308; *C.A.*, 63 (1965) 11682b.

LÜDERITZ, O., SIMMONS, D. A. R. AND WESTPHAL, O.: The immunochemistry of *Salmonella* chemotype VI O-antigens. The structure of oligosaccharides from *Salmonella* group U (O 43) lipopolysaccharides. *Biochem. J.*, 97 (1965) 820–826.

MANZETTI, A. R. AND REICHSTEIN, T.: Die Glykoside von *Streblus asper* LOUR. 4. Mitt. Strukturbestimmung einiger sterk wasserlöslicher Glykoside. *Helv. Chim. Acta*, 47 (1964) 2320–2330.

OKHUMA, S., SHINOHARA, T. AND MIYAUCHI, C.: Chromogen formation and epimerization of N-acetylhexosamines by potassium tetraborate treatment on paper. *Proc. Japan Acad.*, 41 (1965) 185–190; *C.A.*, 63 (1965) 11677f.

RUCKERT, H., PFEIL, E. AND SCHARF, G.: Der sterische Verlauf der Zuckerbildung. *Chem. Ber.*, 98 (1965) 2558–2565 — R_F values of 23 sugars.

SIMMONS, D. A. R., LÜDERITZ, O. AND WESTPHAL, O.: The immunochemistry of *Salmonella* chemotype VI O-antigens. The structure of oligosaccharides from *Salmonella* group N (O 30) lipopolysaccharides. *Biochem. J.*, 97 (1965) 815–819.

STROH, H. H., HEMPEL, H. AND APEL, R.: Die Reaktion heterocyclisch substituierter Hydrazine mit Zuckern. *Chem. Ber.*, 98 (1965) 2500–2503.

11. ORGANIC ACIDS AND LIPIDS

11a. Organic acids

DAGLEY, S., CHAPMAN, P. J. AND GIBSON, D. T.: The metabolism of β-phenylpropionic acid by an *Achromobacter*. *Biochem. J.*, 97 (1965) 643–650 — PC and TLC.

OKUYAMA, M., TSUIKI, S. AND KIKUCHI, G.: α-Ketoglutarate-dependent oxidation of glyoxylic acid catalyzed by enzymes from *Rhodopseudomonas spheroides*. *Biochim. Biophys. Acta*, 110 (1965) 66–80.

PILZ, W.: Untersuchungen über Fermente des menschlichen Blutes. IX. Die Arylesterasen des menschlichen Nabelschnurserums. *Z. Physiol. Chem.*, 338 (1964) 238–250.

WHITFIELD, A. E.: Diazotised sulphanilic acid reagent as an aid to the identification of some oxo acid 2,4-dinitrophenylhydrazones. *J. Chromatog.*, 20 (1965) 401–402.

11b. Lipids and their constituents

DEBUCH, H.: Über die Bildung der Plasmalogene zur Zeit der Myelinisierung bei der Ratte. *Z. Physiol. Chem.*, 338 (1964) 1–9 — PC and TLC.

KALLISTRATOS, G., PFAU, A., FENNER, O. AND TIMMERMANN, A.: Theoretische und experimentelle Untersuchungen über Malignolipin. *Aerztl. Lab.*, 11 (1965) 355–362 — PC and TLC.

MISRA, U. K.: Isolation of lysophosphatidylethanolamine from human serum. *Biochim. Biophys. Acta*, 106 (1965) 371–378.

OP DEN KAMP, J. A. F., HOUSMULLER, U. M. T. AND VAN DEENEN, L. L. M.: On the phospholipids of *Bacillus megatherium*. *Biochim. Biophys. Acta*, 106 (1965) 438–441.

SANDERS, H.: A method for the estimation of choline glycerophosphatides on paper chromatograms. *Biochim. Biophys. Acta*, 106 (1965) 397–402.

SANDHOFF, K., PILZ, H. AND JATZKEWITZ, H.: Über den enzymatischen Abbau von N-acetylneuraminsäure-freien Gangliosidresten (Ceramid-oligosaccharide). *Z. Physiol. Chem.*, 338 (1964) 281–285.

13. STEROIDS

GOSPODAROWICZ, D.: The effect of human chorionic gonadotropin and of follicle-stimulating hormone on the incorporation of [1-^{14}C] acetate into steroids by the rabbit corpus luteum *in vitro*. *Biochim. Biophys. Acta*, 107 (1965) 363–373 — PC and TLC.

JOHNSON, C. A. AND FOWLER, S.: The detection and identification of other 17,21-dihydroxy-20-oxo-steroids in corticosteroids. *J. Pharm. Pharmacol.*, 16, Suppl. (1964) 17T–23T.

14. STEROID GLYCOSIDES AND SAPONINS

AMANMURADOV, K. AND ABUBAKIROV, N. K.: (Glycosides of *Vaccaria segetalis*. III. Enzymatic and alkaline splitting of vacsegoside). *Khim. Prirodn. Soedin., Akad. Nauk Uz. SSR*, (1965) 372–379 — PC and TLC.

AMATO, H.: Paper chromatography of cardiotonic digitalis glycosides. An attempt to rationalize the interpretation of unknown spots. *New Istanbul Contrib. Clin. Sci.*, 8, No. 1 (1965) 35–49; *C.A.*, 63 (1965) 11253d.

BRENNEISEN, K., EUW, J. v., TAMM, C. AND REICHSTEIN, T.: Die Cardenolide der Samen von *Strophantus vanderijstii* Staner. 2. Mitt. Trennung der wasserlöslichen Glykoside. *Helv. Chim. Acta*, 47 (1964) 799–813.

DO NASCIMENTO, Jr., J. M., TAMM, C., JÄGER, H. AND REICHSTEIN, T.: Die Glykoside der Wurzeln von *Asclepias glaucophylla* Schleichter. *Helv. Chim. Acta*, 47 (1964) 1775–1792.

ISELI, E., WEISS, E., REICHSTEIN, T. AND CHEN, K. K.: Papierchromatographische Prüfung der Sekrete von *Bufo melanostictus* Schneider und *Bufo asper* Gravenhorst. *Helv. Chim. Acta*, 47 (1964) 116–119.

MANZETTI, A. R. AND REICHSTEIN, T.: Die Glykoside von *Streblus asper* LOUR. 3. Mitt. Untersuchung der stark wasserlöslichen Anteile. *Helv. Chim. Acta*, 47 (1964) 2303–2320.

MEYER, U., WEISS, E. AND REICHSTEIN, T.: Die Glykoside von *Pachypodium lealii* Welwitsch und *Pachypodium saundersii* N.E.Br. *Helv. Chim. Acta*, 47 (1964) 2330–2357.

MÜHLRADT, P., WEISS, E. AND REICHSTEIN, T.: Die Cardenolide der Samen von *Antiaris toxicaria* Lesch. 1. Mitt. Isolierungen und Identifizierungen. *Helv. Chim. Acta*, 47 (1964) 2164–2186.

17. AMINES, AMIDES AND RELATED NITROGEN COMPOUNDS

FISHBEIN, L. AND CAVANAUGH, M. A.: Detection and paper chromatography of N-substituted hydroxy-, 2-hydroxyethyl-, 2-chloroethyl-, and N,N-bis(2-hydroxyethyl)-derivatives. *J. Chromatog.*, 20 (1965) 283–294 — R_F values and detection reactions for 76 compounds.

GRANTHAM, P. H., WEISBURGER, E. K. AND WEISBURGER, J. H.: Dehydroxylation and deacetylation of N-hydroxy-N-2-fluorenylacetamide by rat liver and brain homogenates. *Biochim. Biophys. Acta*, 107 (1965) 414–424 — PC and TLC.

18. AMINO ACIDS

BELL, E. A. AND TIRIMANNA, A. S. L.: Associations of amino acids and related compounds in the seeds of forty-seven species of *Vicia*: their taxonomic and nutritional significance. *Biochem. J.*, 97 (1965) 104–111.

BROWN, B. L., REITH, W. S. AND TAMPION, W.: Some observations on the use of butanol extracts of human plasma for the determination of iodotyrosines. *Biochem. J.*, 97 (1965) 30P — change of R_F values due to other materials in the extract.

KHARLAMOV, V. T. AND SHUBNYAKOVA, L. P.: (Determination of labelled amino acids by chromatography on paper). *Metody Analiza Radioaktivn. Preparatov, Sb. Statei*, (1965) 168–173; *C.A.*, 63 (1965) 10688d.

LJUNGGREN, J.-G.: The formation of thyroxine from diiodotyrosine in the presence of horseradish peroxidase and hydrogen peroxide. *Biochim. Biophys. Acta*, 107 (1965) 434–440.

RADHAKRISHNAMURTHY, B. AND ROSENBERG, J.: Identification of PTH-amino acids by chromatography on glass paper. *Arch. Biochem. Biophys.*, 112 (1965) 392–393.

SCHNABEL, E. AND SCHUESSLER, H.: (Amide butylation as a side reaction in the synthesis of *tert.*-butyl esters by acid-catalyzed transesterification with *tert.*-butyl acetate). *Ann. Chem.*, 686 (1965) 229–238; *C.A.*, 63 (1965) 11689e.

SNOW, G. A.: Isolation and structure of mycobactin T, a growth factor from *Mycobacterium tuberculosis*. *Biochem. J.*, 97 (1965) 166–175.

WITTMANN, H.: Neue Reagentien zum papierchromatographischen Nachweis von Aminosäuren. *Monatsh. Chem.*, 95 (1964) 1198–1200 — 1,8-trimethylenequinisatin and 1,9-mesoxalylcarbazole.

19. PEPTIDES; CHEMICAL STRUCTURE OF PROTEINS AND PEPTIDES

BROWN, B. L., REITH, W. S. AND TAMPION, W.: A method of separation of dinitrophenyl derivatives of iodotyrosines and thyroid hormones by paper chromatography and gel filtration on Sephadex columns. *Biochem. J.*, 97 (1965) 30P–31P.

DENNIS, P. O. AND LORKIN, P. A.: Isolation and synthesis of balenine, a dipeptide occurring in whale-meat extract. *J. Chem. Soc.*, (1965) 4968–4972.

EASLY, C. W.: Combinations of specific color reactions useful in the peptide mapping technique. *Biochim. Biophys. Acta*, 107 (1965) 386–388.

KATSUKI, S., SCOTT, J. E. AND YAMASHINA, I.: Naphthyl isothiocyanate as a reagent for the Edman degradation of peptides. *Biochem. J.*, 97 (1965) 25C–26C.

MORRISON, M. AND JAYASINGHE, W.: Increased sensitivity and semiquantitative determination of phenylthiohydantoin derivatives of amino acids with a modified spray reagent. *J. Chromatog.*, 20 (1965) 399–401.

RINIKER, B. AND SCHWYZER, R.: Synthetische Analoge des Hypertensins. V. α-L-, β-L-, α-D- und β-D-Asp[1]-Val[5]-Hypertensin II; Desamino-Val[5]-Hypertensin II. *Helv. Chim. Acta*, 47 (1964) 2357–2374 — PC and TLC R_F values for 12 peptides.

21. PURINES, PYRIMIDINES, NUCLEOSIDES, NUCLEOTIDES, NUCLEIC ACIDS, BARBITURATES

BAYEV, A. A., VENKSTERN, T. V., MIRSABEKOV, A. D., KRUTILINA, A. I., LI, L. AND AXELROD, V. D.: Oligonucleotide composition of enzymatic hydrolysates of yeast valine-specific transfer RNA. *Biochim. Biophys. Acta*, 108 (1965) 162–164.

BRADBEER, J. W. AND JARVIS, B. C.: Separation of nucleotides, nucleosides, purines, pyrimidines and some pyrimidine degradation products by two-dimensional paper chromatography. *J. Chromatog.*, 20 (1965) 624–626.

KOBATA, A. AND SUZUOKI-ZIRÒ: The acid-soluble nucleotides of milk. III. Occurrence of UDP-N-acetyllactosamine and UDP-D-xylose in pig's milk and colostrum. *Biochim. Biophys. Acta*, 107 (1965) 405–413.

LINGENS, F. AND SCHNEIDER-BERNLOEHR, H.: (Reaction of naturally occurring pyrimidine bases with hydrazine and methyl-substituted hydrazines). *Ann. Chem.*, 686 (1965) 134–144; *C.A.*, 63 (1965) 11559d.

TYNDALL, R. L., JACOBSON, K. B. AND TEETER, E.: (Characterization of HeLa cell and viral ribonucleic acid by oligonucleotide analysis). *Biochim. Biophys. Acta*, 108 (1965) 11–17.

22. ALKALOIDS

ALBONICO, S. M., KUCK, A. M. AND DEULOFEU, V.: (Isolation and oxidation of (+)-tembetarine from *Fagara naranjillo*). *Ann. Chem.*, 685 (1965) 200–206; *C.A.*, 63 (1965) 11634f.

CHALMERS, A. H., CULVENOR, C. C. J. AND SMITH, L. W.: Characterisation of pyrrolizidine alkaloids by gas, thin-layer and paper chromatography. *J. Chromatog.*, 20 (1965) 270–277 — PC and TLC for 58 alkaloids.

HAUTH, H. AND STAUFFACHER, D.: Die Alkaloide von *Crinum macrantherum* Engl. *Helv. Chim. Acta*, 47 (1964) 185–194.

HESSE, M., PHILIPSBORN, W. V., SCHUMANN, D., SPITELLER, G., SPITELLER-FRIEDMANN, M., TAYLOR, W. I., SCHMID, H. AND KARRER, P.: Die Strukturen von C-Fluorocurin, C-Mavacurin und Pleiocarpamin. *Helv. Chim. Acta*, 47 (1964) 878–911 — R_F values of 22 alkaloids.

MOREIRA, E. A.: (Identification of emetine in some species of the *Boreira* genus). *Bol. Univ. Parana, Farmacognosia*, No. 2 (1963) 1–57.

NIEZGODZKI, L. AND MANIKOWSKI, W.: (Determination of atropine sulfate in a mixture of drug in legal analysis). *Poznan. Towarz. Przyjaciol Nauk, Wydzial Lekar. Prace Komisji Farm.* 3 (1965) 149–154; *C.A.*, 63 (1965) 11253e.

SCHLIENTZ, W., BRUNNER, R., STADLER, P. A., FREY, A. J., OTT, H. AND HOFMANN, A.: Isolierung und Synthese des Ergostins, eines neuen Mutterkorn-Alkaloids. *Helv. Chim. Acta*, 47 (1964) 1921–1933.

23. OTHER SUBSTANCES CONTAINING HETEROCYCLIC NITROGEN

SAWICKI, E. AND PFAFF, J. D.: Analysis for aromatic compounds on paper and thin-layer chromatograms by spectrophotophosphorimetry. Application to air pollution. *Anal. Chim. Acta*, 32 (1965) 521–534 — PC and TLC.

24. ORGANIC SULPHUR COMPOUNDS

GAIND, K. N., DAR, R. N. AND POPLI, S. D.: Spectrophotometric estimation of alliin in *Allium* species. *Indian J. Pharm.*, 27 (1965) 199–200; *C.A.*, 63 (1965) 11255c.

KAPIŠINSKÁ, V. AND KOMPIŠOVÁ, A.: (Qualitative and quantitative estimation of sodium 5-nitro-2-chlorobenzenesulfonate in the presence of sodium 4-nitrodiphenylamine-2-sulphonate). *Chem. Průmysl*, 15 (1965) 33–34; *C.A.*, 63 (1965) 10688g.

QURESHI, M. AND KISHORE, M. N.: Effect of solvent composition on the separation of thiourea, thiocyanate and sulfide by paper chromatography. *Anal. Chem.*, 37 (1965) 1267–1268.

ZINNER, H. AND PESEKE, K.: Glykoside des Benzthiazolthions. *Chem. Ber.*, 98 (1965) 3508–3514.

25. ORGANIC PHOSPHORUS COMPOUNDS

HAY, J. B., ARCHIBALD, A. R. AND BADDILEY, J.: The molecular structure of bacterial walls. The size of ribitol teichoic acids and the nature of their linkage to glycosaminopeptides. *Biochem. J.*, 97 (1965) 723–730.

26. METALLO-ORGANIC COMPOUNDS

SCHLÖGL, K. AND FRIED, M.: Ferrocenasymmetrie. 1. Mitt. Darstellung und Racematspaltung von asymmetrischen Aminen und Aldehyden der Ferrocenreihe. *Monatsh. Chem.*, 95 (1964) 558–575.

27. VITAMINS

SCHOLES, P. B. AND KING, H. K.: Isolation of a naphthoquinone with partly hydrogenated side chain from *Corynebacterium diphtheriae*. *Biochem. J.*, 97 (1965) 766–768.

28. ANTIBIOTICS

BAUDET, P. AND CHERBULIEZ, E.: Sur la saramycétine (= X-5079C), polypeptide antibiotique de *Streptomyces saraceticus*. I. Premières données concernant sa structure. *Helv. Chim. Acta*, 47 (1964) 661–683.

BLINOV, N. O., MOROZOVA, G. R. AND KHOKHLOV, A. S.: (A comparison of coelicomycin with red-violet indicator antibiotics). *Antibiotiki*, 10 (1965) 717–722; *C.A.*, 63 (1965) 11253a.

CIBA, LTD.: (7-Aminocephalosporanic acid and its derivatives). *French Pat.*, 1,392,777 (Cl. C 07*d*), March 19 (1965); Swiss Appl. March 15 (1963); 21 pp.; *C.A.*, 63 (1965) 11580b.

COCKER, J. D., COWLEY, B. R., COX, J. S. G., EARDLEY, S., GREGORY, G. I., LAZENBY, J. K., LONG, A. G., SLY, J. C. P. AND SOMERFIELD, G. A.: Cephalosporanic acids. II. Displacement of the acetoxygroup by nucleophiles. *J. Chem. Soc.*, (1965) 5015–5031.

IGLÓY, M., MIZSEI, A. AND HORVÁTH, I.: Paper chromatography of antibiotics of the macrolide group. *J. Chromatog.*, 20 (1965) 295–298.

PFIZER CORP.: 6-Methylenetetracyclines. *Brit. Pat.*, 995,031 (Cl. C 07*c*), June 10 (1965); U.S. Appl. May 23, June 29, Dec. 1, Dec. 19 (1960) and Febr. 6 (1961); 24 pp.; *C.A.*, 63 (1965) 11464e.

30. SYNTHETIC AND NATURAL DYES

STRAIN, H. H., SHERMA, J., BENTON, F. L. AND KATZ, J. J.: One-way paper chromatography of the chloroplast pigments of leaves. *Biochim. Biophys. Acta*, 109 (1965) 1–15.

STRAIN, H. H., SHERMA, J., BENTON, F. L. AND KATZ, J. J.: Two-way paper chromatography of the chloroplast pigments of leaves. *Biochim. Biophys. Acta*, 109 (1965) 16–22.

STRAIN, H. H., SHERMA, J., BENTON, F. L. AND KATZ, J. J.: Radial paper chromatography and columnar chromatography of the chloroplast pigments of leaves. *Biochim. Biophys. Acta*, 109 (1965) 23–32.

33. INORGANIC SUBSTANCES

BLASIUS, E. AND KRÄMER, R.: Gegenüberstellung der Hochspannungspapierionophorese und der Papierchromatographie bei der Untersuchung einiger Reaktionen von Schwefelverbindungen. *J. Chromatog.*, 20 (1965) 367–373.

KNOCH, W. AND LAHR, H.: Chromatographic separation of Am–Cm on paper. *Radiochim. Acta*, 4 (1965) 114; *C.A.*, 63 (1965) 10651b.

LAGOWSKI, J. M.: Reaction of sulfate ion with the HANES-ISHERWOOD reagent. *J. Chromatog.*, 20 (1965) 420–421.

SAKODINSKY, K. AND LEDERER, M.: The chromatographic properties of paper impregnated with titanium hydroxide. *J. Chromatog.*, 20 (1965) 358–366.

VEN HORST, SISTER, H., ROGERS, M. F. AND SINGH, H. T.: Effect of pH in precipitation chromatography. *Anal. Chem.*, 37 (1965) 1279–1280.

35. MISCELLANEOUS COMPOUNDS AND COMPLEX MIXTURES

MUND, C. AND VENNER, H.: Einfache Methode zur Bausteinanalyse von Polysacchariden, Proteinen und deren Komplexen. *Z. Physiol. Chem.*, 338 (1964) 145–153.

WINTER, M. AND WILLHALM, B.: Recherches sur les arômes. 10. Sur l'arôme des fraises fraîches. Analyse des composés carbonylés, esters et alcohols volatils. *Helv. Chim. Acta*, 47 (1964) 1215–1227.

Thin-layer Chromatography

1. REVIEWS AND BOOKS

GRUENE, A.: (Thin-layer chromatography on cellulose plate). *Riechstoffe, Aromen, Koerperpflegemittel*, 15, No. 6 (1965) 219–223; *C.A.*, 63 (1965) 10652f.

SHOSTENKO, YU. V. AND RACHINSKII, V. V.: (N.A. Ismailov and M.S. Shraiber — inventors of thin-layer chromatographic method). *Zh. Fiz. Khim.*, 39 (1965) 1802–1803.

2. FUNDAMENTALS, THEORY AND GENERAL

ERLENMEYER, H. AND BARTELS, H.: Über das Problem der Ähnlichkeit in der Chemie. Dünnschichtchromatographie mit spezifisch adsorbierenden Silikagelen. I. *Helv. Chim. Acta*, 47 (1964) 46–51.

ERLENMEYER, H. AND BARTELS, H.: Über das Problem der Ähnlichkeit in der Chemie. Über spezifisch adsorbierende Silikagele. II. *Helv. Chim. Acta*, 47 (1964) 1285–1288.

3. TECHNIQUES I

HONEGGER, C. G.: Die Anwendung von Aktivitätsgradienten in der Dünnschichtchromatographie. *Helv. Chim. Acta*, 47 (1964) 2384–2387.

INGLIS, G. R.: Dimethyl sulphoxide as stationary phase in thin-layer partition chromatography on silica gel. *J. Chromatog.*, 20 (1965) 417–419.

JACKSON, R.: Color recording of ultraviolet fluorescence in thin-layer chromatograms. *J. Chromatog.*, 20 (1965) 410–413.

LITT, G. J. AND JOHL, R. G.: A simple apparatus for circular thin-layer chromatography. *J. Chromatog.*, 20 (1965) 605–606.

LUDWIG, E.: (Application of inorganic phosphorescent materials to thin-layer chromatography). *Z. Chem.*, 5 (1965) 186–187; *C.A.*, 63 (1965) 10652a.

PATAKI, G.: Neuere Untersuchungen zur Reproduzierbarkeit der R_F-Werte in der Dünnschichtchromatographie. *Helv. Chim. Acta*, 47 (1964) 784–787.

PRZYBYLOWICZ, E. P., STAUDENMAYER, W. J., PERRY, E. S., BAITSHOLTS, A. D. AND TISCHER, T. N.: Precoated sheets for thin-layer chromatography. *J. Chromatog.*, 20 (1965) 506–513.

SAWICKI, E., STANLEY, T. W. AND ELBERT, W. C.: Direct fluorometric scanning of thin-layer chromatograms and its application to air pollution studies. *J. Chromatog.*, 20 (1965) 348–353.

WARREN, B.: A gradient spreader for thin-layer chromatography. *J. Chromatog.*, 20 (1965) 603–605.

4. TECHNIQUES II

HALPAAP, H.: Grundzüge der präparativen Schichtchromatographie. *Chemiker-Ztg.*, 89 (1965) 835–849.

5. HYDROCARBONS AND HALOGEN DERIVATIVES

GORSHKOVA, G. N., KNYAZEVA, M. S. AND MAIOROV, N. S.: (Spectral and chromatographic analysis of some pure polycyclic compounds by the method of zone melting). *Khim. Pererabotka Smol, Akad. Nauk SSSR, Inst. Goryuch. Iskop.*, (1965) 103–108; *C.A.*, 63 (1965) 10753h.

6. ALCOHOLS

KOCH, H., KOTLAN, J. AND MOHAR, H.: Beiträge zur Chemie des Cyclopentadiens. 2. Mitt. Zur Einwirkung von Perameisensäure auf Cyclopentadien. *Monatsh. Chem.*, 95 (1964) 1257–1265.

7. PHENOLS

FISHBEIN, L. AND FAWKES, J.: The detection and thin-layer chromatography of 3,4-methylenedioxyphenyl- and 3,4-methylenedioxybenzyl carbamates. *J. Chromatog.*, 20 (1965) 521–527 — 43 compounds; comparison between glass-supported DF-5 and Eastman chromagrams.

HOPWOOD, W. J. AND STOCK, J. A.: The benzylation of resorcinol. *J. Chem. Soc.*, (1965) 4972–4975.

TAKACS, F.: Über die Einwirkung von Bleitetraacetat auf Phenole. 11. Mitt. Darstellung neuer *o*- und *p*-Benzochinolacetate und *o*-Chinondiacetate. *Monatsh. Chem.*, 95 (1964) 961–977.

9. OXO COMPOUNDS

BARNARD, J. A. AND IBBERSON, V. J.: Thin-layer chromatography of tarry deposits resulting from the oxidation of aromatic hydrocarbons. *J. Chromatog.*, 20 (1965) 404–405.

BLANC, P., BERTRAND, P., DE SAQUI-SANNES, G. AND LESCURE, R.: (Thin-layer chromatographic separation of vanillin and ethylvanillin). *Chim. Anal.* (*Paris*), 47 (1965) 354–356; *C.A.*, 63 (1965) 10651e.

BYRNE, G. A.: The separation of 2,4-dinitrophenylhydrazones by thin layer chromatography. *J. Chromatog.*, 20 (1965) 528–540 — R_F values for 41 compounds.

LONGO, R.: (Separation and determination of the hydroxy anthraquinones of frangula). *Boll. Chim. Farm.*, 104 (1965) 369–372; *C.A.*, 63 (1965) 11251b.

MOES, A.: (Qualitative investigation of the hydrolysis products of *Senna officinalis* leaflets, by thin-layer chromatography). *J. Pharm. Belg.*, 19, No. 3–4 (1964) 173–189; *C.A.*, 63 (1965) 11248g.

ZAYED, S. M. A. D. AND FAKHR, I. M. I.: Höhere Fettaldehyde und ihre Umsetzung mit 4'-Nitroazobenzol-4-carbonsäurehydrazid. *Monatsh. Chem.*, 95 (1964) 535–540.

10. CARBOHYDRATES

PIFFERI, P. G.: Improved thin layer chromatographic separation of hexoses and pentose using Kieselgel G. *Anal. Chem.*, 37 (1965) 925.

11. ORGANIC ACIDS AND LIPIDS

11a. Organic acids

BENMAMAN, J. D. AND SORBY, D. L.: Procedures for characterization of the degradation products of methyl and ethyl *p*-hydroxybenzoate by thin-layer chromatography. *J. Chromatog.*, 20 (1965) 607–610.

KOLESIŃSKA, J., URBAŃSKI, T. AND WIELOPOLSKI, A.: (Thin-layer chromatography of benzenecarboxylic acids. I). *Chem. Anal.* (*Warsaw*), 10 (1965) 1107–1112 — R_F values of 13 compounds.

LUNT, G. G. AND ROWE, C. E.: Changes in lipid characteristics in brain tissue at 0°. *Biochem. J.*, 97 (1965) 38P–39P.

NUGTEREN, D. H.: The enzymic chain elongation of fatty acids by rat-liver microsomes. *Biochim. Biophys. Acta*, 106 (1965) 280–290.

PIEKACZ, H.: (Determination of dibutyl and dioctyl phthalates in articles of common use, made of plastics; test on migration rates of those compounds into food. I. Detection and study of migration rates into model fluid systems). *Roczniki Panstwowego Zakladu Hig.*, 16 (1965) 281–288; *C.A.*, 63 (1965) 11779f.

RONKAINEN, P.: The separation and staining of methyl esters of the 2,4-dinitrophenylhydrazones of keto acids on thin layers of silica gel. *J. Chromatog.*, 20 (1965) 403–404.

11b. Lipids and their constituents

DITTMER, J.: The identification of cerebroside sulfate as a component of the inositol phosphatide fraction of ox brain. *Biochim. Biophys. Acta*, 106 (1965) 425–427.

MANZOLI, F. A. AND CARINCI, P.: (Thin-layer chromatography of hepatic lipids from chicken embryo). *Boll. Soc. Ital. Biol. Sper.*, 40 (1965) 1283–1286.

MICHALEC, Č.: Biochemistry of sphingolipids. II. Thin-layer chromatography of phytosphingosines. *J. Chromatog.*, 20 (1965) 594–595.

MORGAN, T. E., FINLEY, T. N. AND FIALKOW, H.: Comparison of the composition and surface activity of "alveolar" and whole lung lipids in the dog. *Biochim. Biophys. Acta*, 106 (1965) 403–413.

MULDER, E. AND VAN DEENEN, L. L. M.: Metabolism of red-cell lipids. III. Pathways for phospholipid renewal. *Biochim. Biophys. Acta*, 106 (1965) 348–356.

NICHOLS, B. W.: Light induced changes in the lipids of *Chlorella vulgaris*. *Biochim. Biophys. Acta*, 106 (1965) 274–279.

PENICK, R. J. AND MCCLUER, R. H.: Evidence for a second trisialo ganglioside. *Biochim. Biophys. Acta*, 106 (1965) 435–438.

SKIPSKI, V. P., SMOLOWE, A. F., SULLIVAN, R. C. AND BARCLAY, M.: Separation of lipid classes by thin-layer chromatography. *Biochim. Biophys. Acta*, 106 (1965) 386–396.

VAN DEN BOSCH, H. AND VAN DEENEN, L. L. M.: Chemical structure and biochemical significance of lysolecithins from rat liver. *Biochim. Biophys. Acta*, 106 (1965) 326–337.

VAVER, V. A., PROKAZOVA, N. V., USHAKOV, A. N., POPKOVA, G. A. AND BERGELSON, L. D.: (Chemistry of lipids. V. Derivatives of diols as new kinds of neutral lipids). *Khim. Prirodn. Soedin, Akad. Nauk Uz. SSR*, (1965) 401–409.

13. STEROIDS

CLIFFORD, C. J., WILKINSON, J. V. AND WRAGG, J. S.: Some aspects of the use of thin-layer chromatography in a limit test for related steroids. *J. Pharm. Pharmacol.*, 16, Suppl. (1964) 11T–16T — R_F values of 24 steroids.

CURTIS, P. J.: The transformation and resolution of 8-aza steroids by fungi. *Biochem. J.*, 97 (1965) 148–150.
DITULLIO, N. W., JACOBS, Jr., C. S. AND HOLMES, W. L.: Thin-layer chromatography and identification of free sterols. *J. Chromatog.*, 20 (1965) 354–357.
FILLMANN, B., KNUPPEN, R., LISBOA, B. P. AND BREUER, H.: Stoffwechsel und Aromatisierung von 7-substituierten C_{19}-Steroiden in der Placenta. *Z. Physiol. Chem.*, 338 (1964) 88–99 — TLC and PC.
HALL, A.: Thin-layer chromatography of corticosteroids. *J. Pharm. Pharmacol.*, 16, Suppl. (1964) 9T–10T — R_F values for 28 corticosteroids.
KENT, J. R. AND RAWITCH, A. B.: Thin-layer chromatography of ketosteroid-dinitrophenylhydrazones. *J. Chromatog.*, 20 (1965) 614–615.
LUUKKAINEN, T. AND ADLERCREUTZ, H.: Isolation and identification of 11-dehydro-estradiol-17α, a new type of urinary steroid, in the urine of pregnant women. *Biochim. Biophys. Acta*, 107 (1965) 579–592.
SNATZKE, G.: (19-Nor-5β-methyl steroids. III. Acetolyses of 3-methoxy steroids). *Ann. Chem.*, 686 (1965) 167–181; *C.A.*, 63 (1965) 11653g.
STAINIER, R.: (Application of thin-layer chromatography for the separation of certain keto steroids). *J. Pharm. Belg.*, 20, No. 3–4 (1965) 89–110; *C.A.*, 63 (1965) 11250b.

14. STEROID GLYCOSIDES AND SAPONINS

ZURKOWSKA, J. AND OZAROWSKI, A.: (Cardenolide glycosides. XII. Quantitative thin-layer chromatography on talc of mixtures of lanatosides A,B,C and D). *Acta Polon. Pharm.*, 22 (1965) 83–85; *C.A.*, 63 (1965) 11685h.

15. TERPENE DERIVATIVES

ATTAWAY, J. A., BARABAS, L. J. AND WOLFORD, R. W.: Analysis of terpene hydrocarbons by thin layer chromatography. *Anal. Chem.*, 37 (1965) 1289–1290 — R_F values of 53 terpenes.
DEMOLE, E.: Sur la structure des terpénylphénols obtenus en condensant le camphène avec le phénol, et celle de leurs dérivés hydrogénés à odeur boisée. *Helv. Chim. Acta*, 47 (1964) 319–338.
KAPADI, A. H., SOBTI, R. R. AND DEV, S.: Diterpenoids of *Erythroxylum monogynum*. V. Atisirene, isoatisirene and devadarene. *Tetrahedron Letters*, (1965) 2729–2735; *C.A.*, 63 (1965) 11622b.
MEHLITZ, A. AND MINAS, T.: (Bitter orange peel oil. II. Terpenoids (oxygen-containing compounds)). *Riechstoffe, Aromen, Koerperpflegemittel*, 15 (1965) 204–205, 207, 209 and 211; *C.A.*, 63 (1965) 11243g — TLC and PC.
SCHANTZ, M. v., JUVONEN, S. AND HEMMING, R.: Trennung von Monoterpenen mittels Silbernitrat–Kieselgeldünnschichten. *J. Chromatog.*, 20 (1965) 618–620.

16. NITRO AND NITROSO COMPOUNDS

HANSSON, J.: (Thin-layer chromatographic analysis for the separation of common explosives). *Explosivstoffe*, 11 (1963) 73–77; *C.A.*, 63 (1965) 11237b.
KOHLBECK, J. A.: Determination of nitroglycerin and resorcinol in double-base propellant following separation by thin-layer chromatography. *Anal. Chem.*, 37 (1965) 1282–1283.

17. AMINES, AMIDES AND RELATED NITROGEN COMPOUNDS

GRITTER, R. J.: The separation of diazonium salts by thin-layer chromatography. *J. Chromatog.*, 20 (1965) 416–417.
RINK, M. AND GEHL, A.: Quantitative Bestimmung von Harnstoff im Harn. Spektralphotometrische Bestimmung nach dünnschichtchromatographischer Abtrennung auf Celluloseschichten. *J. Chromatog.*, 20 (1965) 415.

18. AMINO ACIDS

CAMERONI, R., BERNABEI, M. T., FERIOLI, V. AND ALBASINI, A.: (Properties of benzothiadiazine derivatives of amino acids). *Gazz. Chim. Ital.*, 95 (1965) 786–795; *C.A.*, 63 (1965) 11687c.
CHERBULIEZ, E., BAEHLER, B. AND RABINOWITZ, J.: Étude de structures peptidiques à l'aide du phénylisothiocyanate. VI. Chromatographie sur couche mince de phénylthiohydantoïnes dérivées d'acides aminés. *Helv. Chim. Acta*, 47 (1964) 1350–1353.
MICHL, H. AND BACHMAYER, H.: Über freie Aminosäuren im Gifte der Gelbbauchunke *Bombina variegata* L. *Monatsh. Chem.*, 95 (1964) 480–484.
MUNIER, R. L. AND SARRAZIN, G.: Séparation de l'ensemble des dinitrophénylamino-acides éthéro-solubles par chromatographie à deux dimensions et des DNP-amino-acides hydro-acido-solubles par chromato-électrophorèse sur couche mince de poudre de cellulose. *Bull. Soc. Chim. France*, (1965) 2959–2961.

PATAKI, G. AND KELLER, M.: Aminosäuren und Peptide im Urin. *Helv. Chim. Acta*, 47 (1964) 787–789 — TLC of DNP-derivatives.

19. PEPTIDES; CHEMICAL STRUCTURE OF PROTEINS AND PEPTIDES

COLE, M., FLETCHER, J. C. AND ROBSON, A.: The separation of 1-dimethylaminonaphthalene-5-sulphonamido acids (DNS-amino acids) by thin-layer chromatography. *J. Chromatog.*, 20 (1965) 616–618.

DEYL, Z. AND ROSMUS, J.: Thin-layer chromatography of Dansyl amino acid derivatives. *J. Chromatog.*, 20 (1965) 514–520.

PATAKI, G.: Untersuchungen über die Reaktion von Peptiden mit Phenylisothiocyanat. Anwendung der Dünnschichtchromatographie zur Sequenzanalyse von Peptiden. *Helv. Chim. Acta*, 47 (1964) 1763–1765.

21. PURINES, PYRIMIDINES, NUCLEOSIDES, NUCLEOTIDES, NUCLEIC ACIDS, BARBITURATES

LOMAKINA, T. S., GUSKOVA, L. I. AND GRINEVA, N. I.: (Identification, separation and quantitative analysis of nucleoside and nucleotide derivatives by thin-layer chromatography on cellulose). *Khim. Prirodn. Soedin, Akad. Nauk Uz. SSR*, (1965) 335–342 — R_F values of 28 compounds.

22. ALKALOIDS

FRENCH, W. N. AND WEHRLI, A.: Thin-layer chromatography of ergot alkaloids in pharmaceutical preparations. *J. Pharm. Sci.*, 54 (1965) 1515–1516.

JOULE, J. A., OHASHI, M., GILBERT, B. AND DJERASSI, C.: Alkaloid studies. LIII. Structures of nine new alkaloids from *Aspidosperma dasycarpon*. *Tetrahedron*, 21 (1965) 1717–1734; *C.A.*, 63 (1965) 11641h.

KAISER, H., BIEDEBACH, F. AND MANNS, G.: (Atropine sulfate tablets and the use of thin-layer chromatography for purity tests. Communication from the working group of the federal apothecary chamber). *Pharm. Ztg.*, 108 (1963) 1380–1381; *C.A.*, 63 (1965) 11253f.

KUMP, W. G., PATEL, M. B., ROWSON, J. M. AND SCHMID, H.: Indolalkaloide aus den Blättern von *Pleiocarpa pycnantha* (K. Schum.) Stapf, var. *tubicina* (Stapf) Pichon. *Helv. Chim. Acta*, 47 (1964) 1497–1503 — R_F values of 11 alkaloids.

PAULUS, W., GOENECHEA, S. AND WIENERT, V.: (Identification of some ephedrine derivatives by thin-layer chromatography). *Arch. Kriminol.*, 135, No. 3–4 (1965) 84–89; *C.A.*, 63 (1965) 11257b.

STAUFFACHER, D.: Über Buxamin, Nor-buxamin und Buxaminol, neue Alkaloide aus *Buxus sempervirens* L. *Helv. Chim. Acta*, 47 (1964) 968–981.

23. OTHER SUBSTANCES CONTAINING HETEROCYCLIC NITROGEN

GOLD-AUBERT, P., MELKONIAN, D. AND TORIBIO, L.: Synthèse de nouvelles phényl-1-triazoline-1,2,4-ones-5 substituées en 3 et 4. *Helv. Chim. Acta*, 47 (1964) 2068–2071.

KULICKA, J., GREGOROWICZ, Z. AND KARMIŃSKI, W.: (Thin-layer chromatographic analysis of some pyridine derivatives). *Chem. Anal. (Warsaw)*, 10 (1965) 1347–1351.

PETROWITZ, H. J.: (Thin-layer chromatography of heterocyclic nitrogen compounds). *Chimia (Aarau)*, 19 (1965) 426; *C.A.*, 63 (1965) 10652d.

STRÁNSKÝ, Z. AND STUŽKA, V.: Synthese von Derivaten des 2-Hydroxy-9-dimethylamino-9H-benzo(*a*)phenoxazoniumchlorids. *Monatsh. Chem.*, 95 (1964) 1555–1560 — TLC and PC.

24. ORGANIC SULPHUR COMPOUNDS

FOGG, A. G. AND WOOD, R.: Thin-layer chromatography on zinc ferrocyanide. *J. Chromatog.*, 20 (1965) 613–614.

KORCZAK-FABIERKIEWICZ, C., KOFOED, J. AND LUCAS, G. H. W.: The sulfoxides as an additional rapid test of phenothiazine tranquilizers. *J. Forensic Sci.*, 10 (1965) 308–312; *C.A.*, 63 (1965) 11257h.

KOZUKA, H. AND ISONA, H.: Identification of phenothiazine tranquilizers by thin-layer chromatography. *Kagaku Keisatsu Kenkyusho Hokoku*, 17 (1964) 353–356; *C.A.*, 63 (1965) 11257g.

LIN, Y.-T., WANG, K.-T. AND YANG, T.-I.: Polyamide layer chromatography of sulfonamides. *J. Chromatog.*, 20 (1965) 610–612.

25. ORGANIC PHOSPHORUS COMPOUNDS

BEREI, K.: Radio thin-layer chromatography of some aromatic phosphorus and arsenic compounds. *J. Chromatog.*, 20 (1965) 406–407.

BEREI, K.: (Analysis of some organic compounds of P and As by thin-layer chromatography). *Kozlemen.*, 13 (1965) 49–53; *C.A.*, 63 (1965) 10651e.
NEUBERT, G.: Zur Dünnschichtchromatographie von Organophosphiten. *J. Chromatog.*, 20 (1965) 342–347.

26. METALLO-ORGANIC COMPOUNDS

MAZUR, H.: (Determination of selected stabilizers of poly(vinyl chloride) in plastic household articles; test on migration rates with special reference to organotin compounds. I. Detection of dibutyl and dioctyl organotin stabilizers and tests on their migration into model solvents). *Roczniki Panstwowego Zakladu Hig.*, 16 (1965) 275–280; *C.A.*, 63 (1965) 11779b.

27. VITAMINS

CASTREN, E.: (Thin-layer chromatography of vitamins A, D and E in multivitamin preparations). *Anales Farm. Hosp.*, 8, No. 15 (1965) 31–35; *C.A.*, 63 (1965) 11254f.
PASALIS, J. AND BELL, N. H.: Separation of vitamin D esters by thin-layer chromatography. *J. Chromatog.*, 20 (1965) 407–409.

28. ANTIBIOTICS

CIBA, LTD.: (Preparation of 7-aminocephalosporanic acid derivatives). *French Pat.*, Addn. 84,689 (Cl. A 61*h*, C 01*d*), March 26 (1965); Swiss. Appl. Nov. 12 (1962) 13 pp.; *C.A.*, 63 (1965) 11580h.
RUCZAJ, D.: (The thin-layer chromatography of erythromycin and of its decomposition products). *Chem. Anal. (Warsaw)*, 10 (1965) 1317–1320.
RUSTICI, L. AND FERAPPI, M.: (Separation of anhydro derivatives of tetracycline by circular thin-layer chromatography). *Boll. Chim. Farm.*, 104 (1965) 305–308; *C.A.*, 63 (1965) 11249b.

29. INSECTICIDES AND OTHER PESTICIDES

BARNEY, II, J. E.: New spray tests for detecting organophosphorus compounds on thin-layer chromatograms. *J. Chromatog.*, 20 (1965) 334–341.

32. PHARMACEUTICAL AND FORENSIC APPLICATIONS; METABOLISM OF DRUGS

HEYNDRICKX, A., SCHAUVLIEGE, M. AND BLOMME, A.: Photometry and thin-layer chromatography of three tranquilizer N-unsubstituted carbamates. *J. Pharm. Belg.*, 20, Nos. 3–4 (1965) 117–130; *C.A.*, 63 (1965) 11256a.

33. INORGANIC SUBSTANCES

HAYASHI, K. AND OGATA, T.: Thin-layer chromatography of tellurium, selenium and chromium. *Japan Analyst*, 14 (1965) 1146–1149.
HOLZAPFEL, H., LAN, L. V. AND WERNER, G.: Trennung der seltenen Erden durch flüssigen Ionenaustausch. III. Dünnschichtchromatographische Trennung der seltenen Erden. *J. Chromatog.*, 20 (1965) 580–584.
PESCHKE, W.: Beitrag zur Dünnschicht-Chromatographie der Anionen von Halogensauerstoffsäuren. Trennung der Halogenite, Halogenate und Perhalogenate auf Kieselgel-, sowie modifizierten Kieselgur-Aluminium-Oxid-Schichten. *J. Chromatog.*, 20 (1965) 572–579.
SEILER, H. AND ERLENMEYER, H.: Anorganische Dünnschichtchromatographie. 9. Dünnschichtchromatographische Trennung von Sulfaten und Polythionaten. *Helv. Chim. Acta*, 47 (1964) 264–266.

34. RADIOACTIVE AND OTHER ISOTOPIC COMPOUNDS

ERISMANN, K. H.: Hilfsmittel zum Arbeiten mit radioaktiven Substanzen in der Dünnschichtchromatographie. *J. Chromatog.*, 20 (1965) 600–602.

35. MISCELLANEOUS COMPOUNDS AND COMPLEX MIXTURES

ZAHN, H. AND KUSCH, P.: Cyclo-tetrakis-hexamethylenadipinamid. *Chem. Ber.*, 98 (1965) 2588–2595.

Gas Chromatography

1. REVIEWS AND BOOKS

FABIAN, L. W.: Application of gas chromatography in anesthesia. *Clin. Anesthesia*, No. 2 (1964) 146–160.

GÄUMANN, T.: Gas chromatography. *Ann. Rev. Phys. Chem.*, 16 (1965) 125–146.

GUDZINOWICZ, B. J.: *The Analysis of Pesticides, Herbicides, and Related Compounds using the Electron Affinity Detector*, Jarrell-Ash Co., Waltham, Mass., 1965, pp. 7 + 306 — contents: flame ionisation and electron affinity detectors, retention data, correlation of molecular structure and electron capture ability, pesticide extraction and cleanup procedures, references.

LIPSETT, M. B. (Editor): *Gas Chromatography of Steroids in Biological Fluids*, Plenum Press, New York, N.Y., 1965, pp. 19 + 315 — a collection of papers read at Warrenton proceedings (Feb. 25–27, 1965), Virginia; analyses of androgens, corticoids, progestins, and estrogens are presented.

PRESTON, Jr., S. T. AND GILL, M.: Bibliography and index to the literature on gas chromatography–1965. November 1, 1964 to November 1, 1965. *J. Gas Chromatog.*, 3 (1965) 399–440 — 1818 papers registered with complete titles and references; index with 43 items.

2. FUNDAMENTALS, THEORY AND GENERAL

2b. Gas–solid systems

GILMER, H. B. AND KOBAYASHI, R.: The study of gas–solid equilibrium at high pressures by gas chromatography. Part I. Ethane, propane, and *n*-butane at essentially infinite dilution in the methane–silica gel system. *J. Am. Inst. Chem. Engrs.*, 10 (1964) 797–803.

GILMER, H. B. AND KOBAYASHI, R.: The study of multicomponent gas–solid equilibrium at high pressures by gas chromatography. Part II. Generalisation of the theory and application to the methane–propane–silica gel system. *J. Am. Inst. Chem. Engrs.*, 11 (1965) 702–705.

POSHKUS, D. P.: (Theoretical calculation of retention volumes of some unpolar simple gases and vapours on graphitised carbon black). *Zh. Fiz. Khim.*, 39 (1965) 2962–2967 — calculation of the absolute value of the retention volume has been achieved in terms of the virial form of the adsorption isotherms and of the potential function of molecular interaction gas–solid surface.

2c. Thermodynamics and theoretical relationships

DEWAR, M. J. S. AND SCHROEDER, J. P.: Liquid crystals as solvents. Part. 2. Further studies of liquid crystals as stationary phases in gas–liquid chromatography. *J. Org. Chem.*, 30 (1965) 3485–3490 — smectic phases of liquid crystals are more effective than nematic phases for separating aromatic position isomers; the recommended compound n-$C_7H_{15}O \cdot C_6H_4 \cdot COO \cdot C_6H_4 \cdot O \cdot n$-$C_7H_{15}$ has a smectic range of 83–125° and a nematic one of 125–206°.

HAARHOFF, P. C. AND VAN DER LINDE, H. J.: Pressure changes in gas chromatography. *Anal. Chem.*, 37 (1965) 1742–1745 — theoretical explanation in terms of viscosity and flow rate variations.

HORLICK, G., HARRIS, W. E. AND HABGOOD, H. W.: Effects of temperature on carrier-gas flow rates in packed and capillary columns. *Anal. Chem.*, 38 (1966) 7–9 — flow rates (He, H_2, N_2 at 25–200°; packed and capillary columns of metal and glass) at constant temperature may be taken as proportional to $(1/T)^{1.7}$ for packed metal columns and capillary columns of either metal or glass, and as proportional to $(1/T)^{1.8}$ for packed glass columns.

LITTLEWOOD, A. B.: Relative contributions of molecular diffusion and anastomosis in gas chromatographic column. *Anal. Chem.*, 38 (1966) 2–7 — the flow-dependent anastomotic parameter is appreciable at flow rates faster than ~ 20 cm·sec^{-1} in hydrogen and ~ 5 cm·sec^{-1} in nitrogen.

ŠINGLIAR, M., DYKYJ, J., REPÁŠ, M. AND LUKAČOVIČ, L.: Analysis and identification of propylene dimers by gas–liquid chromatography. *Collection Czech. Chem. Commun.*, 31 (1966) 233–242 retention data of hexenes were calculated by MARTIN's procedure (*Anal. Chem.*, 33 (1961) 1143) and compared with experimental results.

3. TECHNIQUES I

3a. Detectors

ABBOTT, D. C. AND DE FAUBERT MAUNDER, M. J.: A simple electron capture detector for the gas chromatographic determination of pesticides. *Chem. Ind. (London)*, (1965) 82–83.

BEVAN, S. C. AND THORBURN, S.: Absolute mass integral detector for gas chromatography. *Chem. Brit.*, 1 (1965) 206–208 — a gravimetric device coupled with sensitive (10^{-7} g) electrobalance.

CAHN, L.: Gravimetric detection. New technique in chromatography. *Res. Develop. Mag.*, 15, No. 10 (1964) 34–37.

CLEMONS, C. A. AND ALTSHULLER, A. P.: Response of electron-capture detector to halogenated substances. *Anal. Chem.*, 38 (1966) 133–136 — responses of 35 freons and fluorinated sulphur compounds.

GRAIFF, L. B.: Hydrogen "singing"-flame ionisation detector. *Nature*, 203 (1964) 856 — flames are in continuous oscillation; flame surface area increases and ion current fluctuates at ~ 1600 c/s; a.c. amplifier can be used.

KEBBEKUS, B. B.: Mixed carrier-gases and the thermal conductivity cell: theory and application to quantitative gas chromatography. *Dissertation Abstr.*, 26 (1965) 661.

3b. Column performance and filling studies

KUSÝ, V.: Effect of solid support and liquid phase on the accuracy of quantitative gas chromatography. *Anal. Chem.*, 37 (1965) 1748–1752 — a nonpolar substance influences the rate of dissolution of a polar substance in the liquid phase, making the approach of polar substances to the solid support much easier and causing quantitative changes.

SÁNCHEZ, L. G.: (Silicones as the stationary phase in the analysis of mixtures of diphenyl and diphenyl oxide by gas chromatography). *Anales Real Soc. Espan. Fis. Quim. (Madrid), Ser. B*, 61 (1965) 993–998.

SCOTT, R. P. W.: Pressure changes in gas chromatography. *Anal. Chem.*, 37 (1965) 1765 — an apparatus for measuring the pressure pulse described in the discussion of the paper by HAARHOFF AND VAN DER LINDE, (*Anal. Chem.*, 37 (1965) 1742).

VAN BERGE, P. C. AND PRETORIUS, V.: The use of gas chromatography for the determination of certain column parameters. *J. S. African Chem. Inst.*, 17 (1964) 133–137.

3c. Apparatus, accessories and materials for GC

BARNES, J. R. AND PARDUE, H. L.: Direct-reading, response-compensating integrator for chromatography. *Anal. Chem.*, 38 (1966) 156–158 — functional and circuit layout given.

KREJČÍ, M. AND JANÍČEK, M.: (Apparatus for measuring surface area by means of the thermal desorption method). *Chem. Listy*, 60 (1966) 93–94 — application of the NELSON-EGGERTSEN technique (*Anal. Chem.*, 30 (1958) 1387).

MONAHAN, C. M. AND PURCHASE, I. F. H.: Gasholding apparatus for use in gas chromatography. *Brit. J. Anaesthesia*, 37 (1965) 210–211 — for 15 ml gas samples on Hg.

SIEGEL, A.: Manifold for the evaporation of chromatographic fractions. *Chemist-Analyst*, 54 (1965) 120–121.

4. TECHNIQUES II

4d. Special microtechniques

DRUSHEL, H. V. AND SOMMERS, A. L.: Combination of gas chromatography with fluorescence and phosphorescence in analysis of petroleum fractions. *Anal. Chem.*, 38 (1966) 10–19 — for S- and N-compounds; phosphorescence, including decay characteristics, provided more information than fluorescence in the study of N-compounds.

EMMETT, P. H.: Adsorption tracers, and microcatalytic–chromatographic techniques. *Anales Real. Soc. Espan. Fis. Quim. (Madrid), Ser. B*, 61 (1965) 195–215.

WILLIAMS, I. H.: Gas chromatographic technique for the identification of low concentrations of atmospheric pollutants. *Anal. Chem.*, 37 (1965) 1723–1732 — a concentration technique based on trapping in an inert substrate at —80°.

4e. Automation

CLEVETT, K. J.: On-line application of vapour-phase chromatography to process plant. *Instr. Rev.*, 12 (1964) 312–315.

IMBER, J. AND GRAY, T. A.: Chromatograph spurs gas-recovery saving. *Chem. Process (Chicago)*, 28 (1965) 116–117.

KUSÝ, V. AND PROCHÁZKA, V.: Erfahrungen mit automatischen Betriebschromatographen für die Analyse von flüssigen Kohlenwasserstoffen. *Brennstoff-Chem.*, 46 (1965) 411–415 — a discussion of errors is given.

4f. Measurement of physico-chemical and related values

BORGSTEDT, H. H. AND GILLIES, A.: Determination of the solubility of nitrous oxide in water by gas chromatography. *Anesthesiology*, 26 (1965) 675–678.

GENKIN, A. N. AND BOGUSLAVSKAYA, B. I.: (Determination of stability constants of C_4–C_5 hydrocarbon — silver nitrate complexes by means of gas chromatography). *Neftekhimiya*, 5 (1965) 897–901.

KNOZINGER, H. AND SPANNHEIMER, H.: Bestimmung der Adsorptionsenthalpie mittels Gaschromatographie. II. *Z. Physik. Chem. (N.F.)*, 45 (1965) 117–126.

5. HYDROCARBONS AND HALOGEN DERIVATIVES

5a. Gaseous hydrocarbons

BLAKEMORE, G. AND HILLMAN, G. E.: Comprehensive fuel-gas analysis by gas chromatography. *Analyst*, 90 (1965) 703–714 — many detailed designs and circuit schemes are given.

JEFFREY, D. A. AND KIRCHNER, J. F.: The direct analysis of the gaseous products from the radiolysis of liquid hydrocarbons. *Intern. J. Appl. Radiation Isotopes*, 16 (1965) 58–60 — on molecular sieve 5A and/or Ucon Polar.

LAASBERG, L. H. AND ETSTEN, B. E.: Gas chromatographic analysis of cyclopropane in whole blood. *Anesthesiology*, 26 (1965) 216–222.

5b. Other hydrocarbons

BALENKOVA, E. E., KHAFIZOVA, N. A. AND KHROMOV, S. I.: (Conversion of *cis*-8-methylhydrindane in the presence of platinum on carbon). *Neftekhimiya*, 5 (1965) 797–800.

DEMAIO, L. AND CORN, M.: Gas chromatographic analysis of polynuclear aromatic hydrocarbons with packed columns. Application to air pollution studies. *Anal. Chem.*, 38 (1966) 131–133 — on Apiezon L and SE-30 by PTGC 220–268° or 240–280°.

GARCÍA, M. P.: (Analysis of polyphenyls by gas chromatography. Part I. Relative performance of biphenyl and terphenyl with respect to triphenylmethane). *Anales Real. Soc. Espan. Fis. Quim. (Madrid), Ser. B*, 61 (1965) 999–1008.

GREEN, J. W., SMITHSON, L. H., SPENCER, C. F. AND TEETER, R. M.: Analysis of alpha olefins using a gas chromatograph–mass spectrometer combination. *J. Am. Oil Chemists' Soc.*, 42, No. 3 (1965) 114A.

JACOBS, E. S.: Rapid gas chromatographic determination of C_1 to C_{10} hydrocarbons in automatic exhaust gas. *Anal. Chem.*, 38 (1966) 43–48 — by PTGC $-55°$ to $+140°$; retention data of 85 hydrocarbons on DC-200.

JOHNSTON, G. W., HOCH, W. S. AND BUTZ, W. C.: Recovery of hydrocarbons from lung tissue in fatal ingestion of furniture polish. Characterization by gas chromatography and infrared spectroscopy. *Am. J. Clin. Pathol.*, 43 (1965) 570–574.

LUDWIG, F. J.: Analysis of microcrystalline waxes by gas–liquid chromatography. *Anal. Chem.*, 37 (1965) 1732–1737 — C_{25}–C_{68} on SE-52 by PTGC.

MARKOVETS, L., GALA, S. AND LANDA, S.: (Isolation of the main components of technical diisobutylene and determination of their structure). *Neftekhimiya*, 5 (1965) 835–844 — retention times of some octenes on 7:8-benzoquinoline at 70° or Apiezon L at 100°.

OTTMERS, D. M., SAY, G. R. AND RASE, H. F.: Low-retention-time gas chromatographic analysis of pentene isomers. *Anal. Chem.*, 38 (1966) 148–149 — on PPG-550 at 25° in 15 min.

TAKAMYA, N. AND SAITO, T.: (Gas chromatography). *Internat. Combust. Engine*, 4, No. 3 (1965) 49–54.

VOROB'EVA, N. S. AND PETROV, A. A.: (Isomerisation of the bicyclo-[3,3,0]-octane (pentalane) series of hydrocarbons in the presence of aluminium bromide). *Neftekhimiya*, 5 (1965) 801–808 — retention data on E-301 at 60°.

5c. Halogen derivatives of hydrocarbons

GERMAN, I. A. AND CIOT, N.: (Gas-chromatographic analysis of chlorinated methane and ethane compounds). *Rev. Chim. (Bucharest)*, 16 (1965) 382–384.

6. ALCOHOLS

CHESUNOV, M. AND MAKAROV-ZEMLYANSKI, YA. YA.: (Gas–liquid chromatography of aqueous solute mixtures). *Nauchn. Tr. Mosk. Tekhnol. Inst. Legkoi Prom.*, No. 31 (1965) 95–97 — on paraffin + PEG.

DRAWERT, F. AND RAPP, A.: Gas-chromatographische Untersuchung der Qualität von Spirituosen. *Z. Lebensm. Untersuch. Forsch.*, 126 (1965) 405–409.

FREIDIGER, J. B. AND WIGNAU, J. A.: Determination of alcohols in body fluids by gas–liquid chromatography. *J. Forensic Sci.*, 10 (1965) 73–76.

FRIEDMAN, S. AND KAUFMAN, M. L.: Preparation of trimethylsilyl ethers of tertiary alcohols. *Anal. Chem.*, 38 (1966) 144–145 — three methods useful for GC separation of alcohols are given.

GALETTO, W. G., KEPNER, R. E. AND WEBB, A. D.: Gas chromatographic analysis of some alcohol 3,5-dinitrobenzoates with application to analysis of acetals. *Anal. Chem.*, 38 (1965) 34–36 — retention data of 23 derivatives on SE-30 at 250°.

NINOMIYA, T., TAKADA, M. AND HASHI, K.: (Gas-chromatographic determination of ethanol in pharmaceutical preparations). *Arch. Pract. Pharm.*, 24 (1964) 114–116 — on Triton X-100 with acetone as internal standard.

7. PHENOLS

ERMAN, W. F. AND LYNESS, W. I.: The isolation, purification, and structure determination of a phenolic acid fraction from slash pine bark. *Tappi*, 48 (1965) 249–256.

9. OXO COMPOUNDS

FURUYA, T., SHIBATA, S. AND IIZUKA, H.: Gas–liquid chromatography of anthraquinones. *J. Chromatog.*, 21 (1966) 116–118 — retention data of 18 compounds as methyl or trimethylsilyl ethers on SE-30 at 240°.

GUDZINOWICZ, B. J. AND JOHNSON, K. A.: Gas chromatographic determination of 2,3-diketones at nanogram concentrations using electron affinity detector. *Anal. Chem.*, 37 (1965) 1745–1747 — 0.1–0.2 nanogram; a discussion of the structure and electron-capturing ability of molecules.

11. ORGANIC ACIDS AND LIPIDS

11a. Organic acids

AAFJES, J. H.: Volatile fatty acid (V.F.A.) in blood of cows with ketosis. *Life Sci.*, 3 (1964) 1327–1334.

ACKMAN, R. G.: Structural correlation of unsaturated fatty acid esters through graphical comparison of gas liquid chromatographic retention times on a polyester substrate. *J. Am. Oil. Chemists' Soc.*, 40 (1963) 558–564.

BAILEY, A. V., PONS, Jr., W. A. AND SKAU, E. L.: Cyclopropenoid fatty acid content and fatty acid composition of crude cottonseed oils from successive solvent extractions. *J. Am. Oil Chemists' Soc.*, 42 (1965) 173–174.

BRUUN, H. H. AND PENSAR, G.: Studies on components in wood. 3. Gas chromatographic separation of resin acids of the pimaric acid type. *Acta Chem. Scand.*, 19 (1965) 531–533.

CAISANO, A. AND CARIBOLDI, L.: Gas chromatographic examination of the fatty acids in coffee oil. *J. Sci. Food. Agr.*, 15 (1964) 619–622.

GELLERMAN, J. L. AND SCHLENK, H.: Pyrolysis for structure determination of cyclopropene and cyclopropene fatty acids. *Anal. Chem.*, 38 (1966) 72–76 — on β-cyclodextrin valerate.

GODDIJN, J. P., VAN PRAAG, M. AND HARDON, H. J.: Quantitative gas-chromatographische Bestimmung der Benzoesäure, deren Derivate und der Sorbinsäure. *Z. Lebensm. Untersuch. Forsch.*, 123 (1963) 300–305.

HINDIN, E., MAY, D. S., MCDONALD, R. AND DUNSTAN, G. H.: Analysis of volatile fatty acids in sewage by gas chromatography. *Water Sewage Works*, 111 (1964) 92–95.

MCKEOWN, C. C. AND READ, S. I.: Esterification and gas chromatography of some acids of the tricarboxylic acid cycle. *Anal. Chem.*, 37 (1965) 1780–1781 — methyl esters on LAC-728 by PTGC 100–180°, 3.3°/min.

NICKERSON, G. B. AND LIKENS, S. T.: Gas chromatographic evidence for the occurrence of hop oil components in beer. *J. Chromatog.*, 21 (1966) 1–5 — procedure of separation given; methyl dec-4-enoate and deca-4,8-dienoate were identified.

PENSAR, G. AND BRUUN, H. H.: Response of flame ionisation detector in the gas-chromatography of methyl esters of resin acids (abietic and pimaric series). *Suomen Kemistilehti*, 38B (1965) 223–225.

VASIL'EV, N. I., GETMANSKAYA, Z. I., KORNILOVA, L. A. AND GERASSIMOVA, N. T.: (γ-Lactones in paraffin oxidation products). *Neftekhimiya*, 5 (1965) 887–891 — retention data of C_5–C_{11} lactones on silicone grease at 150°.

11b. Lipids and their constituents

ACHAYA, K. T., CRAIG, B. M. AND YONGS, C. G. J.: The component fatty acids and glycerides of castor oil. *J. Am. Oil Chemists' Soc.*, 41 (1964) 783–784.

EISNER, J., IVERSON, J. L., MOZINGO, A. K. AND FIRESTONE, D.: Gas chromatography of unsaponifiable matter. III. Identification of hydrocarbons, aliphatic alcohols, tocopherols, triterpenoid alcohols, and sterols present in olive oils. *J. Assoc. Offic. Agr. Chemists*, 48 (1965) 417–433.

GARVER, R. C. AND SWEELEY, C. C.: Methods for methanolysis of sphingolipids and direct determination of long-chain bases by gas chromatography. *J. Am. Oil Chemists' Soc.*, 42 (1965) 294–298.

GENOVA, R.: (The lipemic picture in some lipidic thesaurismoses. Gas chromatographic investigation). *Minerva Pediat.*, 16 (1964) 1517–1525.

GRIECO, D. AND PIEPOLI, G.: Composition of fatty acids obtained in the lipids extracted from oil seeds and fruits. *Riv. Ital. Sostanze Grasse*, 41 (1964) 283–287 — on PEGS at 213°.
O'BRIEN, J. S. AND SAMPSON, E. L.: Fatty acid and fatty aldehyde composition of the major brain lipids in normal human grey matter, white matter, and myelin. *J. Lipid. Res.*, 6 (1965) 545–551.
PATTON, S. AND MCCARTY, R. D.: Conversion of alcohol to ethyl esters of fatty acids by the lactating goat. *Nature*, 209 (1966) 161–167.

13. STEROIDS

BERRETT, C. R. AND MCNEIL, C.: Fractionation of urinary 17-ketosteroids by gas liquid chromatography. *Clin. Chem.*, 11 (1965) 810.
CARLSON, I. H., MEYER, R. K. AND BLAIR, A.: The analysis of progestational compounds by gas chromatography. *J. Clin. Res.*, 12 (1964) 351.
FRANCE, J. T., RIVERA, R., MCNIVEN, N. L. AND DORFMAN, R. I.: (Determination of androsterone, etiocholanolone, and dehydroepiandrosterone in urine by gas–liquid chromatography). *Steroids*, 5 (1965) 687–697.
FRAVOLINI, A. AND BEGLIOMINI, A.: (Gas chromatographic determination of diethylstilbestrol in animal feeds). *Igiene Sanità Pubblica (Rome)*, 20 (1964) 436–443.
KANAI, M.: (Gas chromatographic separation of sterols and its clinical application). *J. Biochem.*, 56 (1964) 266–272.
LURIE, A. O. AND VILLEE, C. A.: Sebum: A common contaminant of samples for gas–liquid chromatography. *J. Chromatog.*, 21 (1966) 113–115 — the retention time of steroidal components (progesterone) varies in a different manner than the common sebum peaks on XE-60 at 210–240°.
PANICUCCI, F. AND TAPONECO, G.: (Gas-chromatographic determination of 17-ketosteroids of the plasma). *Boll. Soc. Ital. Biol. Sper.*, 41 (1965) 874–875.
ROZANSKI, A.: Gas chromatographic determination of campesterol, β-sitosterol and stigmasterol. *Anal. Chem.*, 38 (1966) 36–40 — as trimethylsilyl derivatives on SE-30 at 230°.

15. TERPENE DERIVATIVES

DI GIACOMO, A. AND RISPOLI, G.: (Argentinian lemon essential oils. I. General notices and analysis of terpene constituents). *Riv. Ital. Essenze-Profumi-Plante Offic.-Aromi-Saponi-Cosmet.*, 46 (1964) 381–386.
HUNTER, G. L. K. AND BROGDEN, Jr., W. B.: Analysis of the terpene and sesquiterpene hydrocarbons in some citrus oils. *J. Food Sci.*, 30 (1965) 383–387.
KOGAMI, K., AOKI, K., AISAKA, T., KHTSEGAVA, T. AND KUMANOTANI, D.: (Analysis of methol stereoisomers by gas–liquid capillary chromatography by use of flame ionisation detector). *J. Japan Oil Chemists' Soc.*, 14 (1965) 129–130 — on PEG 4000, Reoplex 400 and LAC 2R-446 at 130°.

16. NITRO AND NITROSO COMPOUNDS

MÉSURE, A. D. AND TILLETT, J. G.: Electron capture analysis of dinitrobenzene isomers. *J. Gas Chromatog.*, 3 (1965) 448 — complete separation of all three isomers on Versamid 90 at 130°.

18. AMINO ACIDS

DARBRE, A. AND BLAU, K.: Trifluoroacetylated amino acid esters: The stability of the derivatives of cysteine, hydroxyproline, serine, threonine and tyrosine. *Biochim. Biophys. Acta*, 100 (1965) 298–300.
HALPERN, B. AND WESTLEY, J. W.: High sensitive optical resolution of D,L-amino acids by gas chromatography. *Biochem. Biophys. Res. Commun.*, 19 (1965) 361–363.

23. OTHER SUBSTANCES CONTAINING HETEROCYCLIC NITROGEN

DRUSHEL, H. V. AND SOMMERS, A. L.: Isolation and identification of nitrogen compounds in petroleum. *Anal. Chem.*, 38 (1966) 19–28 — a separation scheme for isolation is given; GC on SE-30; with one exception, only American references are given.

24. ORGANIC SULPHUR COMPOUNDS

COLEMAN, H. J., THOMPSON, C. J., HOPKINS, R. L. AND RALL, H. T.: Identification of thiols in a Wasson, Texas, crude oil distillate boiling from 110° to 150°. *J. Chem. Eng. Data*, 10 (1965) 80–84.

26. METALLO-ORGANIC COMPOUNDS

FRANC, J. AND MIKEŠ, F.: Micro-determination of Si-H bonds by gas chromatography. *Collection Czech. Chem. Commun.*, 31 (1966) 363–367 — through determination of hydrogen after the reaction $\equiv SiH + H_2O \rightarrow \equiv SiOH + H_2$; sensitivity 0.005 % $C_2H_5SiHCl_2$, errors ± 6.5 % for 0.1 % content.

NAGAI, T. AND MATSUDA, T.: (Gas-chromatographic analysis of methyl borate). *J. Chem. Soc. Japan, Pure Chem. Sect.*, 86 (1965) 965–966.

SOULAGES, N. L.: Simultaneous determination of lead alkyls and halide scavengers in gasoline by gas chromatography with flame ionization detection. *Anal. Chem.*, 38 (1966) 28–33 — GC separation on PEG 400; conversion of $Pb(alkyl)_4$ and CH_2X_2 in effluent on Ni-catalyst to CH_4 or C_2H_6, GC separation on charcoal, and detection of CH_4 and C_2H_6 with FID.

28. ANTIBIOTICS

KAWAI, S. AND HASHIBA, S.: (Gas chromatographic separation of organic acids produced by hydrolysis of penicillins. Analysis of antibiotics by gas chromatography). *Japan Analyst*, 13 (1964) 1223–1226.

29. INSECTICIDES AND OTHER PESTICIDES

BEYNON, K. I.: The positive identification of pesticide residues. *Proc. Soc. Anal. Chem.*, 2 (1965) 180–182 — a critical review including IR and mass spectroscopy, GLC and combination of GLC with other methods.

BOONE, G. H.: Determination of dibrom and DDVP residues in some fruit and vegetable crops by microcoulometric gas chromatography. *J. Assoc. Offic. Agr. Chemists*, 48 (1965) 748–752.

BOWMAN, M. C. AND BEROZA, M.: Analysis of Imidan colorimetrically and by electron-affinity gas chromatography. *J. Assoc. Offic. Agr. Chemists*, 48 (1965) 922–926.

BOWMAN, M. C., SCHECHTER, M. S. AND CARTER, R. L.: Behavior of chlorinated insecticides in a broad spectrum of soil types. *J. Agr. Food Chem.*, 13 (1965) 360–365.

BURKE, J. A.: Gas chromatography of pesticide residues; some practical aspects. *J. Assoc. Offic. Agr. Chemists*, 48 (1965) 1037–1058.

ELGAR, K. E.: Pesticide residues: The analysis of pesticide residues. *Proc. Soc. Anal. Chem.*, 2 (1965) 178–179.

EL-REFAI, A. R. AND GIUFFRIDA, L.: Separation and microquantitative determination of Dipterex and DDVP by gas liquid chromatography. *J. Assoc. Offic. Agr. Chemists*, 48 (1965) 374–379.

KANAZAWA, J.: (Determination of diazinon in water-dispersible powder concentrates by gas–liquid chromatography). *Japan Analyst*, 14 (1965) 20–23.

KANAZAWA, J. AND KAWAHARA, T.: (Determination of O,O-dimethyl 2,2-dichlorovinyl phosphate in emulsifiable concentrates by gas–liquid chromatography). *Japan Analyst*, 13 (1964) 1269–1271.

LANGLOIS, B. E., STEMP, A. R. AND LISKA, B. J.: Analysis of animal-food products for chlorinated insecticide residues. I. Column clean-up of samples for electron-capture gas-chromatographic analysis. *J. Milk Food Technol.*, 27 (1964) 202–204 — on Dow 11 silicone at 185–195°.

ZIELINSKI, JR., W. L. AND FISHBEIN, L.: Gas chromatography of 3,4-methylenedioxyphenyl derivatives. *Anal. Chem.*, 38 (1966) 41–43 — retention data on Carbowax 20M at 160° and QF-1 at 100°.

30. SYNTHETIC AND NATURAL DYES

HARKISS, K. J.: Characterisation of plant pigments by gas chromatography. *Nature*, 205 (1965) 78–79.

31. PLASTICS AND THEIR INTERMEDIATES

JANKOWSKI, S. J. AND GARNER, P.: Determination of carboxylic acids present as esters in plasticizers and polymers by transesterification and gas chromatography. *Anal. Chem.*, 37 (1965) 1709–1711 — a sodium methoxide–methanol–methyl acetate reagent quantitatively converts esters into methyl esters without any influence on free acids.

33. INORGANIC SUBSTANCES

33a. Permanent and rare gases

HIBBITS, J. O.: Gas chromatographic determination of helium in neutron-irradiated beryllium oxide. *Talanta*, 13 (1966) 151–153 — BeO is dissolved in H_2SO_4–H_3PO_4 in the argon atmosphere and chromatographed on a molecular sieve 5A column.

HISSEL, J.: Micro-determination of hydrogen in water for boilers. *Tribune CEBEDEAU (Centre Belge Etude Doc. Eaux)*, 16 (1963) 60–69.

KECKI, Z.: Determination of radiation yields of gaseous products by gas chromatography. *Nucl. Sci. Abstr.*, 19, No. 1 (1965) 50, Abstr. No. 338.

PARK, K. AND CATALFOMO, M.: Gas chromatographic determination of dissolved oxygen in the sea water. *Deep-Sea Res.*, 11 (1964) 917–920 — O_2 is stripped by argon on a molecular sieve 5A column; errors $\pm 1.4\%$.

33b. Volatile inorganic compounds

BERK, E.: Determination of fumigant gases by gas chromatography. *J. Agr. Food Chem.*, 13 (1965) 373–377.

JUVET, Jr., R. S. AND FISHER, R. L.: Gas–liquid chromatography of volatile metal fluorides. *Anal. Chem.*, 37 (1965) 1752–1755 — MoF_6, WF_6, TaF_5, NbF_5, SbF_5 and AsF_3 on Kel-F on Teflon support at 35° and 75° (Mo, W, As) and 125–185° (Ta, Nb, Sb).

34. RADIOACTIVE AND OTHER ISOTOPIC COMPOUNDS

EGGLETON, A. E. J. AND ATKINS, D. H. F.: The identification of trace quantities of radioactive iodine compounds by gas chromatographic and effusion methods. *Radiochim. Acta*, 3 (1964) 151–158.

GERSH, M. E.: Improved separation of isotopic hydrogens by gas chromatography. *Anal. Chem.*, 37 (1965) 1786 — HT and DT separation on Al_2O_3 coated with Fe_2O_3 at 77°K; 97% He + 3% H_2 carrier gas and flow proportional counter.

LIEBENBERG, D. H.: Quantitative analysis of helium-3 by gas chromatography. *Anal. Chem.*, 38 (1966) 149–150.

MUHS, M. A., BASTIN, E. L. AND GORDON, B. E.: Purities of some commercially available ^{14}C-labelled hydrocarbons by gas–liquid radiochromatography. *Intern. J. Appl. Radiation Isotopes*, 16 (1965) 537–544.

35. MISCELLANEOUS COMPOUNDS AND COMPLEX MIXTURES

IGUCHI, S., YAMAMOTO, M. AND AOYAMA, T.: (Studies on medicinal preparations by gas chromatography. II. Detection of preservatives in preparations). *Arch. Pract. Pharm.*, 24 (1964) 41–47 — oxygenated compounds on Triton X-100 at 190°.

KOCH, J., SAJAK, E. AND SCHILLER, H.: Gaschromatographischer Nachweis von Undecalacton in Fruchtsaftlikören. *Mitteilungsbl. GDCh-Fachgr. Lebensmittelchem. Gerichtl. Chem.*, 18 (1964) 269–271 — retention data of oxygen-containing compounds of different structure on Carbowax 20M by PTGC 75–250°, 4.6°/min.

KVENVOLDEN, K. A.: Molecular distributions of normal fatty acids and paraffins in some lower cretaceous sediments. *Nature*, 209 (1966) 573–577 — analogy of compounds according to C-number has been indicated by means of GC.

NATELSON, S. AND STELLATE, R. L.: Instrumentation for the concentration of trace compounds of a mixture for gas chromatography; application to the determination of acetone, ethanol, methanol, and propan-2-ol in blood. *Microchem. J.*, 9 (1965) 245–256.

GAS CHROMATOGRAPHIC ANALYSIS OF HISTAMINE METABOLITES IN URINE

QUANTITATIVE DETERMINATION OF RING METHYLATED IMIDAZOLE-ACETIC ACIDS IN HEALTHY MAN

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One method of studying the endogenous production and release of histamine in man is to estimate the excretion of the amine and its metabolites in urine. Studies on urinary metabolites of subcutaneously administered ^{14}C-histamine have elucidated the catabolic pathways of the amine[1]. About 50 % of injected labelled histamine is excreted as 1-methylimidazole-4-acetic acid (1,4-MeImAA), about 30 % as imidazoleacetic acid, part of which is coupled with ribose, 5 % as 4-(2-aminoethyl)-1-methylimidazole (1,4-methylhistamine) and only about 2 % as unchanged histamine. These studies with labelled material unfortunately do not give any quantitative information about the endogenous production and liberation of histamine.

Several investigators have estimated the excretion of unchanged histamine in an attempt to study the endogenous liberation in physiological and pathological conditions (for a review see ref. 2). Since, however, most of the histamine is transformed into other products, this determination gives a poor indication of histamine production.

Imidazoleacetic acid can be estimated and it has been found to be a normal constituent of urine in man[3,4]. The urinary excretion of this acid is influenced by the histidine contents in food[4,5] and is thus not suitable for studies of the liberation and production of histamine. Methods for estimating the excretion of 1,4-methylhistamine have also been published[6,7].

In previous papers from our laboratory 1,4-MeImAA has been identified by means of gas chromatography as a normal constituent of human urine[8,9]. The metabolite has also been identified in urine with paper chromatographic methods[10]. Since 1,4-MeImAA, according to the isotopic experiments referred to above, is the main metabolite of histamine, it is very likely that the estimation of the excretion of this acid will give a good insight into the endogenous liberation and production of histamine. The present investigation deals with the 24 h excretion of 1,4-MeImAA in healthy human subjects.

In a previous investigation 1-methylimidazole-5-acetic acid (1,5-MeImAA) was shown to be a normal constituent of human urine[9]. The daily excretion of this isomer is also estimated in the present study in order to throw some light on its origin.

EXPERIMENTAL

Materials

For the materials used, see ref. 9.

Separation and esterification of imidazoleacetic acids in urine[9]

Urine was collected in bottles containing hydrochloric acid in amounts sufficient to bring the pH of the urine below 2. The urine was stored at —20° before analysis. An aliquot of urine corresponding to 40–100 mg of creatinine was concentrated *in vacuo* at 50° to 15–20 ml, the pH was brought to 8.8 by addition of 5 *N* NaOH and the volume adjusted to 25 ml. After centrifugation, the supernatant was applied on an ion exchange column, Dowex 1 × 10, 200–400 mesh, acetate form (prepared as in ref. 9), 400 mm × 16 mm i.d. The flow rate was held constant at 15 ml/h by a peristaltic pump at the outlet end of the column. The column was run at room temperature. After the urine had been adsorbed on the column, it was washed with 25 ml of water. Elution was then started with 0.5 *M* acetic acid, the effluent being collected in portions of 5 ml. The pH of the fractions dropped rather suddenly from 4–5 to less than 3. The three fractions immediately preceding this point and the seven consecutive fractions following it were combined in a 100 ml pear-shaped glass flask and freeze-dried. To the dry residue was added 15 ml of methanolic hydrogen chloride[9] and the flask was immediately fitted with a reflux condenser, dried at 150° before use and topped with a calcium chloride drying tube. The solution was then refluxed for 3 h at 90° in an oil bath. It was then cooled in an ice bath and neutralized by rapid addition of a 20% aqueous solution of sodium carbonate. It was evaporated *in vacuo* at 40° to dryness and the residue dissolved in 5 ml of phosphate buffer, *M*/15, pH 8.0. The solution was transferred to a continuous extraction apparatus, the glass flask was rinsed with another 5 ml of phosphate buffer, which was then added to the extraction tube. The solution was then extracted for 4 h with 40 ml of chloroform at 80°. The chloroform extract was collected in a 100 ml pear-shaped glass flask, 2.0 ml of an ethanolic solution of 1-methylimidazole-4-acetonitrile (0.1 mg/ml) was added as internal standard and the solution evaporated *in vacuo* at 40°. The residue was dissolved in 0.1–0.3 ml of methanol and stored at —20° prior to the gas chromatographic analysis.

Gas chromatography

Gas chromatographic analysis was performed with an F & M Model 400 apparatus equipped with a hydrogen flame ionization detection system. The columns consisted of 2.6 m or 3.4 m × 3.2 mm glass tubes, and contained 10% ethylene glycol adipate (EGA) on 100–120 mesh Gas Chrom P, silanized and coated with 1% polyvinylpyrrolidone. The preparation of the column, and the gas chromatographic conditions, were the same as desribed in a previous paper[9]. About 7 μl of the urine extract was injected with a Hamilton syringe. For quantitative determinations, the areas of the peaks representing 1-methylimidazole-4-acetonitrile, 1,4-MeImAA (methyl ester) and 1,5-MeImAA (methyl ester) were measured (height × width of the peak at half height). From the calibration curve (see below) the amount of methyl ester of 1,4-MeImAA and 1,5-MeImAA in the urine extract was determined. The corresponding amount of free acid was calculated, correction being made for losses

occurring during the procedure (the amount of methyl ester was multiplied by the factor (140 × 100)/(154 × 81) = 1.12; see Results and Discussion).

Synthesis of reference compounds

1-Methylimidazole-4-acetonitrile. This was synthesized according to PYMAN[11]. The picrate was converted to the free base by running through an ion exchanger, Dowex 1, converted to the OH^--form.

Methyl ester of 1-methylimidazole-4-acetic acid. 0.55 g of 1-methylimidazole-4-acetic acid hydrochloride was dissolved in a small amount of methanol and cooled to +4°. An ethereal solution of diazomethane[12,13] was added until the yellow colour persisted. After 15 min the excess of diazomethane was removed with a stream of nitrogen and the solution evaporated *in vacuo* at 40°. The residue was dissolved in a small amount of methanol and transferred to a microdestillation apparatus. The solvent was removed *in vacuo* and the residue then destilled at 0.05 mm Hg, bath temperature 105°. The destillate (0.15 g) consisted of a clear, oily, slightly yellow liquid.

Analysis, calculated for $C_7H_{10}N_2O_2$: C 54.5, H 6.54, N 18.17; found: C 53.6, H 7.10, N 18.01. The product proved to be gas chromatographically pure when run on two columns, EGA and F 60-Z[14]. The product also appeared as one distinct spot on thin-layer chromatograms (silica gel), developed with acetone–methanol–water (6:1:3) or in chloroform–methanol–acetic acid (12:6:1) and subsequently placed in a tank with iodine.

Methyl ester of 1-methylimidazole-5-acetic acid. This was prepared from 1-methylimidazole-5-acetic acid hydrochloride in the same way as described above, giving the ester as a clear, oily, slightly yellow liquid.

Analysis, calculated for $C_7H_{10}N_2O_2$: C 54.5, H 6.54, N 18.17; found: C 53.5, H 6.71, N 17.95. The product proved to be gas chromatographically pure when run on two different columns and appeared as one spot on thin-layer chromatograms as described above.

Preparation of calibration curve

To 2.0 ml portions of an ethanolic solution of 1-methylimidazole-4-acetonitrile (0.1 mg/ml) were added varying amounts of ethanolic solutions of the esters of 1,4-MeImAA and of 1,5-MeImAA. The solutions were evaporated *in vacuo* and the residue dissolved in 0.1–0.3 ml of methanol. This was then analyzed gas chromatographically as described above. The ratio of the areas of 1,4-MeImAA (methyl ester) and of 1,5-MeImAA (methyl ester) to that of 1-methylimidazole-4-acetonitrile was measured and a calibration curve constructed. A new calibration curve was prepared whenever a new solution of the internal standard was used or when a new gas chromatographic packing was used.

Estimation of creatinine in urine

This was done with a "Technicon Auto Analyzer"[15]. The method is a modification of the procedure of FOLIN AND WU[16].

RESULTS AND DISCUSSION

Reliability of the method

For quantitative gas chromatographic estimation of 1,4-MeImAA and 1,5-MeImAA it was found convenient to use an internal standard added to the urine extract[17,18]. This will correct for variations in concentration and volume of injected sample and for variations in instrumental response. The requirements for the internal standard are that it should separate completely on the gas chromatographic column from the methyl esters of 1,4-MeImAA and 1,5-MeImAA, but at the same time it should have a retention time within the same range as these compounds. Further it must not occur normally in the urine extract and must not be superimposed on other peaks in the chromatogram. Of several compounds, both imidazoles and others, which were tried as possible internal standards, 1-methylimidazole-4-acetonitrile was found to be the most suitable (Fig. 1). This compound gives a symmetrical peak on the EGA column at 175°. The retention time lies between those of the methyl esters of 1,4-MeImAA and 1,5-MeImAA. In order to get a complete separation from 1.5-MeImAA (methyl ester) it was necessary to use a longer column than that report-

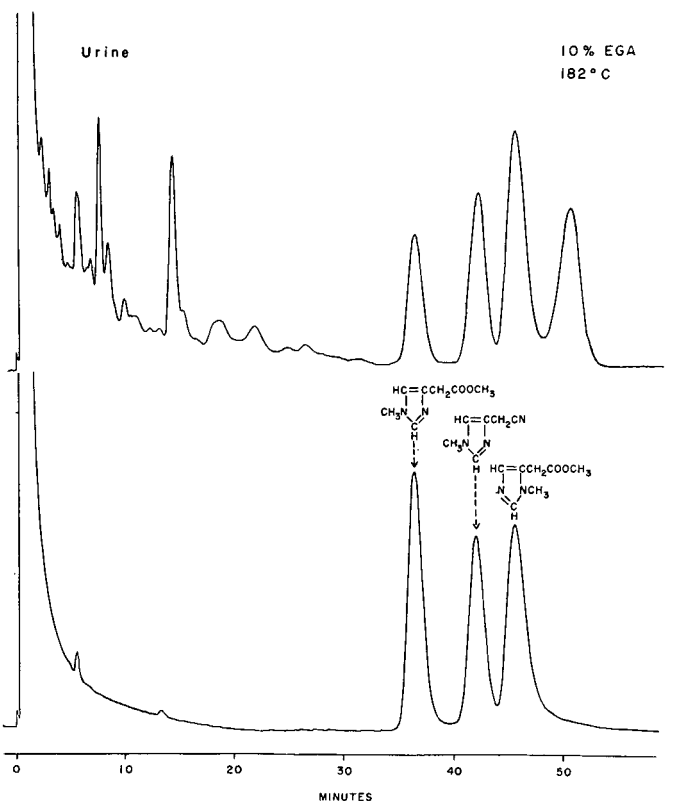

Fig. 1. Upper panel: gas chromatogram of a urine extract treated as described in the text. Internal standard (1-methylimidazole-4-acetonitrile) had been added. Lower panel: gas chromatogram of a mixture of the methyl esters of 1,4-MeImAA and 1,5-MeImAA and of the internal standard. Number of theoretical plates, calculated for 1-methylimidazole-4-acetonitrile: 3500.

ed in a previous publication[9]. 1-Methylimidazole-4-acetonitrile does not interfere with other peaks in the chromatogram, nor does it occur normally in urine extract.

The peaks of 1-methylimidazole-4-acetonitrile and of the methyl esters of 1,4-MeImAA and 1,5-MeImAA were symmetrical, and it was sufficient for quantitative determinations to calculate the product of the height and width of the peak at half height. A calibration curve was constructed as described above (Fig. 2). It is seen that the same amount of 1,4-MeImAA (methyl ester) and of 1,5-MeImAA (methyl ester) gave the same gas chromatographic response.

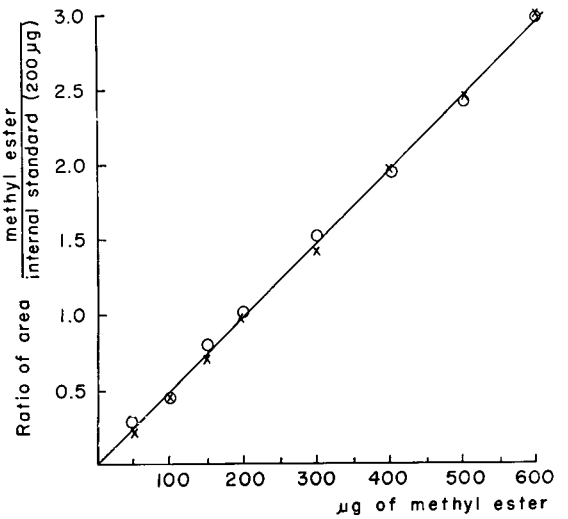

Fig. 2. Gas chromatographic calibration curve for the methyl esters of 1,4-MeImAA and 1,5-MeImAA (see text). (×) 1,4-MeImAA (methyl ester); (○) 1,5-MeImAA (methyl ester).

In order to estimate the losses during the ion exchange procedure, esterification and extraction, experiments were made with addition of known amounts of authentic 1,4-MeImAA and 1,5-MeImAA. In each experiment, two identical samples of urine were analyzed, to one of which a known amount of authentic 1,4-MeImAA and/or 1,5-MeImAA had been added. From the results of the analyses, the recovery of added acid was calculated (Tables I and II). The recovery of 1,4-MeImAA in 31 experiments was 81.6 ± 0.8 %. The recovery of 1,5-MeImAA in 12 experiments was 80.9 ± 1.8 %. No significant difference in the recovery, expressed as % of added authentic acid, was found between samples with a low and with a high content of the corresponding acids. Based upon these experiments, a value of 81 % recovery for both isomers was used throughout the investigation. This recovery is in good agreement with a previous investigation with labelled material[9], where labelled 1,4-MeImAA was added to a urine sample from which the imidazolic acids were subsequently separated and esterified almost exactly in the same way as in the present investigation. The recovery of added labelled acid was found to be about 80 %.

The reproducibility of the method was also investigated. From each of several different urines, 2–4 identical samples (corresponding to an adequate amount of creatinine) were analyzed in the usual way. The original amounts of 1,4-MeImAA and

TABLE I

RECOVERY OF 1,4-MeImAA

1,4-MeImAA found in urine sample without added authentic compound (μg)	*1,4-MeImAA found in urine sample with added authentic 1,4-MeImAA* (μg)	*Amount of added authentic 1,4-MeImAA* (μg)	*Recovery of added authentic 1,4-MeImAA* (%)
27	86	79	74.7
27	155	159	80.5
27	227	238	84.0
68	332	300	88.0
68	336	300	89.3
68	459	500	78.2
75	212	159	86.1
75	427	397	88.7
82	259	200	88.5
82	241	200	79.5
91	150	75	78.7
91	241	200	75.0
91	352	300	87.0
95	127	40	80.0
95	449	450	78.7
123	274	200	75.5
123	545	500	84.4
150	268	150	78.7
150	285	159	84.9
150	309	200	79.5
167	373	250	82.4
173	227	70	77.1
173	282	125	87.2
173	318	175	82.9
182	236	70	77.1
182	261	100	79.0
182	286	125	83.2
182	341	200	79.5
182	491	400	77.3
395	491	125	76.8
445	709	300	88.0
Mean (± standard error of the mean) 81.6 ± 0.8			

1.5-MeImAA in the samples were calculated, correction being made for losses occurring during separation and esterification. The standard deviation and the coefficient of variation were then calculated (Table III). The standard deviation was higher for 1,5-MeImAA than for 1,4-MeImAA. This probably depends on gas chromatographic factors, since in some gas chromatograms the peak corresponding to 1,5-MeImAA (methyl ester) was not completely separated from the following one, the identity of which is unknown. When this occurs, the estimation of the peak area is more uncertain. With some of the EGA-packings, the peak corresponding to 1,5-MeImAA (methyl ester) also showed a slight degree of tailing.

The 24 h excretion of 1,4-MeImAA and 1,5-MeImAA

The 24 h excretion of 1,4-MeImAA and 1,5-MeImAA by healthy individuals of either sex and of ages ranging from 19 to 65 years was determined (Table IV). Diets

TABLE II

RECOVERY OF 1,5-MeImAA

1,5-MeImAA found in urine sample without added authentic compound (μg)	*1,5-MeImAA found in urine sample with added authentic 1,5-MeImAA* (μg)	*Amount of added authentic 1,5-MeImAA* (μg)	*Recovery of added authentic 1,5-MeImAA* (%)
60	200	159	88.1
75	115	50	80.0
80	290	250	84.0
127	182	75	73.3
168	623	600	75.8
273	345	100	72.0
273	405	175	75.4
273	418	175	82.9
273	523	300	83.3
347	484	159	86.2
347	567	238	92.4
450	682	300	77.3

Mean (± standard error of the mean) 80.9 ± 1.8

TABLE III

ANALYSIS OF IDENTICAL SAMPLES

Experiment No.	*Amount of 1,4-MeImAA found in identical samples (values corrected for losses)* (μg)				*Standard deviation** s	*Coefficient of variation* $\frac{s \times 100}{M}$
1	79	92	92	81		
2	96	101	112	90		
3	162	162	148	146	8	4%
4	164	161	170	166		
5	197	177	184	194		
6	549	554				
	Amount of 1,5-MeImAA found in identical samples (values corrected for losses) (μg)					
1	28	43	44	38		
2	84	101	90			
3	106	103				
4	140	129				
5	171	134	151		16	9%
6	157	157				
7	207	179				
8	291	269				
9	392	354	403	352		

* s calculated according to ref. 35.

TABLE IV

EXCRETION OF 1,4-MeImAA AND 1,5-MeImAA BY HEALTHY SUBJECTS

Subject	*Age*	*24 h excretion of 1,4-MeImAA* (μg)	*μg of 1,4-MeImAA per mg of creatinine in urine*	*24 h excretion of 1,5-MeImAA* (μg)	*μg of 1,5-MeImAA per mg of creatinine in urine*
BH ♀	19	1210	1.5	700	0.9
BK ♀	19	1480	2.0	Not measurable*	
KE ♀	21	1120	1.2	Not measurable*	
MM ♀	22	3520	2.8	7580	6.1
LN ♀	22	2330	1.8	710	0.5
AL ♀	24	2420	2.2	1080	1.0
UT ♀	29	3920	2.0	4090	2.1
MH ♀	35	1570	1.6	4940	4.9
JG ♀	38	2360	1.8	1840	1.4
GN ♀	40	3550	2.8	7610	5.9
JK ♀	42	1390	1.0	3550	2.4
BP ♀	47	2100	1.2	3270	1.9
AB ♀	51	3100	2.4	2100	1.5
CH ♀	51	760	1.4	1390	2.5
IJ ♀	62	1460	1.1	560	0.4
EJ ♀	65	3030	1.5	3140	1.9
BF ♂	20	2060	1.7	9250	7.6
TH ♂	23	1560	1.7	11400	12.3
BP ♂	27	2220	1.9	2020	1.7
UA ♂	27	3020	1.7	2100	1.2
CH ♂	29	1790	1.9	Not measurable*	
KN ♂	30	4490	2.7	1110	0.7
RT ♂	30	4050	2.4	2430	1.4
TA ♂	30	1970	1.3	3720	2.5
JL ♂	34	4490	2.6	5610	3.3
RL ♂	35	4260	2.3	6730	3.6
BA ♂	38	3310	1.5	7520	3.5
EH ♂	42	4330	2.2	3080	1.6
GL ♂	44	4260	2.7	Not measurable*	
GK ♂	45	2020	2.0	1120	1.1
BH ♂	47	3610	1.6	1280	0.6
KP ♂	58	2200	1.5	1170	0.8
JA ♂	62	3100	1.4	8570	3.8
ES ♂	65	1930	1.5	810	0.6
Range		760–4490	1.0–2.8	0–11400	0–12.3
Mean ± standard error of the mean		2650 ± 190	1.9 ± 0.1	(3250)	(2.3)
Standard deviation		1100	0.5		

* "Not measurable" means amounts below 0.3 μg/mg of creatinine.

were uncontrolled. Since for various reasons collection of 24 h urine volume may be a rather inexact procedure, it was found convenient to correlate the excretion of the imidazolic acids to that of creatinine. This procedure is frequently used in studies of amino acid metabolism[19,20] and has also been used in gas chromatographic studies of phenolic acids in urine[21].

The mean 24 h excretion of 1,4-MeImAA by 34 healthy individuals was 2.65 mg

(0.76–4.49 mg; Table IV). The relation to the creatinine content of the urine was 1.9 μg (1.0–2.8 μg) per mg of creatinine. It is obvious that the excretion of 1,4-MeImAA by the examined subjects varied less when expressed in terms of creatinine ratios than when expressed in absolute amounts excreted per 24 h. No significant difference in the urinary excretion in men and women was observed, and the mean value was therefore calculated for both groups together.

GREEN *et al.* found that the 24 h excretion by healthy man of 1,4-methylhistamine, the metabolic precursor of 1,4-MeImAA, was 140–480 μg[6,7]. The molar ratio of the mean excretion of 1,4-methylhistamine to 1,4-MeImAA is thus about 1:8. This is in good agreement with experiments with labelled material. Upon injection of ^{14}C-histamine the ratio between excreted labelled 1,4-methylhistamine and 1,4-MeImAA is about 1:10[1,22].

The mean value of the daily excretion of unchanged histamine, reported by different investigators[4,7,23–29], varies from 9–49 μg. The molar ratio to the excretion of 1,4-MeImAA thus lies between 1:230 and 1:40. The ratio of ^{14}C-histamine to ^{14}C-1,4-MeImAA in urine upon injection of ^{14}C-histamine is about 1:17[1,22]. There are of course several possible explanations for this discrepancy; thus it might be possible that besides histamine, there are other precursors to 1,4-methylhistamine and 1,4-MeImAA. Another explanation may be that the excretion pattern of exogenous histamine is not the same as that of histamine from endogenous sources[30].

The 24 h excretion of 1,5-MeImAA was found to be very variable. The excretion by 34 healthy individuals varied from scarcely detectable amounts to 11.40 mg per 24 h (12.3 μg/mg of creatinine). The high degree of variation indicates that this compound may be of dietary origin. Other experiments on individuals fed parenterally give further evidence for this surmise[31]. It is unlikely that the excretion of 1,5-MeImAA reflects the endogenous liberation of histamine. One possible precursor of 1,5-MeImAA is α-amino-β-(1-methyl-5-imidazole)propionic acid (1-methylhistidine). This compound is a normal constituent of human urine[32,33] and has long been known to be a component of the dipeptide anserine[34]. It is notable that also the isomeric α-amino-β-(1-methyl-4-imidazole)propionic acid (3-methylhistidine) is excreted in human urine[33].

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SUMMARY

A gas chromatographic method for the identification of 1-methylimidazole-4-acetic acid and 1-methylimidazole-5-acetic acid in urine, previously described, has been modified to allow the quantitative estimation of the 24 h excretion of the acids by man. The mean excretion of the two acids by healthy individuals was determined. The excretion of 1-methylimidazole-4-acetic acid, expressed in terms of creatinine ratios, varied within a rather narrow range (1.0–2.8 μg/mg of creatinine). It seems

very likely that the estimation of this acid will give valuable information regarding the endogenous liberation and production of histamine. The daily excretion of the isomer, 1-methylimidazole-5-acetic acid was found to be very variable. The metabolic origin of this compound is discussed.

REFERENCES

1 R. W. SCHAYER AND J. A. D. COOPER, *J. Appl. Physiol.*, 9 (1956) 481.
2 H. DUNÉR AND B. PERNOW, *Acta Med. Scand.*, 168 (1960) 307.
3 A. HANSON, *Naturwiss.*, 44 (1957) 470.
4 H. DUNÉR, S. O. LILJEDAHL AND B. PERNOW, *Acta Physiol. Scand.*, 51 (1961) 41.
5 D. D. BROWN, O. L. SILVA, P. B. MCDONALD, S. H. SNYDER AND M. W. KIES, *J. Biol. Chem.*, 235 (1960) 154.
6 J. P. GREEN, D. H. FRAM AND N. KASE, *Nature*, 204 (1964) 1165.
7 D. H. FRAM AND J. P. GREEN, *J. Biol. Chem.*, 240 (1965) 2036.
8 R. THAM, *Life Sci.*, 4 (1965) 293.
9 R. THAM AND B. HOLMSTEDT, *J. Chromatog.*, 19 (1965) 286.
10 J. W. KERR, *Brit. Med. J.*, (1964 II) 606.
11 F. L. PYMAN, *J. Chem. Soc.*, 99 (1911) 2172.
12 A. F. MCKAY, *J. Am. Chem. Soc.*, 70 (1948) 1974.
13 C. M. WILLIAMS, *Anal. Biochem.*, 4 (1962) 423.
14 B. HOLMSTEDT, W. J. A. VANDENHEUVEL, W. L. GARDINER AND E. C. HORNING, *Anal. Biochem.*, 8 (1964) 151.
15 *Technicon Auto Analyzer, Methodology N 11 a*, Technicon Laboratories, Research Park, Chauncey, N.Y., 1963.
16 O. FOLIN AND H. WU, *J. Biol. Chem.*, 38 (1919) 81.
17 E. BROCHMANN-HANSSEN, *J. Pharm. Sci.*, 51 (1962) 1017.
18 W. J. A. VANDENHEUVEL AND E. C. HORNING, in H. A. SZYMANSKI (Editor), *Biomedical Applications of Gas Chromatography*, Plenum Press, New York, 1964, p. 89.
19 D. I. FOWLER, P. M. NORTON, M. W. CHEUNG AND E. L. PRATT, *Arch. Biochem. Biophys.*, 68 (1957) 452.
20 A. HUNTER, *Clin. Chim. Acta*, 12 (1965) 2.
21 C. M. WILLIAMS AND C. C. SWEELEY, in H. A. SZYMANSKI (Editor), *Biomedical Applications of Gas Chromatography*, Plenum Press, New York, 1964, p. 225.
22 K. NILSSON, S.-E. LINDELL, R. W. SCHAYER AND H. WESTLING, *Clin. Sci.*, 18 (1959) 313.
23 M. ROBERTS AND H. M. ADAM, *Brit. J. Pharmacol.*, 5 (1950) 526.
24 H. M. ADAM, R. B. HUNTER AND T. W. G. KINNEAR, *Quart. J. Exptl. Physiol.*, 36 (1950) 49.
25 R. G. MITCHELL, H. R. BUTT AND C. F. CODE, *J. Clin. Invest.*, 33 (1954) 1199.
26 H. DUNÉR AND B. PERNOW, *Scand. J. Clin. Lab. Invest.*, 8 (1956) 296.
27 J. A. OATES, E. MARSH AND A. SJOERDSMA, *Clin. Chim. Acta*, 7 (1962) 488.
28 H. T. GRAHAM, *Pharmacologist*, 5 (1963) 274.
29 G. N. BEALL, *Int. Arch. Allergy*, 26 (1965) 1.
30 A. V. FURANO AND J. P. GREEN, *Nature*, 199 (1963) 380.
31 R. THAM, to be published.
32 J. M. SEARLE, AND R. G. WESTALL, *Biochem. J.*, 48 (1951) PI.
33 H. H. TALLAN, W. H. STEIN AND S. MOORE, *J. Biol. Chem.*, 206 (1954) 825.
34 V. DU VIGNEAUD AND O. K. BEHRENS, *Ergeb. Physiol. Biol. Chem. Exptl. Pharmakol.*, 41 (1939) 917.
35 W. DIXON AND F. MASSEY, *Introduction to Statistical Analysis*, 2nd Ed., McGraw-Hill, New York, 1957.

ESSENTIAL OILS AND THEIR CONSTITUENTS

XXXII. GAS CHROMATOGRAPHY OF SESQUITERPENE HYDROCARBONS*

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INTRODUCTION

In contrast to numerous publications reporting systematically correlated gas chromatographic data for monoterpenes[2–4] no study of comparable magnitude has so far appeared in the literature concerning similar criteria of identity for sesquiterpene hydrocarbons, whose preparation and authentication are delicate and difficult operations. It is the purpose of this paper to present such data, to illustrate their value for the analysis of essential oils and related products and to interpret characteristic gas chromatographic parameters assembled for some of the isolates in terms of molecular structure.

EXPERIMENTAL

Apparatus and procedures

Apparatus and gas chromatographic methods were described previously[5]. In addition to Reoplex 400 (10 %) deposited on acid-washed chromosorb W, silicone rubber SE-30 (10 %) and silicone nitrile XE-60 (5 %) were used as substrate. Monoterpenes were examined at 100° and sesquiterpene hydrocarbons at 150°. Carrier gas (helium) flow rate was maintained at 75 (± 1) ml/min. Three stable aromatic hydrocarbons, namely naphthalene, 2-methylnaphthalene and acenaphthene, which are commercially available (purity 99 %), were used as internal standards.

Isolation of sesquiterpene hydrocarbons

Since these compounds are commercially unavailable, they were isolated from authenticated essential oils by careful rectification and column and/or preparative gas chromatography of specific fractions. Some of the products were courteously supplied by other researchers (see Table I). Their identity was in all instances confirmed by comparison of their infrared spectra with those of genuine reference standards or published data. Column chromatography was generally carried out using neutral grade I alumina (Woelm) (20 times the weight of the sample) as adsorbent and petroleum ether as eluant.

* For Part XXXI, see ref. 1.

TABLE I

ISOLATION OF SESQUITERPENE HYDROCARBONS

No.	*Compound*	*Source and method of isolation*
	Acyclic	
1	β-Farnesene	Farnesol. Column (neutral grade I alumina) and gas chromatography (silicone nitrile XE-60) of dehydration product[6]
	Monocyclic	
2	α-Bisabolene	Oil of opoponax. Column chromatography followed by gas chromatography. Liquid phase: silicone nitrile XE-60
3	β-Bisabolene	
4	γ-Bisabolene	
5	Curcumene	Oil of *Curcuma aromatica*. Column chromatography
6	β-Elemene	Oil of bois de rose. Fractional distillation, column chromatography of high-boiling fraction followed by gas chromatography. Liquid phase: Reoplex 400[7]
7	α-Humulene	Oil of *Zingiber zerumbet* Smith. Column chromatography followed by fractional distillation[8]
8	β-Humulene	Oil of copaiba balsam. Gas chromatography. Liquid phase: Reoplex 400[5]
9	α-Zingiberene	Oil of ginger. Column chromatography followed by gas chromatography. Liquid phase: silicone nitrile XE-60[9]
10	β-Zingiberene	
	Bicyclic	
11	α-Bergamotene	Courtesy Dr. R. B. BATES, University of Arizona, Tucson, Ariz., U.S.A.
12	β-Bergamotene	
13	α-Bulnesene	Oil of patchouli. Column chromatography followed by gas chromatography. Liquid phase: silicone nitrile XE-60
14	γ-Cadinene	Oil of ylang ylang, fraction III. Column chromatography, followed by gas chromatography. Liquid phase: silicone nitrile XE-60
15	δ-Cadinene	
16	Caryophyllene	Oil of copaiba balsam. Gas chromatography. Liquid phase: Reoplex 400[5]
17	Isocaryophyllene	Courtesy Dr. E. WARNHOFF, University of Western Ontario, London, Ont., Canada
18	α-Guaiene	Oil of patchouli. Column chromatography followed by gas chromatography of petroleum ether eluate. Liquid phase: silicone nitrile XE-60
19	α-Himachalene	Courtesy Dr. R. B. BATES, University of Arizona, Tucson, Ariz., U.S.A.
20	β-Himachalene	
21	β-Santalene	Oil of East Indian sandalwood. Column chromatography followed by gas chromatography. Liquid phase: silicone nitrile XE-60
22	*epi*-β-Santalene	
23	α-Selinene	Oil of bois de rose. Fractional distillation, column chromatography of high-boiling fraction followed by gas chromatography. Liquid phase: Reoplex 400[7]
24	β-Selinene	Oil of celery seed. Column chromatography
	Tricyclic	
25	Aromadendrene	Oil of *Eucalyptus globulus*. Column chromatography following removal of cineole by treatment with resorcinol[10]
26	Cedrene	Oil of red cedarwood. Gas chromatography. Liquid phase: Reoplex 400
27	α-Gurjunene	Oil of gurjun balsam. Column chromatography followed by gas chromatography. Liquid phase: Reoplex 400
28	β-Gurjunene	
29	Longifolene	Oil of *Pinus longifolia*. Fractional distillation followed by column chromatography
30	Isolongifolene	Courtesy Dr. S. DEV, National Chemical Laboratory, Poona, India

(*continued on p.* 219)

TABLE I (*continued*)

No.	*Compound*	*Source and method of isolation*
31	β-Patchoulene	Oil of patchouli. Column chromatography followed by gas chromatography. Liquid phase: silicone nitrile XE-60
32	α-Santalene	Oil of East Indian sandalwood. Column chromatography followed by gas chromatography. Liquid phase: silicone nitrile XE-60
33	Ylangene	Oil of ylang ylang, Fraction III. Column chromatography followed by gas chromatography. Liquid phase: silicone nitrile XE-60
	Tetracyclic	
34	Longicyclene	Courtesy Dr. S. DEV, National Chemical Laboratory, Poona, India

DISCUSSION

Performance of internal standards

Experimental data illustrating the gas chromatographic behaviour of the reference standards used are shown in Table II. Naphthalene was found to be most suitable when using Reoplex 400 as the liquid phase, 2-methylnaphthalene performed best when using silicone nitrile XE-60 as the substrate, and acenaphthene was applied to greatest advantage when silicone gum SE-30 served as the stationary phase.

Selectivity of column substrates

Valuable information regarding substrate performance is conventionally obtained by plotting logarithms of relative retention times (RRT) *versus* boiling points for compounds comprising a series of structurally related molecules. Due to lack of boiling point data (valid at 760 mm) such correlations can not be applied to sesquiterpenes. Boiling points at reduced pressures (10 ± 1 mm), available for fourteen of the compounds studied (see Table III), were plotted against log relative retention times, in order to judge the selectivity of the columns employed. A fairly straight line relationship was obtained only for the SE-30 column as shown in Fig. 1. Ses-

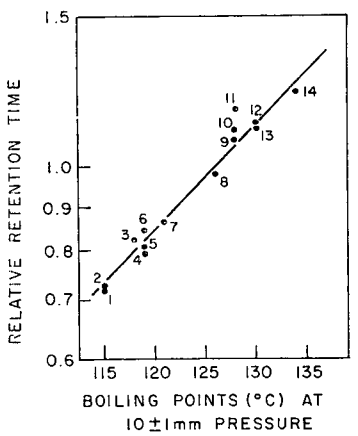

Fig. 1. Relation between boiling points and relative retention times of sesquiterpenes. Column: silicone rubber SE-30; retention data from Table V.

TABLE II

RELATIVE RETENTION TIMES OF REFERENCE STANDARDS
(Column temperature: 150°, Helium: 75 ml/min)

Compound	*Reoplex 400 10%*	*Silicone nitrile XE-60 5%*	*Silicone gum SE-30 10%*
Naphthalene	1.00	0.66	0.29
2-Methylnaphthalene	1.55	1.00	0.46
Acenaphthene	—	2.31	1.00

TABLE III

BOILING POINTS OF SESQUITERPENE HYDROCARBONS AT REDUCED PRESSURE (10 ± 1 mm)*

No.	*Compound*	*Boiling point (°C)*	*No.*	*Compound*	*Boiling point (°C)*
1	β-Elemene	115	8	β-Santalene	126
2	α-Gurjunene	115	9	α-Selinene	128
3	Cedrene	118	10	α-Zingiberene	128
4	β-Gurjunene	119	11	β-Zingiberene	
5	α-Santalene	119	12	β-Bisabolene	130–131
6	Caryophyllene	119	13	γ-Bisabolene	
7	Aromadendrene	121	14	γ-Cadinene	134

* Literature values, see ref. 11.

TABLE IV

SEPARATION FACTORS FOR SOME SESQUITERPENES ON 10% AND 20% REOPLEX 400 COLUMNS

No.	*Sesquiterpene pair*	*Retention time (min)*		*Separation factor*	
		10% column	*20% column*	*10% column*	*20% column*
1	Caryophyllene	9.95	13.75	1.26	1.31
	α-Humulene	12.55	18.10		
2	Caryophyllene	9.95	13.75		
	β-Humulene	12.35	18.00	1.24	1.30
3	*epi*-β-Santalene	9.50	14.00		
	β-Santalene	10.00	14.80	1.05	1.06
4	Longifolene	8.40	12.30		
	Longicyclene	6.75	9.50	1.24	1.29
5	α-Himachalene	10.95	15.70		
	β-Himachalene	13.45	19.45	1.23	1.33

quiterpenes displaying only minor differences with regard to boiling points were, however, more effectively separated by the XE-60 as well as the Reoplex 400 column. In some instances their order of emergence was even reversed.

Separation of peaks was not markedly improved when higher liquid phase concentrations were used. A 20 % Reoplex column, for example, brought about only slightly better separation of sesquiterpene hydrocarbon pairs than the 10 % column (Table IV). In practice the small gain in column effectiveness thus realized was offset by correspondingly longer retention times.

Resolution of sesquiterpene hydrocarbon mixtures occurring in nature

The resolution of sesquiterpene mixtures depends primarily on the vapour pressure and polarizabilities of the individual components. Hence, the nature of different substrates markedly affects column performance. This study has shown that Reoplex 400 and silicone nitrile XE-60 are substrates of choice for separating compounds of close boiling points but different polarizabilities, while silicone rubber SE-30 is most suitable for separating compounds of similar polarizabilities but different boiling points. Thus, Reoplex and silicone nitrile resolve the caryophyllene fraction of oil of copaiba balsam more effectively than silicone rubber as shown in Figs. 2, A, B and C. On the other hand silicone rubber SE-30 resolves β-santalene and *epi*-β-santalene, two epimeric constituents of oil of sandalwood, more effectively than silicone nitrile, no resolution whatever being obtained with Reoplex 400 (see Figs. 2, D, E and F). An example showing better performance of silicone nitrile XE-60 than either SE-30 or Reoplex 400 is the resolution of the sesquiterpene fraction of oil of ginger as illustrated in Fig. 2, G, H and I.

Application of experimental results to identification of essential oil constituents

The data shown in Table V are of value for the analysis of complex mixtures of sesquiterpenes, isolated from essential oils or obtained by synthesis. They allow for the recognition of constituents by comparison of their retention times on two or three columns of different polarities. Supplemented by infrared and coupled gas–thin-layer chromatographic analysis[12] of effluent fractions, the technique described affords a valuable tool for the examination and characterization of complex natural and synthetic compositions. Adopting this approach the authors established the occurrence of β-humulene in the caryophyllene fraction of oil of copaiba balsam[5] and are now reporting the occurrence of ylangene, α-bergamotene and δ-cadinene in this oil for the first time(see Fig. 2).

Dependence of retention time on structural features

Since the advent of gas chromatography, numerous attempts have been made to correlate gas chromatographic data of compounds with molecular structures. A method, first used by MARTIN and co-workers[13], consists of plotting the logarithms of retention volumes obtained with one stationary phase against those obtained with another stationary phase of different polarity. Of special interest in this regard are the studies by ZUBYK AND CONNER[14] and KLOUWEN AND TER HEIDE[4] concerning the correlation of gas chromatographic data with structural features of cyclic and acyclic monoterpenes.

The present investigation indicates that such broad correlations do not apply

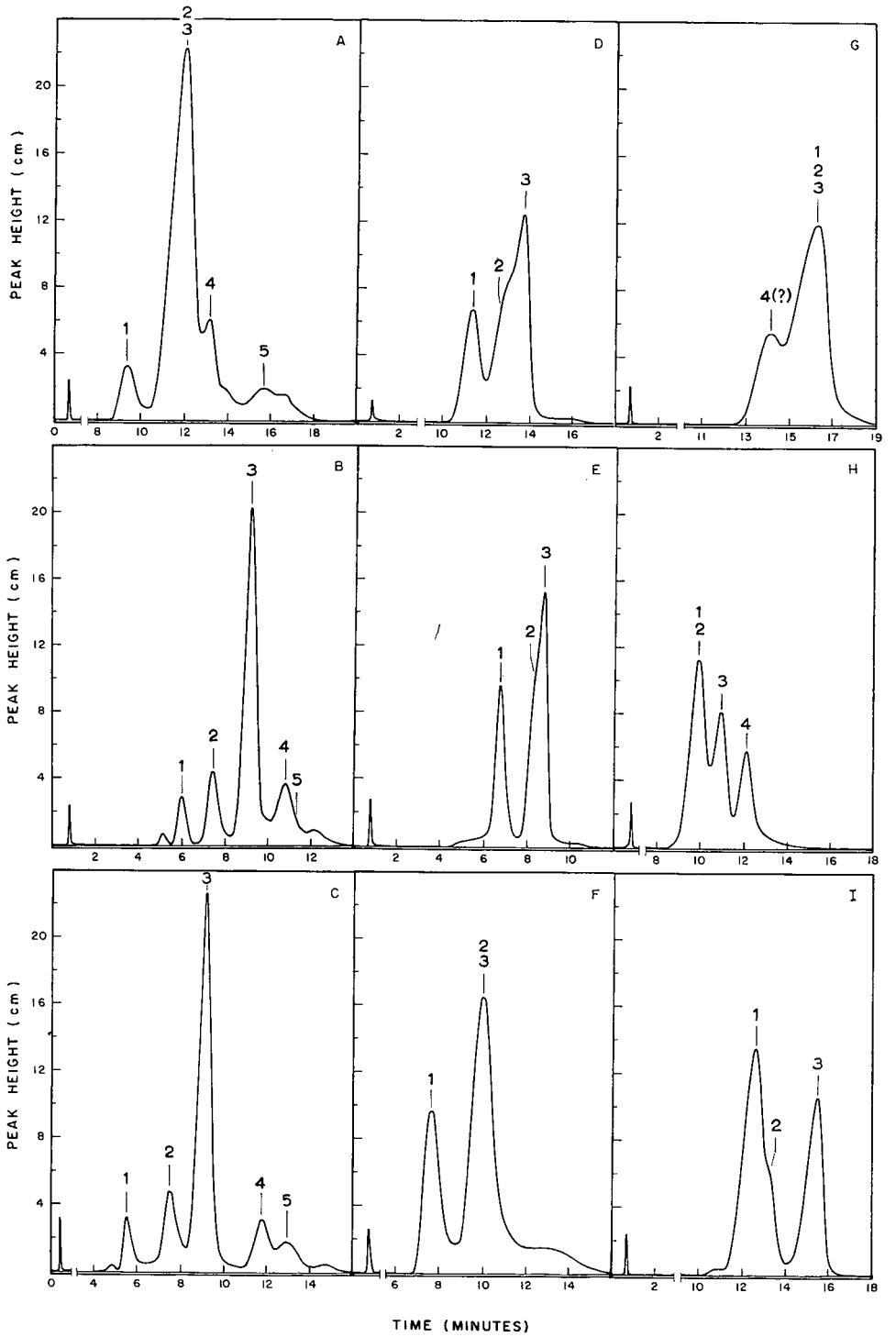

Fig. 2. Separation of sesquiterpene mixtures. Samples: A, B and C = caryophyllene fraction from copaiba balsam; D, E and F = sandalwood oil sesquiterpenes; G, H and I = ginger oil sesquiterpenes. Constituents of A, B and C: 1 = ylangene, 2 = α-bergamotene, 3 = caryophyllene, 4 = β-humulene, 5 = δ-cadinene; D, E and F: 1 = α-santalene, 2 = *epi*-β-santalene, 3 = β-santalene; G, H and I: 1 = α- and β-zingiberene, 2 = β-bisabolene, 3 = curcumene, 4 = farnesene. Columns for A, D and G: silicone rubber SE-30 (10%); B, E and H: silicone nitrile XE-60 (5%); C, F and I: Reoplex 400 (10%). Temperature: 150°. Helium flow: 75 ml/min.

TABLE V

RELATIVE RETENTION DATA FOR SESQUITERPENES

No.	*Sesquiterpene*	*Relative retention times**		
		SE-30 10%	*XE-60 5%*	*Reoplex-400 10%*
	Acyclic			
1	β-Farnesene	0.920	0.718	0.681
	Monocyclic			
2	α-Bisabolene	0.791	0.602	0.526
3	β-Bisabolene	1.120	0.885	0.939
4	γ-Bisabolene	1.113	0.850	0.867
5	Curcumene	1.113	0.975	1.132
6	β-Elemene	0.722	0.627	0.563
7	α-Humulene	0.936	0.883	0.838
8	β-Humulene	0.941	0.912	0.822
9	α-Zingiberene	1.100	0.875	0.889
10	β-Zingiberene	1.165	0.893	0.903
	Bicyclic			
11	α-Bergamotene	0.847	0.631	0.539
12	β-Bergamotene	0.880	0.692	0.623
13	α-Bulnesene	1.157	0.938	0.921
14	γ-Cadinene	1.060	0.958	1.078
15	δ-Cadinene	1.100	0.942	1.039
16	Caryophyllene	0.846	0.779	0.664
17	Isocaryophyllene	0.802	0.673	0.565
18	α-Guaiene	0.875	0.652	0.576
19	α-Himachalene	0.965	0.764	0.729
20	β-Himachalene	1.211	0.950	0.895
21	β-Santalene	0.981	0.759	0.667
22	*epi*-β-Santalene	0.926	0.733	0.633
23	α-Selinene	1.076	0.991	0.998
24	β-Selinene	1.060	0.948	0.960
	Tricyclic			
25	Aromadendrene	0.869	0.719	0.658
26	Cedrene	0.826	0.659	0.553
27	α-Gurjunene	0.727	0.607	0.468
28	β-Gurjunene	0.797	0.696	0.599
29	Longifolene	0.773	0.672	0.560
30	Isolongifolene	0.757	0.600	0.482
31	β-Patchoulene	0.719	0.527	0.411
32	α-Santalene	0.810	0.586	0.507
33	Ylangene	0.700	0.517	0.405
	Tetracyclic			
34	Longicyclene	0.706	0.566	0.450

* Reference standards for SE-30: acenaphthene; XE-60: methylnaphthalene; Reoplex 400: naphthalene.

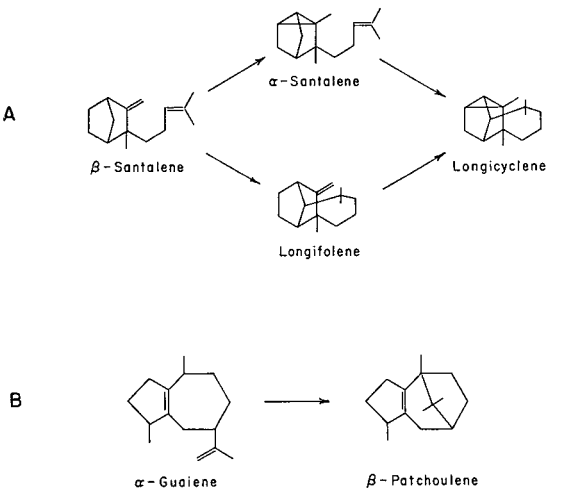

Fig. 3. Cyclisation of sesquiterpenes.

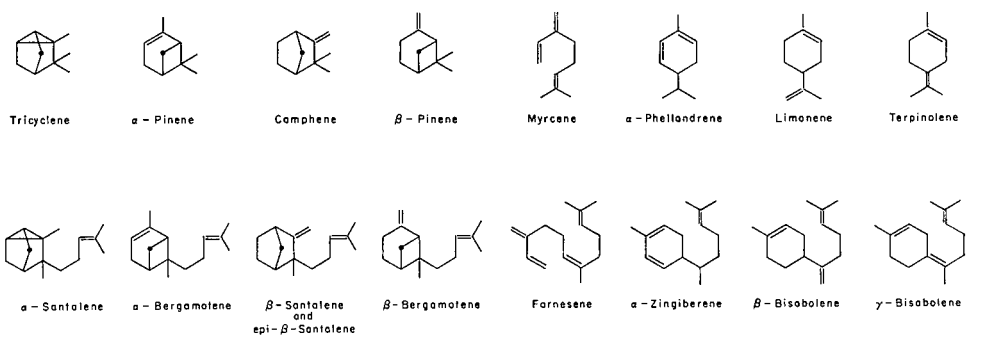

Fig. 4. Structural relationships between monoterpenes and sesquiterpenes examined by gas chromatography.

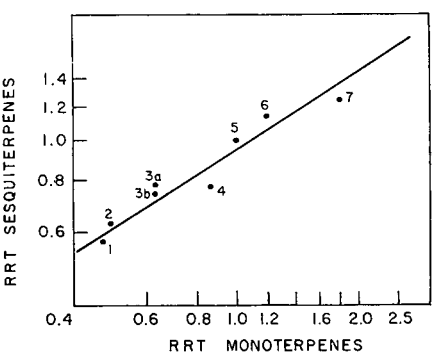

Fig. 5. Correlation of relative retention times of structurally related monoterpenes and sesquiterpenes.

to sesquiterpene hydrocarbons. However, careful examination of experimental data obtained for a number of compounds closely related in structure reveals some interesting relationships.

(*1*) *Aromatic structures.* Aromatisation of a ring possessing two conjugated double bonds leads to an increase in retention time. Thus, curcumene is held more strongly than α- or β-zingiberene by any of the three columns used (Table V).

(*2*) *Effect of cyclisation.* As shown in Fig. 3A distinct structural relationships exist between α-santalene, β-santalene, longifolene and longicyclene. β-Santalene may be visualised to undergo cyclisation involving either of the two unsaturated centres of the molecule and leading to the formation of α-santalene (formation of a cyclopropyl ring) or longifolene (formation of a 7-membered ring). Cyclisation of either of these two compounds may in turn lead to formation of the tetracyclic sesquiterpene, longicyclene. Each of these cyclisation steps is associated with a decrease in retention time. Thus on all three columns α-santalene and longifolene display relative retention times markedly lower than those of β-santalene, and longicyclene is less firmly held by either column than any of its precursors. Similarly β-patchoulene exhibits a shorter relative retention time than α-guaiene (see Table V and Fig. 3B).

(*3*) *Comparison of retention time data of monoterpene and sesquiterpene hydrocarbons.* Sesquiterpenes differ from monoterpenes in possessing an additional isoprene unit (C_5H_8). For example, α-santalene may be visualised to form by the addition of an isoprene unit to one of the geminal methyl groups of tricyclene (see Fig. 4). Relative retention times of some sesquiterpenes and their corresponding monoterpenes are recorded in Table VI.

TABLE VI

RELATIVE RETENTION TIMES FOR SOME SESQUITERPENE HYDROCARBONS AND THEIR CORRESPONDING MONOTERPENES*

No.	*Sesquiterpene*	*RRT* 150°	*Monoterpene***	*RRT* 100°
1	α-Santalene	0.57	Tricyclene	0.47
2	α-Bergamotene	0.63	α-Pinene	0.49
3a	*epi*-β-Santalene	0.74		
3b	β-Santalene	0.78	Camphene	0.63
4	β-Bergamotene	0.76	β-Pinene	0.76
5	β-Farnesene	0.77	Myrcene	0.86
6	α-Zingiberene	1.00	α-Phellandrene	1.00
7	β-Bisabolene	1.15	Limonene	1.19
8	γ-Bisabolene	1.25	Terpinolene	1.82

* Column: Reoplex 400 (20%) on acid washed Chromosorb W.
** Compounds available in author's reference collection.

The results obtained demonstrate that the emergence of sesquiterpene hydrocarbons parallels that of the corresponding monoterpenes. When plotting logarithms of relative retention times of the sesquiterpene hydrocarbons *versus* those of the corresponding monoterpenes a fairly linear relationship is obtained (see Fig. 5). The experimental data, therefore, suggest that the contribution of an isoprene unit toward the retention time of a sesquiterpene hydrocarbon is always of the same order of magnitude.

Relative retention times of a number of sesquiterpene hydrocarbons occurring in essential oils were recently reported by LUKES AND KOMERS[15] using columns other than those reported in the present study. Deductions contemplated by these authors concerning relationships between molecular structure and retention data on different stationary phases will be awaited with keen interest.

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SUMMARY

Characteristic gas chromatographic criteria of identity are reported for 34 authenticated sesquiterpene hydrocarbons, using Reoplex 400, silicone nitrile XE-60 and silicone rubber SE-30 as column substrates and naphthalene, 2-methylnaphthalene and acenaphthene as internal reference standards. The value of the experimental data is demonstrated by application to the analysis of essential oils and correlation with characteristic structural features of some sesquiterpenes. The occurrence of ylangene, α-bergamotene and δ-cadinene in oil of copaiba balsam is reported for the first time.

REFERENCES

1 I. C. NIGAM AND L. LEVI, *J. Pharm. Sci.*, 54 (1965) 1823.
2 H. P. BURCHFIELD AND E. E. STORRS, *Biochemical Applications of Gas Chromatography*, Academic Press, New York, 1962, p. 382.
3 E. GUENTHER, K. KULKA AND J. A. ROGERS, Jr., *Anal. Chem.*, 35 (1963) 39R.
4 M. H. KLOUWEN AND R. TER HEIDE, *J. Chromatog.*, 7 (1962) 297.
5 I. C. NIGAM AND L. LEVI, *Can. J. Chem.*, 40 (1962) 2083.
6 A. BHATI, *Perfumery Essent. Oil Record*, 54 (1963) 376.
7 I. C. NIGAM AND L. LEVI, *Perfumery Essent. Oil Record*, 54 (1963) 814.
8 I. C. NIGAM AND L. LEVI, *Can. J. Chem.*, 41 (1963) 1726.
9 M. C. NIGAM, I. C. NIGAM, L. LEVI AND K. L. HANDA, *Can. J. Chem.*, 42 (1964) 2610.
10 E. GUENTHER, *The Essential Oils*, Vol. II, Van Nostrand, New York, 1949, p. 708.
11 J. SIMONSEN AND D. H. R. BARTON, *The Terpenes*, Vol. III, Cambridge University Press, Cambridge, 1952; E. GUENTHER, *The Essential Oils*, Vol. II, Van Nostrand, New York, 1949.
12 I. C. NIGAM, M. SAHASRABUDHE AND L. LEVI, *Can. J. Chem.*, 41 (1963) 1535.
13 A. T. JAMES, A. J. P. MARTIN AND G. H. SMITH, *Biochem. J.*, 52 (1952) 238.
14 W. J. ZUBYK AND A. Z. CONNER, *Anal. Chem.*, 32 (1960) 912.
15 V. LUKES AND R. KOMERS, *Collection Czech. Chem. Commun.*, 29 (1964) 1599.

TERPENOIDS

LXXXII. EVALUATION OF POLYESTERS AS STATIONARY PHASES IN GAS–LIQUID CHROMATOGRAPHY OF TERPENOIDS*

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(Received December 6th, 1965)

In an earlier communication[1] on our study of polyesters as stationary phases in gas–liquid chromatography (GLC) columns, it was observed that the number of methylene groups between the two carboxyl groups of the acidic fragment of the polyester has a profound influence on the degree of separation. The retention times of hydrocarbons are nearly proportional to the number of methylene groups present. This linearity was found to be valid up to brassylic acid [HOOC-$(CH_2)_{11}$-COOH] and deviated to some extent with higher dicarboxylic acid polyesters. On the same basis of these findings it was felt necessary to study the effect of the temperature gradient more closely and also to find out the effect of equimolar proportion of the different polyesters on the degree of separation. At one stage it was suspected that using equimolar proportion of different polyesters, the retention times for a particular hydrocarbon may become identical; but results of actual experiments presented in this paper will show that this is not really so, and that the number of methylene groups spaced between the carboxyl groups of the dicarboxylic acid moiety plays a more important part than the molecular weight of the polyester. There may be other factors as well.

EXPERIMENTAL

(1) Apparatus: Griffin & George MK II VPC apparatus was used with modifications as described in our previous communication.

(2) The same columns were employed for the study of temperature gradient.

(3) Retention time data were recorded as previously[1] between 70 to 120° at intervals of 5° approximately.

(4) Polyesters of succinic, adipic, azelaic, and brassylic acids were chosen for the present investigation.

(5) α-Pinene, β-pinene, limonene, p-cymene and n-amyl acetate were used as reference compounds.

(6) Specific retention volumes (V_g values) were calculated according to standard procedure[2] and used for the construction of graphs as described in the discussion.

* Communication No. 861, from the National Chemical Laboratory, Poona-8, India.

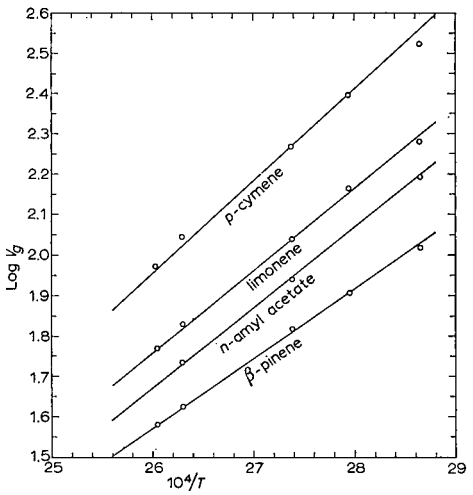

Fig. 1. Succinic acid–diethyleneglycol polyester.

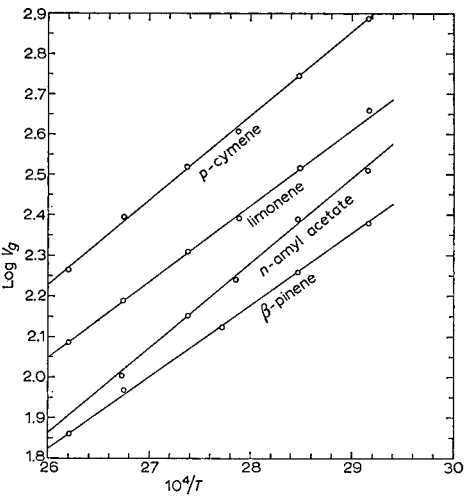

Fig. 2. Adipic acid–diethyleneglycol polyester.

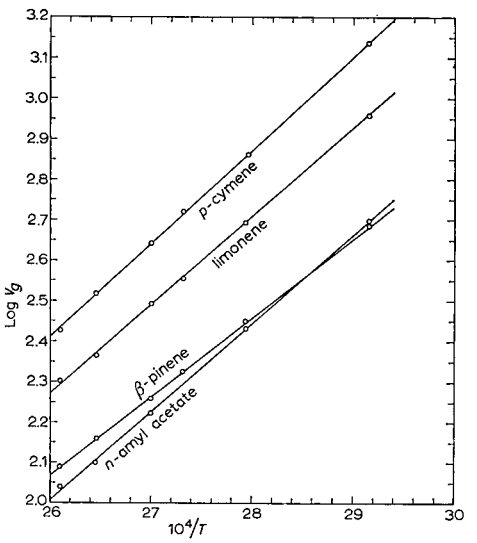

Fig. 3. Azelaic acid–diethyleneglycol polyester.

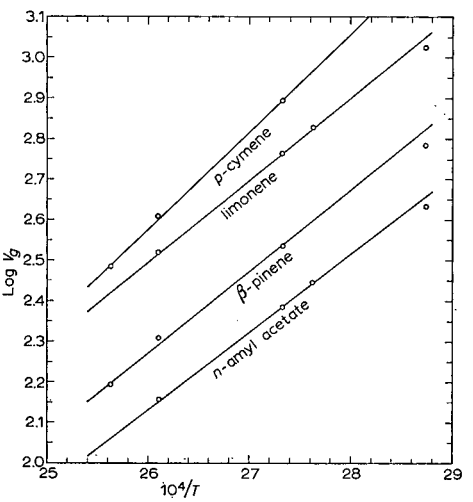

Fig. 4. Brassylic acid–diethyleneglycol polyester.

RESULTS AND DISCUSSION

During the study of the influence of temperature gradient, V_g values were calculated[2] and log V_g against $10^4/T$ was recorded in Figs. 1 to 4. At different temperatures, values of log V_g of the required compound were then read out from each graph. The difference between the two values for a particular compound on two different phases furnished the ratio for the two specific retention volumes. Such ratios for the two terpenic compounds, β-pinene and limonene, have been calculated and are shown in Table I. The maximum deviation in each case has also been calculated and is shown in the same table. The deviation is found to lie generally within the permissible experimental limits and is about 10 % in an extreme case.

TABLE I

Ratio of the number of methylene groups	*Temperature* $10^4/K$					*Maximum deviation*
	26.20	*26.80*	*27.0*	*27.50*	*27.9*	
Compound: β-pinene:						
11/2 = 5.50	5.129	5.346	5.309	5.495	5.697	6.75
11/4 = 2.75	2.851	2.927	2.951	3.020	3.020	9.82
11/7 = 1.57	1.603	1.596	1.614	1.622	1.641	4.52
7/2 = 3.50	3.20	3.273	3.289	3.388	3.467	8.57
7/4 = 1.75	1.778	1.82	1.828	1.862	1.90	8.57
Compound: limonene:						
11/2 = 5.50	5.433	5.383	5.559	5.249	5.129	6.75
11/4 = 2.75	2.851	2.825	2.917	2.950	2.884	6.05
11/7 = 1.57	1.641	1.589	1.648	1.585	1.514	4.97
7/2 = 3.50	3.311	3.388	3.296	3.311	3.888	5.82
7/4 = 1.75	1.738	1.778	1.770	1.862	1.90	8.57

The deviation observed may be due to the following possible reasons:

(i) Fluctuations in the column temperature, which in our case, using the Griffin & George MKII VPC apparatus was about $\pm$ 1° (ref. 3).

(ii) Slight fluctuations in the flow rate[2].

(iii) Since the same columns as were used previously have been used in the present investigation, the weight of the stationary phase may not be exactly the same, because the rate of bleeding of different polyesters is most likely to be different[4].

(iv) It is also worth noting that the lower polyesters are viscous liquids, whereas the higher polyesters are low melting solids. The densities of these polyesters will not be the same at any particular temperature. This is likely to affect the partition coefficient and thus introduce a source of deviation.

The behaviour of *n*-amyl acetate is found to be interesting. On a succinic acid polyester column, this compound emerges after β-pinene; the same occurs with an adipic acid polyester column. However, on an azelaic acid polyester column these two compounds almost overlap and on a brassylic acid polyester column it actually emerges before β-pinene. This gives a good clue to the separation of a mixture of compounds of different natures.

Influence of molar proportion of the polyester

Average molecular weights were taken on the basis of two closely agreeing experimental values and new column fillings were prepared. The columns were adjusted in such a way that each column contained 1/500 mole of the polyester. These columns were conditioned at 80° for 1.5 h and the retention times of reference compounds determined as usual. The results are tabulated in Table II. The retention

TABLE II

Compound	*Number of methylene groups*	*Molecular weight*	*Weight of the liquid*	*Retention time*	*Contribution per gram, per methylene group*
α-Pinene	2	1249	2.448	2.03	0.4146
	3	340	0.676	0.99	0.4879
	4	1154	2.266	3.75	0.4138
	7	398	0.791	2.55	0.4605
β-Pinene	2	1249	2.448	3.60	0.7352
	3	340	0.676	1.55	0.7640
	4	1154	2.266	6.63	0.7316
	7	398	0.791	4.08	0.7369
Δ^3-Carene	2	1249	2.448	4.56	0.9313
	3	340	0.676	1.99	0.9811
	4	1154	2.266	8.50	0.9380
	7	398	0.791	5.31	0.9590
Limonene	2	1249	2.448	6.55	1.338
	3	340	0.676	2.80	1.380
	4	1154	2.266	11.93	1.316
	7	398	0.791	7.18	1.296
Camphene	2	1249	2.448	2.81	0.5738
	3	340	0.676	1.35	0.6655
	4	1154	2.266	5.33	0.5882
	7	398	0.791	3.28	0.5294

times were not identical indicating thereby that molar proportion was not the deciding factor. However, when the retention time was divided by the actual weight of the stationary phase used and then by the number of methylene groups which may be considered as contribution per gram per methylene group, the values were nearly constant. These values are tabulated in Table II under "contribution".

This is in agreement with the linearity factor observed previously, though it may be worth remembering that in the earlier communication equal weights of the stationary phases were used.

SUMMARY

The study of polyesters was extended over a wider range of temperature. The ratio of the specific retention volumes on two different polyesters for a typical hydrocarbon is nearly equal to the number of methylene groups spaced between the two

carboxyl groups of the acidic fragment of the polyester. Further it has also been found that the molecular weight plays a less important part than the number of methylene groups present in the acidic fragment of the polyester.

REFERENCES

1 B. V. BAPAT, B. B. GHATGE AND S. C. BHATTACHARYYA, *J. Chromatog.*, 18 (1965) 308.
2 A. B. LITTLEWOOD, C. S. G. PHILLIPS AND D. T. PRICE, *J. Chem. Soc.*, (1955) 1480.
3 C. PHILLIPS, *Gas Chromatography*, Butterworths, London, 1956.
4 W. GERRARD, S. J. HAWAKES AND E. F. MOONEY, in R. P. W. SCOTT (Editor), *Gas Chromatography* 1960, Butterworths, London, 1961, p. 199.

GAS–LIQUID CHROMATOGRAPHY OF SUBMICROGRAM AMOUNTS OF DRUGS

II. ANALYSIS OF BARBITURATES AND RELATED DRUGS IN BIOLOGICAL MEDIA

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As early as 1960, an attempt was made by JANAK[1] to employ gas chromatography in the identification of barbiturates. His method involved heating the sample to 800° followed by gas chromatographic separation of the pyrolytic products. In 1962, a similar procedure was described by NELSON AND KIRK[2] who presented unique patterns for 22 barbiturates. These methods are probably satisfactory for analyses in which only one unknown compound is involved. In the resolution of mixtures, however, it would appear that correct interpretation of the complicated pattern so obtained would be difficult if not impossible.

Separation of barbiturates by gas chromatography has been described by COOK *et al.*[3,4]. These workers found that they could not separate the barbiturates as such, but by reaction with diazomethane overnight they were able to obtain good separation of the resulting dimethyl derivatives. Such a method will not, of course, resolve compounds which are already N-methylated from their lower non-methylated homologue. Furthermore, using a non-polar stationary phase, it was found that compounds could not be resolved which differed only in the possession of either a hexenyl or a phenyl side-chain, although COOK[4] *was* able to resolve such mixtures using the polar compound polyethylene glycol adipate, as liquid phase.

An attempt to apply gas chromatography to the separation of barbiturates as the free acids was made by PARKER AND KIRK[5] in 1961. It would seem that their solid support of acid-washed firebrick was not silanised and, as a copy of the chromatogram they obtained is not shown, it is not clear whether they experienced tailing and, if so, to what extent. Although they list the retention times for 23 barbiturates, it is not stated whether *any mixture* of these would be resolved. Indeed, they say that mixed barbiturates caused "overlapping peaks", which we interpret as meaning that resolution of certain mixtures was not achieved. A further drawback to their procedure was that they found variation of retention time with sample size; this was probably due to overloading of the column.

In a later article, PARKER, FONTAN AND KIRK[6] used acid-washed Chromosorb W coated with either 5 % SE-30 or 1 % Carbowax 20M in an attempt to provide a gas chromatographic screening procedure for barbiturates, alkaloids, sympatho-

mimetic amines and tranquillisers. Their conditions probably represented a compromise, with the result that their column was not necessarily the best that could have been prepared for the resolution of mixtures containing only barbiturates. These workers did not report any retention times for barbiturates except those included in their Table, which is inadequate. The same workers, in a subsequent article[7], refer, in their brief introduction, to the use of hexamethyldisilazane (HMDS) for silanising the solid support but they do not themselves use a silanising procedure. They use a mixed liquid phase of 1.5 % SE-30 and 2 % Carbowax 20M in, what appears to be, the mistaken belief that incorporation of a more polar compound in the liquid phase will obviate the necessity for silanising the support material. Comparison of results with and without the Carbowax was not reported, and the resolution of 7 barbiturates was not satisfactory.

Interesting results showing good resolution were obtained by CIEPLINSKI[8] who attempted to prevent tailing of barbiturates by the incorporation of dimer acid (0.75 %) with SE-30 (1.5 %) as the liquid phase. In this paper, he also reported results with a liquid phase of 0.75 % trimer acid and 3 % neopentyl glycol adipate (NPGA). In each case, the solid support material was Chromosorb W which had been acid-washed and treated with HMDS. We have repeated his work and found that the column functions satisfactorily at 180°, but when it is raised to, and held at 250° for an hour, dropping the temperature back to 180° gives results for the barbiturates which are not nearly as good as those obtained before the temperature was raised. This means that one would be precluded from carrying out temperature-programming up to 250° or so. We felt that the change we observed might be due to loss of the dimer acid at the higher temperature and wondered whether CIEPLINSKI[8] had tried the trimer acid with SE-30 and, if so, why the results were not reported.

Gas chromatographic detection of barbiturates is briefly discussed by VANDENHEUVEL, HAAHTI AND HORNING[9] in their more general article dealing with drugs and drug metabolites. They found that the barbiturates showed some tailing with a liquid phase of QF-1 and concluded that more than one column was necessary for satisfactory resolution of mixtures.

It seems to us that one of the most outstanding articles dealing with the gas chromatographic separation of barbituric acid derivatives which has so far appeared in the literature is that of BROCHMANN-HANSSEN AND SVENDSEN[10]. It is noteworthy that these workers were able to run the barbiturates at a temperature of about 140° (some 40° lower than that found necessary by other workers), which means that they were using a column showing much less adsorption of barbiturates. Although they show chromatograms of the resolution of some 9 to 12 barbiturates on columns using four different stationary phases, it is clear from their list of 22 barbituric acid derivatives that any *one* of their five columns would not be capable of resolving *any* mixture of barbiturates. The amounts of barbiturates which they injected varied from 5 to 10 μg. Their solid support was 60–80 mesh Chromosorb W which had been washed with concentrated hydrochloric acid and methanolic potassium hydroxide and then treated with HMDS prior to application of the stationary phase.

In a recent article, MCMARTIN AND STREET[11] have described their investigations into the treatment of solid support material, the preparation of columns showing considerably reduced adsorption, the effect of tristearin on such columns, and the scope and limitations of these columns. It is the purpose of the present paper to de-

scribe the application of these investigations to the analysis of barbituric acid derivatives both in pure solution and in extracts of biological material. With our procedure, most of the barbiturates can be detected down to the 0.04 μg level; in some cases, 0.01 μg can be detected. This description also includes analysis of certain other compounds related to the barbiturates either structurally, *e.g.*, the glutarimides Doriden and Megimide, or because they may appear in the same analytical fraction, *e.g.* salicylic acid.

METHODS, APPARATUS AND MATERIALS

Gas–liquid chromatography details

All the results were obtained using a Perkin-Elmer Model 800 gas chromatograph fitted with a flame-ionisation detector and equipped for temperature-programming. Nitrogen was used as the carrier gas at a flow rate of about 30 ml per min. The flow rates of hydrogen and air were optimised. The temperature of the injector block was usually between 50° to 100° above column temperature. Detector temperature was approximately the same as column temperature. The signal was recorded on a Honeywell —0.05 to +2.5 mV recorder. The recorder chart has 10 small squares to the inch.

Stainless steel columns, 6 ft. × 0.085 in. I.D., $^1/_8$ in. O.D., were packed with Chromosorb W (100–120 mesh) which had been treated and coated with either a mixture of silicone gum rubber (SE-30) 2 %, and tristearin, 0.1 %, as described by McMARTIN AND STREET[11], or by a similar procedure in which the SE-30 was replaced by a fluorosilicone polymer (QF-1). Samples were injected with a 10 μl Hamilton glass syringe, graduated in divisions of 0.2 μl, fitted with a fixed 2 in. stainless steel needle.

Preparation of samples

Blood. Extracts were prepared either by the method of STREET AND McMARTIN[12] or by a scaled-down version of this method starting with 1 ml of blood and reducing the volumes of reagents by a factor of 5. Residues were dissolved in 50, 100 or 200 μl of ethanol and from 1 to 5 μl of these solutions were injected into the gas chromatograph.

Liver. Extracts were prepared by the procedure described by STREET[13] but starting with 25 g of liver in place of 100 g, and using proportionately less volumes of reagents. One tenth of the dried ether extract from such a preparation was taken carefully to dryness and the residue dissolved in 200 μl of ethanol. From 1 to 5 μl of this solution were injected into the gas chromatograph.

Standards. Solutions were prepared from the free acids of the barbituric acid derivatives kindly supplied by Messrs. May and Baker, Ltd., England. These barbiturates were made up in either ethanol or methanol as 0.1, 0.02, 0.004 and 0.001 % solutions containing respectively, 1, 0.2, 0.04 and 0.01 μg of the drug per μl. For nomenclature of the drugs, that given in the Merck Index (1960) has been adopted.

The compounds studied were:
Barbital (Barbitone), 5,5-diethylbarbituric acid;
Di-allylbarbituric acid (Allobarbitone), 5,5-di-allylbarbituric acid;

Butethal (Butobarbitone), 5-butyl-5-ethylbarbituric acid;
Pentobarbital (Pentobarbitone), 5-(1-methylbutyl)-5-ethylbarbituric acid;
Amobarbital (Amylobarbitone), 5-(3-methylbutyl)-5-ethylbarbituric acid;
Aprobarbital, 5-allyl-5-isopropylbarbituric acid;
Rutonal, 5-phenyl-5-methylbarbituric acid;
Secobarbital (Quinalbarbitone), 5-allyl-5-(1-methylbutyl)-barbituric acid;
Cyclobarbital (Cyclobarbitone), 5-(1-cyclohexen-1-yl)-5-ethylbarbituric acid;
Heptabarbital (Medomin), 5-(1-cyclohepten-1-yl)-5-ethylbarbituric acid;
Hexobarbital (Hexobarbitone), 3,5-dimethyl-5-(1-cyclohexen-1-yl)-barbituric acid;
Salicylic acid, *o*-hydroxybenzoic acid;
Acetylsalicylic acid (Aspirin), 2-acetoxybenzoic acid;
Doriden (Glutethimide), α-ethyl-α-phenyl glutarimide;
Bemigride (Megimide), β-methyl-β-ethyl glutarimide;
Caffeine, 1,3,7-trimethylxanthine.

RESULTS AND DISCUSSION

Analysis of pure solutions of drugs

Figs. 1 and 2 show the results obtained by injection of 0.2 μg and 0.01 μg, respectively, of mixtures containing each of the five barbiturates, di-allylbarbituric acid, butethal, amobarbital, pentobarbital and secobarbital. Even at the level of 10 nanograms, partial resolution of the two isomers amo- and pentobarbital is effected. Sensitivity around the 10 nanogram region means that it should be possible to determine the "free" (as distinct from protein-bound) plasma levels of certain drugs which could lead to an attempt to correlate such levels with toxicity and therapeutic effectiveness. Using only a single compound, *e.g.* in clinical trials, gas-chromatographic conditions could be chosen to suit that particular compound rather than those to suit resolution of a mixture. In this connexion too, solid sampling (which we have not investigated) would seem to be the answer to possible interference by solvent, and would also allow smaller initial samples of biological material to be used.

The results obtained by injection of 4 μl of a solution containing 2 μg each of a mixture of 10 barbiturates run at 160° at an attenuation of $\times$ 100 are shown in Fig. 3. It will be noted that, at this temperature, 34 min are required before the last peak (heptabarbital) emerges. The same mixture of barbiturates can be separated in 12 min by temperature-programming from 150° at 5° per min, when the peaks are actually sharpened. These results are shown in Fig. 4 where 0.5 μg of each of 10 barbiturates was injected at an attenuation of $\times$ 50. Fig. 5 shows the results obtained by temperature-programming a mixture of 0.05 μg of each of the barbiturates, secobarbital (1), hexobarbital (2), rutonal (no peak), cyclobarbital (4) and heptabarbital (5), at attenuation $\times$ 5. The tailing of rutonal which is apparent in both Figs. 3 and 4 and its disappearance as shown in Fig. 5, is difficult to account for in view of the fact that cyclobarbital shows less tailing. We have noticed that certain other compounds display anomalous results. For example, 4-hydroxyacetanilide shows considerably more tailing even at 200° than either *p*-aminophenol or salicylic acid which run well at 150°.

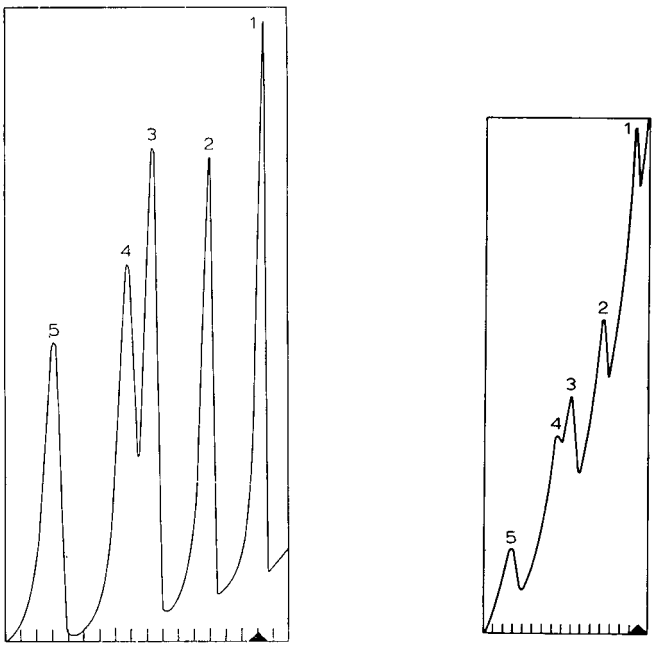

Fig. 1. Chromatogram obtained by injection of 0.2 μg each of a mixture of di-allylbarbituric acid (1), butethal (2), amobarbital (3), pentobarbital (4), and secobarbital (5) in 1 μl of ethanol. SE-30–tristearin column at 160°. Attenuation: × 10. Y-axis = detector response. X-axis = time after injection (increasing to left). 1 division ≡ 0.1 in. ≡ 24 sec. ▲ is 1 in. from point of injection.

Fig. 2. Details as in Fig. 1 but 0.01 μg of each of the 5 barbiturates injected in 1 μl of ethanol at attenuation × 1. Axes and ▲ as in Fig. 1.

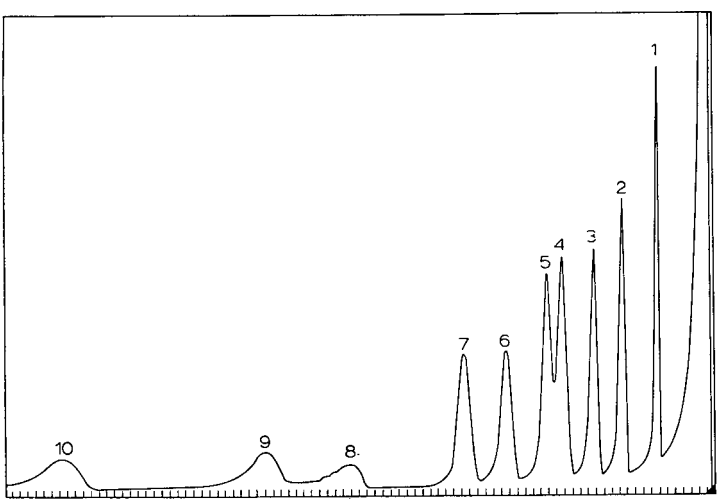

Fig. 3. Chromatogram obtained by injection of 2 μg each of a mixture of barbitone (1), di-allylbarbituric acid (2), butethal (3), amobarbital (4), pentobarbital (5), secobarbital (6) hexobarbital (7), rutonal (8), cyclobarbital (9), and heptabarbital (10) in 4 μl of ethanol. SE-30–tristearin column at 155°. Attenuation: × 100. Axes as in Fig. 1. ◢ is at point of injection.

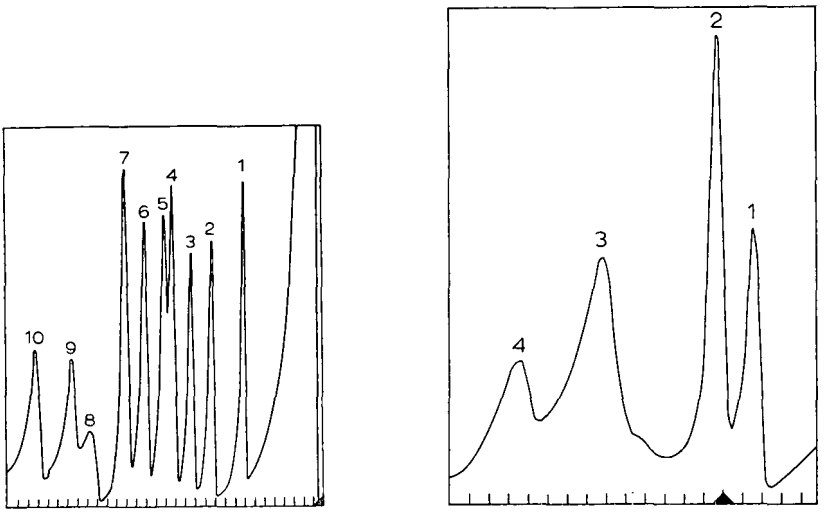

Fig. 4. Chromatogram obtained by injection of 0.5 μg each of a mixture of those barbiturates referred to in Fig. 3, in 2 μl of ethanol. SE-30–tristearin column temperature-programmed from 150° at 5° per min. Attenuation: × 50. Axes as in Fig. 1. ◢ is at point of injection.

Fig. 5. Chromatogram obtained by injection of 0.05 μg each of a mixture of secobarbital (1), hexobarbital (2), rutonal (no peak), cyclobarbital (3), and heptabarbital (4), in 1 μl of ethanol. SE-30–tristearin column temperature-programmed from 150° at 5° per min to 200° then isothermal. Attenuation: × 5. Axes as in Fig. 1. ▲ is 2 in. from point of injection.

Separation of the mixture of 10 barbiturates referred to above can be effected in 6 min by running at 200° but in this case the resolution of amobarbital and pentobarbital at 200° is not as good as either the 160° or the temperature-programmed run.

On the SE-30–tristearin column, it is not possible to achieve good resolution of a mixture of aprobarbital and di-allylbarbituric acid but, by using the more polar QF-1 phase, (also containing tristearin), these two barbiturates may be satisfactorily resolved. On the other hand, a mixture of rutonal and cyclobarbital is not resolved at all on QF-1–tristearin but, on SE-30–tristearin, the two drugs are separated quite cleanly.

Analysis of extracts from biological media

Initially, barbiturates were added to biological samples known not to contain any barbiturate. One result, typical of these experiments, is shown in Fig. 6. For comparison purposes under the same conditions, Fig. 7 shows the tracing from a standard barbiturate mixture. In this case, the barbiturates barbital, di-allylbarbituric acid, butethal, amobarbital and pentobarbital were added to 5 ml of blood to give a concentration for each barbiturate of 1 mg per 100 ml of blood. Three-fifths of the extract was taken to dryness and the residue dissolved in 100 μl of ethanol. The 5 μl of this solution which was injected would contain 1.5 μg of each barbiturate if there were 100 % recovery. By comparison of peak heights with the standard (Fig. 7), the recovery would appear to be about 50 % for butethal, amobarbital and pentobarbital.

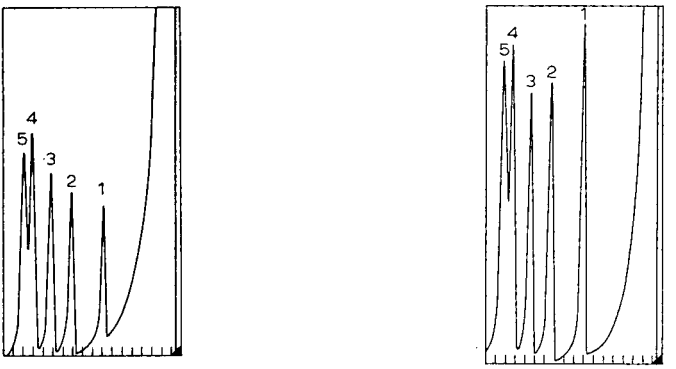

Fig. 6. Chromatogram obtained by injection of 5 μl of a solution of a residue obtained from an extract of blood (see text). In this case to 5 ml of blood had been added 50 μg each of a mixture of barbital (1), di-allylbarbituric acid (2), butethal (3), amobarbital (4), and pentobarbital (5). If recovery were 100%, 1 μl of final solution would contain 0.3 μg of each barbiturate. SE-30–tristearin column temperature-programmed from 150° at 5° per min. Attenuation: × 100. Axes as in Fig. 1. ◢ is at point of injection.

Fig. 7. Chromatogram obtained by injection of 1 μg each of a mixture of those barbiturates referred to in Fig. 6 in 1 μl of ethanol. SE-30–tristearin column temperature-programmed from 150° at 5° per min. Attenuation: × 100. Axes as in Fig. 1. ◢ is at point of injection.

One interesting point emerges by comparing *relative* peak heights, when it is seen that the recovery of barbital and of di-allylbarbituric acid is less than that of the other barbiturates. This is in keeping with previous observations regarding these two drugs and is probably related to the fact that their partition coefficients in ether–water are lower than those of most other barbiturates. The same blood sample carried through the procedure without added barbiturate gave a trace which was free from peaks in the region under observation. In each case, the runs were made at attenuation × 100 and the column was temperature-programmed from 150° at 5° per min.

Using the same batch of blood, the above experiment was repeated but with the barbiturates added to the blood this time to give a concentration of 0.1 mg per 100 ml of blood. 5 ml of blood were extracted and the residue was dissolved in 50 μl of ethanol. 2 μl of this solution were injected into the gas chromatograph and produced the results shown in Fig. 8. The column in this case was temperature-programmed from 155° at 5° per min and the attenuation was × 10. This increased sensitivity now reveals a number of peaks in the blood blank but the barbiturate peaks (corresponding to about 0.1 μg of each drug, *i.e.* again about 50% recovery) are clearly distinguished from these peaks, which we have found to be due to impurities in the ether used in the extraction procedure. Attempts to purify the ether by standard procedures did not eliminate these peaks. It is pertinent to note here that the low recoveries in each of the above two experiments may be related to the method of evaporating down the ether solutions, which may have resulted in the residue being distributed over an area too large to be covered by the small volumes of ethanol used to dissolve the residues. There is obviously room for improvement here, although the use of an internal standard, added to the sample prior to extraction, would be of value in using the procedure quantitatively. It is probable that some form of solid sampling would

obviate losses. We have not investigated this because our apparatus is not designed to permit solid sampling other than by syringe. However, the results of Fig. 8 show that it would be quite easy to follow blood barbiturate levels in patients receiving therapeutic doses of the drugs. Again, such a study would be even simpler and could be made more sensitive for a single, *known* barbiturate.

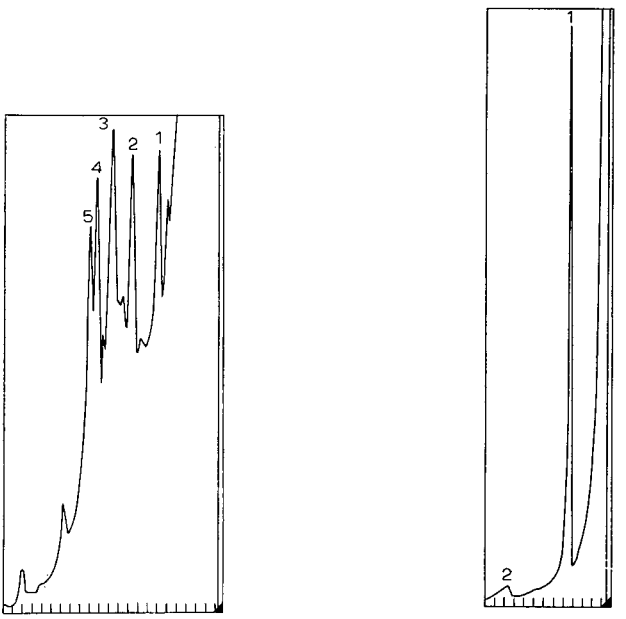

Fig. 8. Chromatogram obtained by injection of 2 μl of an ethanolic solution of a residue obtained from an extract of blood (see text). In this case, to 5 ml of blood had been added 5 μg each of a mixture of barbital (1), di-allylbarbituric acid (2), butethal (3), amobarbital (4), and pentobarbital (5). If recovery were 100%, 1 μl of final solution would contain 0.1 μg of each barbiturate. SE-30–tristearin column temperature-programmed from 155° at 5° per min. Attenuation: × 10. Axes as in Fig. 1. ◢ is at point of injection.

Fig. 9. Chromatogram obtained by injection of an ethanolic solution of a residue from an extract of liver from a case where death was due to an overdose of amobarbital (see text). (1) amobarbital, (2) phenobarbital. QF-1–tristearin column at 200°. Attenuation: × 100. Axes as in Fig. 1. ◢ is at point of injection.

Fig. 9 shows the results obtained from an actual routine case in our department. By ultraviolet spectroscopy and elevated temperature paper-chromatography (see STREET[14,15]) it had previously been established that death was due to ingestion of an overdose of barbiturates. The liver was found to contain 26.7 mg of barbiturates per 100 g and paper chromatography and treatment with hot sulphuric acid (see STREET AND MCMARTIN[16]) suggested that amobarbital was present. The paper chromatogram also showed a very faint absorbing spot (in 254 mμ light) in the region of phenobarbital. Part of this extract was subjected to gas–liquid chromatography (QF-1–tristearin) at 200°, and gave the results shown in Fig. 9, the retention times of which peaks agree with those of amobarbital and phenobarbital. It was concluded that death was due to an overdose of amobarbital and that the phenobarbital was probably present in therapeutic amount.

Interfering compounds

In the analysis of blood for barbiturates in poisoning cases it is important to remember that there are a few compounds which may be present in the sample which are not barbiturates and yet may appear in the same extract. Precisely which compounds will be present in any given extract will, of course, depend upon the extraction procedure used. In any event, it is well to be aware that Doriden, aspirin, salicylic acid, and caffeine may be present in the extract and may confuse the analysis. Furthermore, Bemegride is often used in the treatment of barbiturate poisoning and this drug, being a weak acid, will be found in the same extract as the barbiturates.

0.1 μg of Bemegride is readily picked out in a mixture of 5 barbiturates (in 1 μg amounts). Barbital has the shortest retention time of the barbiturates studied; Bemegride shows a retention time of about half that of barbital when the compounds are temperature-programmed from 150° at 5° per min on an SE-30–tristearin column. Under these conditions, Doriden shows almost the same retention time as hexobarbital and a mixture of these two drugs is not resolved. This difficulty can be circumvented either by using a more polar phase or by making use of the fact that Doriden is unstable in dilute alkali at room temperature.

At 140°, aspirin and salicylic acid can be separated from each other. Their retention times are shorter than Bemegride so that they can easily be picked up and do not interfere, even in relatively large amounts, with the barbiturates.

Caffeine is such a weak base that it is generally present in the "neutral" fraction of the extraction procedure and it may, therefore, be found along with the barbiturates if a separation of "neutral" drugs has not been effected prior to chromatography. On the SE-30–tristearin column, caffeine shows the same retention time as secobarbital but resolution of the two drugs is achieved again by using QF-1 in place of SE-30.

Variation of retention time with concentration of injected solution

In a previous article (see McMARTIN AND STREET[11]) we noted that we had found identical retention times for a number of drugs for concentration ranges from 0.2 μg/μl to 0.04 μg/μl and, in cases where the drug could be detected in smaller amounts, from 0.2 μg/μl to 0.01 μg/μl. Two articles dealing with the gas chromatographic analysis of low concentrations of barbiturates have appeared very recently after our work had been completed. In the first of these articles, by GUDZINOWICZ AND CLARK[17], it was found that, for hexobarbital, their peak maxima shifted to longer retention times as the amount of sample introduced was decreased, and they state that "this effect, together with peak tailing and loss of sample when less than 0.1 μg is injected, is undoubtedly caused by adsorption on the solid support". We are inclined to agree with their statement and this is precisely why we investigated the way of reducing such adsorption (see McMARTIN AND STREET[11]). We further suggest that, as a result of our investigation, the column we have produced shows, at least for the compounds we have studied, much less adsorption than any previously reported columns, as is evidenced by the fact that the retention time is independent of concentration of injected sample over a relatively large range in the sub-microgram region.

At first sight, there might appear to be a discrepancy between the retention times of heptabarbital and cyclobarbital as shown in our Fig. 4 and those shown in our Fig. 5. However, it will be observed that in one case temperature-programming

was carried out up to 200° and then the run was isothermal, whereas in the other case temperature-programming was carried out until the last drug had emerged from the column.

BRADDOCK AND MAREC[18], working with pentobarbital and thiopental (and with barbital as "a possible internal standard") observed that their peaks all showed considerable tailing, especially at the lower concentrations. They found that the minimum levels of detection were about 0.002, 0.003 and 0.005 μg per μl for barbital, pentobarbital and thiopental, respectively. They then go on to say that "At these concentrations, the shape of the response peak and the amount of background noise interact to make the peak just barely discernible on some runs and non-existent on others." In view of this statement, we feel that their minimum levels of detection should be somewhat higher than the figures they have stated. These workers also found that at concentrations below 0.05 μg per μl, barbital, pentobarbital and thiopental all displayed a lengthening of retention time as the concentration was reduced.

It would seem, therefore, that our remarks concerning the article by GUDZINOWICZ AND CLARK[17] are also applicable to the article by BRADDOCK AND MAREC[18].

ACKNOWLEDGEMENT

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SUMMARY

This paper describes the gas–liquid chromatographic separation of mixtures of barbiturates in pure solution and in extracts of biological material. Using a special preparation of SE-30–tristearin on acid-washed Chromosorb W in a stainless steel column it is shown that successful resolution can be obtained with submicrogram amounts of the drugs. In some cases, 10 ng of barbiturates can be detected. Interference of other drugs and methods of eliminating this interference are described.

REFERENCES

1 J. JANAK, *Nature*, 185 (1960) 684.
2 D. F. NELSON AND P. L. KIRK, *Anal. Chem.*, 34 (1962) 899.
3 J. G. H. COOK, C. RILEY, R. F. NUNN AND D. E. BUDGEN, *J. Chromatog.*, 6 (1961) 182.
4 J. G. H. COOK, *Proc. Assoc. Clin. Biochem.*, 2 (1963) 199.
5 K. D. PARKER AND P. L. KIRK, *Anal. Chem.*, 33 (1961) 1378.
6 K. D. PARKER, C. R. FONTAN AND P. L. KIRK, *Anal. Chem.*, 35 (1963) 356.
7 K. D. PARKER, C. R. FONTAN AND P. L. KIRK, *Anal. Chem.*, 35 (1963) 418.
8 E. W. CIEPLINSKI, *Anal. Chem.*, 35 (1963) 256.
9 W. J. A. VANDENHEUVEL, E. O. A. HAAHTI AND E. C. HORNING, *Clin. Chem.*, 8 (1962) 351.
10 E. BROCHMANN-HANSSEN AND A. B. SVENDSEN, *J. Pharm. Sci.*, 51 (1962) 318.
11 C. MCMARTIN AND H. V. STREET, *J. Chromatog.*, 22 (1966) 274.
12 H. V. STREET AND C. MCMARTIN, *Nature*, 199 (1963) 456.
13 H. V. STREET, in A. STOLMAN (Editor), *Progress in Chemical Toxicology*, Vol. 2, Academic Press, New York, 1965.
14 H. V. STREET, *J. Forensic Sci. Soc.*, 2 (1962) 118.
15 H. V. STREET, *J. Forensic Sci.*, 7 (1962) 222.
16 H. V. STREET AND C. MCMARTIN, *Clin. Chim. Acta*, 9 (1964) 301.
17 B. J. GUDZINOWICZ AND S. J. CLARK, *J. Gas Chromatog.*, 3 (1965) 147.
18 L. I. BRADDOCK AND N. MAREC, *J. Gas Chromatog.*, 3 (1965) 274.

SULFUR-CONTAINING AMINO ACIDS: CHROMATOGRAPHY ON CATION AND ANION EXCHANGE RESINS WITH AN AUTOMATIC ANALYZER*

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INTRODUCTION

In a recent publication, DE MARCO *et al.*[1] described a method for examining a number of sulfur-containing amino acids with emphasis on sulfinic, sulfonic, thiosulfonic and thiosulfuric acids. However, some of the compounds could not be separated by their method, which involved chromatography on a column of cation-exchange resin, and had to be determined indirectly. We have been using columns of anion-exchange resin to separate and estimate compounds of this type in conjunction with an amino acid analyzer[2]. This paper reports the results obtained together with the chromatographic behaviour of a number of mixed disulfides and related compounds on cation-exchange resin. The following abbreviations are used in the formulation of compounds:

Cy = $-CH_2CH(NH_2)COOH$;
Pen = $-C(CH_3)_2CH(NH_2)COOH$;
R′ = $-CH_2CH_2NH_2$;
R″ = $-CH_2CH_2COOH$.

The usual abbreviations, GSSG and GSH, are used for oxidised and reduced glutathione, respectively.

EXPERIMENTAL AND RESULTS

Materials

Cystine, cysteine sulfinic acid ($CySO_2H$), cysteic acid, cysteine, homocystine, homocysteic acid, penicillamine, penicillaminic acid ($PenSO_3H$), glutathione (ox.), glutathione (red.) and taurine were commercial products obtained from CalBiochem or Mann Research Laboratories.

The following compounds were prepared by methods described in the literature: $CySSO_3Na$[3], $(CySO_2S)_2Ba$[4], $CyCH_2SSO_3Na$[3], $(CySSSO_3)_2Ba$[5], and $CySSSCy$[6].

Homocysteine sulfinic acid ($CyCH_2SO_2H$) was prepared by decomposing homocystine-S-monoxide with dilute ammonium hydroxide solution and isolating the product by chromatography on Dowex 1 × 8 ion-exchange resin with formic acid[7]. Penicillamine sulfinic acid ($PenSO_2H$) was produced by irradiating an aqueous

* DCBRL Report No. 491.

solution of PenSH with γ-rays and separating the products by chromatography on Dowex 1 resin. The compound was not characterized but it was eluted in the expected position and gave a positive test with iodoplatinic acid. Penicillamine (ox.) (PenSSPen) was prepared by bubbling oxygen through an aqueous solution of PenSH till a test for thiols was negative (3 days) then crystallizing the product. Mixed disulfides were prepared using the following reaction[8,9]: RSOSR + 2 R'SH → 2 RSSR' + H_2O. The intermediates required, cystine-S-monoxide and homocystine-S-monoxide were prepared as described by SAVIGE *et al.*[6].

The trisulfide CySSSCy was prepared from the monoxide by reaction with H_2S[6].

The mixed disulfide, CySSG, was kindly supplied by W. F. FORBES.

Cation-exchange chromatography

The amino acid analyzer (Technicon Chromatography Corp.) had 15 mm tubular flow cells and a 0.6 × 140 cm column. The resin supplied was "Chromobeads" Type A (Cation-exchange). The instrument was used as recommended by the manufacturer except that the glass reaction coil was replaced by a Teflon capillary tube (18 gauge, 100 ft.) which was heated in a refluxing water bath[10]. Introduction of nitrogen into the stream of reagents was then unnecessary and all of the sample from the ion-exchange column reacted with ninhydrin and passed through the flow cells.

Stock solutions of disulfides in 1 % HCl (2.5 mE per litre) were stored at 5° and diluted 5 times before use. (E is the equivalent weight with respect to ninhydrin positive groups). Solutions of sulfhydryl compounds such as CySH were prepared with thiodiglycol present as an antioxidant. Table I gives the elution times and yields of some disulfides etc.

TABLE I

ELUTION OF DISULFIDES ETC. FROM CHROMOBEADS CATION-EXCHANGE RESIN

Column temperature = 60° throughout

Compound	*Time (min)*	*Yield (hw, 0.25 μE)*	*Absorption ratio 440/570 mμ*	*Remarks*
Norleucine	660	13.8	0.2	Reference standard
L-CySH	265	2.5 (440 mμ)	2.3	
D-PenSH	240	—	0.3	Oxidised to PenSSPen
GSH	142	4.3	0.25	
L,L-CySSCy	510	7.0	0.6	
DL,DL-$CyCH_2SSCH_2Cy$	720	12.6	0.3	
DL,DL-PenSSPen	590, 600	—	0.2	Twin peaks
D,D-PenSSPen	590	15.0	0.2	Single peak
GSSG	300	9.7	0.2	Broad peak, 9 cm
L,D-CySSPen	540	8.1	0.35	
L-CySSR'	900	—	0.6	Impure
L-CySSR''	460	9.7	0.5	Broad peak, 7 cm
DL,D-$CyCH_2SSPen$	690	7.0	0.25	Trace of PenSSPen
DL,L-$CyCH_2SSCy$	660	8.8	0.5	
DL-$CyCH_2SSG$	610	—	0.25	Impure
L-CySSG	410	9.5	0.35	
L,L-CySSSCy	640	5.1	0.8	

Anion-exchange chromatography

Dowex 1-x8, minus 400 mesh (BioRad Laboratories) anion-exchange resin has been used successfully as described previously[2]. The resin was converted to the chloracetate form with sodium monochloroacetate solution, rinsed and packed in a glass column 0.6 × 140 cm identical to that used for the "Chromobeads". For standards, operation of the column was similar to the procedure used for the "Chromobeads" column. The compounds being examined were put on in aqueous solution and the column was eluted with a linear gradient of water to 1 *M* sodium monochloroacetate solution (600 ml). The gradient mixture was prepared from water containing 10 ml BRIJ-35 and 5 ml thiodiglycol per litre and neutral (pH 6) sodium monochloroacetate solution containing 10 ml BRIJ-35 per litre. Thiodiglycol was necessary to prevent oxidation of sulfinic acids. The column was operated at room temperature throughout with a flow rate of 30 ml per h. Elution times of some of the compounds examined are reported in Table II.

TABLE II

ELUTION OF ACIDS FROM DOWEX 1 ANION-EXCHANGE RESIN AT ROOM TEMPERATURE

Compound	*Time (min)*	*Yield (hw, 0.25 μE)*	*Remarks*
Glutamic	200	15.3	Reference standard
$CySO_2H$	240	12.6	
$CySO_3H$	320	12.0	
$CySO_2SH$	540	—	Impure, broad, 8 cm
$CySSO_3H$	580	8.6	Broad peak, 8 cm
$CySSSO_3H$	880	—	Very broad peak, 15 cm
$CyCH_2SO_2H$	225	—	Impure
$CyCH_2SO_3H$	320	12.2	
$CyCH_2SSO_3H$	585	—	Impure
$PenSO_2H$	245	—	Impure
$PenSO_3H$	300	9.2	
$R'SO_3H$	80	11.2	
$R'SO_2H$	50	12.0	
GSSG	320	9.7	Broad peak, 8 cm
CySSR''	250	9.6	
Aspartic	210	12.6	

Fig. 1 illustrates the separation obtained with cystine and a number of related acids (0.25 μE of each). Glutamic acid was added as a reference standard. A shallower gradient with a total volume of 700 ml was used for the chromatograms illustrated as this improved the separation in the region of the sulfinic acids.

Fig. 2 shows the results obtained with a number of acids related to homocysteine and penicillamine.

Application of Dowex 1 column

During a study of the γ-radiolysis of cystine in dilute aqueous solution (3×10^{-4} *M*) at low doses[11], it was necessary to separate and estimate the yield of a number of acidic products. This was achieved using the column of Dowex 1 described above as follows. The reference acid (glutamic), and thiodiglycol (5 drops) were added to 25 ml of the solution after irradiation and the mixture was pumped through the

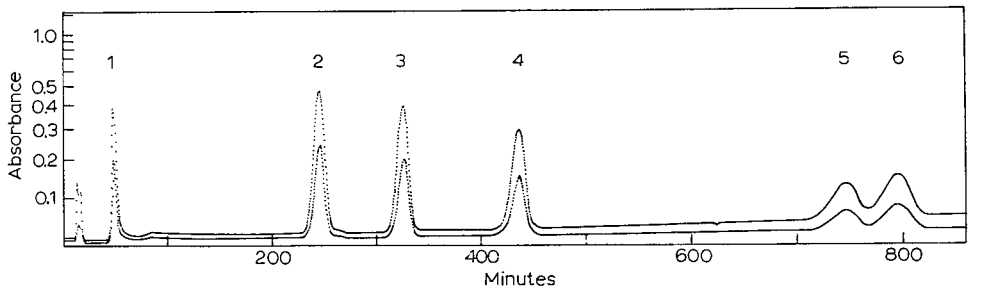

Fig. 1. Chromatography on Dowex 1: 1 = CySSCy; 2 = glutamic acid; 3 = $CySO_2H$; 4 = $CySO_3H$; 5 = $CySO_2SH$; 6 = $CySSO_3H$. Chart speed: 3 in. per h.

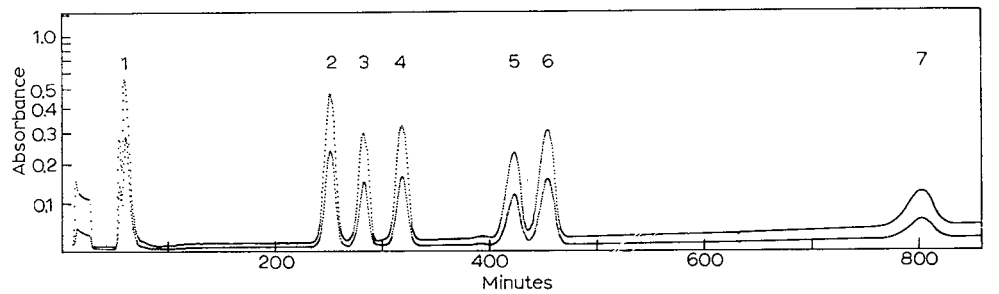

Fig. 2. Chromatography on Dowex 1: 1 = Homocystine; 2 = glutamic acid; 3 = aspartic acid; 4 = $CyCH_2SO_2H$; 5 = $PenSO_3H$; 6 = $CyCH_2SO_3H$; 7 = $CyCH_2SSO_3H$.

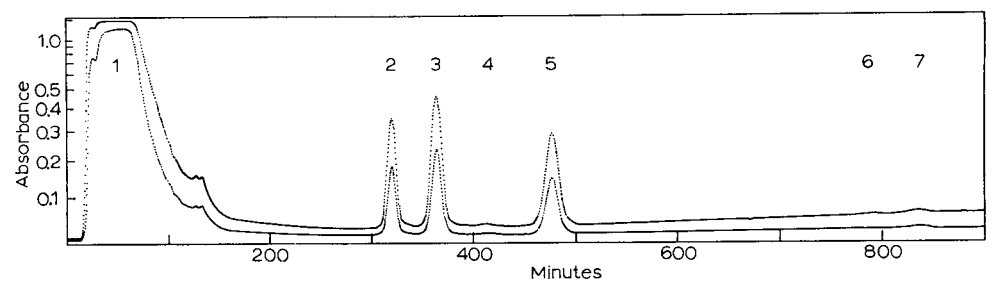

Fig. 3. Irradiated cystine solution (10,000 rads): 1 = Cystine and neutral products; 2 = glutamic acid; 3 = $CySO_2H$; 4 = unidentified product; 5 = $CySO_3H$; 6 = $CySO_2SH$; 7 = $CySSO_3H$.

Dowex 1 column without concentration. The resin retained the acidic compounds and cystine passed through the column. After rinsing with a further 25 ml of water containing BRIJ-35 and thiodiglycol, the column was eluted with aqueous sodium monochloroacetate solution as described above. A typical separation is illustrated in Fig. 3.

In practice the two columns, Dowex 1 and "Chromobeads", were usually operated simultaneously. Two colorimeters (570 mμ and 440 mμ) were used in conjunction with the "Chromobeads" column and the other (570 mμ) was used for the Dowex 1 column[2]. The results were recorded with the standard three point recorder.

DISCUSSION

Chromatography of disulfides and neutral amino acids was straightforward and standard procedures were sufficient. Mixed disulfides were eluted at positions intermediate between those of the corresponding symmetrical disulfides. The ratio of the absorptions at 440 and 570 mμ were useful for identifying derivatives of cystine which had high 440/570 absorption ratios. Cysteine had a particularly high value[12] and the trisulfide CySSSCy was also distinctive in this way (Table I).

Cation-exchange resins were only partially successful for separating mixtures of acids. The method of DE MARCO *et al.*[1], while successful for mixtures of cysteic acid, cysteine sulfinic acid, taurine and hypotaurine, did not resolve mixtures containing cysteic acid, alanine thiosulfonic acid, and alanine thiosulfuric acid since these compounds were not retained by the resin. Anion-exchange resins, on the other hand, retain these strong acids and they can be eluted by buffer solutions or acids approximately in order of their pK_a values[7,13]. MOORE and co-workers[14,15] used a column of Dowex 1 resin to determine cysteic acid produced by oxidation and hydrolysis of proteins. Recently the same method has been used to determine penicillaminic acid[16]. In order to separate a complex mixture of products, however, gradient elution is necessary. Neutral sodium monochloroacetate solution was the most successful of the eluants investigated in the present work. Sodium formate was an equally good eluant but it appeared to interfere with the ninhydrin reaction. Acetate buffer, pH 5.5 eluted the acids from the column but glutamic acid and cysteine sulfinic acid gave peaks with extended shoulders. A similar effect was observed with sodium monochloroacetate solution when the pH was about 4. Most of the sulfinic acids examined were eluted in approximately the same time and this is also true of the sulfonic acids. A separation of at least 15 min is desirable if the yields are to be calculated in the usual way. Improved separation could probably be achieved with a more complex gradient and higher operating temperature but the labile nature of the compounds makes the latter undesirable. The method described has been used successfully in studying the γ-radiolysis of cystine[11].

SUMMARY

Some thiols and mixed disulfides related to cysteine were chromatographed on a cation-exchange resin and the results are reported.

Separation of cysteine sulfinic acid, cysteic acid, alanine thiosulfonic acid, alanine thiosulfuric acid and other compounds of this type on Dowex 1 anion-exchange resin is described.

REFERENCES

1 C. De Marco, R. Mosti and D. Cavallini, *J. Chromatog.*, 18 (1965) 493.
2 J. W. Purdie, *Technicon Symposium, Technicon, Chauncey, New York, September, 1965*, Paper No. 21.
3 B. Sorbo, *Acta Chem. Scand.*, 12 (1958) 1990.
4 C. De Marco, M. Coletta, B. Mondovi and D. Cavallini, *Ital. J. Biochem.*, 9 (1960) 3.
5 T. W. Szczepkowski, *Roczniki Chem.*, 35 (1961) 563.
6 W. E. Savige, J. Eager, J. A. Maclaren and C. M. Roxburgh, *Tetrahedron Letters*, (1964) 3289.
7 C. Davies, R. D. Hartley and G. J. Lawson, *J. Chromatog.*, 18 (1965) 47.
8 La V. D. Small, J. H. Bailey and C. J. Cavallito, *J. Am. Chem. Soc.*, 69 (1947) 1710.
9 A. Schöberl and H. Gräfje, *Ann.*, 617 (1958) 71.
10 P. B. Hamilton, *Anal. Chem.*, 35 (1963) 2055.
11 J. W. Purdie, in preparation.
12 A. Wainer, personal communication.
13 G. J. Lawson and J. W. Purdie, *Mikrochim. Acta*, 3 (1961) 415.
14 E. Schram, S. Moore and E. J. Bigwood, *Biochem. J.*, 57 (1954) 33.
15 S. Moore, *J. Biol. Chem.*, 238 (1963) 235.
16 K. Borner, *Z. Physiol. Chem.*, 341 (1965) 264.

A DEMOUNTABLE TUBE* FOR LARGE-SCALE CHROMATOGRAPHY AND ITS APPLICATION TO THE ISOLATION OF HEMOGLOBIN A_{Ic}

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For chemical investigations of the structure of the minor hemoglobin component A_{Ic} (ref. 1) which occurs to the extent of 5 to 7 % in normal adult human hemoglobin[2,3], it has been necessary to isolate several grams of material. Although this amount may be isolated by repeated small-scale chromatograms, a large-scale chromatographic column (10 × 100 cm) has been successfully operated with a load of 50 g of hemoglobin. The construction and use of this chromatographic apparatus is described herewith; some results of the chemical investigations of hemoglobin A_{Ic} have been presented in part[4] and will be described in detail elsewhere[5].

CONSTRUCTION OF THE CHROMATOGRAPHIC TUBE

A photograph of the filled chromatographic tube, its supports and accessories is shown in Fig. 1. Because of the chromatographic properties of hemoglobin A_{Ic} under the specific conditions employed, the tube was constructed in two approximately equal lengths which were held together with a pipe flange. In this way, hemoglobin A_{Ic} could be developed until it alone was present in the lower half of the column. The two parts could then be separated, and the hemoglobin could be rapidly eluted from each by altering the conditions. (In other applications, a different proportion of lengths may be advantageous.)

The upper half A (Fig. 1) was constructed of 10-cm (I.D.) Pyrex tubing which was fused to a 4-in. Pyrex pipe joint (Corning Glass Works, Corning, N.Y.). The water jacket was of such dimensions that the water annulus was 6 mm thick; the water inlet is E (Fig. 1). The socket joint M at the top was 65/40 in size.

The lower half B was constructed in similar fashion. To support the resin, a coarse sintered funnel K was fused into the tube. An 18/9 ball joint L at the bottom served as a connection to direct the effluent to the desired place.

This apparatus was designed for easy portability, and the pipe flange which connected the two sections was incorporated into the support. A triangular pressboard base G (approx. 65 cm on a side) with swivel wheels H supported the three legs of the steel tripod F. Each leg was bolted directly to the lower half of the pipe flange D (Pyrex pipe to Pyrex pipe flange, 4-in. size, Style 1, aluminum, with inserts,

* The chromatographic *tube* should be distinguished from the chromatographic *column*. The former contains the solid support or *column* on which the chromatographic separation takes place.

** Contribution No. 3316.

Corning Glass Works, Corning, New York). Part B rested on the pipe flange D and was further supported by the rubber covered metal ring I with extensions 120° apart that were connected to the turnbuckles J for adjusting the position of I. A Koroseal gasket C (Type R-3 for 4-in. flange, Corning Glass Works, Corning, N.Y.) separated the two parts of the tube and provided a leak-proof seal as well as a cushion to prevent breakage. Six stainless steel bolts held the two halves of the flange and, hence, of the tube together.

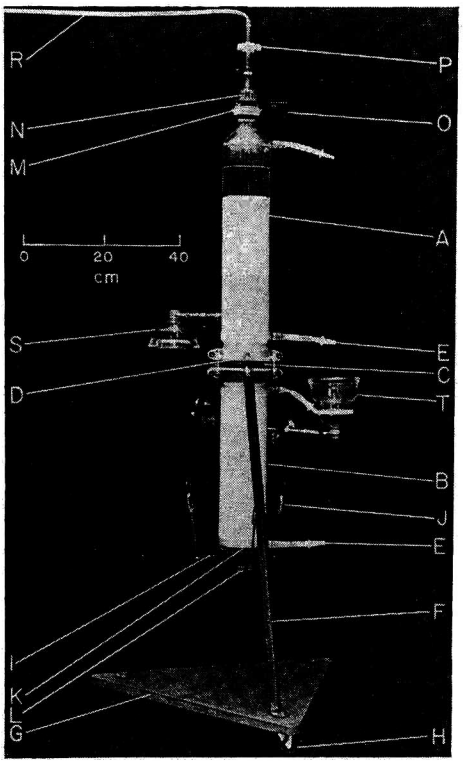

Fig. 1. The 10 × 100 cm chromatographic tube, its supports and accessories.

Developer was directed to the chromatogram through tubing R, Teflon stopcock P (with an 18/9 ball joint), and adapter N (ball joint 65/40, socket joint 18/9). A pressure clamp O held the parts of the joint together. A thin silicone rubber gasket was placed between the parts of a large joint such as M in order to prevent leakage.

Parts S and T are accessories that were used after the two sections of the column had been separated at the end of the chromatogram. In Fig. 1, they are supported by clamps for illustrative purposes only. Part S, thus, is an adapter of 4-in. Pyrex pipe and an 18/9 socket joint that is connected to B in order to direct developer through this portion of the column alone. Likewise, T is an adapter of 4-in. Pyrex pipe, a coarse sintered disc, and an 18/9 ball joint which, when it was attached to A, permitted this section to be eluted separately.

OTHER EQUIPMENT

For chromatography on this scale, several items of equipment are necessary for handling large volumes of ion exchange resin and developer. As a reservoir for developer, a 13-gallon (56-l) aspirator bottle (Q, Fig. 2) was used (Polyethylene aspirator bottle, 13-gallon capacity, with tubulation, Nalge No. 2302, The Nalge Co., Inc., Rochester, N.Y.). Two other bottles of the same size (Polyethylene carboy, 13-gallon capacity, with hand-grips, Nalge No. 2210) were required for preparation and storage of developer. Small carts were convenient for the transportation of the filled carboys In addition, a polyethylene tank (rectangular polyethylene tank, 10-gallon capacity, Nalge No. 11000, with cover No. 11003) was utilized for the preparation of ion exchange resin before the column was packed.

In the application of this apparatus to the isolation of hemoglobin A_{Ic}, the developer contained potassium cyanide (0.6 g/l) which at neutral pH is present in solution as hydrogen cyanide. In a closed cold room without addition of fresh air, a lethal concentration of cyanide could easily develop. In the interest of safety, it is advisable to monitor the concentration of hydrogen cyanide with a detector kit for hydrogen cyanide gas (No. DH 73493) which is available from the Mine Safety Appliance Company (201 N. Braddock Ave., Pittsburg 8, Pa.). For use in an emergency, a positive pressure type gas mask and amyl nitrite should be readily available. When all containers were carefully closed, the concentration was easily kept to a safe level of 2 p.p.m.

PROCEDURE

The procedure differs from that previously described[1,2,4,6] essentially only in the scale. The following description, then, is concerned only with those factors that must be considered because of the increased scale.

Approximately 15 lb. of (wet) resin were required for a 10 × 100 cm column. Bio-Rex 70 (which is equivalent to IRC-50) was purchased from Bio-Rad Laboratories, Richmond, Calif. in actual wet mesh size 200–325. The resin in the sodium form was suspended by vigorous stirring in 10 times its settled volume of distilled water and then allowed to settle for 30 min after which time the supernatant fluid with suspended fine particles was removed. Although the amount of fine material was small, three settlings were made. The resin was then suspended in twice its volume of the buffer which was to be used for equilibrating the column and was adjusted to the pH of the equilibrating buffer with phosphoric acid. The column was poured from this suspension at room temperature in a single section: as the resin in the tube settled under gravity and the liquid drained from the bottom, additional suspension was added.

Fig. 2 shows schematically a convenient arrangement for handling the necessarily large volumes of developer. The 56-l aspirator bottle Q should be placed at maximum height to take advantage of the hydrostatic pressure thus produced. The tubing R (Figs. 1 and 2) led directly to the column from the tubulation at U. A stopcock and an inlet tube with an 18/9 socket joint V passed through the rubber stopper in the neck of the bottle. A pressure collar retained the rubber stopper in the neck when the bottle was under pressure. A small centrifugal pump was used to

transfer developer to reservoir Q from the carboy in which it was mixed. During this operation, the line W from the pump was connected to V. During equilibration and development, the line X from the tank of compressed nitrogen was attached to V and the stopcock was closed.

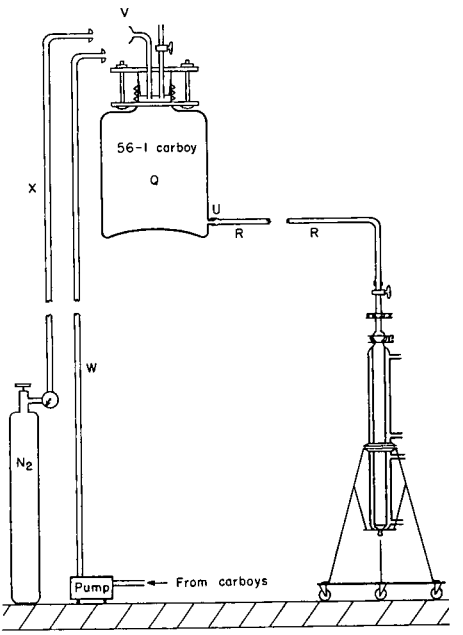

Fig. 2. Arrangement of apparatus.

Before use, the column was equilibrated (in this application with Developer No. 6 (ref. 7)) in the cold room at 2° with 160 l of buffer. With a hydrostatic head of approximately 3 m from the reservoir to the bottom of the column and the additional application of 0.5 atm of pressure from compressed nitrogen, the developer flowed through the column at approximately 2 l/h. (Compressed gas pressure should be applied to polyethylene carboys with caution. The application of pressure to the carboy that was used caused the concave bottom to become convex and tended to tip the carboy over.)

After the column had been equilibrated, the buffer was removed from the top, and the sample (about 50 g of adult carbon monoxyhemoglobin from 1 pint of blood in 500 ml of Developer No. 6 after thorough dialysis) was poured onto the resin bed and stirred into the resin to a depth of 5 cm to give an even boundary between the stirred and unstirred portions. After the sample had entered the column, the sides of the tube were rinsed carefully three times with about 25 ml of buffer. Finally, the remainder of the tube was filled with buffer, and development was carried out at approximately 2 l/h.

With Developer No. 6, "non-heme proteins" and the minor components A_{Ia} and A_{Ib} (ref. 1) separate from and move down the column ahead of A_{Ic}. Because the isolation of A_{Ic} alone was of interest, the effluent which contained these faster moving components was directed to a drain and diluted with a stream of water to reduce

the cyanide concentration. After approximately 36 h of development (72 l), the front of hemoglobin A_{Ic} reached the sintered disc K at the bottom, of the column. At this point, the chromatogram was stopped by closing outlet L and stopcock P. After the bolts had been removed from the flange A, the top half of the column which contained hemoglobin A_{II} (the main component), was lifted off and set aside on an aluminum plate. The dimensions of the column and the chromatographic conditions had been chosen so that only A_{Ic} would normally be present in the lower half of the column. Occasionally when a small amount of A_{II} had moved into the lower half, it could be readily scooped out. A_{Ic} and A_{II} usually were separated by several centimeters of almost white resin.

The lower half column B and its support system (D, F, G, H, I, J) were removed from the cold room, adapter S was attached and held in place with the flange, developer was added to the top (although passage of developer was still prevented at outlet L), and this section of the column was warmed by circulating water at 38° through the jacket. After the column had warmed to 38° (2 to 3 h), A_{Ic} was eluted at that temperature as rapidly as possible into a collecting vessel which was chilled in ice. Approximately 2 g of A_{Ic} were contained in a volume of about 2 l; the solution was concentrated at 2° through collodion bags (membrane filter, collodion bags type CB, porosity less than 5 mμ, size 8 ml, from the Membranfiltergesellschaft, Göttingen, Germany) to a final volume of 5 to 10 ml.

In the same manner, hemoglobin A_{II} was eluted from the top half of the columm by the use of adapter T. Complete elution required elevation of the temperature to 50°.

Prior to the next chromatogram, all resin was removed and suspended in water. After the column had been repoured, it was equilibrated as described.

DISCUSSION

By means of the apparatus described, it has been possible to increase the scale of operations in the chromatographic isolation of hemoglobin by a factor of 20 over previous experiments. Although the saving in time and effort is appreciable, it is by no means a factor of 20.

Although this chromatographic tube has been used successfully for a specific purpose, the special features of demountability and transportability may make it valuable with modification for other applications.

ACKNOWLEDGEMENTS

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SUMMARY

The construction and operation of a demountable 10 × 100 cm chromatographic tube is described. In a specific application of this apparatus, a load of 50 g of adult human hemoglobin has been chromatographed for the isolation of 2 g of the minor hemoglobin component A_{Ic}.

REFERENCES

1 D. W. ALLEN, W. A. SCHROEDER AND J. BALOG, *J. Am. Chem. Soc.*, 80 (1958) 1628.
2 M. D. CLEGG AND W. A. SCHROEDER, *J. Am. Chem. Soc.*, 81 (1959) 6065.
3 B. F. HORTON AND T. H. J. HUISMAN, *Brit. J. Haematol.*, 11 (1965) 296.
4 W. R. HOLMQUIST AND W. A. SCHROEDER, *Biochim. Biophys. Acta*, 82 (1964) 639.
5 W. R. HOLMQUIST AND W. A. SCHROEDER, *Biochemistry*, in press.
6 R. T. JONES AND W. A. SCHROEDER, *J. Chromatog.*, 10 (1963) 421.
7 A. G. SCHNEK AND W. A. SCHROEDER, *J. Am. Chem. Soc.*, 83 (1961) 1472.

CELLULOSE COLUMN CHROMATOGRAPHY FOR THE FRACTIONATION AND ISOLATION OF ACID MUCOPOLYSACCHARIDES

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A number of techniques are available for fractionation and isolation of acid mucopolysaccharides. In one procedure, AMPS** is precipitated from an aqueous solution containing different metal ions (Ca, Mg, Ba) with organic solvents[1,2]. Ion exchange chromatography with Dowex[3,4], DEAE[5] or ECTEOLA cellulose[6] have been used by other investigators. The complex formed between polyanions and quaternary ammonium salts, described by SCOTT[7,8] has since been utilized as the basis for the isolation of individual fractions by several investigators[9–11]. It has been our experience that none of these methods provides sharp separations.

ANTONOPOULOS and co-workers[12] recommended a method combining the SCOTT procedure with column chromatography. This provided clean separations of HA, the CS group and Hep, but was not capable of distinguishing members of the chondroitin sulfate group on Dowex 1, DEAE or ECTEOLA columns[13–15].

To permit identification of members of the chondroitin sulfate group in investigations of arterial AMPS, a two-step procedure was adopted. The first step served to separate HA, HMS, and the CS group by elution from a Dowex 1 column[4,15] or the CP–AMPS complexes from a cellulose column[16], in the second step, the CS group was subjected to alcohol fractionation on a Hyflo supercel column, an adaptation of KAPLAN AND MEYER'S technique[17]. It proved feasible to combine these two steps on a single cellulose column for the sharp separation of individual components from the mixture in isolated aortic AMPS.

MATERIALS

Reference standards consisted of: HA (Sigma Co., St. Louis, Mo., Grade I) purified by CPC fractionation: CS-A (Nutritional Biochemicals Co., Cleveland, Ohio) purified twice by alcohol fractionation; CS-B was kindly furnished by Dr. J. A. CIFONELLI, Chicago University; CS-C (Kakenaku-Kako Co. Ltd., Kyoto, Japan); HMS extracted from human aorta and purified by alcohol fractionation after Dowex 1 column fractionation; Hep (Upjohn Co., Kalamazoo, Mich.) purified by Dowex 1 column fractionation.

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** Abbreviations used: AMPS = acid mucopolysaccharides; HA = hyaluronic acid; CS = chondroitin sulfate; HMS = heparitin sulfate; Hep = heparin; CP(C) = cetyl pyridinium (chloride).

(*a*) *Preparation of the column*

The cellulose column was a modification of those described by GARDELL[18] and ANTONOPOULOS *et al.*[12,19]. Glass tubes with an inner diameter of 1.0 cm and a length of 20 cm extending from a pear-shaped 200 ml reservoir, were filled with cellulose powder (Standard grade, Whatman Co.) using a slurry that had been washed four times in distilled water.

(*b*) *Fractionation*

Two to five mg of AMPS dissolved in 20 ml of 0.05 *M* NaCl, were precipitated quantitatively with 2–3 ml of 2 % aqueous CPC (K & K Laboratory Co.) (see Table I).

After the addition of about 0.3 g of Celite (Johns-Manville) the mixture was allowed to stand overnight at 25°; the CP–AMPS complex was then harvested by centrifugation, resuspended in washing solution (0.05 *M* NaCl in 0.01 % CPC aqueous

TABLE I

FRACTIONATION AND ISOLATION OF ACID MUCOPOLYSACCHARIDES

1. Dissolve sample in 0.05 *M* NaCl.
2. Add 2 % CPC aqueous solution.
3. Add 0.3 g of Celite.
4. Allow to precipitate overnight at 25°.
5. Centrifuge, discard supernatant.
6. Apply precipitate to cellulose column.
   Elute in sequence with:
7. 0.35 *M* NaCl (40 ml).
8. 0.6 *M* NaCl (40 ml).
9. Abs. ethanol (5 ml) and 90 % ethanol sat. Ca acetate (35 ml).
10. 30 % ethanol with 5 % Ca acetate 3 % acetic acid (40 ml).
11. 10 % ethanol with 5 % Ca acetate 3 % acetic acid (40 ml).
12. Dialyse each fraction against distilled water, 2 days.
13. Add 4 volumes of 95 % ethanol containing 1 % K acetate and 1 % acetic acid.
14. Store 2 days at 20°.
15. Centrifuge at 2,000 r.p.m. for 20 min.
16. Wash 2 times with 95 % ethanol.
17. Air dry.

solution) and transferred to the column. The flow rate from the column was adjusted between 0.2 and 0.4 ml/min by addition of Celite suspended in washing solution. Stepwise elution at 25° was conducted using in turn 40 ml each of 0.35 *M* and 0.6 *M* NaCl. An automatic fraction collector (LKB) was used to collect the effluent in 5 ml quantities. The column was then washed with 5 ml of absolute ethanol and with 35 ml of 90% ethanol saturated with calcium acetate, disrupting the CP–AMPS complex. Further elution was conducted using successively 40 ml quantities of 55 %, 45 %, 30 % and 10 % ethanol in a solution of 5% calcium acetate and 3% acetic acid. When kerato sulfate is known to be absent, the 55% elution may be omitted; similarly elution with 45 % ethanol is necessary only when chondroitin sulfate A is present. The presence of AMPS in the collection tubes was ascertained by the precipitation reaction with CPC. Uronic acid was measured in each positively reacting effluent tube using both the carbazole[20] and orcinol[21,22] methods. The presence of CPC, NaCl and calcium in the effluent caused errors in the uronic acid determination of less than 5%.

(*c*) *Identification of acid mucopolysaccharide fractions*

Identification of each eluent was performed by chemical analysis, acetate paper electrophoresis, infrared spectroscopy, digestion with hyaluronidase and paper chromatography of the product of acid hydrolysis.

Chemical analyses included the carbazole and orcinol reactions for uronic acid, as previously mentioned, the BOAS[22] modification of the Elson-Morgan reaction for hexosamine, and the method of EGAMI AND TAKAHASHI[24] for sulfate groups.

Electrophoresis was performed with acetate paper (Sepraphore III, Gelman Co.) in a horizontal chamber using pyridine–acetic acid–water (5:100:895, v/v) buffer pH 3.6[25] and a constant current of 0.5 mA/cm for 120 min at 25°. The air dried strip was stained with alcian blue (0.1 % alcian blue and 5 % acetic acid in 20 % ethanol). The Beckman Analytrol, Model RB, was used at 660 mμ for determination of the percentage composition of the mixture[26].

Infrared spectrum. A few drops of aqueous AMPS solution were allowed to dry on the surface of a cell plate formed of 56 % thallium iodide, 4 % thallium bromide and 40 % sodium chloride (Hitachi Co.). After further dehydration in a desiccator overnight, the infrared spectrum was recorded from 1,330 cm^{-1} to 400 cm^{-1} using a Perkin-Elmer model 337 spectrophotometer.

Hyaluronidase (EC 4.2.99.1) digestion of AMPS was performed in dialysis tubing with testicular hyaluronidase (Nutritional Biochemicals Co.) in 0.1 *M* phosphate buffer, pH 7.0 constituted with a physiologic concentration of NaCl. After 4 days of dialysis at 37° (against the same buffer), unaffected AMPS retained in the dialysis tube was measured by the orcinol method.

Identification of products of acid hydrolysis was accomplished by paper chromatography.

Hexosamine was determined in the following manner: after hydrolysis with 4 *N* HCl at 100° for 12 h in sealed tubes, the amino sugar component of each AMPS was ascertained by descending paper chromatography on Whatman No. 1 paper with *n*-butanol–pyridine–water (5:3:2, v/v) for 30 hours at 25°. The chromatograms were stained with ninhydrin (0.1 %) in acetone and with a water-saturated butanol solution (30 ml) of aniline (3 ml) and phthalic acid (4.8 g)[27], D-glucosamine and D-galactosamine hydrochlorides (Nutritional Biochemicals Co.) were used as control standards.

Uronic acids in AMPS were identified by the method of RADHAKRISHNAMURTHY AND BERENSON[28] following hydrolysis for 6 h at 100° in sealed tubes with formic acid. Descending paper chromatograms (Whatman No. 1) obtained with *n*-butanol–acetic acid–water (4:1:2, v/v for 18 h, were developed with aniline–phthalic acid solution and silver nitrate. D-Glucuronic acid (Sigma Co.) and L-iduronic acid derived from hydrolysed CS-B were used as standards.

(*d*) *Recovery of individual standard acid mucopolysaccharides*

Five mg of each AMPS (HA, HMS, CS-A, CS-B, CS-C and Hep) were dissolved in 25 ml of 0.05 *M* NaCl. Five ml of each solution were retained as a control while 2 ml of 2 % aqueous CPC and 0.3 g of Celite were added to the remaining 20 ml which was then kept overnight at 25°. The CP–AMPS precipitate separated by centrifugation was applied to a cellulose column and fractionated in sequence with NaCl and with ethanol as previously described.

TABLE II

CELLULOSE COLUMN FRACTIONATION OF ACID MUCOPOLYSACCHARIDES

Fraction	*Recovery of individual standards* (%)					
	HA	*HMS*	*CS-A*	*CS-B*	*CS-C*	*Hep*
0.35 *M* NaCl	98.2	0	0	0	0	0
0.6 *M* NaCl	0	97.3	2.0	0	3.0	2.0
55% ethanol	—	—	—	—	—	—
45% ethanol	0	0	22.7	0	65.5	0
30% ethanol	0	0	74.8	2.1	30.1	0
10% ethanol	0	0	0	96.6	0	98.7
Total recovery	98.2(±0.5)	97.3(±0.5)	99.5(±1.2)	98.7(±1.1)	98.6(±1.2)	100.7(±1.2)

The recovery rate for each substance exceeded 97 % as shown in Table II.

(*e*) *Recovery of standard acid mucopolysaccharides from a mixture*

Four mg of HA, HMS, CS-B and CS-C were mixed and dissolved in 40 ml of 0.05 *M* NaCl, 10 ml of 2 % aqueous CPC and 0.5 g of Celite were added. The mixture was left overnight at 25° and subjected to the fractionation procedure previously described. AMPS recovered in each fraction was measured and identified. The results shown in Table III indicate very effective separation and recovery of the individual standards.

TABLE III

RECOVERY OF STANDARDS FROM A MIXTURE OF ACID MUCOPOLYSACCHARIDES WITH CELLULOSE COLUMN FRACTIONATION

Fraction	*Substance*	*Purity as revealed by acetate paper electrophoresis* (%)	*Recovery* (%)
0.35 *M* NaCl	HA	100	98.5
0.6 *M* NaCl	HMS	100	98.3
90% ethanol (Ca acetate saturated)	No AMPS		0
30% ethanol	CS-C	97	98.2
	CS-B	3	
10% ethanol	CS-B	97	97.7
	CS-C	3	

(*f*) *Human aortic acid mucopolysaccharides*

Sodium chloride was added to aqueous solutions of AMPS extracted from aortas of twenty to forty year old normal humans (20–30 ml containing 1.0 to 2.0 mg of orcinol reacting uronic acid) to a concentration of 0.05 *M* (Table IV). Two or three ml of 2 % aqueous CPC and about 0.3 g of Celite were added and allowed to react overnight at 25°. The resultant CP–AMPS complexes were isolated and fractionated. Each fraction was measured and identified by acetate paper electrophoresis (those not completely resolved were refractionated). Electrophoretically homogeneous

TABLE IV

CELLULOSE COLUMN FRACTIONATION OF ACID MUCOPOLYSACCHARIDES OF HUMAN AORTAS

Eluent	*AMPS* (%)	*Carbazole–orcinol ratio*	*Uronic acid*–hexosamine ratio*	*Sulfate–hexosamine molar ratio*	*Paper chromatography*		*Digested*** (%)	*Identity****
					Hexosamine	*Hexuronic acid*		
0.35 *M* NaCl	16.3	0.98	1.01	0.11	Glucosamine	Glucuronic acid	98	HA
0.6 *M*	11.8	1.73	0.75	0.65	Glucosamine (2% galactosamine)	Glucuronic acid	4	HMS
30% ethanol	58.9	1.04	1.15	0.97	Galactosamine (2% glucosamine)	Glucuronic acid	95	CS-C
10% ethanol	10.2	0.58	0.98	0.98	Galactosamine	Iduronic acid	0	CS-B
Recovery	97.2							

* Uronic acid determined by the orcinol method.
** With testicular hyaluronidase.
*** Verified by electrophoretic mobility and infrared spectral absorption.

fractions were further identified by paper chromatography of amino sugars and uronic acids, by sulfate group determinations and by infrared spectroscopy.

DISCUSSION

As the accompanying Table II shows, the recovery of any of six individual acid mucopolysaccharides from the fractionation procedure exceeds 97 %; with a mixture of four, such as those normally present in arteries[17,29,30], highly effective separation is accomplished with no impairment of the yield. Acetate paper electrophoresis indicated mutual contamination of CS-C and CS-B of about 3 %.

Additional steps are necessary if the AMPS mixture included CS-B and Hep, both of which are eluted in the 10 % ethanol fraction, and if CS-A and CS-C are present together. Since the salt solubilities of the CP-complexes of CS-B and Hep are different[4,31], they can be easily separated using a cellulose column and two eluting solutions differing in salt concentration. The incomplete partition of CS-A and CS-C in the 45 % and 30% ethanol fractions can be rectified by precipitation of CS-A with 45 % ethanol containing 5 % calcium acetate and 3 % acetic acid.

The results of the fractionation of human aortic AMPS are summarized in Table IV and Fig. 1. A small amount of chondroitin sulfate eluted with 0.6 *M* NaCl accounted for the presence of 2% galactosamine in the HMS fraction. A greater

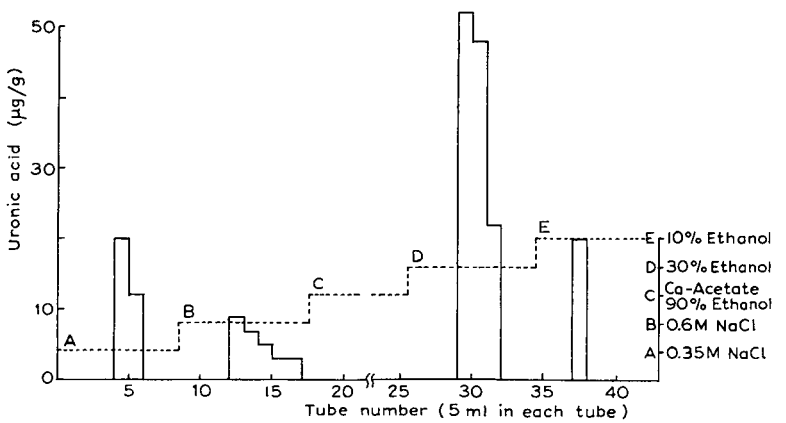

Fig. 1. Fractionation of aortic acid mucopolysaccharides.

potential error may arise because quantities of HMS at times as much as 10 %, are incompletely eluted with 0.6 *M* NaCl and then appear with CS-B in the 10 % ethanol fraction. Electrophoresis and paper chromatography permit such occurrences to be recognized. They are then corrected by careful refractionation of the CP-complex with 0.6 *M* NaCl. This difficulty seems attributable to reported variations in sulfate content and the degree of polymerization[32–34]. LAURENT AND SCOTT[35] observed that CS–CP complexes of low molecular weight are more readily soluble in salt solutions than complexes of high molecular weight. It is to be noted that neither heparin nor kerato sulfate were identified in analyses accounting for more than 97 % of human aortic AMPS contrary to other published reports[29,36].

SUMMARY

An acid mucopolysaccharide (AMPS) mixture may be separated into its individual constituents by eluting the cetyl pyridinium complexes from a cellulose column with 0.35 *M* and 0.6 *M* NaCl, disrupting the complexes with 90% ethyl alcohol and eluting further with 55, 45, 30 and 10% concentrations of ethanol. Depending upon the specific AMPS in the mixture, the procedure can be simplified further. Human arterial AMPS, for example, can be adequately partitioned with only two concentrations of alcohol, 30 and 10%. Acetate paper electrophoresis, infrared spectra, chemical analysis, hydrolysis with hyaluronidase and paper chromatography of products of acid hydrolysis of AMPS are used to identify the separated components and to ascertain the adequacy of separation. Different situations encountered during separation of various mixtures of AMPS are discussed.

REFERENCES

1 J. E. JORPES AND S. GÅRDELL, *J. Biol. Chem.*, 176 (1948) 267.
2 K. MEYER, E. DAVIDSON, A. LINKER AND P. HOFFMAN, *Biochim. Biophys. Acta*, 21 (1956) 506.
3 E. DAVIDSON AND K. MEYER, *J. Biol. Chem.*, 211 (1954) 605.
4 S. SCHILLER, G. A. SLOVER AND A. DORFMAN, *J. Biol. Chem.*, 236 (1961) 983.
5 M. SCHMIDT, *Biochim. Biophys. Acta*, 63 (1962) 346.
6 N. R. RINGERTZ AND P. REICHARD, *Acta Chem. Scand.*, 13 (1959) 1467.
7 J. E. SCOTT, *Chem. Ind. (London)*, (1955) 1568.
8 J. E. SCOTT, *Biochim. Biophys. Acta*, 18 (1955) 428.
9 L. RODEN, *Arkiv Kemi*, 10 (1956) 383.
10 E. BUDDECKE, *Z. Physiol. Chem.*, 318 (1960) 33.
11 E. KIMOTO, Y. TANAKA, Y. IMOTO AND Y. MATSUSHITA, *Kurume Med. J.*, 7 (1960) 97.
12 C. A. ANTONOPOULOS, E. BORELIUS, S. GARDELL, B. HAMNSTRÖM AND J. E. SCOTT, *Biochim. Biophys. Acta*, 54 (1961) 213.
13 Y. TANAKA, Y. MATSUSHITA, K. KOYANAGI, H. KAWASAKI, M. TAKEUCHI AND Y. KOYANAGI, *J. Kurume Med. Assoc.*, 24 (1961) 1559.
14 M. TAKEUCHI AND Y. TANAKA, *Kurume Med. J.*, 10 (1963) 37.
15 I. GORE, Y. TANAKA AND H. A. WHITE, *Arch. Pathol.*, 78 (1964) 181.
16 I. GORE, Y. TANAKA, T. FUJINAMI AND M. L. GOODMAN, *J. Nutr.*, 87 (1965) 311.
17 D. KAPLAN AND K. MEYER, *Proc. Soc. Exptl. Biol. Med.*, 105 (1960) 78.
18 S. GARDELL, *Biochem. Soc. Symp. (Cambridge, Engl.)*, 20 (1961) 39.
19 C. A. ANTONOPOULOS, S. GARDELL, J. A. SZIRMAI AND R. DE TYSSONSK, *Biochim. Biophys. Acta*, 83 (1964) 1.
20 Z. DISCHE, *J. Biol. Chem.*, 167 (1947) 189.
21 A. J. BOLLET, D. V. ANDERSON AND W. F. SIMPSON, *J. Clin. Invest.*, 37 (1958) 858.
22 Y. TANAKA, Y. MATSUSHITA, K. KOYANAGI, M. TAKEUCHI AND Y. KOYANAGI, *J. Kurume Med. Assoc.*, 24 (1961) 1671.
23 N. BOAS, *J. Biol. Chem.*, 204 (1953) 553.
24 F. EGAMI AND N. TAKAHASHI, *Bull. Chem. Soc. Japan*, 30 (1957) 443.
25 K. D. VON BERLEPSCH, *Federation Proc.*, 21 (1962) 171.
26 E. KIMOTO, H. BABA, H. MASUDA AND M. TAKEUCHI, in the press.
27 S. M. PARTRIDGE, *Nature*, 164 (1949) 443.
28 B. RADHAKRISHNAMURTHY AND G. S. BERENSON, *Arch. Biochem. Biophys.*, 101 (1963) 360.
29 E. BUDDECKE, *J. Atherosclerosis Res.*, 2 (1962) 32.
30 O. V. SIREK, S. SCHILLER AND A. DORFMAN, *Biochim. Biophys. Acta*, 83 (1964) 148.
31 C. A. ANTONOPOULOS, S. GARDELL AND B. HAMNSTRÖM, *J. Atherosclerosis Res.*, 5 (1965) 9.
32 J. A. CIFONELLI AND A. DORFMAN, *J. Biol. Chem.*, 235 (1960) 3283.
33 A. LINKER, P. HOFFMAN, P. SAMPSON AND K. MEYER, *Biochim. Biophys. Acta*, 29 (1958) 443.
34 S. SCHILLER, *Biochim. Biophys. Acta*, 32 (1959) 315.
35 T. C. LAURENT AND J. E. SCOTT, *Nature*, 202 (1964) 661.
36 S. Y. YU AND H. T. BLUMENTHAL, *J. Gerontol.*, 13 (1958) 366.

IMPROVED METHODS FOR THIN-LAYER CHROMATOGRAPHIC SEPARATION OF 2,4-DINITROPHENYLHYDRAZONES

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INTRODUCTION

2,4-Dinitrophenylhydrazones (2,4-DNPH) have often been used for the identification of carbonyl compounds. As these derivatives are intensely colored, they are also well suited for quantitative determination of the carbonyl compounds by colorimetric analysis and for separation by chromatographic procedures. Many methods involving column, paper or thin-layer chromatography have already been published; the thin-layer chromatographic methods seem to be the most promising, as they are in general characterized by simplicity and speed, see for example refs. 1–6*.

The original aim of the present investigation was to develop a rapid and simple procedure for separation and identification of the lower aliphatic carbonyl compounds. However, many aromatic carbonyl compounds have also been included. Thin-layer chromatography is of importance for many practical as well as educational purposes and the methods discussed in this paper have been included in the course in organic identification at the Technical University of Denmark.

EXPERIMENTAL

Preparation of the derivatives

The 2,4-DNPH's were prepared by the "diglyme method"[7], using pure, commercial carbonyl compounds as starting materials. The melting points were determined and agreed fairly well with the recorded values. In some cases, the nitrogen contents were also determined (by Mr. P. HANSEN, The Microanalytical Laboratory, University of Copenhagen). In most cases, the products prepared were very pure, but a few of the compounds contained isomers which, however, did not influence the investigation.

Adsorbents

The following adsorbents were used:

(A) Silica Gel H or HF 254 (the latter containing a luminescent indicator), both obtained from Merck.

(B) Alumina (Fluka, 5 % plaster of Paris).

(C) Kieselguhr G (Merck).

(D) Avicel-SF (technical grade microcrystalline cellulose from FMC Corp.).

(E) Eastman Chromagram sheet (K301R2).

* Note added in proof: See also ref. 9.

Preparation of the plates

The microchromatoplates (40 × 76 mm) were coated by dipping them into a well stirred suspension of 75 g adsorbent in 350 ml of chloroform. In the case of (B), (C) and (D), 2 % methanol was added to the suspension.

Procedure

The plates were developed in a simple 250 ml beaker covered with a cork. When using adsorbent A, the mobile phase was petroleum ether (62–82° from Shell). With adsorbent A, it was also found suitable to employ reversed phase chromatography; this was effected by allowing a solution of 20 % by vol. of dimethylformamide in chloroform, to impregnate the plates for 15 min. The now fully impregnated plates were dried in air for 1 min and were then used immediately.

The alumina plates (B) were developed in the same way as (A) but the mobile phase in this case was a mixture of ethyl acetate and petroleum ether (62–82°) saturated with water.

For the kieselguhr (C) and the microcrystalline cellulose (D) adsorbents both mobile phases mentioned above were used. Similar experiments were carried out in the case of the Chromagram (E); this material was impregnated with dimethylformamide in chloroform as was adsorbent (A).

Detection of the spots

As 2,4-DNPH's are intensely colored, they can often be observed on the plates without further treatment. Smaller amounts are more easily detected by using an U.V.-lamp—especially when HF 254 (containing a luminescent indicator) is employed as the adsorbent.

In the present work, 2,4-DNPH's were detected on the chromatoplates by placing the latter in a beaker containing a small amount of a liquid lower, aliphatic amine. Propylamine, isopropylamine and diethylamine have all been used for this purpose, and cause an increase in color of the 2,4-DNPH's. The color developed disappears in a few seconds after the plates are removed from the beaker; the aliphatic aldehydes—especially methanal—may give rise to a permanent color change on treatment with the amine vapour.

RESULTS AND DISCUSSION

(A) Silica gel plates

Silica gel plates have been employed for separation of the lower aliphatic aldehydes and ketones, but the results obtained were not very satisfactory.

In the literature on the paper chromatographic separation of 2,4-DNPH's good results have been obtained by prior impregnation of the paper with polar compounds, as *e.g.* dimethylformamide[8]. Similar results were observed in the present work, using 10–20 ml of ethyl acetate per 100 ml of petroleum ether as the mobile phase. Using this concentration ethanal, propanal, and butanal were fully separated. The ketones moved a little faster than the aldehydes with the same number of carbon atoms, *e.g.* acetone faster than propanal and 2-butanone faster than butanal. A branched chain causes the compound to move more slowly (2-methylpropanal is slower than butanal).

Double bonds retard the movement, *e.g.* 3-buten-2-one moves more slowly than 2-butanone, and 2-butenal is slower than butanal.

Ethanal moves only a little faster than methanal, but separation can be obtained by repeating the chromatographic procedure once or twice. In this case, it will often be advantageous to allow the mobile phase to evaporate from the upper edge of the plate; the time necessary is 30–45 min. The last-mentioned method also allows the separation of benzaldehyde and ethanal (the former moving faster than the latter).

(B) Alumina

With this adsorbent, different amounts of ethyl acetate in petroleum ether were used for the chromatography of a mixture of four aldehydes (methanal, ethanal, propanal, and butanal); the results appear in Fig. 1. For further work a mobile phase consisting of 15 ml of ethyl acetate in 100 ml of petroleum ether was chosen, as this concentration of ethyl acetate also gave good results when used for the other lower, aliphatic carbonyl compounds. The time of development was 15 min.

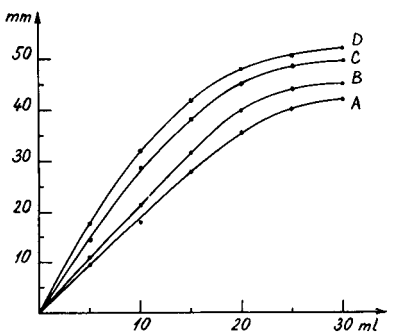

Fig. 1. Relation between the amount of ethyl acetate per 100 ml petroleum ether and the movement on alumina of some lower, aliphatic aldehydes. A = methanal; B = ethanal; C = propanal; D = butanal.

With the procedure used, it is not possible to characterize the movement of the individual compounds by R_F values as the chromatography continues after the front has reached the upper edge of the plate. Therefore, the data reported in this paper represent relative values, with the movement of butanal taken as the standard. In this way, good reproducibility of the results is obtained, as deviations from the normal due to variations in temperature, composition of mobile phase, layer thickness etc. are largely eliminated. The reproducibility is illustrated by the results from 15 plates, chromatographed one after the other, with 2 reference samples of the four aldehyde 2,4-DNPH's one on each edge of the plate. The relative distances and the standard deviations for the 30 runs are given in Table I.

In Table II, relative values are given for all the 2,4-DNPH's dealt with, and color reactions with the amines are recorded as well. The compounds were chromatographed on 3–6 different plates with a standard sample of the four aldehydes mentioned above on each edge.

It can be seen in Table II that in most cases the phenolic compounds do not

TABLE I

REPRODUCIBILITY IN CHROMATOGRAPHING 2,4-DNPH's

2,4-DNPH	*Average value* $\left(\pm\sqrt{\frac{\Sigma_i^2}{n\,(n-1)}}\right)$	*Standard deviation* $\left(\pm\sqrt{\frac{\Sigma_i^2}{n-1}}\right)$
Methanal	0.629 ± 0.004	± 0.019
Ethanal	0.729 ± 0.004	± 0.023
Propanal	0.900 ± 0.003	± 0.015
Butanal	1.00 ± 0	—

TABLE II

R_X VALUES (RELATIVE TO BUTANAL) ON ALUMINA AND REACTIONS FOR 2,4-DINITROPHENYLHYDRAZONES

No.	*2,4-DNPH*	R_X *(relative to butanal)*	*Colour with amine vapour*
1	Methanal	0.63	brown
2	Ethanal	0.73	brown
3	Propanal	0.90	brown
4	Acetone	0.90	brown
5	Butanal	1.00	brown
6	2-Butanone	1.03	brown
7	2-Methylpropanal	1.03	brown
8	Pentanal	1.04	brown
9	2-Pentanone	1.09	brown
10	3-Pentanone	1.09	brown
11	3-Methyl-2-butanone	1.07	brown
12	2-Hexanone	1.12	brown
13	4-Methyl-2-pentanone	1.20	brown
14	Heptanal	1.11	brown
15	2-Heptanone	1.07	brown
16	3-Heptanone	1.19	brown
17	4-Heptanone	1.31	brown
18	5-Methyl-2-hexanone	1.08	brown
19	4,4-Dimethyl-2-pentanone	1.20	brown
20	Acrolein	0.86	red-brown
21	3-Buten-2-one	1.01	red-brown
22	2-Butenal	0.88	red-brown
23	5-Hexen-2-one	1.03	brown
24	Glyoxal	0.06	red
25	Butane-2,3-dione	0.70	red
26	Pentanedial	0.10	brown
27	3-Hydroxy-2-butanone	0.59	red
28	Cyclopentanone	1.00	brown
29	Cyclohexanone	1.03	brown
30	Benzaldehyde	0.87	red
31	2-Chlorobenzaldehyde	1.01	red
32	4-Chlorobenzaldehyde	0.86	red
33	2,4-Dichlorobenzaldehyde	1.04	red
34	4-Bromobenzaldehyde	0.86	red
35	2-Nitrobenzaldehyde	0.51	violet
36	3-Nitrobenzaldehyde	0.47	violet
37	4-Nitrobenzaldehyde	0.23	blue

(continued on p. 265)

TABLE II (*continued*)

No.	*2,4-DNPH*	R_F *(relative to butanal)*	*Colour with amine vapour*
38	Acetophenone	0.95	red
39	2,5-Dichloroacetophenone	1.12	red
40	3,4-Dichloroacetophenone	0.88	red
41	2-Nitroacetophenone	0.39	purple
42	3-Nitroacetophenone	0.38	red
43	4-Nitroacetophenone	0.38	purple
44	2-Methylbenzaldehyde	0.94	red
45	4-Methylbenzaldehyde	0.90	red
46	Propiophenone	1.05	purple
47	1-Phenyl-2-propanone	0.95	brown
48	Butyrophenone	1.10	red
49	4-Phenyl-2-butanone	0.92	brown
50	Isobutyrophenone	1.12	brown
51	4-Methylpropiophenone	1.08	purple
52	Cinnamaldehyde	0.80	red
53	Crotonophenone	1.11	red-brown
54	1,3-Diphenyl-2-propanone	1.04	red-brown
55	2-Hydroxybenzaldehyde	0.07	red
56	2-Methoxybenzaldehyde	0.83	red
57	3-Hydroxybenzaldehyde	0	red
58	3-Methoxybenzaldehyde	0.71	red
59	4-Hydroxybenzaldehyde	0	red
60	4-Methoxybenzaldehyde	0.61	red
61	2-Hydroxyacetophenone	0	red
62	4-Hydroxyacetophenone	0	red
63	4-Methoxyacetophenone	0.77	red
64	Benzoin	0.17	blue
65	2,4-Dihydroxybenzaldehyde	0	red
66	3,4-Dihydroxybenzaldehyde	0	red
67	2,6-Dihydroxyacetophenone	0	red
68	2,4,6-Trihydroxybenzaldehyde	0	red
69	3,4,5-Trihydroxyacetophenone	0	red
70	4-Dimethylaminobenzaldehyde	0.69	red

move. The only exception is 2-hydroxybenzaldehyde, which moves a little. Aliphatic hydroxy groups also retard the compounds, but to a much lesser degree than do phenolic systems. Nitro groups also retard the movement to some degree; methoxy groups have no great effect in this direction. The *ortho*-compounds in all cases move faster than the *meta*- and *para*-compounds. The *para*-compounds move more slowly than the *meta*-compounds; in some cases the *para*-compounds seem to be so insoluble in the mobile phase that poor chromatograms with pronounced tailing result. Nevertheless good chromatograms can be prepared by taking small samples as the color reaction allows detection of small amounts. The reagent, 2,4-dinitrophenylhydrazine, does not move.

(C), (D) and (E)—other methods

The kieselguhr (C) and the microcrystalline cellulose (D) were used in a manner similar to that described for silica gel and alumina, but the materials did not effect a separation sufficient for the present purposes.

The Chromagram (E) yielded separations comparable to those on silica gel

plates. When impregnated with dimethylformamide, the results obtained were nearly as good as those of the impregnated silica gel plates.

SUMMARY

A simple method for the separation of 2,4-dinitrophenylhydrazones of aldehydes and ketones has been developed. The separation is achieved on thin layers of alumina using 15 ml of ethyl acetate per 100 ml of petroleum ether (62–82°) saturated with water. Detection of the spots is carried out by a color reaction with either propylamine, isopropylamine or diethylamine. R_X values relative to butanal are given for 70 carbonyl compounds and the colors with the amines are presented.

REFERENCES

1 J. H. DHONT AND C. DE ROOY, *Analyst*, 86 (1961) 74.
2 J. ROSMUS AND Z. DEYL, *J. Chromatog.*, 6 (1961) 187.
3 E. F. L. J. ANET, *J. Chromatog.*, 9 (1962) 291.
4 G. URBACH, *J. Chromatog.*, 12 (1963) 196.
5 G. RUFFINI, *J. Chromatog.*, 17 (1965) 483.
6 E. DENTI AND M. P. LUBOZ, *J. Chromatog.*, 18 (1965) 325.
7 H. J. SHINE, *J. Org. Chem.*, 24 (1959) 252.
8 L. HORNER AND W. KIRMSE, *Ann.*, 597 (1955) 48.
9 G. A. BYRNE, *J. Chromatog.*, 20 (1965) 528.

SÉPARATION ET IDENTIFICATION DE STÉROLS SUBSTITUÉS SUR LES CYCLES A ET B PAR CHROMATOGRAPHIE EN COUCHES MINCES ET CHROMATOGRAPHIE EN PHASE GAZEUSE

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La molécule de cholestérol est capable de donner naissance à des composés, conservant la configuration générale de la molécule initiale, mais qui en diffèrent par certaines modifications (adjonction de fonctions alcooliques ou cétoniques, apparition ou déplacement de doubles liaisons), atteignant les cycles A et B. On les considère le plus souvent comme des produits d'oxydation d'origine biologique[1–5] dont le rôle est encore obscur[6,7]; mais certains peuvent aussi se former spontanément *in vitro* sous l'influence d'agents physicochimiques[5,8–10]. C'est le cas du 7-hydroxycholestérol qui pourrait jouer cependant un rôle métabolique important[11]. Ce travail décrit les procédés qui, à l'aide de la chromatographie en couche mince (CCM) et de la chromatographie en phase gazeuse (CPG), permettent de séparer ces principaux stérols dont la structure est souvent très voisine.

MÉTHODE D'ÉTUDE DES STÉROLS SUBSTITUÉS SUR LES CYCLES A ET B

Les Tableaux I et II groupent les données relatives à l'isolement et à la caractérisation de divers stérols modifiés sur les cycles A et B.

Chromatographie en couches minces

Réalisées sur Kieselgel*** en utilisant les solvants benzène–acétate d'éthyle 9:1, 2:1 et 1:2 v/v selon des protocoles antérieurement décrits[10] pour éviter les divers risques d'artéfacts.

La mise en évidence des spots est obtenue par observation directe en lumière ultra violette, ce qui permet de déceler déjà quelques corps tel le $\Delta^{3,5}$-cholestadiène, et par pulvérisation des réactifs suivants.

(a) *Acide phosphomolybdique à 10 % dans l'éthanol,* colorant en bleu intense les stérols porteurs de groupements hydroxyles. Des stérols possédant des fonctions cétoniques peuvent être également révélés, mais avec moins d'intensité. Pour ces derniers, la coloration obtenue n'est pas toujours stable et évolue après conservation des plaques 24 h à l'obscurité, à l'inverse de la coloration des stérols hydroxylés, qui demeure inchangée. Le $\Delta^{4,6}$-cholestadiène-3-one et le Δ^{4}-cholestène-3,6-dione en

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TABLEAU I

CARACTÉRISTIQUES DE LA SÉPARATION ET DE LA RÉVÉLATION PAR CHROMATOGRAPHIE EN COUCHE MINCE

Les R_C sont calculés par rapport au cholestérol.

Dénomination	*BAE 9–1*	*BAE 2–1*	*BAE 1–2*	*Fluor.*	*Acide phosphomolybdique*	*$SbCl_3$*	*Liebermann*	*$FeCl_3$*
Δ^5-Cholestène-3β,4β-diol	0.23	0.42	0.69	o	+ bleu	bleu à froid et à chaud	bleu à froid chauffage 8 min: bleu-noir	bleu à froid, violet à chaud
Δ^5-Cholestène-3β,7α-diol	0.10	0.28	0.46	o	+ + bleu	bleu à froid et à chaud	bleu à froid et à chaud	bleu à froid et à chaud
Δ^5-Cholestène-3β,7β-diol	0.11	0.33	0.55	o	+ + bleu	bleu à froid et à chaud	bleu à froid et à chaud	bleu à froid et à chaud
Δ^5-Cholestène-3-one	1.60	1.36	1.09	± vert	+ bleu	rose pâle à chaud, fugace	chauffage 8 min: brun clair	brun
Δ^5-Cholestène-3β-ol-7-one (7-Céto-cholestérol)	2.32	1.53	1.10	o	+ bleu	violet à chaud	chauffage 8 min: mauve	violet
$\Delta^{3,5}$-Cholestadiène-7-one	2.16	1.46	1.09	+ jaune	± gris-bleu	brun stable à chaud	chauffage 8 min: brun-vert	bistre
Δ^4-Cholestène-3,6-dione	1.60	1.35	1.09	+ jaune	± gris bleu, brun après 24 h à l'obscurité et redevient bleu par exposition aux U.V.	brun stable à chaud	chauffage 8 min: brun	rouge violacé
$\Delta^{1,4}$-Cholestadiène-3-one	1.38	1.25	1.07	o	+ + bleu, violet-rouge après 24 h à l'obscurité	gris vert à chaud, mauve après 24 h	chauffage 8 min: bistre	gris-rosé
$\Delta^{4,6}$-Cholestadiène-3-one	1.51	1.32	1.08	+ vert	± gris bleu, brun après 24 h à l'obscurité. Redevient bleu par exposition aux U.V.	brun-mauve à chaud	chauffage 8 min: gris brun	rose

olestane-3β-ol(Cholestanol)	0.93	1	1.02	o	+ + bleu	rouge bistre par chauffage virant au mauve	chauffage: non coloré en 3 min, brun-mauve en 8 min	violet
5α-Cholestane-3α-ol (Epicholestanol)	1.46	1.26	1.08	c	+ + bleu	rouge bistre par chauffage virant au mauve	chauffage: non coloré en 3 min, brun-mauve en 8 min	violet
5β-Cholestane-3β-ol (Coprostanol)	1.31	1.20	1.04	o	+ + bleu	rouge bistre par chauffage virant au mauve	chauffage: non coloré en 3 min, brun-mauve en 8 min	violet
5α-Cholestane-3β-ol-6-one	0.25	0.54	0.73	o	±	jaune pâle	chauffage 8 min: jaune-brun	brun orangé
5α-Cholestane-3β,5α,6β-triol	o	0.05	0.17	o	+ + bleu	bistre puis gris noir après chauffage	chauffage 8 min: bistre	bistre
5α-Cholestane-3β,5α-diol-6-one	o	0.05	0.17	o	+ + bleu	bistre puis gris noir après chauffage	chauffage 8 min: bistre	bistre
$\Delta^{3,5}$-Cholestadiène	2.58	1.53	1.10	+ vert	+ + bleu	vert à froid violet après chauffage	vert à froid bleu vert à chaud	bleu-violet à froid et à chaud
$\Delta^{5,7}$-Cholestadiène-3β-ol (7-Déhydrocholestérol)	1	1	1	+ + vert	+ + bleu	rose à froid violet à chaud	gris vert à froid et après chauffage	gris vert
Δ^{7}-Cholestène-3β-ol (Lathostérol)	1	1	1	o	+ + bleu	brun à chaud	gris vert à froid et après chauffage	gris vert

TABLEAU II

SÉPARATION PAR CHROMATOGRAPHIE EN PHASE GAZEUSE

Colonne de XE 60 à 3 % et SE 52 à 10 %. Les temps de rétention sont calculés par rapport au cholestérol.

Dénomination	*XE-60 3 %*	*SE-52 10 %*
Δ^5-Cholestène-3β,4β-diol	1.87	1.43
Δ^5-Cholestène-3β,7α-diol Δ^5-Cholestène-3β,7β-diol	0.39	0.58
Δ^5-Cholestène-3-one	1.73	1.36
Δ^5-Cholestène-3β-ol-7-one (7-Cétocholestérol)	1.28	1.37
$\Delta^{3,5}$-Cholestadiène-7-one	1.45	1.28
Δ^4-Cholestène-3,6-dione	4.18	2.07
$\Delta^{1,4}$-Cholestadiène-3-one	2.37	1.58
$\Delta^{4,6}$-Cholestadiène-3-one	2.06	1.50
5α-Cholestane-3 β-ol (β-Cholestanol)	1	1
5α-Cholestane-3α-ol (Epicholestanol)	0.94	1
5β-Cholestane-3β-ol (Coprostanol)	0.84	0.89
5α-Cholestane-3β-ol-6-one (6-Céto-cholestanol)	4.38 début de décomposition	1.86
5α-Cholestane-3β,5α,6β-triol	—	2.91
5α-Cholestane-3β,5α-diol -6-one	—	2.66
$\Delta^{3,5}$-Cholestadiène	0.40	0.62
$\Delta^{5,7}$-Cholestadiène-3β-ol (7-Déhydrocholestérol)	1.15	décomposé
Δ^7-Cholestène-3β-ol (Lathostérol)	1.15	1.14

particulier virent du bleu gris pâle au brun, et le $\Delta^{1,4}$-cholestadiène-3-one au violet rouge dans ces conditions.

En outre, la coloration gris bleu initiale est susceptible de réapparaître par exposition à la lumière ultra violette, ce qui constitue une très bonne contre-épreuve.

(b) *Trichlorure d'antimoine* en solution saturée dans le chloroforme.

(c) *Réactif de* LIEBERMANN-BURCHARD, préparé selon le procédé décrit par ABELL *et al.*[12]

(d) *Réactif au perchlorure de fer*:

— Solution de perchlorure de fer anhydre à 10 % dans l'acide acétique cristallisable: 0.2 ml.

— Acide acétique cristallisable: 30 ml.

— Acide sulfurique concentré: 20 ml.

Les colorations obtenues à l'aide de ces trois derniers réactifs sont souvent voisines ou identiques (cholestérol par exemple). Leur utilisation conjuguée est cependant indispensable pour différencier certains stérols de R_F voisin qui fournissent des colorations différentes (voir Tableau I).

Ces colorations peuvent apparaître spontanément à froid (7-hydroxycholestérol), mais nécessitent le plus souvent un chauffage, dont l'intensité et la durée ont une

influence capitale sur l'apparition et la nature de la couleur observée. Nous avons finalement retenu un chauffage uniforme à 110° pour tous les réactifs, seul variant le temps d'exposition.

Ces temps sont les suivants: réactif a: 10 min; réactif b: 8 min; réactif c: 3 min et 8 min; réactif d: 10 min.

De tous ces agents, le plus sensible est à coup sûr le réactif au perchlorure de fer, mais celui qui fournit les colorations les plus stables est le trichlorure d'antimoine.

La quantité minimale de substance pour laquelle la coloration est encore perçue varie non seulement en fonction du réactif, mais aussi selon les stérols: elle est de l'ordre du microgramme pour les plus sensibles (cholestérol; $\Delta^{3,5}$-cholestadiène; $\Delta^{3,5}$-cholestadiène-6-one; cholestane-3β,5α,6β-triol, par exemple), et de quelques microgrammes (5 à 10) pour les moins sensibles (Δ^{5}-cholestène-3-one, $\Delta^{3,5}$-cholestadiène-7-one; $\Delta^{4,6}$-cholestadiène-3-one; Δ^{4}-cholestène-3,6-dione; par exemple).

En opérant avec les précautions indiquées antérieurement[10] et en réalisant en particulier les migrations à l'obscurité, nous n'avons jamais observé d'altération au cours de l'analyse par CCM.

Chromatographie en phase gazeuse

L'appareil utilisé est du type Aérograph 600 C à détecteur à ionisation de flamme, muni d'un double four permettant l'analyse successive rapide sur deux types de colonne des échantillons. On a retenu:

(a) Une colonne de 10 pieds × 2 mm en verre, de silicone XE 60 ("nitrile gum") à 3 % sur Gas Chrom Z (lavé aux acides et désactivé au diméthyl chlorosilane) 80–100 mesh. Température de colonne 220°, température de l'injecteur 260°.

(b) Une colonne de 5 pieds × 2 mm en inox de phényl méthyl silicone (SE 52) à 10 % sur chromosorb W 80 à 100 mesh lavé aux acides. Température de la colonne: 260°, température de l'injecteur: 280°.

Les débits d'azote employés sont de l'ordre de 35 à 50 ml/min, les stérols sont injectés en solution dans le sulfure de carbone ou le dioxanne.

Les temps de rétention indiqués au Tableau II sont calculés par rapport au cholestérol et non au 5α-cholestane comme il est habituel de le faire pour deux raisons:

(1) Le cholestérol est toujours présent dans les échantillons d'origine biologique que nous soumettons à l'analyse et constitue ainsi en quelque sorte un auto étalon interne.

(2) Les temps de rétention de certains stérols sont trop élevés pour être évalués par rapport au 5α-cholestane.

Dans un but de simplicité et de rapidité d'exécution, ainsi que pour éviter l'addition d'une manipulation supplémentaire qui ne serait pas forcément inactive sur ces molécules particulièrement peu stables, nous n'avons pas cherché à améliorer nos séparations par l'utilisation de dérivés (esters trifluoracétiques, triméthylsilyques, etc.).

De plus, l'utilisation *successive* de la CCM préparative, comme il a été décrit dans un travail antérieur[10], et de la CPG permet d'achever une séparation qui peut être imparfaite par un seul de ces procédés. L'élution de la zone où se trouvent deux spots de R_F voisins, suivie de l'injection en CPG est en effet souvent fructueuse.

REMARQUES

Comme le montre l'examen des Tableaux I et II, certains stérols sont très bien séparés par CCM comme par CPG, mais dans un certain nombre de cas, leur utilisation est complémentaire. On peut alors distinguer 3 possibilités:

(1) *Résolution incomplète par CCM, complète par CPG*

Quatre groupes de stérols entrent dans cette catégorie:

(a) 5α-Cholestane-3β,5α,6β-triol et 5α-cholestane-3β,5α-diol-6-one qui sont uniquement distingables sur SE 52 à 10 %.

(b) Δ^5-Cholestène-3β,4β-diol et 5α-cholestane-3β-ol-6-one.

(c) $\Delta^{1,4}$-Cholestadiène-3-one et coprostanol.

(d) Δ^5-Cholestène-3-one, epicholestanol, Δ^4-cholestène-3,6-dione et $\Delta^{4,6}$-cholestadiène-3-one. Pour ces deux derniers stérols en particulier, la très bonne séparation par CPG contraste avec leur grande analogie de comportement en CCM.

Il faut souligner également que le caractère différentiel des réactifs de révélation proposés permet souvent d'aider au dépistage de deux de ces stérols voisins (Δ^5-cholestène-3β,4β-diol et 5α-cholestane-3β-ol-6-one).

(2) *Résolution incomplète par CPG, complète par CCM*

Entrent dans cette catégorie 4 autres groupes:

(a) Δ^5-Cholestène-3β,7α-diol, Δ^5-cholestène-3β,7β-diol et $\Delta^{3,5}$-cholestadiène.

(b) Coprostanol, epicholestanol et bloc cholestérol–cholestanol.

(c) Δ^5-Cholestène-3β,4β-diol et Δ^5-cholestène-3-one.

(d) 5α-Cholestane-3β-ol-6-one et Δ^4-cholestène-3,6-dione.

(3) *Résolution incomplète par CCM et CPG*

Certains stérols portés aux Tableaux I et II sont mal séparés par les deux procédés. Il s'agit du cholestanol, du 7-déhydrocholestérol, et du lathostérol. Ils ont été indiqués en fait ici pour mémoire afin de ne pas négliger la possibilité de leur interférence, mais leur séparation a été décrite en détail dans des travaux antérieurs[13–15], souvent à l'aide de plaques imprégnées de nitrate d'argent.

On peut néanmoins faire remarquer que la CPG sur les types de colonne préconisés permet, au moins pour le 7-dehydrocholestérol et le lathostérol, de présumer de leur présence.

CONCLUSION

L'utilisation parallèle ou successive de la CCM ou de la CPG dans certaines conditions permet la séparation et la caractérisation de divers stérols substitués ou modifiés sur les cycles A et B par rapport au cholestérol.

Ceci doit permettre leur recherche précise dans les milieux biologiques après avoir réalisé leur isolement dans des conditions rigoureuses[10].

RÉSUMÉ

L'utilisation de la chromatographie en couche mince sur plaque silicagel à

l'aide de 3 solvants différents, suivie de la révélation par divers réactifs, liée à celle de la chromatographie en phase gazeuse sur deux types de colonnes, permet la séparation et l'identification de divers stérols substitués ou modifiés sur les cycles A et B par rapport au cholestérol. Cette méthode doit permettre l'étude de ces stérols dans divers milieux biologiques où la réalité de leur existence présente un grand intérêt métabolique.

SUMMARY

The use of thin layer chromatography on silica gel plates with three different solvents, followed by visualization by means of various reagents, together with gas chromatography on two types of columns, permits the separation and identification of various sterols in which rings A and B are substituted or modified as compared with cholesterol. This method permits the study of these sterols in many different biological media where their presence is of great metabolic interest.

BIBLIOGRAPHIE

1 G. A. D. HASLEWOOD, *Nature*, 154 (1944) 29.
2 H. B. MACPHILLAMY, *J. Am. Chem. Soc.*, 64 (1942) 1732.
3 E. HARDEGGER, L. RUZICKA ET E. TAGMANN, *Helv. Chim. Acta*, 26 (1943) 2205.
4 O. WINTERSTEINER ET J. R. RITZMANN, *J. Biol. Chem.*, 136 (1940) 697.
5 V. PRELOG, L. RUZICKA ET P. P. STEIN, *Helv. Chim. Acta*, 26 (1943) 222.
6 S. BERGSTROM, *Arkiv Kemi, Mineral. Geol.*, 16A (1943) 1.
7 P. BLADON, dans R. P. COOK, *Cholesterol Chemistry, Biochemistry and Pathology*, Academic Press, New York, 1958, p. 76.
8 I. M. HAIS ET N. B. MYANT, *Biochem. J.*, 94 (1965) 85.
9 J. R. CLAUDE ET J. L. BEAUMONT, *Compt. Rend.*, 260 (1965) 3204.
10 J. R. CLAUDE ET J. L. BEAUMONT, *J. Chromatog.*, 21 (1966) 189.
11 G. S. BOYD, *Federation Proc.*, 21 (1962) 86.
12 C. L. ABELL, B. B. LEVY, B. B. BRODIE ET F. E. KENDALL, *J. Biol. Chem.*, 195 (1952) 357.
13 J. AVIGAN, D. S. GOODMAN ET D. STEINBERG, *J. Lipid Res.*, 4 (1963) 100.
14 J. R. CLAUDE, *J. Chromatog.*, 17 (1965) 596.
15 J. R. CLAUDE ET J. L. BEAUMONT, *Ann. Biol. Clin. (Paris)*, 22 (1964) 815.

AROMATIC AMINES AS SPRAY REAGENTS IN THE THIN-LAYER CHROMATOGRAPHY OF CHLORINATED ORGANIC PESTICIDES

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INTRODUCTION

Since thin-layer chromatography has been used for analyses of chlorinated pesticide residues[1–4], it has been found that silver nitrate reagent, the reagent most frequently used in paper chromatography[5–15], did not give satisfactory results: the background was dark, the spots with low concentrations were not sufficiently defined, etc. Other reagents such as methyl yellow[16], indophenol blue[17], or N,N-dimethyl-*p*-phenylenediamine hydrochloride in sodium ethoxide[18] were also not satisfactory for various reasons.

However, the "spot test" for amine compounds[19] can be modified (organochlorine pesticides being used instead of fluorescein chloride, and diphenylamine and zinc chloride as reagents) and can be used for the detection of chlorinated pesticides. LEMLEY[20] was one of the first to suggest the possibility of detecting DDT with zinc chloride, phenol and diphenylamine. CUETO[21] melted dieldrin with diphenylamine and zinc chloride at 205° and used the coloured compound so obtained for colorimetric determination of dieldrin. GRAUPNER[22] treated toxaphene in a similar way. MCKINLEY[23] described a procedure for the detection of captan by spraying the chromatogram with diphenylamine and zinc chloride in acetone and irradiating with U.V. light. BONDI[24] described the detection of aldrin, dieldrin, toxaphene and DDT by a "spot test" technique, but got a negative reaction for lindane. KATZ[25] used diphenylamine–zinc chloride and diphenylamine–iodine as spray reagents in thin-layer chromatography and after heating at 200° he obtained coloured compounds with DDT, methoxylchlor, kelthane, captan and toxaphene.

All the above references are to reactions of chlorinated insecticides with diphenylamine in the presence of zinc chloride or iodine. We also used diphenylamine, but found[26] that DDT, lindane, aldrin, dieldrin, endrin, toxaphene, together with 2,4-D, 2,4,5-T, MCPA and MCPP, under the influence of U.V. light react with diphenylamine, even if zinc chloride or iodine are absent, to give characteristically coloured products.

Investigation of the behaviour of other aromatic amines showed that those compounds having a free or substituted amino-group react, under the above mentioned conditions, with chlorinated insecticides, giving more or less characteristically coloured reaction products.

EXPERIMENTAL

Reagents

1. Silica gel according to STAHL (*"Kemika", Zagreb*). 30 g of adsorbent and 72 ml of water are shaken for 2 min in an Erlenmeyer flask with a ground stopper and then applied to glass plates by means of an applicator; the thickness of the layer is 0.25 mm. After air-drying the adsorbent is activated at a temperature of 120° for 30 min and then cooled in a desiccator over silica gel.

2. Solvent. Benzine fraction (b.p. 90 to 100°), purified by shaking with sulphuric acid (20 % SO_3), water and a 2 % solution of sodium bicarbonate and again water. The solvent obtained in this way is dried over anhydrous sodium sulfate and distilled.

3. Standard insecticide solutions. 0.1 % solutions of recrystallized active material: *p,p'*-DDT, lindane, aldrin, dieldrin and endrin in ethyl acetate.

4. Chromogenic reagents. 0.5 % v/v or 0.1 % w/v ethanol solution of the following amine compounds freshly distilled or recrystallized: aniline; *o*-, *m*-, and *p*-toluidine; *o*-aminophenol; *p*-anisidine·HCl; *p*-nitroaniline; dimethylaniline; *p*-phenylenediamine; *p*-aminodimethylaniline; *p*-dimethylaminobenzaldehyde; benzidine; *o*-tolidine; 3,3'-diaminobenzidine; diphenylamine; α-naphthylamine; N-1-naphthylethylenediamine·2 HCl; N-phenyl-1-naphthylamine.

All chromogenic reagents have to be freshly prepared before they are used.

APPARATUS

The ultraviolet light source was an Analysen Quarzlampe, Hanau, 2536 Å.

PROCEDURE

Known amounts of the standard insecticide solutions were spotted to the prepared plates from a 10 μl micro-syringe, and after air-drying the plate was developed in a jar saturated with the vapor from the solvent. The solvent front was allowed to reach a previously marked line 15 cm from the origin and the plate was then removed. Development time was 40–50 min.

The plate was dried at room temperature, then sprayed with the chromogenic reagent and exposed to the U.V. light without a filter at a distance of 25 cm for 1–15 min, depending on the chromogenic reagent being used.

RESULTS AND DISCUSSION

Before obtaining the results described in Table I we tested the amine compounds listed above under *Chromogenic reagents* under the conditions given by MCKINLEY[23] and KATZ[25], but the results were unsatisfactory; the sensitivity was shown to be very low and some of the reactions failed completely. However, when we used diphenylamine in the form of a "spot test"[26], we got sensitivities ranging in limits between 0.2 and 0.5 μg. With some of the compounds which we have just described an even higher sensitivity could be achieved with the "spot test", and this will be the subject of a future publication.

As regards the stability of the colours obtained diphenylamine is particularly

TABLE I

COLOUR OF THE SPOTS FORMED AND DETECTABLE LIMITS IN MICROGRAMS

No.	*Aromatic amine*	*Aldrin*	*p,p'-DDT*	*Lindane*	*Dieldrin*	*Endrin*	*Background colour*
1	Aniline	yellow-brown (0.5–1)	yellow-brown (5)	yellow-brown (0.5–1)	yellow-brown (0.5–1)	yellow-brown (0.5–1)	bright-cream
2	*o*-Toluidine	reddish brown (1)	reddish brown (3–5)	reddish brown (0.5–1)	reddish brown (1)	reddish brown (1)	cream
3	*m*-Toluidine	reddish brown (1)	reddish brown (3)	reddish brown (0.5–1)	reddish brown (1)	reddish brown (1)	cream
4	*p*-Toluidine	yellowish brown (1)	reddish brown (3)	reddish brown (0.5)	yellowish brown (1)	yellowish brown (1)	bright-rose
5	*o*-Aminophenol	—	—	reddish brown (10)	reddish brown (10)	reddish brown (10)	cream
6	*p*-Anisidine·HCl	reddish brown (3–5)	violet (1)	reddish brown (3–5)	reddish brown (3–5)	reddish brown (3–5)	white (brown)
7	*p*-Nitroaniline	—	reddish brown (5–10)	—	—	—	yellow-green
8	Dimethylaniline	bright-green (0.5)	brown (1)	grey-violet (1)	bright-green (0.5)	bright-green (0.5)	white
9	*p*-Phenylenediamine	grey-olive green (0.5)	grey-olive green (0.5)	violet (0.5)	grey-olive green (0.5)	grey-olive green (0.5)	almost white
10	*p*-Aminodimethylaniline	rose (1)	rose (1)	rose (1)	rose (1)	rose (1)	dirty-red
11	*o*-Dimethylaminobenzaldehyde	orange (1)	yellow (1)	orange (1)	orange (1)	orange (1)	bright-yellow-green
12	Benzidine	yellow-green (0.5)	yellow-green (0.5)	blue-green (0.5)	yellow-green (0.5)	yellow-green (0.5)	bright-cream
13	*o*-Tolidine	green (0.5)	yellow-green (0.5)	blue (0.5)	green (0.5)	green (0.5)	white-grey
14	3,3'-Diaminobenzidine	bright brown (3)	bright-brown (2)	bright-brown (4)	bright-brown (3)	bright-brown (3)	yellowish green
15	Diphenylamine	emerald-green (0.5)	reddish brown (1–2)	dark-violet (0.5)	green-yellow (0.5)	green-yellow (0.5)	almost white
16	α-Naphthylamine	reddish brown (1)	reddish yellow (0.5)	reddish brown (0.5)	reddish brown (1)	reddish brown (1)	almost white
17	N-1-Naphthylethylenediamine·2 HCl	grey-blue (1–2)	grey-blue (2)	grey-blue (0.5)	grey-blue (1–2)	grey-blue (1–2)	bright-yellow
18	N-Phenyl-1-naphthylamine	grey-blue (5)	green (3)	violet (3)	grey-blue (5)	grey-blue (5)	bright-orange

outstanding, and it is possible to read the chromatogram even after 60 days, with only a slight darkening of the background. Such stability of the coloured spots was not obtainable with other amine compounds, but in their case it is possible to revive the colour by means of further U.V. irradiation (benzidine, *o*-tolidine, dimethylamine and others).

In addition to the data in Table I some additional information concerning each of the amines used is given below.

Aniline

The irradiation time necessary after spraying with this reagent is 5–7 min. DDT is more difficult to detect than the other insecticides (sensitivity limit 5 μg).

o-, m-, and p-Toluidine

All three isomers behave similarly. Apart from the DDT spot, the colour of which fades, the other spots remain stable even after several hours.

o-Aminophenol

As distinct from the other reagents, *o*-aminophenol is dissolved in petroleum ether. To produce any coloured spots it was necessary to irradiate for 15 min, but because of lack of sensitivity the reagent has no practical value.

p-Aminodimethylaniline and p-nitroaniline

These reagents are of no account either, as with the first an intensely coloured background is obtained, and the second does not possess the required sensitivity.

p-Anisidine

This reagent is only of use with DDT, with which it forms a violet-red spot on a white background after 30–60 sec irradiation. In order to develop the spots of the other insecticides, 5 min irradiation are required, but this darkens the background so that the spots are barely discernible.

Dimethylaniline

This has been shown to be a very sensitive reagent, and with it a certain amount of selectivity is possible in the following manner. The irradiation usually lasts 2–7 min, but if the plate is irradiated for only 2 min the aldrin, dieldrin and endrin spots are blue-green, DDT brown and lindane violet. On further irradiation only the colour of the DDT spots remains unchanged. Dimethylaniline reacts with chlorphenoxy acids as well, producing violet-coloured spots.

p-Phenylenediamine

Irradiation of the chromatogram for 30–60 sec is sufficient to produce a hardly noticeable background with well defined spots; the sensitivity is better than 0.5 μg. If the irradiation is continued for 5 min the background becomes dirty-pink and the spots grey-blue, excepting lindane and toxaphene, which are coloured violet.

p-Dimethylaminobenzaldehyde

If the chromatogram sprayed with *p*-dimethylaminobenzaldehyde is irradiated

for 5–7 min, only a lemon-yellow spot due to DDT appears on the white background; after 15 min irradiation the background becomes more intensively coloured and the insecticide spots become orange. Under the unfiltered U.V. light the coloured spots become more noticeable.

Benzidine and o-tolidine

Both of these compounds behave similarly, and are equally sensitive and suitable for use as chromogenic reagents, not only for the insecticides mentioned but also for toxaphene and derivatives of chlorophenoxy acids. The chromatogram need only be irradiated for 5–10 sec to cause the appearance of coloured spots; further irradiation intensifies the colours but the background also becomes coloured. In daylight the colours change quickly, the background becomes brighter and the insecticide spots sandy-yellow; a subsequent brief irradiation produces the same effect. If the chromatogram is exposed to sunlight the same result is obtained after somewhat longer interval (several minutes).

3,3'-Diamino-benzidine

In contrast to benzidine and *o*-toluidine, 3,3'-diaminobenzidine cannot be used so successfully.

Diphenylamine

Diphenylamine is regularly used as a chromogenic reagent by us, not only for the cases when standard insecticide solutions are being examined but when impure extracts from cereals, vegetable, clover or milk are being investigated; residues of fat, waxes and other coloring pigments do not interfere. In addition to the insecticides mentioned toxaphene, MCPA, MCPP, 2,4-D and 2,4,5-T also react with diphenylamine; their spots are coloured grey after an irradiation of 5 min. The colour is very stable, making the identification of 0.5 μg of insecticide possible even after 2 months. The same effect can be produced by sunlight.

α-Naphthylamine

It takes only 30 seconds to develop the coloured spots with α-naphthylamine; if the irradiation is continued up to 2 min the background becomes rosy and the spots less discernible. The colours are unstable in daylight and after 5–10 min only the lindane spot is visible.

N-1-Naphthylethylenediamine and N-phenyl-1-naphthylamine

Both these give similarly coloured reaction products; the first reagent is somewhat more sensitive; the irradiation time is about 10 min.

CONCLUSION

Eighteen aromatic amines have been tested for their ability to identify chlorinated insecticides in thin-layer chromatography. Of these *o*-aminophenol, *p*-anisidine, *p*-nitroaniline, *p*-aminodimethylaniline and 3,3'-diaminobenzidine have no practical value; either their sensitivity is inadequate, they do not react at all, or the chromatogram background is too dark. The other amines can all be used practically as chromo-

genic reagents; dimethylaniline, *p*-phenylenediamine, benzidine, *o*-tolidine, diphenylamine and α-naphthylamine are particularly useful.

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SUMMARY

A simple and rapid procedure for identification of some chlorinated insecticides on thin-layer chromatograms by irradiating the sprayed chromatogram with U.V. light is described. Eighteen aromatic amines were used as chromogenic reagents; the following showed the highest sensitivity and, consequently, a certain practical value: dimethylaniline, *p*-phenylenediamine, benzidine, *o*-tolidine, diphenylamine and α-naphthylamine.

REFERENCES

1 H. J. PETROWITZ AND J. G. PASTUSCHKA, *Chemiker Ztg.*, 85 (1961) 867.
2 D. C. ABBOT, H. EGAN AND J. THOMSON, *J. Chromatog.*, 16 (1964) 481.
3 K. C. WALKER AND M. BEROZA, *J. Assoc. Offic. Agr. Chemists*, 46 (1963) 250.
4 H. V. MORLEY AND M. CHIBA, *J. Assoc. Offic. Agr. Chemists*, 47 (1964) 306.
5 L. C. MITCHELL, *J. Assoc. Offic. Agr. Chemists*, 35 (1952) 920.
6 L. C. MITCHELL AND W. I. PATTERSON, *J. Assoc. Offic. Agr. Chemists*, 36 (1953) 553.
7 L. C. MITCHELL AND W. I. PATTERSON, *J. Assoc. Offic. Agr. Chemists*, 37 (1954) 996.
8 R. G. BRIDGES, A. HARRISON AND F. P. W. WINTERINGHAM, *Nature*, 177 (1956) 86.
9 L. C. MITCHELL, *J. Assoc. Offic. Agr. Chemists*, 40 (1957) 296.
10 L. C. MITCHELL, *J. Assoc. Offic. Agr. Chemists*, 41 (1958) 781.
11 L. C. MITCHELL, *J. Assoc. Offic. Agr. Chemists*, 44 (1961) 643.
12 L. C. MITCHELL, *J. Assoc. Offic. Agr. Chemists*, 45 (1962) 682.
13 P. A. MILLS, *J. Assoc. Offic. Agr. Chemists*, 42 (1959) 734.
14 P. A. MILLS, *J. Assoc. Offic. Agr. Chemists*, 44 (1961) 1.
15 P. A. MILLS, *J. Assoc. Offic. Agr. Chemists*, 44 (1961) 175.
16 L. F. KRZEMINSKI AND W. A. LANDMANN, *J. Chromatog.*, 10 (1963) 515.
17 S. O. GRAHAM, *Science*, 139 (1963) 835.
18 J. BÄUMLER AND S. RIPPSTEIN, *Helv. Chim. Acta*, 44 (1961) 1162.
19 F. FEIGL, *Spot Tests in Organic Analysis*, Elsevier, Amsterdam, 1960.
20 J. D. LEMLEY, *Mass. Inst. Technol. M.R. No. 176, Project A 11.1* (1945).
21 C. CUETO, *J. Agr. Food Chem.*, 8 (1960) 273.
22 A. J. GRAUPNER AND C. L. DUNN, *J. Agr. Food Chem.*, 8 (1960) 286.
23 W. MCKINLEY AND S. J. GRAHAM, *J. Assoc. Offic. Agr. Chemists*, 43 (1960) 89.
24 A. BONDI, *Talanta*, 10 (1963) 679.
25 D. KATZ, *J. Chromatog.*, 15 (1964) 269.
26 V. M. ADAMOVIĆ, *Symp. Role Pesticides Fields Agr. Nutr., 1st, May 10th and 11th, 1965, Novi Sad, Yugoslavia.*

ANION EXCHANGE CHROMATOGRAPHY OF OXIDIZED INSULIN PEPTIDES

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The separation of the A and B chains of performic acid-oxidized insulin is frequently desired for various biochemical investigations, such as the determination of bond specificities of newly-isolated proteinases[1-4]. An interest in determining the bonds hydrolyzed by some proteinases we have isolated prompted a search for a convenient procedure to separate the oxidized A and B chains (glycyl and phenylalanyl peptides, respectively) in amounts sufficient to permit their use as substrates. It was desirable that the procedure obviate such specialized equipment as the countercurrent distribution train required by the procedure of CRAIG, KONIGSBERG AND KING[5]; it was mandatory that it produce peptides of satisfactory purity and that it be quick and reproducible, inasmuch as production of the peptides was to be routine. We describe herein a convenient chromatographic procedure for the separation of A and B chains of oxidized insulin in a highly pure, salt-free state.

EXPERIMENTAL

Materials

Insulin, six-times crystalline, was obtained from Boots Pure Drug Co., Ltd., Nottingham, England. DEAE-Sephadex A-25, fine particle size, capacity 2.9 mequiv./g, was purchased from Pharmacia Fine Chemicals, Inc. Performic acid was prepared from 97–100 % formic acid (Matheson, Coleman and Bell), and Merck Reagent Grade Superoxol (approximately 33 % H_2O_2). Tris-(hydroxymethyl)-aminomethane was purchased from Mann Research Laboratories. The 6 *N* hydrochloric acid used for hydrolysis was prepared either by diluting freshly opened Reagent Grade HCl (Fisher Scientific Co.) or by distillation in glass. All other chemicals used were Reagent or Analytical grade.

Oxidation of insulin

The method of CRAIG *et al.*[5] was used without modification for the performic acid oxidation of insulin at 0°. The oxidized product was lyophilized and stored at −15°.

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Preparation of DEAE-Sephadex columns

A number of experiments were performed using both DEAE-cellulose and DEAE-Sephadex columns with a variety of buffer systems. Although good separations were obtained with both types of exchangers, most of the experiments were performed with DEAE-Sephadex. Therefore, only the procedure using DEAE-Sephadex is described herein.

DEAE-Sephadex was allowed to swell in an excess of distilled water; the water was removed by vacuum filtration and the filter cake was suspended in 0.5 *N* HCl. The HCl was removed immediately by vacuum filtration, and the DEAE-Sephadex was washed several times in water. The filter cake was suspended in 0.5 *N* NaOH and allowed to stand for about 15 min. The suspension was filtered, washed several times with water and finally washed several times with the initial buffer. The exchanger was then packed into a chromatographic tube of a size selected on the basis of the sample size. A column of 1.2 × 21 cm was poured for samples of oxidized insulin of 25–50 mg; for samples of 50–250 mg, a column of 2 × 27 cm was used.

Chromatographic procedures

A sample of the desired amount of oxidized, lyophilized insulin was dissolved in distilled water by the dropwise addition of 0.5 *N* base; NaOH was used when the sample was to be eluted with NaCl, and NH_4OH was used when an ammonium formate gradient was to be used. The sample was applied to the column in the minimum volume.

Elution was accomplished with gradients of either ammonium formate or NaCl. For elution with ammonium formate, a linear gradient was used, with 300 ml of 0.01 *M* ammonium formate (adjusted to pH 9.5 with NH_4OH) in the first chamber of an Autograd (Technicon Chromatography Corp.), and with 300 ml of 1.0 *M* ammonium formate (pH 9.5) in the second chamber. For elution with NaCl, a compound gradient was obtained by loading the Autograd as follows: Chamber 1, 0.005 *M* Tris·HCl; Chambers 2 and 3, 0.032 *M* Tris·HCl, 0.3 *M* in NaCl; Chambers 4 and 5, 0.041 *M* Tris·HCl, 0.4 *M* in NaCl; Chamber 6, 0.050 *M* Tris·HCl, 0.5 *M* in NaCl. The Tris buffer was pH 8.0 in all chambers, each of which contained 75 ml. Flow rates were approximately 1 ml/min; column effluents were monitored at 280 mμ with an Ultraviolet Absorption Meter (Gilson Medical Electronics) and a "Rectiriter" recorder (Texas Instruments, Inc.). Fractions (10 ml) were collected in a G.M.E. fraction collector. In some experiments the positions of the fractions were located by reading the effluent in a Beckman DU Spectrophotometer at 280 mμ, or by the alkaline hydrolysis–ninhydrin method of HIRS *et al.*[6].

Desalting

Samples eluted by the ammonium formate were desalted by sublimation in a freeze-drying apparatus whose condenser was chilled in a bath of solid CO_2 and methylcellosolve. A pressure of 0.05 mm or less was maintained, and heat from infrared lamps was applied to the flasks containing the samples to aid in sublimation of the volatile salt.

Fractions from the NaCl elution were desalted by passage through a 1.2 × 30 cm column of Sephadex G-25.

Amino acid analyses

The samples of fractions to be analyzed for amino acids were hydrolyzed *in vacuo* with 6 *N* HCl by the method of MOORE AND STEIN[7]. The de-aeration procedure recommended by these authors was used. After hydrolysis for 22 or 96 h, the excess HCl was removed by repeated evaporation in a rotary evaporator, the samples were taken to dryness, then dissolved in 1 % HCl for application to the column of the automated amino acid analyzer.

A Technicon Amino Acid Analyzer with a 130 cm column of Chromobeads B was used for amino acid analysis. Standards were run before and after each series of unknowns, and norleucine was used as an internal standard with each unknown sample.

RESULTS

Separation of the oxidized A and B chains of insulin was readily achieved with either the ammonium formate or the Tris–NaCl gradients. The former, however, was simple and convenient, and was more frequently used. Fig. 1 shows a chromatogram in which the ammonium formate gradient was applied. The chromatograms were quite reproducible, with the B chain (Fraction II) emerging at approximately 0.4 *M* ammonium formate, and the A chain (Fraction III) at approximately 0.6 *M*. The identity of Fraction I was not extensively investigated, but it appeared to consist of undefined mixtures of the oxidized A and B chains. A single experiment revealed the presence of all amino acids of both chains, but the molar ratios of amino acids in

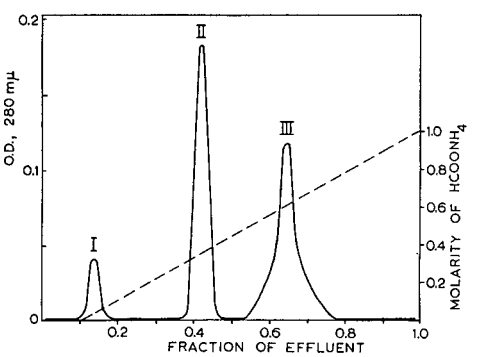

Fig. 1. Chromatography of oxidized insulin on DEAE-Sephadex, with elution by ammonium formate gradient. Fraction I is an unidentified impurity, Fraction II is the B chain, and Fraction III is the A chain.

the hydrolysate suggested that there was relatively more B chain than A chain. The material from Fraction I in this experiment also contained a non-protein component which was not identifiable by spectrophotometric analysis. The absence of methionine from hydrolysates of the preparations ruled out the possibility that glucagon contributed to the impurity. CRESTFIELD, MOORE AND STEIN[8] also found an impurity in the separation of reduced, S-carboxymethylated insulin peptides by gel filtration.

The shape of the peak representing the B chain suggested the presence of a

single component. This is borne out by the amino acid analyses shown in Table I, whose values are in satisfactory agreement with the known composition of this peptide, and with the values reported from the B chain by CRAIG *et al.*[5], who separated the peptides by counter-current distribution. Isoleucine, the "marker" amino acid for A chain, was barely detectable.

TABLE I

AMINO ACID COMPOSITION OF OXIDIZED INSULIN B CHAIN (PHENYLALANYL PEPTIDE)*

Amino acid	*μmoles found*	*No. of residues per mole*	
		Found	*Theoretical*
Cysteic acid	0.053**	1.83**	2
Cystine	0.000	0.00	0
Aspartic acid	0.031	1.08	1
Threonine	0.033	1.14	1
Serine	0.027	0.93	1
Glutamic acid	0.079	2.72	3
Proline	0.033	1.14	1
Glycine	0.088	3.01	3
Alanine	0.065	2.22	2
Valine	0.088	3.01	3
Isoleucine	0.002	0.07	0
Leucine	0.114	3.91	4
Tyrosine	0.054	1.86	2
Phenylalanine	0.088	3.01	3
Lysine	0.034	1.15	1
Histidine	0.056	1.93	2
Arginine	0.028	0.96	1
Ammonia	0.1725	5.94	2

* A 22-h hydrolysate was prepared as described in the text.

** Corrected by dividing by 0.94 (ref. 9). The values for other amino acids have not been corrected for destruction during hydrolysis.

Amino acid analyses of Fraction III (Fig. 1) normally revealed the presence of small amounts of amino acids known not to be in the A chain, but present in the B chain (Table II). The amount of contamination was obviously low, but it appeared desirable to attempt its removal. Consequently, the A chain was rechromatographed on DEAE-Sephadex with the same gradient used in the initial separation of the two

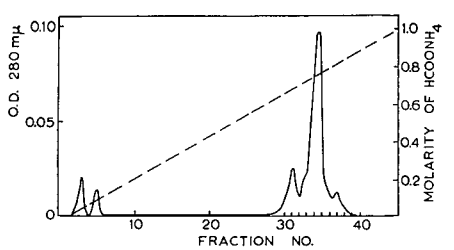

Fig. 2. Rechromatography of oxidized A chain of insulin on DEAE-Sephadex. The sample taken for amino acid analysis consisted of Fractions 34–36.

TABLE II

AMINO ACID COMPOSITION OF OXIDIZED INSULIN A CHAIN (GLYCYL PEPTIDE)*

Amino acid	*μmoles found*	*No. of residues per mole*	
		Found	*Theoretical*
Cysteic acid	0.234**	3.75**	4
Cystine	0.000	0.00	0
Aspartic acid	0.134	2.15	2
Threonine	trace	trace	0
Serine	0.117	1.88	2
Glutamic acid	0.241	3.86	4
Proline	0.000	0.00	0
Glycine	0.071	1.13	1
Alanine	0.071	1.14	1
Valine	0.116	1.85	2
Isoleucine	0.044	0.70	1
Leucine	0.136	2.17	2
Tyrosine	0.120	1.92	2
Phenylalanine	0.004	0.06	0
Lysine	0.004	0.06	0
Histidine	0.001	0.02	0
Arginine	trace	trace	0
Ammonia	0.384	6.16	4

* A 22-h hydrolysate was prepared as described in the text.
** Corrected by dividing by 0.94 (ref. 9). The values for other amino acids have not been corrected for destruction during hydrolysis.

TABLE III

AMINO ACID COMPOSITION OF RECHROMATOGRAPHED A CHAIN OF OXIDIZED INSULIN*

Amino acid	*μmoles found*	*No. of residues per mole*	
		Found	*Theoretical*
Cysteic acid	0.176**	4.15**	4
Cystine	0.000	0.00	0
Aspartic acid	0.093	2.19	2
Threonine	0.000	0.00	0
Serine	0.083	1.96	2
Glutamic acid	0.165	3.89	4
Proline	0.000	0.00	0
Glycine	0.047	1.10	1
Alanine	0.048	1.12	1
Valine	0.078	1.83	2
Isoleucine	0.031	0.72	1
Leucine	0.092	2.16	2
Tyrosine	0.084	1.98	2
Phenylalanine	0.000	0.00	0
Lysine	0.000	0.00	0
Histidine	0.000	0.00	0
Arginine	0.000	0.00	0
Ammonia	0.229	5.40	4

* A 22-h hydrolysate was prepared as described in the text.
** Corrected by dividing by 0.94 (ref. 9). The values for other amino acids have not been corrected for destruction during hydrolysis.

peptides. Fig. 2 shows the rechromatography of the A chain, and in Table III are the results of amino acid analyses on the rechromatographed peptide. Those amino acids distinctive to the B chain (threonine, phenylalanine, proline, and the basic amino acids) were not detected, and the values for the other residues are close to the theoretical values for the A chain. The slightly low values for valine and isoleucine in Tables II and III can probably be ascribed to the resistance of the isoleucyl–valyl bond to acid hydrolysis[8].

The higher-than-theoretical values for ammonia shown in Tables I–III are doubtless an indication that the sublimation procedure did not completely remove the ammonium formate present in the effluent. Nevertheless, the ammonia values are sufficiently low to show the effectiveness of the desalting for most purposes. Desalting the effluent from NaCl elution by gel filtration on Sephadex G-25 was not completely effective in our hands. The procedure was not pursued extensively, however, and there is no reason to assume that it cannot be made satisfactory. Furthermore, removal of NaCl by ion retardation resins[10] should be feasible.

Inasmuch as our purpose was to use the peptides as substrates for proteolytic enzymes, we were primarily concerned with the purity of the separated chains rather than with yields; therefore, the fractions were cut quite sharply by retaining only a few tubes near the centers of the peaks for analyses and for use as substrates. In one experiment the total recovery of all fractions from the column was 77 %. Failure to achieve higher recovery in this experiment can be ascribed largely to mechanical losses incurred in removing the dried material from the lyophilizer flasks.

Preliminary experiments using the bead forms of DEAE-Sephadex A-25 indicated that the gradient would need to be altered somewhat in order to achieve separations comparable to those obtained with the block-polymerized, fine particle size material used in the experiments described herein.

DISCUSSION

The separation of the two peptides resulting from the performic acid oxidation of insulin has been effected by several non-chromatographic procedures, involving precipitation[11,12], counter-current distribution[5], and zone electrophoresis[13]. Previously described chromatographic procedures have been based on the use of a cation exchange resin (Dowex 50) to separate performic acid-oxidized insulin peptides[14] and S-sulfonate peptides derived from treatment of insulin with sulfite[15,16]. The use of cation exchange resins permits the glycyl peptide to pass through the column unretarded[14–16]. Our rationale in selecting anion exchange materials for the separation of the oxidized insulin peptides was based on the difference in the number of sulfonic acid residues in the two chains, to permit the retention of both peptides on the column, from which they could be selectively eluted by a gradient.

We recently became aware of a short communication by FITTKAU[17], published while our work was in progress, in which he reported the separation of performic acid-oxidized insulin peptides by chromatography on 1 × 2 cm columns of DEAE-Sephadex. The peptides were identified by zone electrophoresis and end group analysis, but no analytical values for amino acids in the products were given. FITTKAU's procedure involved a step-wise elution, using 20 % formic acid to remove the B chain, and 1 *N* HCl to elute the A chain. Our procedure appears to have an advantage over

the step-wise elution of the peptides, as the latter would not be expected to separate such impurities as that represented by Fraction I (Fig. 1). Moreover, the gradient elution permits the sharp cutting of fractions, which emerge in an easily-removed volatile buffer that avoids extremes of pH values; CRAIG *et al.*[5] have pointed out the desirability of buffering the peptides for stability.

The procedure described in this paper has proved to be simple, reproducible, and adaptable to relatively large quantities. It requires a minimum of equipment; even the Autograd and the ultraviolet monitor are dispensable conveniences.

ACKNOWLEDGEMENTS

We are indebted to Mrs. BILLIE HEFTI for her assistance with the amino acid analyses, and to Mr. CARL M. CATER for independently checking the procedure. The financial support of the Robert A. Welch Foundation (Grant A-003) is gratefully acknowledged.

SUMMARY

The A and B chains of performic acid-oxidized bovine insulin were separated by a rapid, simple procedure involving chromatography on DEAE-Sephadex. Elution was accomplished with a gradient of ammonium formate, which was removed from the final products by sublimation to yield salt-free preparations of the two peptides. The elution gradient produced good resolution of the A and B chains, which were collected in sharp fractions. The B chain emerged in a high state of purity, as judged by quantitative amino acid analysis. The A chain frequently contained traces of contamination which could be removed by rechromatography on the DEAE-Sephadex.

REFERENCES

1 S. M. MARTIN, K. SINGH, H. ANKEL AND A. H. KAHN, *Can. J. Biochem. Physiol.*, 40 (1962) 327.
2 G. PFLEIDERER AND G. SUMYK, *Biochim. Biophys. Acta*, 51 (1961) 482.
3 M. SATAKE, T. OMORI, S. IWANAGA AND T. SUZUKI, *J. Biochem.*, 54 (1963) 8.
4 T. MURACHI AND H. NEURATH, *J. Biol. Chem.*, 235 (1960) 99.
5 L. C. CRAIG, W. KONINGSBERG AND T. P. KING, *Biochem. Prep.*, 8 (1961) 70.
6 C. H. W. HIRS, S. MOORE AND W. H. STEIN, *J. Biol. Chem.*, 219 (1956) 623.
7 S. MOORE AND W. H. STEIN, *Methods Enzymol.*, 6 (1963) 819.
8 A. M. CRESTFIELD, S. MOORE AND W. H. STEIN, *J. Biol. Chem.*, 238 (1963) 622.
9 S. MOORE, *J. Biol. Chem.*, 238 (1963) 235.
10 C. ROLLINS, L. JENSEN AND A. N. SCHWARTZ, *Anal. Chem.*, 34 (1962) 711.
11 F. SANGER, *Biochem. J.*, 44 (1949) 126.
12 H. HAYASHI, *Seikagaku*, 32 (1960) 411; *C.A.*, 60 (1964) 3244a.
13 W. GRASSMAN, R. STROBEL, K. HANNIG AND M. DEFFNER-PLOCKL, *Z. Physiol. Chem.*, 305 (1956) 21.
14 M. J. MYCEK, D. D. CLARKE, A. NEIDLE AND H. WAELSH, *Arch. Biochem. Biophys.*, 84 (1959) 528.
15 J. L. BAILEY AND R. D. COLE, *J. Biol. Chem.*, 234 (1959) 1733.
16 G. H. DIXON AND A. C. WARDLAW, *Nature*, 188 (1960) 721.
17 S. FITTKAU, *Naturwiss.*, 50 (1963) 522.

CHROMATOGRAPHIC TECHNIQUES USING LIQUID ANION-EXCHANGERS

II. STRONG MONOBASIC ACID SYSTEMS*

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INTRODUCTION

In our previous paper[1] dealing with the use of high-molecular-weight amines and substituted ammonium salts in paper and thin-layer chromatography, the investigation was limited to HCl systems. The results so obtained showed the necessity to test further some of the selected liquid anion-exchangers with respect to their behaviour with other strong monobasic acids.

This paper deals with systems using HNO_3, HBr, HI, HSCN and $HClO_4$, and some conclusions are given regarding the value of the "adsorption sequence" tentatively proposed by us and the possible applicability of the systems considered here for further qualitative and quantitative work.

MATERIALS AND METHODS

For a full description of materials and methods, one is referred to ref. 1. Ions not mentioned there were also applied using solutions containing 2–8 μg cation per μl. The ions chosen for each acid system were selected with regard to their expected divergent behaviour and results already known in the literature on anion-exchange and neutral organophosphorus systems.

All high-molecular-weight amines and substituted ammonium salts were converted into the appropriate salt form by equilibrating a 0.10 (thin layer) or 0.15 (paper) molar solution in $CHCl_3$, or occasionally benzene, for 10 min in a separatory funnel with 3 vol. of approx. 2 *N* acid. The ammonium salts — purchased as chlorides —were converted into the desired salt form by shaking the solution in $CHCl_3$ for some minutes with a 5-fold excess of freshly precipitated Ag_2O, decanting the supernatant solution and treating the hydroxide with the acid in question.

All solutions of acids except those of HSCN were made by diluting the concentrated acid of p.a. quality to the desired normality and subsequent standardization. Concentrated HBr and HI contained only traces of Br_2 and I_2, respectively; these were not removed prior to use. This omission caused no difficulties even with concentrated hydriodic acid, because any I_2–I^- complexes formed in low concentration were tightly held by the liquid anion-exchanger-treated support (*cf.* RESULTS).

* For the first part of this series, see ref. 1.
** This paper forms part of the work done by U. A. TH. BRINKMAN for his Ph. D. Thesis.

HSCN solutions were made by acidifying KSCN solutions of appropriate molarity with $HClO_4$ to a final acid concentration of 0.1 *N* (*cf.* ref. 2) and filtering off the precipitated $KClO_4$; HSCN was standardized after this treatment.

RESULTS AND DISCUSSION

HNO_3 was selected as a first choice for our present investigation, mainly because some work had already been done in this direction by CERRAI, TESTA and coworkers[3–6] and others[7,8].

Based on the results previously obtained in HCl systems[1], seven high-molecular-weight amines and substituted ammonium salts were investigated using HNO_3 as an eluant. In consequence of this work (see below), the number of liquid anion-exchangers tested with the remaining four strong monobasic acid systems was limited to five.

HNO_3 systems

Systematic investigations were carried out using 8 ions, *viz.* UO_2(II), Th(IV),

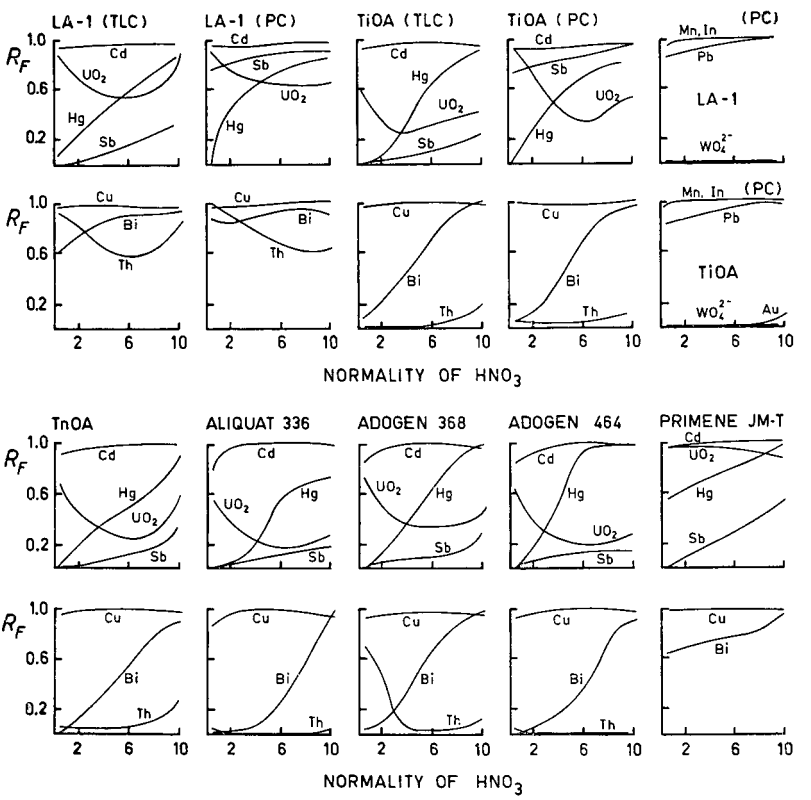

Fig. 1. R_F spectra for 12 ions using various amine·HNO_3 and quaternary ammonium nitrate systems in thin-layer (TLC) and paper (PC) chromatography; the lower set of diagrams entirely pertains to TLC. To complete the data presented in the diagrams the following is observed. Detection of UO_2 and Th was difficult with Primene JM-T; the UO_2 curve here was found in PC, and Th (omitted) most probably had an R_F of 0.9–1.0 over the whole range. Slight streaking of the spots was occasionally observed with Hg. Sb generally gave fairly long spots, while WO_4^{2-} showed weak comets attached to the main portion at R_F 0.0.

Sb(III), Cu(II), Cd(II), Bi(III) and Hg(II), with 0.5–10 N HNO_3 as an eluant. Incidentally, R_F curves were also recorded for some further ions, WO_4^{2-}, Pb(II), Mn(II), In(III) and Au(III). The corresponding data, obtained for both thin-layer and paper chromatographic experiments, are summarized in Fig. 1. Results found with both techniques for TiOA* and Amberlite LA-1 show good mutual analogy except for Sb(III). This last phenomenon was also encountered in experiments with $HClO_4$, *i.e.* another case where Sb has high R_F values in paper chromatography. In the HNO_3 system the use of chloride and nitrate solutions of Sb yielded similar results, so that the differences between paper and thin-layer chromatography cannot be explained by the presence of chloro complexes of Sb.

The data on Th and UO_2 correspond well with those published by CERRAI AND TESTA[3] when using TnOA. When our experimental work had been finished, SASTRI *et al.*[9] published R_F spectra for HNO_3 systems in paper chromatography using among other things Aliquat 336 and dilaurylamine. Their results also correspond favourably with those given by us; this holds for both the absence of adsorption found with Cu, Cd, Pb, Mn and In and for the more interesting curves obtained with Th, UO_2, Au, Bi and Hg. The data for most ions tested moreover correspond well with those given by FARIS AND BUCHANAN[10] for solid anion resins, though large discrepancies occur with Bi and Hg. On the other hand, the curves for Bi (and for most other elements) closely agree with those published by O'LAUGHLIN AND BANKS[11,12] and CERRAI AND TESTA[13] when using neutral organophosphorus compounds; with Hg, though there is less agreement.

Attention may also be called to a study by OSSICINI[14] on the adsorption of metal ions on solid anion resin paper Amberlite SB-2 using 0.5–8.0 N HNO_3. Her data confirm essentially those reported by us, though deviations do occur, especially with Bi.

We may conclude, therefore, that in HNO_3—as in HCl—systems, the chromatographic data found when using various liquid anion-exchangers are closely analogous (refs. 3, 9 and this paper). On the other hand, discrepancies between results so obtained, and those found for either solid anion resins or paper treated with neutral organophosphorus compounds, seem to be somewhat more serious than in HCl systems[1]. Secondly, as regards the "adsorption sequence" of the liquid anion-exchangers tested, Primene JM-T again has the weakest adsorption strength, while Amberlite LA-1, though being much stronger, clearly falls behind the five others, which do not show significant differences; Aliquat 336 being probably slightly stronger than the other four. Therefore, the sequence recently given for HCl systems seems to be reliable for general guidance. Minor variations occurring when going from one (acid) system to another (see also below), may be due to small experimental inconstancies.

For qualitative and quantitative separations, HNO_3 systems are most probably of limited value only. However, UO_2 and Th, Sb and Bi or Hg, some noble metals[9] or a limited number of rare earths elements[4] may be separated from each other and from many other elements. Moreover, in quantitative analysis, many ions will easily be eluted by changing the composition of the eluant to dilute HNO_3 or nitrate (*cf.* refs. 5 and 6).

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* Abbreviations: TnOA = tri-*n*-octylamine; TiOA = triisooctylamine; HDEHP = hydrogen di(2-ethylhexyl) phosphate.

HBr systems

Systematic investigations were carried out using the 12 ions previously selected for HCl systems[16]. Al(III), Cd(II) and Hg(II) were, however, only examined with Amberlite LA-1, where their R_F curves hardly differed from our earlier results with HCl as an eluant (R_F = 1.0, 0.0 and 0.0, respectively). Elution was done with 0.5–8.0 *N* HBr.

Thin-layer data for the HBr system are graphically represented in Fig. 2; paper chromatographic experiments carried out with 2 and 4 *N* HBr as an eluant for Cu, Zn and Pb did not show any significant discrepancies and this technique therefore was not applied further. The thin-layer data show a rather close agreement with those previously published by us for HCl systems[1,16], though Cu is distinctly more strongly adsorbed in the bromide system, which effect is to a lesser extent apparent with Pb and Bi and seems to be reversed with Co and Fe.

Comparison of our data can best be done with those of BAGLIANO *et al.*[15], who reported R_F values of metal ions on various anion-exchange papers using 0.5–6.0 *N* HBr. The curves found for Cu, Cd, Pb, Bi, Hg and Fe closely resemble those given by us. Thin layers impregnated with Aliquat 336, TiOA and the Adogens have approximately the same adsorption strength as the strong base resin paper Amberlite SB-2, which clearly surpasses layers treated with Amberlite LA-1; Whatman DE-20 and AE-30 cellulose anion-exchange papers fall far behind (*cf.* ref. 1). In connection with these results, there seems to be no motive for a differentiation between adsorption in the network and ion-exchange as detailed as was given by BAGLIANO *et al.*[15], except in the case of Au.

As regards the picture outlined above for some ions in HBr systems, further reference may be made to a study by HERBER AND IRVINE[17]. Using Dowex-1, these

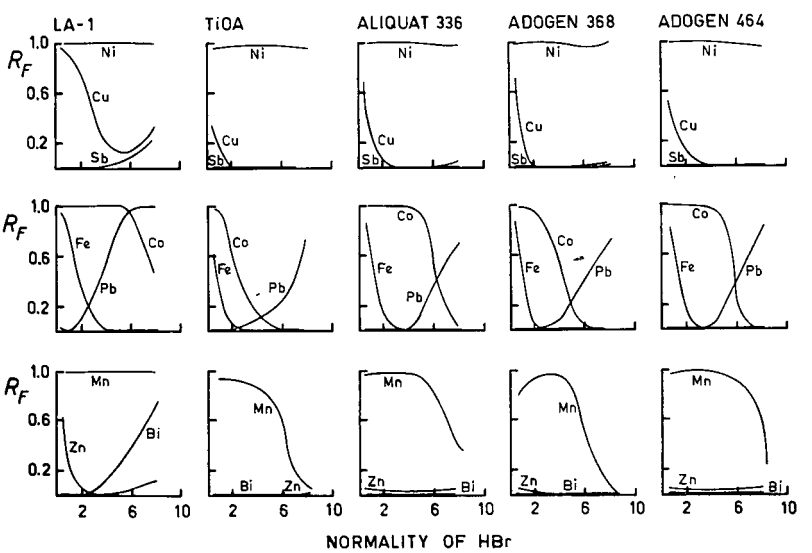

Fig. 2. R_F spectra for 9 ions using various amine·HBr and quaternary ammonium bromide systems in thin-layer chromatography. Owing to partial reduction to Fe(II) (R_F 1.0), Fe(III) showed weak comets to the solvent front. Partial reduction of Cu(II) to Cu(I) yielded tailing spots for Cu at low acid strengths.

authors found only small deviations between the behaviour of Ni, Cu, Co and Zn in HCl and HBr media, respectively.

No special conclusions concerning the adsorption sequence of the liquid anion-exchangers seem possible, TiOA and Adogen 368 being this time slightly more strongly adsorbing than Adogen 464 and Aliquat 336, with Amberlite LA-1 again clearly in the last place. As an analytical tool, the HBr systems in general will closely resemble the HCl analogues. However, higher cost, a limited concentration range and especially reduction of oxidizing ions (*e.g.* Cu^{2+} and Fe^{3+}; see legend to Fig. 2) present serious drawbacks. Apart from the last aspect use of the HBr system will not meet with any practical difficulties, making incidental application possible.

HI systems

Systematic investigations were carried out using the ions previously tested in HCl systems[16], excepting Al(III), Cd(II) and Hg(II), which still showed fairly constant R_F values of 1.0, 0.0 and 0.0, respectively, with Amberlite LA-1. Elution was generally done with 0.5–7.4 *N* HI, made by appropriate dilution of conc. HI p.a. However, in one instance, *viz.* with Amberlite LA-1, comparative experiments were carried out using KI–H_2SO_4 solutions for both equilibration and elution. The results of all thin-layer experiments have been recorded in Fig. 3. Paper chromatography, done at 2 and 4 *N* HI for reasons of comparison, did not show any advantage and was therefore abandoned.

As regards the experimental aspects of the use of HI solutions, some results are worth mentioning. Though the HI solutions turn their colour to brown-red on standing owing to oxidation of I^- to I_2, the thin-layer plates are only lightly coloured. Moreover, in all identification procedures involving either H_2S or NH_3, these re-

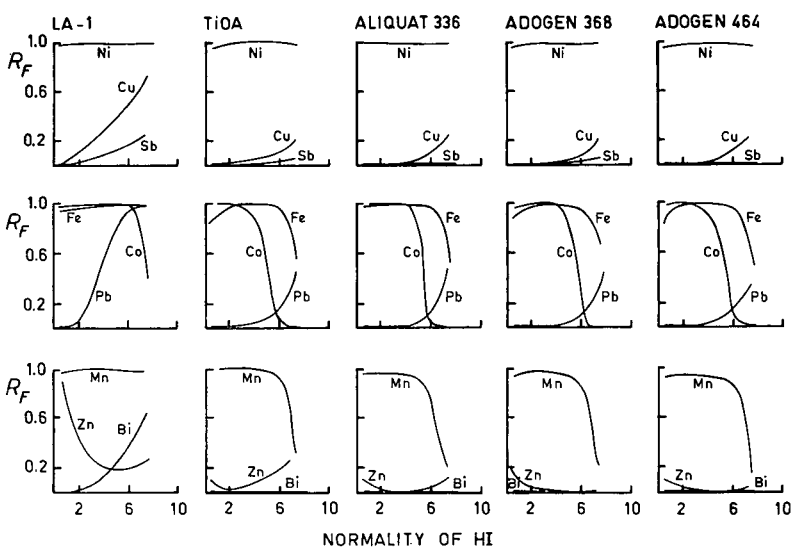

Fig. 3. R_F spectra for 9 ions using various amine·HI and quaternary ammonium iodide systems in thin-layer chromatography. The curves for Cu and Fe represent those of Cu(I) and Fe(II), respectively; at low acid strengths, the Fe(II) spots tailed weakly to the origin, reduction obviously not being complete.

ductants cause a complete disappearance of the disturbing colour. Secondly, it was observed that any I_2–I^- complexes present in the eluant are held tightly by the anion-exchanger-treated support, forming a small 2–3-mm wide zone at the lower end of the plate. When, therefore, the spots are applied a few mm higher than usual, no further precautions are necessary as regards the presence of traces of I_2 in HI. The use of the more concentrated HI solutions generally interferes to some extent with the identification procedures of the ions tested, but no insurmountable difficulties are encountered.

The spectra in Fig. 3 are generally quite analogous to those obtained with either HCl or HBr systems; good agreement also exists between the HI and the KI–H_2SO_4 data. The results found for Cu(II) and Fe(III) are, however, noteworthy. Here reduction to Cu(I) and Fe(II), respectively, appears to be fairly complete, which satisfactorily explains the greatly changed R_F curves.

Summarizing, we may state that generally the anion separation of iodo complexes of the elements studied—and most probably of many others—does not offer special advantages over those reported for the more convenient chloride system, while placing an upper limit of approx. 7.5 *N* (conc. HI) on the acid concentration which can be employed in the separations. Moreover, with both HI and HBr systems, the ions have a tendency to streak. On the other hand, the handling of the HI system appears to be relatively simple, thus encouraging further investigation, *e.g.*, in cases where procedures using other (acid) systems are not successful.

HSCN systems

Systematic investigations were limited to six ions, Cu(II), Cd(II), Pb(II), Co(II), Mn(II) and Ag(I), but in the case of Amberlite LA-1 most ions tested with the HCl system were studied. Thin-layer experiments were carried out using NH_4SCN acidi-

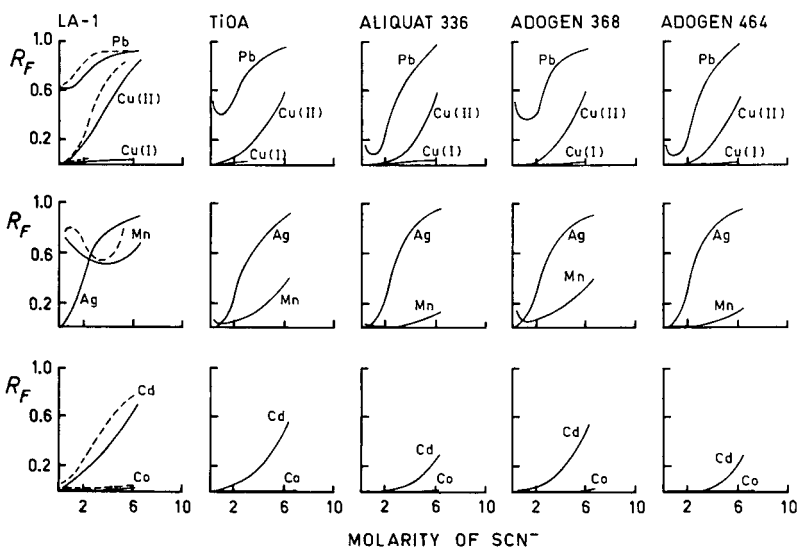

Fig. 4. R_F spectra for 6 ions using various amine·HSCN and quaternary ammonium thiocyanate systems in paper (———) and thin-layer (– – –) chromatography. At low and high thiocyanate molarities, some tailing occurred with Pb and Cu(II), respectively.

fied with HCl and KSCN–$HClO_4$ solutions of varying composition as eluants. In all cases fairly irregular solvent fronts were observed, while identification was more difficult than with *e.g.* HCl systems. Another slight disadvantage of the silica gel thin-layer system concerns the presence of traces of Fe(III) in the treated support, which imparts a red colour to the thin layer. Therefore, though the data obtained are decidedly reliable as regards the general outline of the curves (see below), they are less reproducible than with other acid systems. Contrary to the procedure generally followed, the main portion of the systematic work was therefore carried out using paper chromatography, where reproducible R_F values were obtained and no special difficulties were encountered.

The results of the experiments are summarized in Fig. 4. They were carried out using the KSCN–$HClO_4$ solutions described in MATERIALS AND METHODS, but incidentally a HSCN solution was made by adding an equivalent amount of $HClO_4$ to the KSCN solution and filtering off[2]. Results so obtained were qualitatively comparable to those given in Fig. 4 and therefore the latter technique was rejected, because the more concentrated HSCN solutions are subject to a fairly rapid deterioration. Some results of thin-layer experiments using Amberlite LA-1 are included in Fig. 4 and it is seen that these indeed correspond agreeably with those reported for the paper chromatographic system. Thin-layer chromatography of the ions not represented in Fig. 4 showed that Ni and Al give R_F values of 1.0, while Zn, Hg and Sb have R_F = approx. 0.0 over the whole concentration range. The curve found for Bi closely resembles that of Cd.

As regards the R_F curves found for the ions tested, attention may be called to the two clearly distinguished curves found for Cu, which must be ascribed to Cu(II) and Cu(I), the latter ion originating from a partial reduction of Cu(II). Secondly, it is emphasized that for some ions the adsorption in the HSCN system is significantly different from that found in the halogenide systems. For instance, Co, Mn and Zn are more strongly adsorbed in the thiocyanate system, while the opposite holds for Cd and Pb. Therefore, the HSCN system certainly may be of value for qualitative analytical work, *e.g.* when separating Co, Mn and Ni or Cd and Zn.

Further research of the HSCN system appears to be more justified than that where HBr or HI are used as eluants. The paper chromatographic technique may be used as such, but more work will have to be done on the thin-layer experiments. As regards the liquid anion-exchangers tested, the data in Fig. 4 clearly indicate that with HSCN as an eluant Aliquat 336 and Adogen 464 are somewhat stronger than Adogen 368 and TiOA.

$HClO_4$ systems

Systematic investigations were carried out using the ions UO_2(II), Th(IV), Cu(II), Pb(II), Hg(II), Sb(III), Zn(II), In(III) and Fe(III). Thin-layer experiments were done using 0.5–10 *N* $HClO_4$ as an eluant, but paper chromatography had to be limited to 0.5–8.0 *N* acid, because 10 *N* $HClO_4$ clogs the paper.

The experiments as a whole had no really satisfactory results. No difficulties were encountered with UO_2, Cu, Zn, Fe and In, which gave R_F values of 0.85–1.0 in all paper and thin-layer experiments; Th also yielded a fairly consistent picture of slight adsorption, though here tailing of the spots occurred in thin-layer work at

high acid strengths. The remaining three ions, however, showed a somewhat irregular behaviour (*cf.* Fig. 5). As was the case in HNO_3 systems, Sb gave low R_F values in thin-layer work contrasted with fairly high ones on paper. Pb yielded satisfactory R_F spectra on paper, but the thin-layer experiments were less successful. Here, and still more with Hg, long tailing spots were observed in most instances, so that the

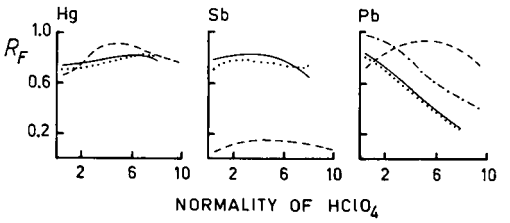

Fig. 5. R_F spectra for Hg(II), Sb(III) and Pb(II) in $HClO_4$ systems using supports treated with liquid anion-exchangers and supports merely dipped in $CHCl_3$ and dried. – – – = thin-layer and ——— = paper chromatography using Amberlite LA-1; –·–·–· = thin-layer chromatography using Adogen 464; ··· = paper chromatography using paper not treated with a liquid anion-exchanger. The curve on Adogen 464 demonstrates the divergent behaviour of Pb in thin-layer work, simultaneously showing good agreement with the paper chromatographic curves.

R_F values reported are not too significant. For that matter, it was observed that especially on paper, where the most reliable results were obtained, hardly any R_F differences were noted between papers treated with each of the five anion-exchangers tested and paper not treated with anion-exchanger, but merely dipped in $CHCl_3$ and dried. Obviously, the role of the basic exchangers generally is negligible here; *i.e.*, the fairly complete absence of complex formation in the $HClO_4$ system precludes the possibility of anion-exchange playing an active part. Other factors will now determine the R_F picture, sometimes giving rise to unexpected (and badly defined) curves.

In this context, a tentative comparison may be made with the results of O'LAUGHLIN AND BANKS[11,12] who used 0.5 and 3.0 *N* $HClO_4$ only for most ions. With the notable exception of UO_2, which had fairly low R_F values in sharp contrast to the complete absence of adsorption found by us, their paper chromatographic data reasonably correspond with those recorded by us. However, we must still conclude that as yet liquid anion-exchanger·$HClO_4$ systems appear to be rather unsuccessful in thin-layer and paper chromatography. To a certain extent, this result parallels that of O'LAUGHLIN AND BANKS, who obtained some striking discrepancies when investigating various neutral organophosphorus compounds.

CONCLUSION

The results presented in our recent papers show what can be accomplished in the field of chromatography when using supports impregnated with liquid anion-exchangers and eluting with various strong monobasic acids.

It has been shown that the great majority of the liquid anion-exchangers tested gives satisfactory results both in paper and thin-layer chromatography, irrespective of the acid system used. The adsorption sequence previously given is moreover useful for general guidance, at least for the strong monobasic acid systems tested. The sharp changes in R_F values noted by SASTRI *et al.*[9] when comparing

tertiary and quaternary basic extractants, are distinctly absent from our work. Technical-grade products may be recommended, though some purification will be necessary when contaminants mar the identification procedures at the solvent front, as was found *e.g.* with Primene JM-T in the HNO_3 system. For the rest, practical aspects such as quality of the impregnated support and short elution time will determine the ultimate choice. In this connection, both the favourable results generally obtained in thin-layer experiments and the practical advantages with regard to the solid resin-impregnated supports (*cf.* ref. 1) may be once more emphasized.

Secondly, as regards the acid system(s) to be recommended, preference must be distinctly given to the HCl system, this being more convenient than both the HBr and HI system, which generally gave comparable results. On the other hand, HSCN may be recommended for further research, though it has some practical disadvantages (see foregoing section). Moreover, it is feared that a rather large number of elements will be held at or close to the origin owing to strong complexation with SCN^-*. Lastly, HNO_3 must be clearly preferred to $HClO_4$ when selecting a system with comparatively low adsorption; HNO_3 will also be a good choice in problems concerning rare earth separations[4].

Therefore, liquid anion-exchangers and acid systems having been fairly thoroughly investigated, a few selected combinations may now be tested for a large number of cations. Work in this direction is in progress now and has already shown that the HCl system is indeed advantageous for primary investigation. A subsequent paper[19] will deal more systematically with this aspect and some interesting qualitative separations will be recorded.

Next, the tentative interpretation of the R_F curves previously given[1] is briefly considered again. As regards the HCl system, the data in ref. 1—especially in combination with those on the notable absence of adsorption found for supports merely dipped in $CHCl_3$ and dried—clearly point to the role of the basic extractants as anion-exchangers. This theory is supported on broad lines by our present work, *viz.* by the good mutual analogy between the data on the HCl, HBr and HI systems, which is presumably caused by the opposing action of the general increase of complex formation and competition of excess ligand, when increasing the ligand size, *i.e.* when going from Cl^- to I^-. The correspondence between our data and those reported in the literature on solid anion-exchangers points in the same direction. Strong adsorption (*i.e.* complexation) in the HSCN system fits onto the picture and the same holds for the HNO_3 system, where adsorption, though being generally low, is found with UO_2 and Th, both known to form negatively charged nitrato complexes. Lastly, the correspondence between data on impregnated and untreated supports in the $HClO_4$ system, where generally high R_F values are found, may be mentioned.

On the other hand, several important aspects need further investigation. Among these range: (1) The composition of the species involved in the ion-exchange process, especially that of the complex metal anions. (2) The factor(s) governing the desorption of the adsorbed complex metal anions, *e.g.* competition of excess ligand, formation of higher charged negative ions and formation of undissociated complex metal acids. (3) Various points such as the discrepancy between the Sb curves in

* When preparing this manuscript, a paper[18] came to our notice which briefly summarizes results obtained with SCN^- systems for many ions. Agreement with our data is good, and most of the ions having interesting R_F curves with HSCN as an eluant are included in our present work.

HNO_3 and $HClO_4$ systems when using either paper or silica gel as support; the adsorption found for Bi and Hg in the HNO_3 system, which does not correspond too well with some of the literature data; and the adsorption found for Pb in the $HClO_4$ system. In these and similar cases the influence of hydrolysis and precipitation cannot simply be neglected.

When trying to solve some of the problems outlined, comparative experiments using supports treated with cation-exchangers, anion-exchangers and neutral supports[20,21] may be enlightening, *i.e.* the use of liquid cation-exchangers such as HDEHP and the stronger acidic dinonylnaphthalene sulphonic acid will be valuable. In a preliminary study some interesting observations regarding the role of the anion in the case of supports treated with liquid cation-exchangers have been made by us and it is hoped that these investigations will be continued shortly. Involvement of these cation-exchangers is also interesting because excellent results have already been achieved when using them in the field of *e.g.* rare earth[6] and alkaline earth[21] separations. Therefore, a more thorough investigation of these acidic extractants in chromatography, especially for the more commonly encountered ions, is certainly justified.

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SUMMARY

Paper and thin-layer chromatography—which generally give analogous results—have been carried out in HNO_3, HBr, HI, HSCN and $HClO_4$ systems, using supports impregnated with high-molecular-weight amines and substituted ammonium salts. Based on results previously obtained in the HCl system, 5–7 liquid anion-exchangers were investigated, using approx. 10 cations for each acid system.

The HBr and HI systems generally give results comparable to those found with HCl as an eluant. A more divergent picture is shown by HSCN, with which eluant it is preferable to use paper chromatography for the time being. The HNO_3 system shows fairly low adsorption for most ions, but some interesting applications are outlined. Results with $HClO_4$, on the other hand, are not particularly successful. The use of HI and HBr solutions containing some free I_2 and Br_2, respectively, has no unfavourable effects.

The R_F spectra in the HBr and HNO_3 systems show good agreement with literature data on solid anion-exchangers. Most curves may be explained assuming an anion-exchange mechanism, adsorption to the support being important incidentally.

The adsorption sequence previously reported for the liquid anion-exchangers appears to be reliable for general guidance.

REFERENCES

1 U. A. TH. BRINKMAN, G. DE VRIES AND E. VAN DALEN, *J. Chromatog.*, 22 (1966) 407.
2 L. C. LOCK AND E. C. MARTIN, *J. Chromatog.*, 7 (1962) 120.
3 E. CERRAI AND C. TESTA, *J. Chromatog.*, 5 (1961) 442.
4 C. TESTA, *Anal. Chem.*, 34 (1962) 1556.
5 E. CERRAI AND C. TESTA, *J. Chromatog.*, 8 (1962) 232.
6 E. CERRAI, *Chromatog. Rev.*, 6 (1964) 129.
7 S.-W. PANG AND S.-C. LIANG, *Hua Hsueh Hsueh Pao*, 30 (1964) 401.
8 S.-W. PANG AND S.-C. LIANG, *K'o Hsueh T'ung Pao*, (1964) 156; *C.A.*, 61 (1964) 15324f.
9 M. N. SASTRI, A. P. RAO AND A. R. K. SARMA, *J. Chromatog.*, 19 (1965) 630.
10 J. P. FARIS AND R. F. BUCHANAN, *Anal. Chem.*, 36 (1964) 1157.
11 J. W. O'LAUGHLIN AND C. V. BANKS, *U.S. At. Energy Comm. Rept., IS-737*, 1963.
12 J. W. O'LAUGHLIN AND C. V. BANKS, *Anal. Chem.*, 36 (1964) 1222.
13 E. CERRAI AND C. TESTA, *J. Chromatog.*, 7 (1962) 112.
14 L. OSSICINI, *J. Chromatog.*, 17 (1965) 625.
15 G. BAGLIANO, G. GRASSINI, M. LEDERER AND L. OSSICINI, *J. Chromatog.*, 14 (1964) 238.
16 U. A. TH. BRINKMAN AND G. DE VRIES, *J. Chromatog.*, 18 (1965) 142.
17 R. H. HERBER AND J. W. IRVINE, Jr., *J. Am. Chem. Soc.*, 76 (1954) 987;
R. H. HERBER AND J. W. IRVINE, Jr., *J. Am. Chem. Soc.*, 78 (1956) 905.
18 A. WAKSMUNDZKI AND S. PRZESZLAKOWSKI, in K. MACEK AND I. M. HAIS (Editors), *Stationary Phase in Paper and Thin-Layer Chromatography*, Elsevier, Amsterdam, 1965, p. 199.
19 U. A. TH. BRINKMAN, G. DE VRIES AND E. VAN DALEN, *J. Chromatog.*, in press.
20 M. LEDERER AND F. RALLO, *J. Chromatog.*, 7 (1962) 552.
21 E. CERRAI AND G. GHERSINI, *J. Chromatog.*, 13 (1964) 211 and 15 (1964) 236.

Notes

Gas chromatography of halogenated carbamates

II. N-Trichloroacetyl derivatives

The gas chromatographic analysis of N-chloroethyl and N,N-bis-(2-chloroethyl) carbamates has been reported earlier. References to the analysis of nitrogen mustard derivatives and chlorinated carbamates were cited in that paper[1]. The preparation of halogen-containing substituted urethanes via reaction of halogen-substituted fatty acid acyl chlorides with compounds of type RNHCOOR' has been reported[2]. Other derivatives containing the trichloroacetyl moiety have been prepared by the reaction of trichloroacetyl chloride with amino acids[3] and aromatic amino acids[4]. Trichloroacetanilides were made by condensation of aromatic amines with trichloroacetic acid[5].

The preparation of N-trichloroacetyl alkyl and halogenated aryl carbamates has afforded the opportunity of investigating the thermal stability of an N-acyl carbamoyl grouping during gas chromatographic assay. A secondary result of this study was the elaboration of the relative electron capturing capability of trichloroacetamide *vs.* the appropriate halogenated phenol or alcohol formed by the thermal rupture of the highly labile precursor carbamates during chromatography.

Experimental

The N-trichloroacetyl carbamates were prepared via reaction of trichloroacetyl isocyanate* with the respective alkyl, aryl, and cyclic alcohols and halogenated phenols, and recrystallized from hexane. Melting points were determined on a Fisher-Johns melting point apparatus.

Gas chromatography was carried out on acetone solutions, both with an electron capture detector (Aerograph Hy-FI Model 600-B containing a column of 5 ft. by 0.125 in. O.D. Teflon tubing packed with 2 % neoglycol succinate (NGS) and 1 % polyvinylpyrrolidine (PVP) on 80–100 mesh acid-washed Chromosorb P) and with a flame ionization detector (F & M Model 1609 containing a column of 6 ft. by 0.25 in. O.D. glass coil packed with 4 % QF-1 fluorosilicone on 80–100 mesh HMDS-pretreated Chromosorb W). Specific analytical operating conditions are given in the footnotes to Table I.

Results and discussion

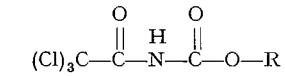
The analytical results for the N-trichloroacetyl chlorophenyl and trihaloethyl carbamates are given in Table I.

The 2,2,2-trifluoroethyl derivative appeared as a single peak under the conditions employed for the QF-1 column, without degradation to trichloroacetamide

* Obtained from Eastman-Kodak, Rochester, N.Y., U.S.A.

TABLE I

GAS CHROMATOGRAPHY OF N-TRICHLOROACETYL CARBAMATES



R	*m.p.* (°C)	*Relative retention*[a]		*Relative sensitivity*[b]		
		E.C.[c]	*F.I.*[d]	*E.C.*	*F.I.*	*F.I./E.C. ratio*
		Alcohol	*Alcohol*	*Alcohol*	*Alcohol*	
2,2,2-Trifluoroethyl	95–97	[e]	[e]	[e]	[e]	—
2,2,2-Trichloroethyl[f]	101–102	0.067	0.072	7.5	2.6[g]	0.35
2,2,2-Tribromoethyl	124–125	0.34	0.33	3.4	4.1	0.83
		Phenol	*Phenol*	*Phenol*	*Phenol*	
o-Chlorophenyl	94–95	0.22	0.19	0.14	9.7[g]	69
m-Chlorophenyl	92–93	0.52	0.58	0.089	10.8	121
p-Chlorophenyl	105–106	0.58	0.62	0.14	30.1	215
2,3-Dichlorophenyl	84–85	0.91	0.50	2.4[g]	33.7	14
2,4-Dichlorophenyl	49–51	0.79	0.48	2.1[g]	5.7	2.7
2,5-Dichlorophenyl	95–97	0.88	0.48	1.2[g]	7.5	6.2
2,6-Dichlorophenyl	81–83	0.50	0.66	1.4[g]	4.0	2.9
3,4-Dichlorophenyl	55–57	[h]	1.8	[i]	23.1	—
3,5-Dichlorophenyl	123–125	0.80[j]	1.4	[i]	20.4	—
2,4,5-Trichlorophenyl	55–57	4.3	1.2	27.5	5.2	0.19
2,4,6-Trichlorophenyl	98–100	1.3	1.3	22.7	7.4	0.33
2,3,4,6-Tetrachlorophenyl	58–60	10.8	3.3	35.9	3.5	0.098
Pentachlorophenyl	122–123	[k]	7.3[m]	[k]	1.1[m]	—

[a] Relative to trichloroacetamide as 1.00. Trichloroacetamide eluted at 3.4 minutes on QF-1 (flame detector) and at 5.3 minutes on NGS–PVP (E.C. detector).

[b] Relative to trichloroacetamide as 1.0. Sensitivity of trichloroacetamide was approximately 3.8 $mm^2/\mu g$ on flame ionization and 2.740 $mm^2/\mu g$ on electron capture.

[c] Electron capture detector. Column: 2% NGS and 1% PVP on 80–100 mesh acid-washed Chromosorb P, 5 ft. by 0.125 in. O.D. Teflon column. Conditions: column 160°; injector 65 V; detector 160°; 10 input impedance; 1 × output sensitivity; nitrogen carrier 60 ml/min; 250 millicuries tritium.

[d] Flame ionization detector. Column: 4% QF-1 on 80–100 mesh HMDS-pretreated Chromosorb W, 5 ft. by 0.25 in. O.D. glass coil column. Conditions: column 110°; injector 70 V; range 1000; air 300 ml/min; hydrogen 75 ml/min; nitrogen carrier 86 ml/min.

[e] Intact carbamate had a relative retention of 3.6 on QF-1. Trifluoroethanol eluted at the solvent front and could not be assigned at $t_R°$ value for either column or a sensitivity value on NGS–PVP. The relative sensitivity of standard 2,2,2-trifluoroethanol was 1.6 on QF-1.

[f] Trichloroacetamide peak did not appear with the flame ionization system.

[g] Determined by separate chromatography of trichloroacetamide, chlorophenol and trihaloethanol standards.

[h] Only trichloroacetamide peak was obtained.

[i] 3,4- and 3,5-dichlorophenols did not produce peaks under the conditions employed.

[j] Appeared on analysis of the carbamate. See footnote i.

[k] Absence of pentachlorophenol peak.

[m] Relative retention changes to 5.1 at a column temperature of 160° and a carrier flow of 70 ml/min, with a concomitant change in relative sensitivity to 1.6.

and 2,2,2-trifluoroethanol. The tribromo and trichloro derivatives on the other hand, underwent rupture with the release of the amide and the respective trihaloethanol. All of the chlorophenyl derivatives evidenced cleavage to trichloroacetamide and the respective chlorophenol. The retention data indicate the expected trend of increased

retention with increased molecular weight for the chlorophenols. Separations appeared best on the NGS–PVP column. Comparison of the results obtained on this column with those of the QF-1 column indicates that mono-, di-, and tri-chlorophenols are more readily resolved on polar substrates.

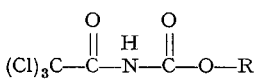
In the instances where cleavage to 2,2,2-trichloroacetamide and the chlorinated alcohol or phenol occurred, no third peak for residual intact carbamate was noted. If it is assumed that cleavage to the amide and phenol (or alcohol) components is stoichiometrically 1:1, one may calculate the sensitivity of the phenols and alcohols relative to trichloroacetamide via the respective chromatogram peak areas. The electron-capturing capacities thus obtained for the chlorophenols were in the order of tetra > tri > di > mono; and in general, trichlorophenols > trihaloethanols > dichlorophenols.

The relative sensitivities observed with the flame ionization detector were roughly mono-, dichlorophenols > trichlorophenols > trihaloethanols > tetrachlorophenol > pentachlorophenol. These latter results suggest a general decline in sensitivity as the halogen content of the molecule increases. It is of interest that considerable variation in relative sensitivity exists among the dichlorophenol isomers, the higher sensitivities being associated with a chlorine atom attached in the 3 position. It is questionable, however, whether this alone is the governing factor for sensitivity, since the 5 position is also *meta* to the hydroxy group and the 2,5-isomer does not evidence such a level of sensitivity.

It has been stated by other investigators[6] that a flame/EC response ratio could

TABLE II

MELTING POINT DATA OF MISCELLANEOUS ALKYL, ACYLIC AND ARYL N-TRICHLOROACETYL CARBAMATES



R	*Mol. wt.*	*M.p.* (°C)
Methyl	220	100–101
Ethyl	234	57–58
2-Propynyl	244	54–55
Allyl	246	32–33
n-Propyl	248	44–45
Isopropyl	248	73–74
n-Butyl	262	38–39
sec.-Butyl	262	61–63
Isobutyl	262	80–81
tert.-Butyl	262	99–100
2-Chloroethyl	269	62–63
Cyclopentyl	274	65–66
Phenyl	282	108–109
Cyclohexyl	288	89–90
Benzyl	296	83–85
Heptyl	304	28–29
Phenethyl	310	43–44
2-Bromoethyl	313	54–55
Piperonyl	340	88–89

be taken as a characteristic value of a particular electron-capturing molecule. This ratio is given in the last column of Table I and shows the general trend: monochlorophenols $\gg$ dichlorophenols $>$ trihaloethanols $>$ trichlorophenols $>$ tetrachlorophenol.

A variety of N-trichloroacetyl alkyl carbamates, including the cyclic pentyl and hexyl, and the aromatic phenyl, benzyl, phenethyl, and piperonyl derivatives, were synthesized and analyzed. Only the 2,2,2-trichloroacetamide peak appeared on the recorder chart for each of these compounds using either detector system. The absence of the alcohol component for the electron-capture system was due to lack of component sensitivity, and for the flame ionization system to rapid elution of the alcohol component which in most cases was lost in the solvent peak under the analytical conditions employed. The melting points of these derivatives are reported in Table II.

Concerning the lability of the N-trichloroacetyl carbamates to gas chromatographic analysis, it is felt that alternate analytical conditions such as column temperature considerations could influence the stability of these derivatives. Stability, to be sure, has been observed during thin-layer chromatography[7]. The relationship of thermal rupture to analytical temperature is presently under investigation.

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1 W. L. ZIELINSKI, JR. AND L. FISHBEIN, *J. Chromatog.*, 23 (1965) 175.
2 J. R. GEIGY, A. G., *Brit. Pat.*, 498,506, Dec. 21, 1938; *C.A.*, 33 (1939) 3814.
3 E. RONWIN, *J. Org. Chem.*, 18 (1953) 127.
4 M. NAGASAWA, M. YOSHIDO AND R. KUBOTA, *Japan. Pat.*, 8650, Oct. 7, 1950; *C.A.*, 52 (1950) 12310f.
5 F. A. BERTI AND L. M. ZITI, *Arch. Pharm.*, 285 (1952) 372.
6 H. HARTMANN, D. OAKS AND K. P. DIMICK, *Aerograph Research Notes*, Fall Issue, 1963.
7 L. FISHBEIN, unpublished data.

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Gas chromatography of metallic derivatives of ethylenebis(dithiocarbamic acids)

The dithiocarbamates are well-recognized fungicides. The general method of analysis of these compounds is based upon the liberation of carbon disulfide[1–5]. Gas chromatographic analysis has been used to measure carbon disulfide and hydrogen sulfide collected from decomposition of thiocarbamic compounds[6–8]. They have been measured as well by ultraviolet[9] and infrared[10] spectrophotometric techniques, and by paper chromatography[11]. Recent work has described the thin-layer chromatography of Zineb and Maneb[12].

The instability of these derivatives is quite well known, undergoing degradation to ethylenethiuram monosulfide (ETM) and its polymer, and ethylenethiourea (ETU) with the evolution of carbon disulfide.

This communication reports the feasibility of gas chromatographic analysis of the metallic ethylenebis(dithiocarbamates) Zineb, Maneb and Nabam, based on the thermal release of ETU on sample injection.

Experimental

The zinc, manganese and disodium ethylenebis(dithiocarbamates) (Zineb, Maneb and Nabam, respectively) were obtained from Dr. D. E. ROSEN, E.I. du Pont de Nemours and Co., Wilmington, Dela. The ETM sample was received from Dr. I.A.M. FORD, Robinson Bros., West Bromwich, Staffs., England. The ETU standard was purchased from Eastman-Kodak, Rochester, N.Y. The fungicides evidenced decomposition prior to melting. The melting points of ETM and ETU were 116° and 199.5°, respectively.

Gas chromatography was carried out utilizing an F & M Model 1609 flame ionization instrument containing a modified flow system (Applied Science Labs., Inc., State College, Pa.) incorporating a 6 ft. by 0.25 in. glass coil column packed with 4% QF-1 fluorosilicone on 80–100 mesh HMDS-pretreated Chromosorb W. The analytical operating conditions are given in Table I.

TABLE I

GAS CHROMATOGRAPHY OF ZINEB, MANEB AND NABAM

Compound	*Corrected elution*[a] *(min)*			$t_R°$
Zineb	0.2		0.6	3.75[d]
Maneb			0.6	3.75[d]
Nabam	0.2	0.45	0.6[c]	3.75[d]
ETM			0.6	
ETU				3.75
Ethylenediamine	0[b]			

[a] Column: see Experimental. Conditions: column temperature 180°; detector temperature 225°; injector 70 V; nitrogen carrier 86 ml/min; hydrogen 72 ml/min; air 300 ml/min; range 1000; hydrogen flame detector.

[b] Eluted at solvent front.

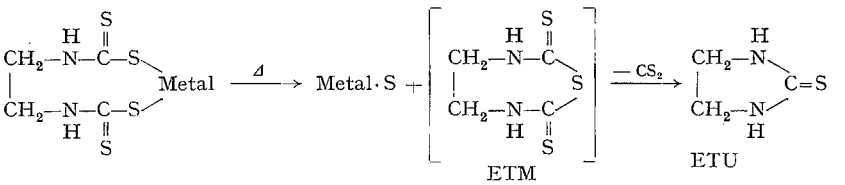
[c] Trace.

[d] Major peak.

Results and discussion

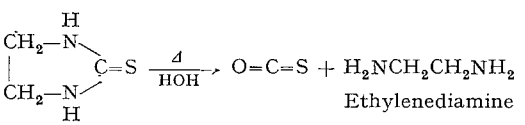
It is interesting to note that chromatography was carried out below the melting point of ETU. Lability of the fungicides was indicated by the predominant appearance of ETU and minor amounts of ETM and several other components as reported in Table I.

The thermal breakdown to ETU is suggested as follows:



It is felt that thermal cleavage of the metallic ethylenebis(dithiocarbamates) to ETU is hastened through the ETM stage owing to the observation that ETM itself may be chromatographed with little or no conversion to ETU. The solution medium for the analysis of ETM and ETU was dimethylsulfoxide. An aqueous medium was provided for Zineb and Nabam, having the form of an emulsion, and a clear solution, respectively. It should be pointed out that attempts to chromatograph ETM in pyridine resulted in a considerable loss in ETM with the production of two minor peaks (one being ETU) and two major peaks, the largest of which had elution of 3.93 relative to ETM.

The gaseous decomposition products of dithiocarbamate fungicides have been investigated by several workers[13–16]. COX, SISLER AND SPURR[13] concluded that carbon disulfide and ethylenediamine may be liberated from Nabam solutions, while MOJE, MUNNECKE AND RICHARDSON[17] described carbonyl sulphide as a volatile fungitoxicant from Nabam in soil. The acid hydrolysis suggested by the latter investigators indicated the concomitant formation of ethylenediamine. The appearance of this compound by gas chromatographic analysis might be expected to occur as follows:



Chromatography of ethylenediamine, however, resulted in its elution at the solvent front and not coincidal to any of the sample peaks.

In order to appreciate the quantitative relationships of ETU formation, a standard curve for Nabam degradation is given in Fig. 1. While the sensitivity units are in hundreds of micrograms of Nabam, the corresponding high attenuation (× 1000) of the signal should be denoted.

A general extension of this preliminary investigation to the analysis of the dithiocarbamate class by gas chromatography via *in situ* degradation during sample injection is presently under study.

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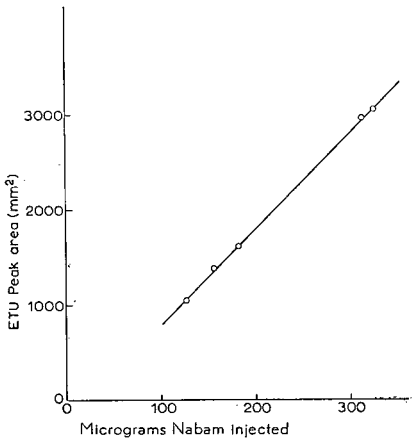

Fig. 1. Standard curve for Nabam based on ETU liberation. Attenuation: × 1000.

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1 T. CALLAN AND N. J. STRAFFORD, *J. Soc. Chem. Ind. (London)*, 43 (1924) 8T.
2 D. G. CLARK, H. BAUM, E. L. STANLEY AND W. F. HESTER, *Anal. Chem.*, 23 (1951) 1842.
3 W. K. LOWEN, *J. Assoc. Offic. Agr. Chemists*, 36 (1953) 484.
4 M. LEVITSKY AND W. K. LOWEN, *J. Assoc. Agr. Chemists*, 37 (1954) 555.
5 H. L. PEASE, *J. Assoc. Offic. Agr. Chemists*, 40 (1957) 1113.
6 C. BIGHI, *Atti Acad. Sci. Ferrara*, 38 (1961) 1.
7 C. BIGHI, *Ann. Chim. (Rome)*, 53 (1963) 1061.
8 C. BIGHI AND G. SAGLIETTO, *J. Chromatog.*, 17 (1965) 13.
9 K. E. KRESS, *Anal. Chem.*, 23 (1951) 1313.
10 D. FIRESTONE AND P. J. VOLLMER, *J. Assoc. Agr. Chemists*, 39 (1956) 866.
11 W. P. MCKINLEY AND S. A. MAGARVEY, *J. Assoc. Offic. Agr. Chemists*, 43 (1960) 717.
12 L. FISHBEIN AND J. FAWKES, *J. Chromatog.*, 19 (1965) 364.
13 C. E. COX, H. D. SISLER AND R. R. SPURR, *Science*, 114 (1951) 643.
14 L. E. LOPATECKI AND W. NEWTON, *Can. J. Botany*, 30 (1952) 131.
15 S. RICH AND J. G. HORSFALL, *Am. J. Botany*, 37 (1950) 643.
16 R. M. WEED, S. E. A. MCCALLAN AND L. P. MILLER, *Contrib. Boyce Thompson Inst.*, 17 (1953) 299.
17 W. MOJE, D. E. MUNNECKE AND L. T. RICHARDSON, *Nature*, 202 (1964) 831.

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Thin-layer chromatographic separation of preservatives

The increasing use of preserving agents in foodstuffs, both the overall use and the number of agents in use, brings in its wake the necessity for laboratories working in the field to be able to carry out qualitative and quantitative analyses with standard apparatus and under normal working conditions.

Recent advances have been made in this field by COPIUS-PEEREBOOM AND BEEKES[1], who separated several agents using thin-layer chromatography, and COVELLO AND SCHETTINO[2], who employed a thin layer of silica gel for the separation of a similar series of preservatives. The development length used by COPIUS-PEEREBOOM, however, was about 20 cm, which meant that standard plates (20 × 20 cm) could not be employed. The work of COVELLO AND SCHETTINO, on the other hand, did not give a complete separation and the method of detection using sublimation is not suitable for routine analysis.

SALO[3] separated *p*-hydroxybenzoic acid and thirteen of its esters on a mixture of acetylated cellulose and polyamide utilising Shell Sol A–glacial acetic acid (50:50, v/v) as solvent. LUECK[4] effected a separation of benzoic and sorbic acids after prior bromination.

The aim of the present work was to effect a separation of nine commonly used preserving agents using a standard technique. By employing a 1:1 mixture of Kieselgel G and Kieselguhr G (Merck)[1,2,5–7] and developing twice to a length of 15 cm with a petroleum ether–chloroform–formic acid (10:4:1, v/v/v) solvent mixture, a complete separation was obtained. All the agents under analysis were visible under U.V. (366 nm) after adding the fluorescent indicator Ultraphor W.T. (BASF) (0.02 %).

Experimental

A 1:1 mixture of Kieselgel G and Kieselguhr G was employed as adsorbent. Fifteen grams of each material were mixed with 60 ml of a 0.02 % solution of Ultraphor W.T. in water. A thickness of 0.25 mm of adsorbent was utilised on standard (20 × 20 cm) plates. The plates were dried in a warm air current for 10 min before activation by heating at 110° for 30 min.

The acetic acid concentration in the solvent mixture employed by COPIUS-PEEREBOOM AND BEEKES was raised slightly and this gave an increased separation.

TABLE I

No.	*Preservative*	*No. on photograph*	R_F	*Quantity spotted on the plate (γ)*
1	Benzoic acid	2	0.77 — 0.86	100
2	Sorbic acid	3	0.72 — 0.80	25
3	Salicylic acid	1	0.63 — 0.74	25
4	Dehydroacetic acid	9	0.57 — 0.66	50
5	Bromoacetic acid	6	0.41 — 0.44	25
6	Propyl-*p*-hydroxybenzoate	4	0.29 — 0.31	25
7	Ethyl-*p*-hydroxybenzoate	5	0.24 — 0.26	25
8	Methyl-*p*-hydroxybenzoate	7	0.19 — 0.20	25
9	*p*-Hydroxybenzoic acid	M lower spot	0.10 — 0.11	25

Substitution of acetic acid by formic acid and ether by $CHCl_3$ gave preferable results in our hands, and the eluting solvent finally employed was petroleum ether–$CHCl_3$–formic acid (100:40:10, v/v/v). We eluted twice on the same plate to a length of 15 cm for each elution.

We found that temperature control during elution was of prime importance. At 24° salicylic acid and dehydroacetic acid were not separated. At 22°, however, all nine preserving agents under study were plainly distinguishable from one another (see Fig. 1 and Table I).

A further refinement in our technique was the method of sample application. Depositing the extracts of preserving agents in a streak rather than in the more customary spot gave a clearer separation.

All nine preserving agents used were visible for several minutes under U.V. irradiation (366 nm) ("Fluotest"—original Hanau) (see Fig. 1). The use of Leuchtpigment ZS Super (Riedel de Haën) as a fluorescence indicator at 254 nm was not

Fig. 1. Separation of preservatives by thin-layer chromatography. Conditions: 15 g Kieselgel G + 15 g Kieselguhr G (1:1) + 60 ml 0.02% Ultraphor; solvent: petroleum ether (25–70°, p.a.)–$CHCl_3$–HCOOH (100:40:10); elution twice for 15 cm; temperature maximum: 22°.

so successful due to the interference from Kieselguhr G. (Leuchtpigment ZS is recommended for DESAGA U.V. lamps.)

The above method has been used in the detection of preserving agents in a wide variety of foodstuffs and found to be successful on all occasions.

Detection reagents

1. Benzoic acid. (a) 4.5 ml H_2O_2 (30 %) + 4.5 ml H_2O + 1 ml saturated $MnSO_4$ solution; then (b) 0.3 % $FeSO_4$ aqueous solution.

2. Sorbic acid. (a) 5 ml 0.5 % $K_2Cr_2O_7$ + 5 ml 0.3 *N* H_2SO_4; followed by (b) saturated thiobarbituric acid solution.

3. Salicylic acid. 0.1 % $FeCl_3$ in water.

4. Dehydroacetic acid. 3 % $TiCl_3$ aqueous solution; or 0.1 % $FeCl_3$ aqueous solution.

5. Bromoacetic acid[8] (see under next section). The following mixture (a) 3 vol. phenol red (24 mg phenol red in 2.4 ml 0.1 *N* NaOH made up to 100 ml with acetone) and 1 vol of a CH_3COONa solution (6 g CH_3COONa + 3 ml CH_3COOH + water to 100 ml) is sprayed on the chromatogram and followed by a spray of (b) chloramine T solution (25 mg chloramine T in 15 ml of water–acetone, 1:1).

6–9. The esters and the free p-hydroxybenzoic acid[5]. Millon's reagent: 1 part of Hg by weight + 2 parts of fuming HNO_3 + 2 vol. water.

Detection

Following the separation, the various components of the mixture were confirmed using suitable reagents for each constituent. The order of use of the reagents is as indicated in the list above.

Small amounts of the reagent were sprayed on the plate, exposing only the part under scrutiny. If this was insufficient to give an unequivocable decision, the plate was heated and re-sprayed, small amounts of reagent again being employed.

Benzoic acid. Nearly all the detection reactions described in the literature employ H_2O_2 as an oxidising agent followed by $FeCl_3$ to give a colour[1,2,5–7]. KRÖLLER[9], however, added a saturated $MnSO_4$ solution to the peroxide to give a catalytic effect. He also used a mixture of $FeCl_3$ and $FeSO_4$ on paper chromatograms. In our hands a 0.3 % $FeSO_4 \cdot 7H_2O$ solution gave the best results. After initial spraying with this reagent the plate was redried for 3 min and resprayed. The reagent is oxidised on the plate and gives a light brown spot on a white background. Redrying and respraying with aqueous $FeSO_4$ gives a white spot which turns brown on drying.

Sorbic acid. After oxidising with 0.5 % $K_2Cr_2O_7$ solution the plate was dried and sprayed with a saturated solution of thiobarbituric acid. On further drying a pink spot on a white background is revealed. The dilute solution of $K_2Cr_2O_7$ employed by SCHMIDT[10] is preferred to the 10 % solution used by COPIUS-PEEREBOOM AND BEEKES[1], the yellow background being given by the latter solution making identification more difficult.

PEKKARINEN AND PORKKA[6] mention interference with this detection by various oxidation products in rancid fats, when carried out on the extract directly.

Salicylic acid. This gives a brown-violet spot after spraying with a dilute aqueous 0.1 % $FeCl_3$ solution. Another possibility is to spray with the Millon's reagent[5], the latter giving a yellow-orange spot.

Dehydroacetic acid. An aqueous 3 % $TiCl_3$ solution[1] gives a purple blue colour. We also used dinitrophenylhydrazine as a chromogenic agent. The difference between the yellow spot and the yellow background of the detection agent was not clear. We prefer a very dilute aqueous 0.1 % $FeCl_3$ solution which gives a yellow spot on a white background.

Bromoacetic acid. The plate is exposed to ammonia vapour for 10 min and heated afterwards for 10 min to eliminate the excess ammonia. After spraying with a mixture of phenol red and CH_3COONa the spot was immediately revealed with chloramine T. A blue spot on a white or a yellow background resulted[8].

Propyl, ethyl and methyl-p-hydroxybenzoate. The best detecting reagent was Millon's reagent. The three esters and the free acid give a red or red-brown colour after spraying with Millon's reagent and heating. If after the first spray the spots were not clear, the plate was sprayed for a second time and heated again for a few minutes at 100°.

p-Hydroxybenzoic acid. On account of the position of the esters and the acid on the chromatogram the most simple detection agent is Millon's reagent. We also used diazotized *p*-nitroaniline to detect the acid to give a yellow spot which turned to orange on exposure to ammonia vapours.

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1 J. W. COPIUS-PEEREBOOM AND H. W. BEEKES, *J. Chromatog.*, 14 (1964) 417.
2 M. COVELLO AND O. SCHETTINO, in G. B. MARINI-BETTÓLO (Editor), *Thin-Layer Chromatography*, Elsevier, Amsterdam, 1964, pp. 215–219.
3 T. SALO, *Lebensm. Untersuch. Forsch.*, 124 (1964) 448.
4 E. LUECK, *Deut. Lebensm. Rundschau*, 61 (1965) 78.
5 C. GENEST AND R. A. CHAPMAN, *J. Assoc. Offic. Agr. Chemists*, 42 (1959) 436.
6 L. PEKKARINEN AND E. PORKKA, *Z. Anal. Chem.*, 201 (1964) 423.
7 R. PINZON, A. MIRINANOFF AND I. KAPETANIDIS, *Pharm. Acta Helv.*, 40 (1964) 141.
8 A. SCHALLER, *Fruchtsaft-Ind.*, 2 (1957) 94, 138.
9 E. KRÖLLER, *Deut. Lebensm. Rundschau*, 59 (1963) 319.
10 H. SCHMIDT, *Z. Anal. Chem.*, 178 (1960) 173.

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Two-dimensional thin-layer chromatography of amino acids on microcrystalline cellulose

Protein hydrolyzates may be resolved, and the constituent amino acids detected, identified and determined, by ion-exchange column chromatography with an amino acid autoanalyzer[1]. For qualitative work, two-dimensional chromatography on a support of paper[2,3], moisture-equilibrated silica gel[4,5], or cellulose[6], offers the advantage of low cost. The thin-layer techniques[4–6] have the added advantage of greater speed than separations on paper[2,3]. In our hands the silica gel technique[4,5] suffers from disadvantages due to difficulty in standardizing the plates, fragility of the layer, and poor reproducibility in the R_F values obtained. Cellulose supports[6,7] give improved separations, but layers showing good adhesion and physical strength may be difficult to obtain. A form of microcrystalline cellulose known as "Avicel"-Technical Grade (formerly termed "Avirin"), produced commercially by acid treatment of cellulose, gives tenacious layers on glass plates, with excellent physical characteristics for thin-layer chromatography[7]. This support has been used for qualitative separations of sugars and amino acids[8], and for quantitative separations of sugars[9].

We report herein a two-dimensional thin-layer chromatographic procedure on an "Avicel"-Technical Grade support for the separation, detection and identification of amino acids in mixtures containing up to twenty different amino acids.

Experimental

Chromatoplates. "Avicel", Technical Grade (formerly termed "Avirin"), obtained from the Avicel Sales Division of American Viscose Division, FMC Corp., Marcus Hook, Pennsylvania, is blended with water, and applied to clean glass plates (20 × 20 × 0.4 cm) by the method of WOLFROM, PATIN AND DE LEDERKREMER[8], except that a layer thickness of 0.5 mm is used. The plates are allowed to dry without heating, are kept for at least 24 h at room temperature before use, and are not kept in a desiccator. Different commercial lots may require different amounts of water to obtain the same consistency.

Developers. The solvent system for development in the first direction is 1-butanol–acetic acid–water (3:1:1, w/w). For development in the second direction, phenol–water (3:1, w/w) is used.

Separation procedure. Light pencil lines are ruled parallel to, and 1.5 cm from two adjacent edges of the plate. Two more lines are ruled parallel to, and 12 cm from, each of the first two lines. The intersection of the first two lines is the starting point for the two-dimensional chromatogram. A solution containing the mixture to be resolved is applied to the starting point with a fine capillary as a compact zone <0.5 cm in diameter. The solution contains 0.1–0.5 μg of each amino acid standard, or 10–50 μg of a protein hydrolyzate.

A glass jar (29.5 × 27 × 10 cm) containing the first solvent to a depth of 0.5 cm is allowed to equilibrate for at least 6 h. The plate is introduced, with the starting point in the lower left corner, and the solvent is allowed to ascend to the line 12 cm above the starting line (about 1.5 h). The plate is then removed, and is allowed to dry in the air for at least 6 h at room temperature. It is then introduced, with the starting point in the lower right corner, into a second, pre-equilibrated jar containing the second solvent to a depth of 0.5 cm. The plate is removed when the solvent has

ascended to the line 12 cm above the starting line (about 2 h), and is allowed to dry in a hood for at least 12 h, without heating.

For control experiments by one-dimensional chromatography, a row of starting points 1.5 cm from one edge of the plate are marked out with a pencil. Amounts of 0.01–0.05 μg of each amino acid standard, or 1–5 μg of protein hydrolyzate, are used.

Hydrochloric acid hydrolyzates of protein or peptide preparations are freed from acid before chromatography. Hydrolyzates are evaporated in a desiccator over sodium hydroxide, the residues are redissolved in distilled water, and the solutions are re-evaporated over sodium hydroxide. The procedure is repeated at least once more.

Indication of the zones. The dried plates are sprayed lightly with a 0.2 % solution of ninhydrin in 95 % ethanol, and they are kept in the dark for 24 h at room temperature. The amino acids are revealed as purple-blue zones (proline gives a yellow zone), and background color is negligible.

A slight increase in sensitivity is attainable if, directly after spraying, the plates are heated for 5 min at 80°, but it is difficult to avoid the development of colored background, and artifacts resulting from finger contact with the plate.

Results and discussion

Fig. 1 shows the appearance of a chromatoplate on which a mixture of 20 amino acids had been applied, and resolved by the two-dimensional technique. Table I lists the R_F values for 20 amino acids in each of the solvent directions, and gives the limit of visual detection for each amino acid, in the first solvent system with indication at room temperature. Also listed are the detection limits recorded[4,5]

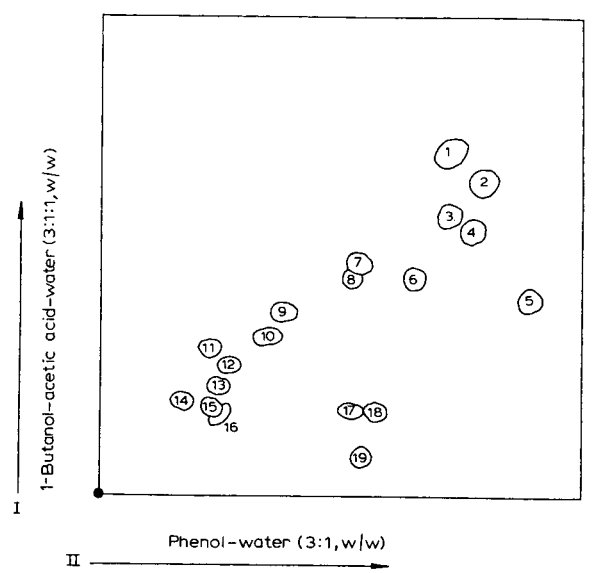

Fig. 1. Two-dimensional thin-layer chromatogram on "Avicel"-Technical Grade of a mixture of amino acids; 1 = isoleucine and leucine; 2 = phenylalanine; 3 = valine; 4 = methionine; 5 = proline; 6 = 4-aminobutyric acid; 7 = tryptophan; 8 = tyrosine; 9 = alanine; 10 = threonine; 11 = glutamic acid; 12 = glycine; 13 = serine; 14 = aspartic acid; 15 = taurine; 16 = lysine; 17 = arginine; 18 = histidine; 19 = cysteine.

TABLE I

R_F VALUES OF AMINO ACIDS AND LIMITS OF DETECTION

Amino acid	*BuOH–AcOH–H_2O (3:1:1, w/w)*		*PhOH–H_2O (3:1, w/w)*	*Limit of detection on silica gel G (μg)* *
	R_F	*Limit of detection (μg)*	R_F	
Alanine	0.37	0.02	0.38	0.009
4-Aminobutyric acid	0.44	0.01	0.64	
Arginine	0.17	0.005	0.49	0.01
Aspartic acid	0.19	0.03	0.18	0.1
Cysteine	0.08	0.03	0.56	
Glutamic acid	0.28	0.01	0.23	0.04
Glycine	0.25	0.01	0.32	0.001
Histidine	0.17	0.01	0.60	
Isoleucine	0.70	0.02	0.71	
Leucine	0.71	0.02	0.71	0.01
Lysine	0.17	0.005	0.24	0.005
Methionine	0.55	0.01	0.76	0.01
Phenylalanine	0.64	0.02	0.77	0.05
Proline	0.41	0.05	0.89	0.1
Serine	0.22	0.003	0.24	0.008
Taurine	0.18	0.01	0.22	
Threonine	0.32	0.02	0.34	0.05
Tryptophan	0.47	0.03	0.52	0.05
Tyrosine	0.45	0.02	0.52	0.03
Valine	0.58	0.01	0.73	0.01

* Data from refs. 4 and 5.

for some of these amino acids by one-dimensional chromatography on moisture-equilibrated silica gel G (E. Merck, Darmstadt, Germany). It is noteworthy that the sensitivity of the present method is superior to the silica gel method, except for alanine, glycine, and leucine. The R_F values recorded in Table I for 3:1:1 1-butanol–acetic acid–water, in a weight rather than in a volume ratio, are somewhat larger than those few which were previously reported[8]. We do not find that leucine and isoleucine are separable in admixture.

The R_F values are influenced by the moisture content of the plate, the thickness of the layer, and the amount of sample applied. Plates having 0.5 mm coating, dried as indicated, and with sample amounts in the given ranges, gave R_F values reproducible within $\pm$ 0.02. The one-dimensional R_F values are not noticeably changed by development perpendicular to, rather than along, the direction in which the plates are spread.

Support layers 0.25 mm or 1.0 mm thick gave somewhat less satisfactory results than 0.5 mm layers. "Avicel", the pharmaceutical grade produced by the same manufacturer, gave inferior results.

The technique is in use in this laboratory for identification of amino acids present in biological materials and in hydrolyzates of protein and peptide materials[10]. Compared with thin-layer techniques on moisture-equilibrated silica gel, and other commercial grades of cellulose, it offers the advantage of greater reproducibility, and a tenacious coating which can be marked with a pencil and which does not disintegrate at the point where the sample is applied. On samples of protein hydrolyzates,

the technique gives excellent qualitative agreement with compositions determined with an amino acid autoanalyzer.

The simplicity and low cost of this technique commend its use for instructional, as well as research purposes.

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1 D. H. SPACKMAN, W. H. STEIN AND S. MOORE, *Anal. Chem.*, 30 (1958) 1190; C. H. KIRKPATRICK AND R. A. ANDERSON, *J. Chromatog.*, 14 (1964) 295.
2 R. CONSDEN, A. H. GORDON AND A. J. P. MARTIN, *Biochem. J.*, 38 (1944) 224.
3 R. J. BLOCK, E. L. DURRUM AND G. ZWEIG, *A Manual of Paper Chromatography and Electrophoresis*, 2nd Ed., Academic Press, New York, 1958, p. 110.
4 M. BRENNER AND A. NIEDERWIESER, *Experientia*, 16 (1960) 378; A. R. FAHMY, A. NIEDERWIESER, G. PATAKI AND M. BRENNER, *Helv. Chim. Acta*, 44 (1961) 2022.
5 K. RANDERATH, *Thin-Layer Chromatography*, Academic Press, New York, 1963, p. 93; J. M. BOBBITT, *Thin-Layer Chromatography*, Reinhold, New York, 1963, p. 166.
6 E. VON ARX AND R. NEHER, *J. Chromatog.*, 12 (1963) 329.
7 P. WOLLENWEBER, *J. Chromatog.*, 9 (1962) 369; G. PATAKI, *J. Chromatog.*, 16 (1964) 541.
8 M. L. WOLFROM, D. L. PATIN AND R. M. DE LEDERKREMER, *Chem. Ind. (London)*, (1964) 1065; M. L. WOLFROM, D. L. PATIN AND R. M. DE LEDERKREMER, *J. Chromatog.*, 17 (1965) 488.
9 M. L. WOLFROM, R. M. DE LEDERKREMER AND G. SCHWAB, *J. Chromatog.*, 22 (1966) 474.
10 D. HORTON, M. R. SCHMEER AND A. TANIMURA, unpublished data.

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Purification of polycyclic hydrocarbons by chromatographic decomposition of their picrates, trinitrobenzolates and styphnates

The secondary reactions caused in adsorption chromatography on alumina consisting, for example, in the saponification of glycerides[1] and deacetylation of acetylated sugars[2,3] and other types of decomposition have been reported in the literature.

LEDERER AND LEDERER[4] have referred to the decomposition of the picrates of aromatic hydrocarbons as a practical method for their purification and indicated its application to styphnates and trinitrobenzolates[5,6].

However, LEDERER *et al.* in their publication[5] on ambergris have mentioned the decomposition of the picrate of a hydrocarbon obtained from ambreinolide by passing its solution through alumina and describe the styphnate but not its decomposition.

Further, LEDERER, MERCIER AND PÉROT[6], in connection with their studies on the structure of ambreine, describe the purification of 1,2,5,6-tetramethylnaphthalene *via* decomposition of its picrate but not that of its styphnate.

The purification of hydrocarbons by decomposition of their molecular derivatives by passing through a column of alumina seems to have been mentioned first by PLATTNER AND PFAU[7], who purified azulene and some of its derivatives by chromatographic decomposition of the corresponding picrate and trinitrobenzolate on Brockmann alumina.

The present work describes the systematic application of chromatographic decomposition of the molecular acid derivatives, *viz.* picrates, trinitrobenzolates and styphnates, on basic alumina, to the purification of some representative polycyclic hydrocarbons on a quantitative scale.

The incentive for this work came principally from the difficulties faced in the purification of 1,2,3,4,9,10,11,12-octahydrotriphenylene in connection with our work on a total synthesis of triphenylene[8]. This could only be obtained in a crystalline form, by the decomposition of its picrate on basic alumina.

Materials

The picrates of naphthalene (I), anthracene (II), phenanthrene (III), acenaphthene (IV), 1,2,3,4-tetrahydrophenanthrene (V) and 1,2,3,4,9,10,11,12-octahydrotriphenylene (VI) and the styphnates and trinitrobenzolates of I–V were prepared by heating equimolecular solutions of the hydrocarbons I–VI and picric acid, styphnic acid or trinitrobenzene in benzene, acetic acid and ethanol respectively for 15 min on the water bath with subsequent crystallization at room temperature, except the styphnate of anthracene, which was prepared in benzene solution. They were purified by recrystallization from benzene–petroleum ether mixture, acetic acid and ethanol respectively, with the exception of the picrate of V which was recrystallized from ethanol and of anthracene styphnate, which was best recrystallized from benzene.

Method

Chromatographic decomposition. Weighed quantities of the respective picrates, styphnates or trinitrobenzolates were dissolved in a minimum amount of benzene and allowed to pass over a column of a weighed quantity of basic alumina (Woelm, activity I). The hydrocarbons were eluted by measured quantities of petroleum ether (60–80°). The trinitrobenzolates were applied on the column in a minimum quantity of benzene–petroleum ether (1:2) (60–80°) solution. Special care was taken to use more than a minimum quantity of benzene in order to avoid the presence of trinitrobenzene in the eluates.

The above method was also extended with success to the purification of α- and β-naphthols and α- and β-naphthylamines by decomposing their picrates. These picrates were applied on the column in a minimum quantity of ethyl acetate and eluted with the same solvent.

Results

Tables I to III give the qualitative and quantitative data for the purification and the yields of the purified hydrocarbons finally obtained.

TABLE I

DECOMPOSITION OF PICRATES

In each case 7–8 g of basic alumina was used for preparing the column.

Hydrocarbons	*Picrates*		*Volume of eluent petroleum ether (ml)*	*Purified hydrocarbons*		*Yield (%)*
	M.p. (°C)	*Weight (mg)*		*M.p. (°C)*	*Weight (mg)*	
(I)	148–149	100	150	79– 80	32	91
(II)	139–140	160	150	212–213.5	65	93
(III)	144–145	185	130	99–100	75	92
(IV)	161–162	200	150	94– 94.5	76	92
(V)	110–111	100	120	31– 32	40	89
(VI)	196–198	100	150	121–122	48	94

TABLE II

DECOMPOSITION OF STYPHNATES

In each case 4–5 g of basic alumina was used for preparing the column.

Hydrocarbons	*Styphnates (recrystallized)*		*Purified hydrocarbons (eluted with 80 ml* of petroleum–ether, 60–80°)*		*Yield (%)*
	M.p. (°C)	*Weight (mg)*	*M.p. (°C)*	*Weight (mg)*	
(I)	162–164	100	79– 80	32	94
(II)	180–181	150	211–214	60	95
(III)	142–144	80	98–100	32	94
(IV)	152–154	100	93– 94	36.5	96
(V)	136–138	60	31– 32	24	94

* In the case of V only 60 ml of eluent was required.

TABLE III

DECOMPOSITION OF TRINITROBENZOLATES

In each case 10 g of basic alumina was employed for each 100 g of the trinitrobenzolate.

Hydrocarbons	*Trinitrobenzolates (recrystallized)*	*Volume of eluent (petroleum ether)*	*Purified hydrocarbons*		*Yield (%)*
	M.p. (°C)		*M.p. (°C)*	*Weight (mg)*	
(I)	148–149	60 ml	79.5– 80	35	96
(II)	162–163	60 ml	212–213	42	93
(III)	163–164	80 ml	96– 97	43	95
(IV)	164–166	60 ml	93– 94	40	95
(V)	128.5–129	80 ml	29– 30	44	95

TABLE IV

U.V. ABSORPTION SPECTRA

	Max ($m\mu$)	*a* (ε)	*b* (ε)
(1) *Acenaphthene*			
	227	76200	91800
	243	1075	1145
	270	3720	3920
	279	5630	5920
	288.5	6330	6650
	299.5	3940	4100
	305.5	2600	2700
	313	910	955
	320	1340	1365
(2) *Anthracene*			
	245	100700	111100
	251.5	181000	197000
	309	1145	1160
	323.5	2680	3120
	339	5070	5320
	356	7300	7750
	374	7170	7620
(3) *Phenanthrene*			
	211	31750	32400
	245	48200	49700
	250.5	63800	64300
	273.5	12480	13600
	280.5	9600	10420
	292	11580	12680

The hydrocarbons I to IV used as starting materials were commercial poducts with melting points 78–80°, 209–212°, 95–98°, 93–94°, and V and VI were intermediates obtained in this laboratory both as coloured oily products. The melting points of the purified hydrocarbons mentioned in the tables are those of products recrystallised in ethanol (I–IV), petroleum ether (60–80°) (V) and benzene (VI).

Table IV, showing the ultraviolet spectra of acenaphthene, anthracene and phenanthrene, both for (a) impure and (b) purified products, gives an idea of the grade of purification obtained by chromatographic decomposition of the picrates of the same.

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1 W. TRAPPE, *Biochem. Z.*, 305 (1940) 150.
2 H. BREDERECK, H. DÜRR AND K. RUCK, *Chem. Ber.*, 87 (1954) 526.
3 E. A. TALLEY, D. R. REYNOLDS AND W. L. EVANS, *J. Am. Chem. Soc.*, 65 (1943) 575.
4 E. LEDERER AND M. LEDERER, *Chromatography*, Elsevier, Amsterdam, 1957, p. 65.
5 E. LEDERER, F. MARX, D. MERCIER AND G. PEROT, *Helv. Chim. Acta*, 29 (1946) 1354.
6 E. LEDERER, D. MERCIER AND G. PEROT, *Bull. Soc. Chim. France*, (1947) 345.
7 PL. A. PLATTNER AND A. S. PFAU, *Helv. Chim. Acta*, 20 (1937) 224.
8 A. RAHMAN AND O. L. TOMBESI, *Chem. Ber.*, 1966, in press.

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The separation and identification of some alkanolamines and their salts by thin-layer chromatography

Alkanolamines, in particular ethanolamines and isopropanolamines, are often used in hydraulic brake fluids and cutting oils as corrosion inhibitors. Also when combined as soaps with fatty acids (usually oleic and stearic acids) they are used extensively as emulsifiers and detergents. The separation and identification of alkanolamines and their salts in such products can be time-consuming and it is advantageous to have a rapid method for doing this.

Amines have been separated by paper and thin-layer chromatography[1–6] and the R_F value for monoethanolamine in various solvents has been reported. The paper chromatographic methods suffer from the disadvantages of long development times and diffuse substance zones. Thin-layer chromatography, however, is ideally suited to the separation of alkanolamines and the development of a suitable procedure for separating and identifying ethanolamines and isopropanolamines and some of their salts in commercial formulations is described below.

Experimental and discussion

Solvent system/adsorbent/locating agent. Most of the solvent systems reported in the literature for separating amines consist of an alcohol and a base (usually ammonia). For the separation of simple mixtures of ethanolamines and isopropanolamines these systems work fairly well but they do not give a very good separation of complex mixtures. However, if methylene chloride is incorporated into the alcohol–base solvent mixture a much better separation is obtained and also the developing time

TABLE I

COLOURS AND R_F VALUES OF SOME ALKANOLAMINES AND THEIR CARBOXYLIC ACID SALTS

Compounds	*Colour of zones*		*R_F values*
	Ninhydrin	*Ninhydrin, then alizarin*	
(1) Monoethanolamine	Crimson	Crimson	0.26
(2) Diethanolamine	White	Blue-purple	0.43
(3) Triethanolamine	Grey	Grey-purple	0.60
(4) Monoisopropanolamine	Crimson	Crimson	0.47
(5) Diisopropanolamine	White	Blue-purple	0.63
(6) Triisopropanolamine	Green	Grey-purple	0.71
Oleates of alkanolamines (1)–(6)*	Blue/yellow fringe	Blue	Baseline
	Red	Red	0.60
Naphthenates of alkanolamines (1)–(6)*	Yellow	Blue	Baseline
	Red	Red	0.60
Oleic acid**	Yellow	Blue	Baseline
	Faint red	Faint red	0.60
Naphthenic acids** (equivalent weight: 300)	Yellow	Blue	Baseline
	Faint red	Faint red	0.60

* A zone due to the particular alkanolamine used was also observed.
** The R_F values of the carboxylic acids are recorded for comparison.

is decreased. The solvent system finally adopted was methylene chloride–ethanol (95 %)–ammonia (0.880) in the proportions 43:43:15 by volume. Of the various adsorbents examined for the separation neutral silica gel was the most suitable. Solutions of 0.2 wt. % ninhydrin and alizarin in acetone were used to locate the separated alkanolamines.

Procedure. The usual thin-layer chromatographic (TLC) procedure was used to separate the alkanolamines. 0.1 % solutions of the alkanolamines in ethanol were spotted on the TLC plate of neutral silica gel (250 μm thickness), 1 cm apart. The plates were then developed by the ascending technique, heated for 10 min at 110°, sprayed with ninhydrin solution and finally heated for a further 5 min at 110° to locate the separated alkanolamines. Respraying with alizarin solution gave a further identification.

When an alkanolamine salt is subjected to chromatographic techniques zones are obtained for both the alkanolamine and acid moieties. The alkalonamine salts used included oleates and naphthenates. All of these salts gave a distinct yellow zone at the baseline and a red zone at about R_F 0.60. The R_F values of the alkanolamines separated are given in Table I and the separation is illustrated in Fig. 1.

Application of the method to commercial formulations. A number of hydraulic brake fluids and cutting oils were examined by the procedure.

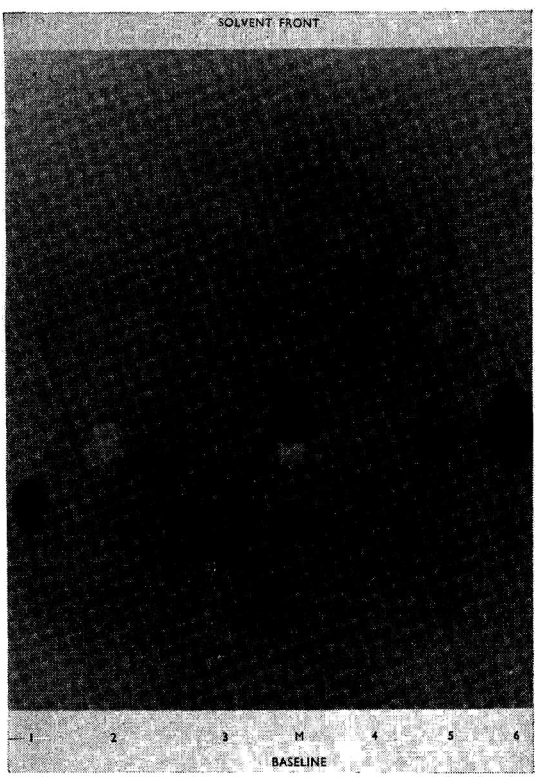

Fig. 1. Thin-layer chromatogram of alkanolamines. 1 = Monoethanolamine; 2 = diethanolamine; 3 = triethanolamine; 4 = triisopropanolamine; 5 = diisopropanolamine; 6 = monoisopropanolamine; M = mixture of 1–6.

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TABLE II

THE DETECTION OF ALKANOLAMINES AND THEIR SALTS IN VARIOUS COMMERCIAL FORMULATIONS

Formulation	*Colour of zones*		R_F *values*	*Inference*	*Alkanolamine found by chemical analysis*
	Ninhydrin	*Ninhydrin, then alizarin*			
1	Crimson	Crimson	0.26	Monoethanolamine	
	White	Blue/purple	0.43	Diethanolamine	
	Red	Red/purple	0.60	Triethanolamine Alkanolamine salt	Triethanolamine oleate
	Yellow	Blue	Baseline	Alkanolamine salt	
2	Crimson	Crimson	0.26	Monoethanolamine	
	White	Blue/purple	0.43	Diethanolamine	Diethanolamine
	Red	Red	0.60	Alkanolamine salt	Diethanolamine oleate
	Yellow	Blue	Baseline		
3	Crimson	Crimson	0.26	Monoethanolamine	
	White	Blue/purple	0.43	Diethanolamine	Triethanolamine
	Grey	Grey/purple	0.60	Triethanolamine	
	Yellow	Blue	0–0.20	Unknown	
4	Crimson	Crimson	0.47	Monoisopropanolamine	
	Red	Red	0.60	Monoisopropanolamine salt	Monoisopropanolamine naphthenate
	Yellow	Blue	Baseline		
5	Crimson	Crimson	0.26	Monoethanolamine	
	White	Blue/purple	0.43	Diethanolamine	
	Red	Red/purple	0.60	Triethanolamine Alkanolamine salt	Triethanolamine Triethanolamine naphthenate
	Yellow	Yellow	0.36	None	
	Yellow	Blue	0–0.20	Unknown	

The results given in Table II show that whenever triethanolamine was incorporated in a formulation, mono- and di-ethanolamine were present as impurities. When the chromatogram is sprayed with ninhydrin the red zone (R_F 0.60) due to a carboxylic acid overlays and conceals the zone of triethanolamine (R_F 0.60) when both the acid and amine are present in a mixture. However, if triethanolamine is present it can be confirmed by the distinct purple hue of the red zone when sprayed with alizarin solution. Sometimes the other components of a formulation cause the acid constituent to appear as a red streak from $R_F \sim 0.3$ to $R_F \sim 0.6$ but this does not prevent identification of the alkanolamine constituents.

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1 H. ROSENBERG AND A. N. ENNOR, *Biochem. J.*, 73 (1959) 521.
2 C. E. ROWE, *Biochem. J.*, 73 (1959) 438.
3 J. FRANC AND M. HAJKOVA, *Coll. Czech. Chem. Commun.*, 24 (1959) 423.
4 J. BLASS AND A. SARRNOFF, *J. Chromatog.*, 3 (1960) 168.
5 E. A. MISTRYUKOV, *J. Chromatog.*, 9 (1962) 314.
6 K. TEICHERT, E. MUTSCHLER AND H. ROCHELMEYER, *Deut. Apotheker-Ztg.*, 100 (1960) 283.

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Paper chromatography of sugar phosphates and three-carbon phosphates. Extension and modification of the Agarwal procedure*

Many methods[1–6] have been developed for the paper chromatographic separation of phosphorylated metabolic intermediates. While using one of these procedures[6], originally designed for the chromatography of hexose phosphates, in the study of organic phosphates in honey[7], it was found that this procedure would also separate sugar phosphates from some three-carbon phosphates as a group. In addition, with the modification described here, some separation of the individual three-carbon phosphates was obtained.

Experimental

Reagents. All reagents were analytical grade and used as supplied.

Standard sugar phosphates and three-carbon phosphates. These standards were converted to their ammonium salts by the method of AGARWAL *et al.*[8]. The amount of salt used produced a 0.05 *M* solution of free acid or ester. The standards are listed with their name, source and abbreviation used in the text**.

* From a thesis submitted by MARY H. SUBERS in partial fulfillment of the requirements for the Degree of Master of Science in Chemistry at Saint Joseph's College.

** Mention of trade or company names does not imply endorsement by the Department over others not named.

Fructose 1-phosphate, barium salt, Sigma Chemical Co.	F-1-P
Fructose 6-phosphate, barium salt, Sigma Chemical Co.	F-6-P
Fructose 1,6-diphosphate, barium salt, courtesy of J. W. WHITE, Jr.	F-1,6-P
Glucose 1-phosphate, dipotassium salt, Calbiochem	G-1-P
Glucose 6-phosphate, disodium salt, Calbiochem	G-6-P
Ribose 5-phosphate, sodium salt, Sigma Chemical Co.	R-5-P
Dihydroxyacetone phosphate, dimonocyclohexylamine salt, Sigma Chemical Co.	DHAP
2,3-Diphosphoglyceric acid, barium salt, Sigma Chemical Co.	2,3-PGA
DL-Glyceraldehyde 3-phosphate, diethyl acetal, barium salt, Sigma Chemical Co.	G-3-P
DL-α-Glycerophosphate, disodium salt, hexahydrate, Sigma Chemical Co.	α-GP
DL-β-Glycerophosphate, disodium salt, hexahydrate, Sigma Chemical Co.	β-GP
2-Phosphoglyceric acid, barium salt, Sigma Chemical Co.	2-PGA
3-Phosphoglyceric acid, barium salt, Nutritional Biochem. Corp.	3-PGA
Phospho(enol)pyruvic acid, barium salt, monohydrate, Sigma Chemical Co.	PEP

Solvent systems. AGARWAL formic acid[6]: *tert.*-butanol–50 % formic acid–water (16:1:4).

ALBON AND GROSS[9]: *n*-propanol–ethyl acetate–water (7:1:2).

Spray reagent. The molybdate reagent of HANES AND ISHERWOOD[1] was used to locate the phosphate spots.

Apparatus. Glass tanks, 61 cm high × 30 cm square, designed for descending chromatography and accommodating 4 glass troughs, each holding a 21 × 56 cm chromatogram.

Paper washer, 49.5 × 61 × 16.5 cm (made of Plexiglas) with a perforated plate 8.5 cm from the bottom.

Whatman No. 1 filter paper, after proper washing, was used for all chromatography.

General Electric 15-W germicidal ultraviolet lamp. Papergrams were irradiated with this lamp[2] to produce the blue phosphomolybdate spots.

Purification of the filter paper. The filter paper washing was a very critical step, because calcium and magnesium salts in the paper cause irreversible adsorption of the phosphates[10] and retard their migration. Two methods of washing were used: the first involved soaking in formic acid (1 *N*) followed by a soak in 0.5 % versene, pH 8.5 (ref. 5); the second omitted the soak in versene.

Chromatographic procedures

(1) *General.* All chromatograms were developed in the descending direction at 25–30°.

A uniform atmosphere was provided in the tank by use of solvent-wetted sheets (10 × 26.5 cm) of Whatman No. 3MM paper around the sides at the bottom. In addition, a blank sheet of Whatman No. 1 paper was suspended from one of the troughs and developed in the same manner as the chromatograms. The tank was al-

lowed to equilibrate at least 4 h for the fast-moving propanol system and overnight for the slower-moving formic acid system. A serrated edge was cut into the bottom end of the paper so that the solvent could flow off evenly.

The phosphates were used as their ammonium salts. Well defined spots were obtained when a 3 μl portion of a 0.05 M (as free acid or ester) solution was applied to the paper. Nine compounds, spotted 2 cm apart, could be studied at one time on a 21 × 56 cm chromatogram. The distance traveled was measured from the origin to the center of the spot. Orthophosphate (2 μl of 0.05 M $Na_3PO_4 \cdot 12H_2O$) was spotted on every chromatogram as a reference standard. The distance traveled by the organic phosphates was compared with the distance traveled by the orthophosphate. This ratio is the position constant, Rp.

(2) *Original Agarwal procedure.* The original AGARWAL procedure[6] was followed with two exceptions. Phosphates were applied as ammonium salts and formic acid-washed paper was used.

(3) *Modified Agarwal procedure.* The chromatogram was developed on formic acid-washed paper using successive irrigations with the propanol system and the formic acid system. First, the paper was put into the tank containing the propanol system and, after a 2-h equilibration, was irrigated for 15.5 h. The solvent was allowed to run off the paper. The sheet was removed and air-dried in a hood for 3 h. It was then put into the formic acid system tank, equilibrated 4 h, and irrigated for 20 h in the same direction traveled by the propanol system. This solvent front was not allowed to run off the paper. The sheet was removed when the front reached the beginning of the serrated edge.

Detection of phosphates. The phosphates were located with the molybdate reagent in the following way. Papers were heated at 85–90° for 3–5 min, then sprayed thoroughly, but not soaked, to insure complete hydrolysis of some of the very stable three-carbon phosphates. Papers were allowed to air-dry completely then heated again as above for about 1 min. The papers were carefully watched during this heating. If they began to change color, the heating was discontinued. After heating, the papers were illuminated with the U.V. lamp at a distance of about 15 cm until blue spots appeared against a white or buff background. Inorganic phosphate produced a blue-green spot and G-1-P showed a blue-green-purple spot. All of the other organic phosphates showed blue spots.

Results

In the original AGARWAL formic acid system[6], α-GP, 2-PGA and 3-PGA all moved the same distance. β-GP moved just a little faster (Fig. 1). When the development with AGARWAL formic acid system was preceded by irrigation with the ALBON AND GROSS propanol system[9], the glycerophosphates moved ahead of the phosphoglyceric acids. In addition, the β-GP traveled faster than the α-GP. The 2-PGA and 3-PGA showed a slight separation with 2-PGA moving a little faster (Fig. 2). These effects are also shown by the position constants listed in Table I.

In the original AGARWAL procedure, both G-1-P and G-6-P produced double spots whose movement was highly reproducible (Fig. 1). When the propanol treatment was used G-1-P still formed double spots consistently, but G-6-P now produced a single spot (Fig. 2). The movement of the hexose phosphates after the propanol irrigation was in the same order as that found by AGARWAL[6].

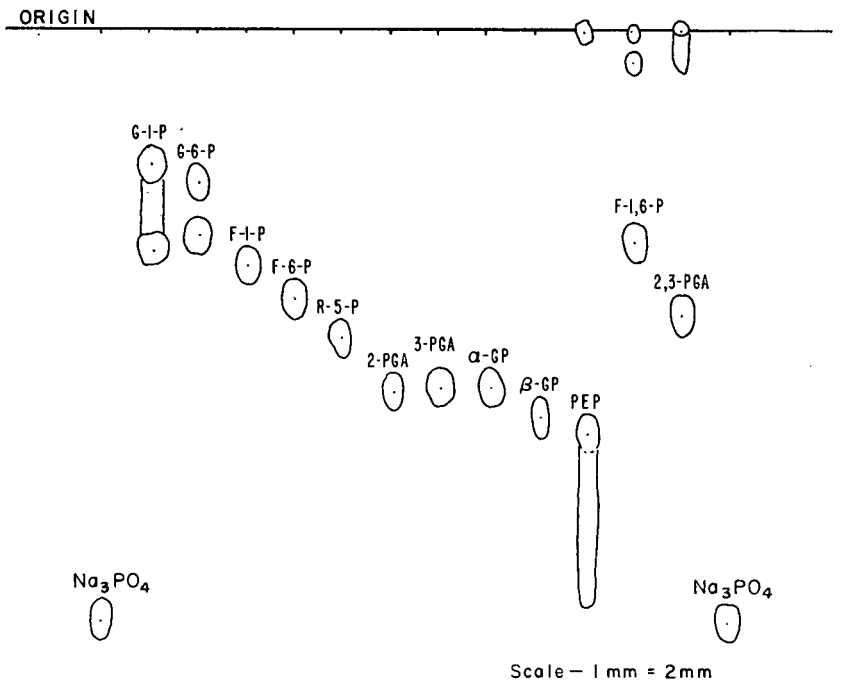

Fig. 1. Paper chromatogram of sugar phosphates and three-carbon phosphates developed in AGARWAL system for 39.5 h. Formic acid-washed paper was used.

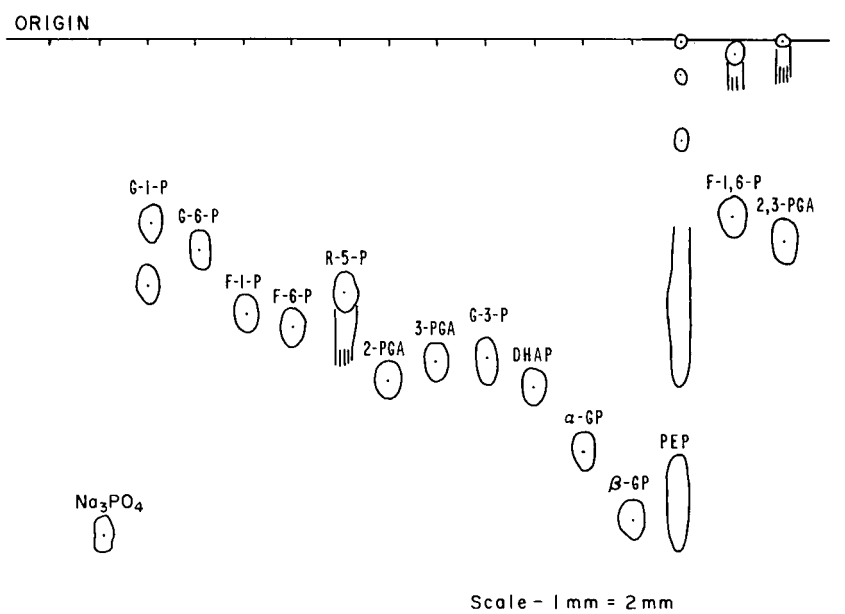

Fig. 2. Paper chromatogram of sugar phosphates and three-carbon phosphates irrigated with ALBON AND GROSS system for 15.5 h, then developed in AGARWAL system for 20 h. Formic acid-washed paper was used.

TABLE I

EFFECT OF SUCCESSIVE DEVELOPMENT WITH ALBON AND GROSS PROPANOL SYSTEM AND AGARWAL FORMIC ACID SYSTEM ON SEPARATION OF SUGAR PHOSPHATES FROM THREE-CARBON PHOSPHATES

Phosphate material	*Rp**	
	Agarwal solvent only	*Albon and Gross solvent Agarwal solvent*
G-1-P	23, 38 **	37, 53 **
G-6-P	26, 37 **	44
F-1-P	43	55
F-6-P	48	58
R-5-P	52	52
DHAP	—	74
G-3-P	—	67
2-PGA	57	72
3-PGA	57	69
α-GP	57	86
β-GP	64	99

* Figures are averages except for F-1-P, F-6-P, R-5-P, DHAP and G-3-P, which were from one run only; *Rp*, position constant $= \frac{\text{distance traveled by phosphate material}}{\text{distance traveled by orthophosphate}}$.

** Two spots from one origin.

The diphosphates, F-1,6-P and 2,3-PGA, formed multiple spots whose travel was unpredictable in both procedures.

The rate of travel of all of the phosphate compounds, including orthophosphate, was greatly increased by the irrigation with the propanol system. The propanol system alone did not move the phosphate compounds significantly from the origin. When the paper was irrigated with the propanol system before the phosphates were applied, there was no separation of three-carbon phosphates from each other. Their rate of travel was the same as that shown when the formic acid system alone was used.

Results were unsatisfactory when acid–versene-washed paper was used instead of formic acid-washed paper. All of the phosphates decomposed except 2-PGA, 3-PGA, β-GP and G-6-P.

The AGARWAL ammonia system[8] did not separate sugar phosphates from three-carbon phosphates and a preliminary irrigation with the propanol system had no effect.

Discussion

The propanol system irrigation probably made the conditions of chromatography less acidic than they were when the formic acid system was used alone. Hence there would be enough phosphate groups ionized to allow the solubility of the three-carbon phosphates to influence their migration. The sugar phosphates moved more slowly than the three-carbon phosphates, because the environment was still acid enough for the non-phosphate moieties to govern their rate of travel.

In addition to these chemical effects, the propanol system, which was designed originally for the chromatography of disaccharides[9], may have washed some carbohydrate impurities out of the paper or out of the phosphate samples themselves.

Several thin-layer methods for the separation of sugar phosphates were considered. The method of DIETRICH *et al.*[11], using ECTEOLA layers, was tried and it produced multiple spots for the three-carbon phosphates. The methods of WARING AND ZIPORIN[12] and SANDERSON *et al.*[13] were not applicable, because of reported insufficient separations and, therefore, were not investigated further. The proposed paper procedure provides a well-defined separation due to greater migration distance, and is simpler to use. About the same time is required as for preparation of plates and analysis with the thin-layer methods.

Acknowledgement

The authors wish to thank Dr. JONATHAN W. WHITE, Jr. for his suggestions, encouragement, and interest throughout this investigation.

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1 C. S. HANES AND F. A. ISHERWOOD, *Nature*, 164 (1949) 1107.
2 R. S. BANDURSKI AND B. AXELROD, *J. Biol. Chem.*, 193 (1951) 405.
3 D. C. MORTIMER, *Can. J. Chem.*, 30 (1952) 653.
4 H. E. WADE AND D. M. MORGAN, *Biochem. J.*, 60 (1955) 264.
5 V. C. RUNECKLES AND G. KROTKOV, *Arch. Biochem. Biophys.*, 70 (1957) 442.
6 D. P. AGARWAL, G. G. SANWAL AND P. S. KRISHNAN, *Analyst*, 87 (1962) 758.
7 M. H. SUBERS, A. I. SCHEPARTZ AND R. P. KOOB, *J. Apicult. Res.*, in press.
8 D. P. AGARWAL, G. G. SANWAL AND P. S. KRISHNAN, *Analyst.*, 88 (1963) 969.
9 N. ALBON AND D. GROSS, *Analyst.*, 77 (1952) 410.
10 A. A. BENSON, in S. P. COLOWICK AND N. O. KAPLAN (Editors), *Methods in Enzymology*, Vol. 3, Academic Press, New York, 1957, p. 111.
11 C. P. DIETRICH, S. M. C. DIETRICH AND H. G. PONTIS, *J. Chromatog.*, 15 (1964) 277.
12 P. P. WARING AND Z. Z. ZIPORIN, *J. Chromatog.*, 15 (1964) 168.
13 A. SANDERSON, C. E. BODWELL AND A. M. PEARSON, *Nature*, 206 (1965) 938.

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A convenient migration chamber for electrophoresis in solid gels*

Reproducible electrophoretic conditions are provided by a conveniently designed migration chamber, which was constructed at the Instrument Service Shop of Montana State University. This apparatus eliminates many of the difficulties encountered in making and handling both starch and acrylamide gels. The apparatus has been extensively used in our laboratory for routine electrophoretic analysis of milk proteins in starch gel and has the following advantages over comparable units: (1) Gel solutions are poured directly into the assembled unit while it is in the vertical position and the gel is not exposed or removed until it is ready for staining. (2) No filter paper wicks or salt bridges are used, thus eliminating any changes in the buffer or gel composition due to evaporation or contamination. (3) Samples are conveniently applied through a sample introduction opening in the apparatus. (4) Removal of the gel for staining after completion of electrophoresis is greatly simplified without the danger of tearing or breaking the gels, which are fragile. (5) The need for slicing the gel or covering the samples with liquid petrolatum is eliminated. (6) No sponge support for flexible gels such as acrylamide is needed to support the gel in vertical position. (7) Leakage of the buffer from the upper buffer vessel onto the surface of the vertical gel is eliminated. (I found this leakage to be troublesome when the apparatus reported by RAYMOND[3] was used.) (8) The gel thickness can be easily varied between 3, 5 or 9 mm simply by changing the gel frame in the apparatus.

Fig. 1a shows the various components of the unassembled unit while Fig. 1b presents a profile of the assembled unit.

Assembling the unit

The gel frame, 4, together with the back cooling plate, 6, are held tightly against the front cooling plate, 3, with the aid of two clamps, 7, on both sides of the migration chamber. The gel is formed into the shape, 19, on the gel frame, 4. The dimensions of the gel are 10 × 30 cm. The thickness of the gel may be varied between 3, 5 or 9 mm by using a separate frame for each. Both the front and the back cooling plates are made of 1.5 cm thick block of plexiglass with internal channels, 17, 37, for circulation of cooling water. These channels are formed by milling grooves 0.50 in. wide and 1/8 in. deep on one face and cementing a cover plate over the grooves. Inlet, 28, connects to the main supply of cooling water, while outlet, 29, allows the passage of exhaust water. The borders of the two cooling surfaces on the cooling plates, which come in direct contact with the gel, are lined with a 2 mm thick rubber gasket, 34, to prevent leakage of the liquid gel from the assembled unit. The front cooling plate, 3, has two oblong openings, 32 and 38, at both ends measuring 2.5 × 14 cm. These two openings face the two buffer vessels, 1 and 2, which are cemented at both ends of the front cooling plate, 3. Openings 32 and 38, when exposed, provide direct contact of the gel ends with the buffer in the buffer vessels, thus eliminating the need for filter paper wicks. Before introducing the liquid gel into the apparatus, the two openings, 32 and 38, are plugged tightly with two gel end plugs, 11 and 14, which are lined with rubber at the side facing the gel ends. The two gel end plugs, 11 and 14, are secured tightly in place with the thumb screws, 16, on the two gel end

* Contribution from Montana State University, Agricultural Experiment Station, Paper No. 126, Project No. 1151.

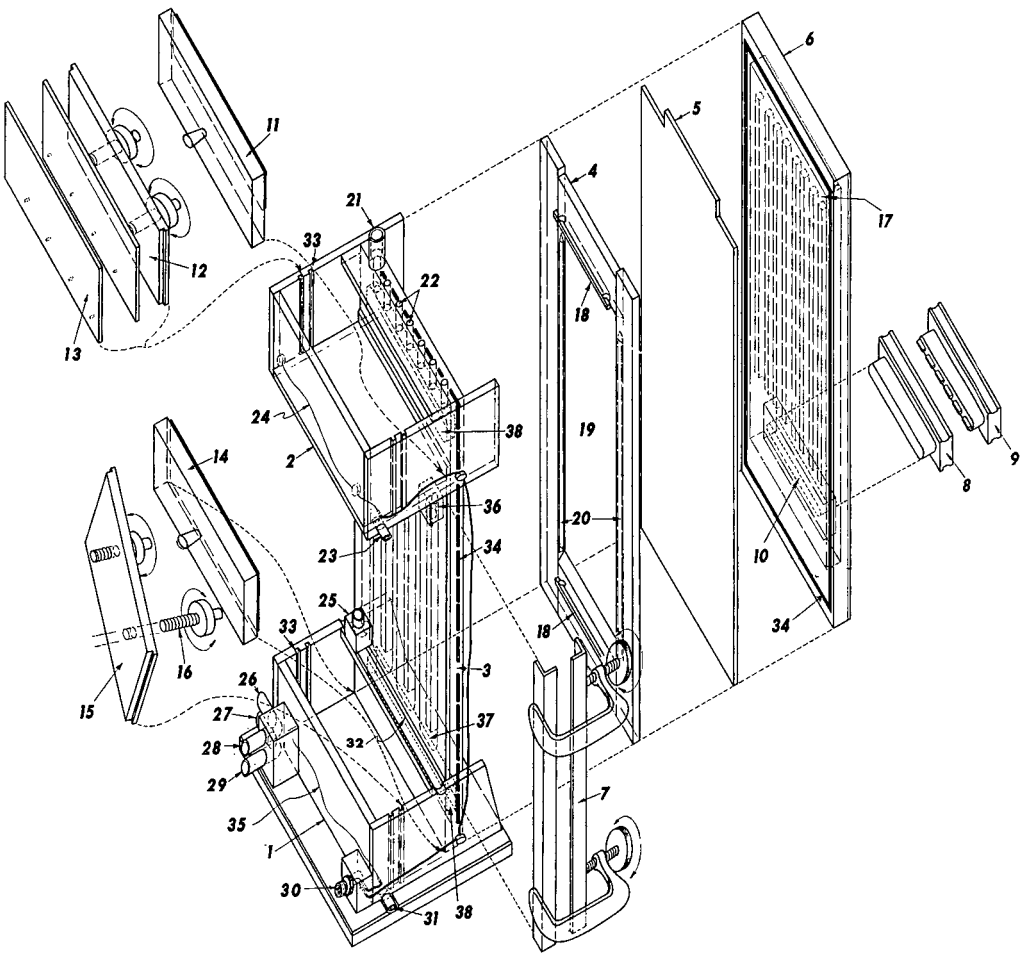
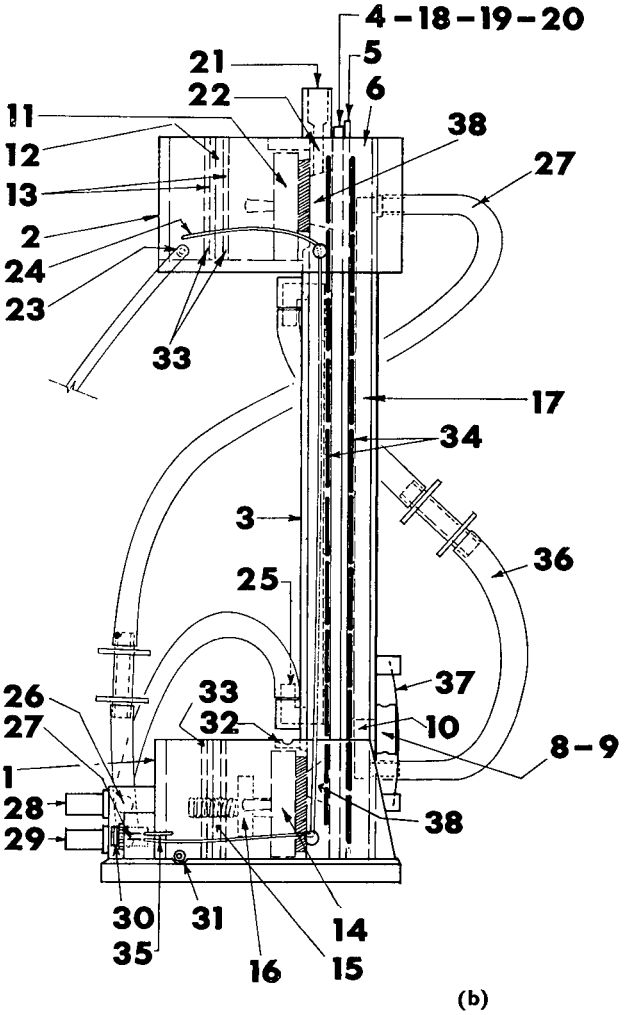


Fig. 1. Diagram of vertical electrophoresis migration chamber: (a) unassembled, (b) assembled unit. Components: 1 and 2 = buffer vessels; 3 = front cooling plate; 4 = gel frame; 5 = gel frame support for gel removal; 6 = back cooling plate with an opening, 10, for sample application; 7 = apparatus clamp; 8 = sample opening plug; 9 = sample slot former; 11 and 14 = plugs for enclosure of openings at both ends of front cooling plate where contact between buffer and gel is made; 12 and 15 = supports for plugs 11 and 14; 13 = buffer vessel partitions; 16 = thumb screws on supports 12 and 15 for holding plugs 11 and 14 tightly in place; 17 and 37 = cooling channels for circulation of water into cooling plates; 18 = gel retainers on gel frame, 4; 19 = space where gel is formed on gel frame; 20 = side channels on gel frame to hold gel in place; 21 = inlet for introducing liquid gel; 22 = air escape vents; 23 and 31 = outlets for emptying buffer vessels; 24 and 35 = platinum electrodes; 25, 26, 27, 28, 29 and 36 = inlets and outlets for cooling water circulation; 30 = electric outlet for connection to the power supply; 32 and 38 = oblong openings at both ends of front cooling plate to expose the gel ends to the buffer; 33 = grooves on sides of buffer vessels to hold either the buffer partitions, 13, or plug supports, 12 and 15, in place; 34 = rubber gaskets on sides of cooling plates.



(b)

plug supports, 12 and 15. The back cooling plate, 6, has an opening for introducing the sample, 10, measuring 12 × 2.5 cm at one of its ends. This opening could be plugged either with the sample opening plug, 8, or with the slot former, 9, before introducing the gel. Plug 8 or slot former 9 are held tightly in place with strong adhesive paper.

When the apparatus is completely assembled the space, 19, on the gel frame, 4, is completely enclosed and safeguarded from leakage. The gel frame, 4, has a bar handle, 18, at each end for holding the gel ends in place. On both sides of the gel frame there are two 0.60 cm deep grooves, 20, to secure the sides of the gel in place on the frame.

Use

Before assembling the unit, the two sides of the cooling plates which come in direct contact with the gel should be covered with a very thin film of liquid petro-

latum. This prevents the surface of the gel block from sticking to the surface of the cooling plates. The back cooling plate, 6, can be assembled with the sample introduction opening, 10, at the upper end of the vertical unit for downward migration of proteins or in the lower most position for upward migration. Before introducing the liquid gel, a funnel is placed into the gel inlet, 21, and the liquid gel is poured directly into the unit while it is in the vertical position. The air displaced by the gel escapes through the vent holes, 22. When using starch gel it is very important to insure that the gel temperature is within the range of 25–30°. If the temperature is higher than that the gel shrinks after setting, allowing condensate to form between the cooling surface and the surface of the gel. With acrylamide gel the gel setting temperature should be 22 ± 1°. Drastic changes in temperature during gel setting cause gel shrinkage and localized sweating which are some of the major causes of irregular and defective electrophoretic patterns. Starch gel should be allowed to set for a minimum of 12 h and a maximum of 24 h for optimum performance. Acrylamide gels require 20–30 min for setting.

For sample application the sample opening plug, 8, or the slot former, 9, is carefully removed and the samples are applied either in liquid form in the slots or in the form of dry filter paper inserts previously impregnated with the samples by a technique reported by the author[2]. The sample plug is again secured in place. The gel end plugs, 11 and 14, and their support plates, 12 and 15, are carefully removed to expose the gel ends facing the buffer vessel, 1 and 2. The buffer partitions, 13, are then placed in the grooves, 33, in the buffer vessels and the buffer removal outlets, 23 and 31, are closed by tightening the pinch cocks on the tygon tubing connected to them. The buffer (500 ml) is placed in each of the buffer vessels and the power supply is connected to the migration chamber through the electric socket, 30. The proper potential gradient is applied through the two platinum electrodes, 24 and 35. The electrodes are made of two 5-mil platinum wires stretched across the buffer vessels. The electrophoresis is either conducted in the cold room at 2° or cooling water at 15 ± 1° for starch gel, or 22 ± 1° for acrylamide gel, is circulated through the cooling plates. Fluctuations in the temperature of cooling water should be avoided, otherwise localized sweating of the gel occurs, leading to defective electropherograms.

At the termination of electrophoresis the buffer is emptied through the outlets, 23, 31, on the buffer vessels. The two clamps, 7, are loosened and removed and the back cooling plate is carefully removed. The gel frame with the gel block stretched across it is then carefully removed after supporting the back of the gel with the gel plate support, 5. Staining and destaining of the gel is carried out while the gel is on the frame. After destaining the gel assumes a solid consistency which makes it easy to remove and handle.

Results

Typical electropherograms obtained through the use of the above discussed migration chambers are presented in Fig. 2. In Fig. 2a patterns 1 and 2 represent K-casein prepared and purified by the method of ZITTLE AND CUSTER[4]. Pattern 3 represents a γ-rich casein fraction prepared by a technique previously reported[1]. Pattern 5 represents α_s casein prepared and purified by the method of ZITTLE AND CUSTER[4]. Pattern 6 represents whole casein prepared from pooled milk. Fig. 2b presents five samples of whole casein prepared from the milk of individual cows.

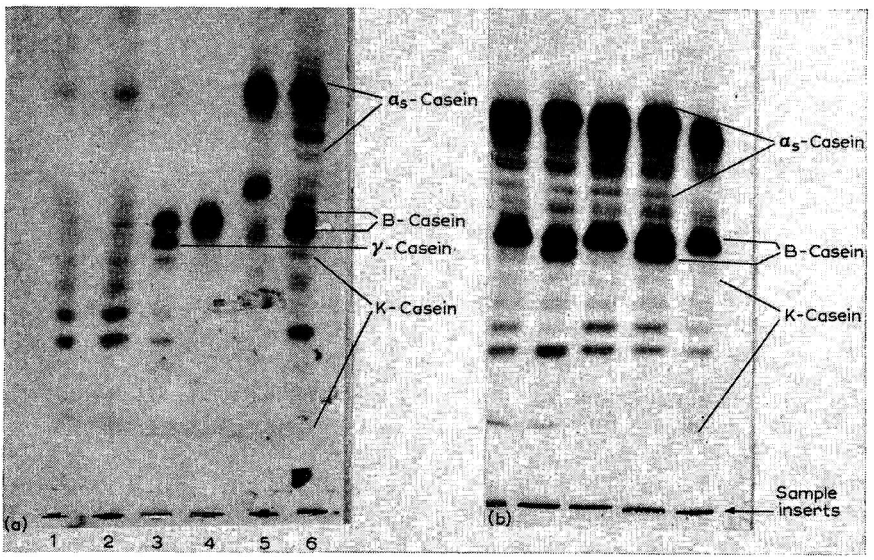


Fig. 2. Starch-gel electropherograms of casein and its components. (a) 1 and 2 = K-casein; 3 = γ-rich casein fraction; 4 = B-casein; 5 = α_s-casein and 6 = whole casein. (b) Five samples of whole casein from individual cows.

The genetic variants of α_s, B-, and K-caseins are clearly demonstrated in these patterns. The starch gel technique used for preparation of these electropherograms has been previously reported by the author[2].

Consistently reproducible starch gel electropherograms have been obtained in the author's laboratory using the above discussed migration chamber.

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1 A. M. EL-NEGOUMY, *J. Dairy Sci.*, 46 (1963) 768.
2 A. M. EL-NEGOUMY, *J. Dairy Sci.*, 58 (1965) 781 (Abst.).
3 S. RAYMOND, *Clin. Chem.*, 8 (1962) 455.
4 C. A. ZITTLE AND J. H. CUSTER, *J. Dairy Sci.*, 46 (1963) 1183.

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The influence of Tween 60 on the microelectrophoretic patterns of human serum on nitrocellulose and acetylcellulose membranes

In a preliminary note[1] on the microelectrophoresis of human serum on nitrocellulose membranes[2] it was shown that a pretreatment of the membranes with polyglycol sorbitol monostearate (Tween 60) was necessary for successful separations

of protein fractions. Various types of nitrocellulose membranes, differing by their pore sizes, have been tested[3] and microelectrophoresis on this supporting medium was used for the characterisation of modified bovine serum[4]. In comparison with electrophoresis on acetylcellulose strips[5], a smaller electro-endo-osmotic flow was observed on nitrocellulose impregnated with Tween 60[1,4] and also certain differences in the protein patterns were noticed. According to other orientation experiments[3], a part of the Tween 60 is firmly adsorbed on the nitrocellulose, most probably forming a film which covers the inner surface of the membrane pores; thus the interfering adsorption of proteins on nitrocellulose[1,2] was prevented. Another part of the Tween 60, however, seemed to be only mechanically occluded in the microporous structure of the membrane under the conditions used.

In the present paper, we wanted to reexamine these results in more detail and to test whether the contact of serum proteins with Tween 60 during electrophoresis would cause some changes in the electrophoretic patterns of certain fractions known to be sensitive to the detergent[6]. The results of the experiments on nitrocellulose were compared again to those obtained on acetylcellulose both untreated and treated with Tween 60.

Experimental

Material and methods. Nitrocellulose membrane filters "VUFS" (produced by VCHZ Synthesia, n.p., Uhříněves, Czechoslovakia)[2] and "Oxoid" electrophoresis strips (produced by Courtaulds, Ltd., Coventry)[5] were used as supporting media for electrophoresis. A veronal (25 m*M*)–citrate (2.5 m*M*)–oxalate (1 m*M*). buffer[7], pH 8.6, was used throughout these experiments. Human serum was taken from a sample stored at —20°.

5 × 1 cm strips were soaked before electrophoresis[1,5] either with the buffer alone or with a solution of 2 % Tween 60 in this buffer, for 5 min[1,3]. The strips were then rinsed on both sides as before[1,3,4], either with 5 ml of the detergent-free buffer, or thoroughly washed with 5–10 ml of the buffer on a Büchner funnel, to remove all excess of "unbound" detergent. Electrophoresis was performed without cooling in a moist chamber[1] with a bridge gap of 3.5 cm, using 0.4–0.5 mA/cm and 15–20 V/cm for 15 min. Sample volumes of the order of 10^{-4} ml were applied from the tip of a square wick of acetylcellulose soaked with sample and through which a perforation had been made by a pin (Fig. 1). The area between the tip and the perforation was blotted gently with a filter paper, while above the perforation a small droplet of the serum (stained with bromophenol blue) was left. This method of application gave the best and most reproducible results among various techniques tested even on acetylcellulose strips. After electrophoresis the strips were dried at 75–85° for 10 min and stained with nigrosine (Ed. Gurr, Ltd.)[1,5].

The adsorption of Tween 60 to the membranes was tested by ascending chromatography, using the electrophoretic buffer as solvent. About 10^{-3} ml of a 2% solution of the detergent was applied at the start as a streak close behind the advancing front of the solvent. Dragendorff's reagent was used to detect the detergent on the strips[3,8].

Results

Fig. 2 shows schematically the strong adsorption of Tween 60 on the "VUFS" nitrocellulose strip during ascending chromatography and the minimal adsorption

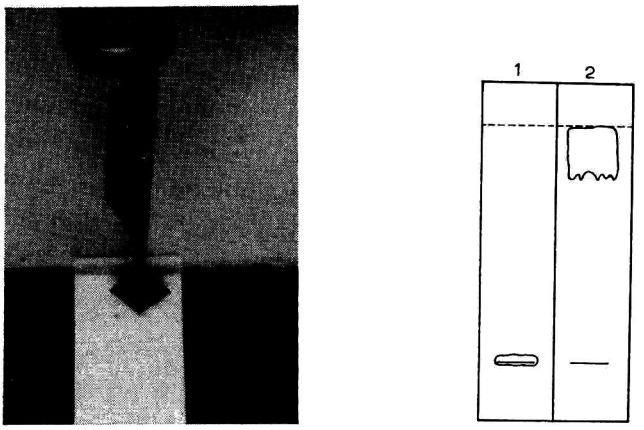

Fig. 1. Application of a sample on the start line. A wick of "Oxoid" membrane soaked with the sample was fitted to a steel pen.

Fig. 2. Ascending chromatography of Tween 60. 1 = nitrocellulose membrane; 2 = acetylcellulose membrane. Solvent: veronal–citrate–oxalate buffer, pH 8.6. Detection: Dragendorff's reagent[8].

of this detergent on the "Oxoid" acetylcellulose. Some strips were pretreated with Tween 60 in the usual way and then only slightly rinsed with buffer, and then the excess detergent was eluted until no more detergent could be detected in the eluted solution. However, even after this operation a strongly positive reaction with Dragendorff's reagent was obtained with nitrocellulose membranes, indicating the presence of firmly bound Tween 60.

Some changes of the electrophoretic patterns of human serum, caused by Tween 60, can be seen in Fig. 3. The "Oxoid" strip (No. 1) served as the "normal" standard pattern. After treatment of acetylcellulose with Tween 60 (strip No. 2), the prealbumin zone gained in mobility but lost in intensity. Furthermore, there was a gap

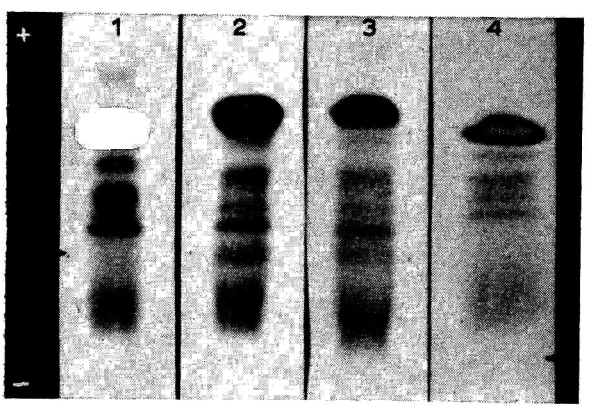

Fig. 3. Comparative microelectropherograms of human serum. 1 = acetylcellulose without detergent; 2 = acetylcellulose pretreated with Tween 60; 3 = nitrocellulose pretreated with Tween 60; 4 = nitrocellulose pretreated with Tween 60, excess of unbound detergent removed. 0.4–0.5 mA/cm, 15–20 V/cm; 15 min runs; nigrosine stain. The position of the start on strips No. 1 and 2 is indicated on the left, on strips No. 3 and 4 on the right.

in the α-globulin region and a new zone appeared in the region of the β-globulins. The γ-globulin also showed the presence of at least two zones. Similar changes were also observed on the nitrocellulose membranes. Thus strip No. 3 (with an excess of unbound detergent present) showed an electrophoretic pattern analogous to that on acetylcellulose strip No. 2. On strip No. 4 (with firmly bound detergent only), however, a "normal" pattern was achieved which could be compared to that on strip No. 1. A somewhat greater adsorption of proteins was observed sometimes on the "VUFS" strips treated in this way as well as a slight coloration of the background by nigrosine. The zones, however, were often more distinct than on acetylcellulose.

Discussion

The results mentioned above led to the conclusion that changes of the "normal" electrophoretic pattern of human serum were most probably caused by the presence of unbound Tween 60 in the membranes. Similar changes have been described[6] with sera incubated with detergents. These artefacts, however, consisting in a changed mobility of certain fractions, might be expected to have a special analytical value, for instance when analysing sera of patients suffering from different diseases.

Nevertheless, it was interesting to find that Tween 60, when firmly bound to the supporting medium, did not cause the changes mentioned above. This result seemed to confirm a previous assumption[3] that the electrophoretic separation of proteins took place in a buffer solution which was supported by the microporous framework of nitrocellulose, coated thoroughly by a film of Tween 60. In this connection it would be possible to speak about an electrophoresis on Tween 60 rather than on nitrocellulose. Further experiments involving quantitative evaluations will be necessary, however, before an adequate interpretation can be given to these findings.

The results presented here reaffirmed our previous observations of a minimal electro-endo-osmotic flow on nitrocellulose as compared to that on acetylcellulose (*cf.* Fig. 3).

It seemed also reasonable to assume that other impregnating substances could be bound to the nitrocellulose membranes instead of Tween 60, to form supporting media with appropriate properties and special binding capacities. Experiments concerning these questions are now under study in our laboratory.

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1 T. I. PŘISTOUPIL, *Biochim. Biophys. Acta*, 117 (1966) 475.
2 ULTRAFILTERS UFS, VCHZ Synthesia, n.p. Uhříněves, 1962.
3 T. I. PŘISTOUPIL, in preparation.
4 T. I. PŘISTOUPIL, *Clin. Chim. Acta*, in press.
5 J. KOHN, *Ärztl. Lab.*, 10 (1964) 233.
6 S. GRUNDY, H. L. DOBSON AND A. C. GRIFFIN, *Proc. Soc. Exptl. Biol. Med.*, 98 (1958) 313.
7 J. PECHAR, *Sb. Pathofysiol. Trav. Výž.*, 8 (1954) 165.
8 R. KLIFFMÜLLER, *Z. Anal. Chem.*, 164 (1958) 462.

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Kombination von Hochspannungselektrophorese und Rundfilterchromatographie

Für komplexe Gemische gestattet die von KICKHÖFEN UND WESTPHAL[1] beschriebene Methode der Kombination von Hochspannungselektrophorese und Papierchromatographie, die später durch INGRAM's[2] "finger prints" besonders bekannt wurde, nicht immer eine sichere Trennung von Aminosäuren und Peptiden. Unter gewissen Umständen wird keine übersichtliche Abgrenzung der Komponenten erreicht, insbesondere ist dies nach der chromatographischen Trennung zu beobachten. Eine unzulängliche Trennung der Banden wird entweder durch Diffusionserscheinungen bei sehr langer Versuchsdauer bedingt, oder aber die Substanzen besitzen in dem verwendeten Fliessmittel sehr ähnliche R_F-Werte. Zu ungenügender Abgrenzung der Flecken kann es auch kommen, wenn Fraktionen geringerer Konzentration in unmittelbarer Nachbarschaft von solchen mit hoher Konzentration liegen. Durch derartige Probleme wurde die Untersuchung von Fleischextrakten und tryptisch gespaltenem Protein erschwert, die der eine von uns (K.) zur Herstellung bakterieller Nährböden gewonnen hatte.

Durch den Einsatz der Rundfilterchromatographie anstelle der auf- oder absteigenden Chromatographie konnten wir jedoch auch in diesen Fällen auf Grund der radial wachsenden Entwicklungsfläche eine scharfe Bandenbildung erzielen. Diese Trennung lässt sich in bekannter Weise durch zweimaligen Lauf des Fliessmittels verbessern[3].

Die Rundchromatographie erlaubt wegen der radialen Entwicklung und der dadurch bedingten Ausbreitung der Banden in Kreissektoren normalerweise keine Kombination mit einem zweiten Trennverfahren. Wenn aber durch die Hochspannungselektrophorese in der ersten Laufrichtung eine wenigstens teilweise Auftrennung erfolgt ist, so lassen sich die guten Trenneffekte der Radiärchromatographie zur weiteren Differenzierung schwieriger Stoffgemische ausnutzen.

Zur Trennung eines Gemisches von Peptiden und Aminosäuren mit sauren, basischen und neutralen Komponenten wurde die zu untersuchende Substanz als 5 mm breiter Startstrich in einem Abstand von 10 mm von der Mitte eines Papierbogens (400 × 300 mm) für die Hochspannungselektrophorese aufgetragen und parallel dazu ein Gemisch von Vergleichssubstanzen.

Die Hochspannungselektrophorese erfolgte bei pH 6.0 [Pufferlösung: Pyridin–Eisessig–Wasser (100:10:890, v/v)], 50 V/cm, etwa 2 mA/cm, Temperatur in der Kühlkammer zwischen −1 und +2°, Versuchsdauer 40 Min. Als geeignete Papiersorte erwies sich das FN 4-Papier des VEB Spezialpapierfabrik Niederschlag/Erzgeb. Nach Beendigung des Versuches werden die Elektropherogramme getrocknet. In der Mitte zwischen den beiden Startlinien markiert man das Zentrum der Radialentwicklung (vgl. Fig. 1).

Als Fliessmittel für die Chromatographie diente ein Gemisch aus Butanol–Eisessig–Wasser (4:1:1, v/v). Die Chromatographie erforderte im Durchlaufverfahren etwa 12 St. Die weitere Behandlung und Anfärbung des Chromatogramms erfolgte mit den bekannten Methoden.

Auf diesem Wege ist die einwandfreie Trennung der sauren, basischen und fast aller neutralen Aminosäuren möglich. Zur intensiveren Auftrennung der Gruppe

von neutralen Aminosäuren und Peptiden wird diese Fraktion nach der Hochspannungselektrophorese isoliert und durch die "strip transfer method"[4] erneut aufgetragen. Zur Elektrophorese verwendet man nun eine Pufferlösung vom pH 1.1 [Ameisensäure–Eisessig–Wasser (15:10:75, v/v)]. Da die Substanzen in dieser Pufferlösung ausschliesslich kathodisch wandern, wird der Startpunkt 100 mm von der Anodenseite gewählt. Zur Festlegung des Zentrums für die Radialentwicklung, das etwa in

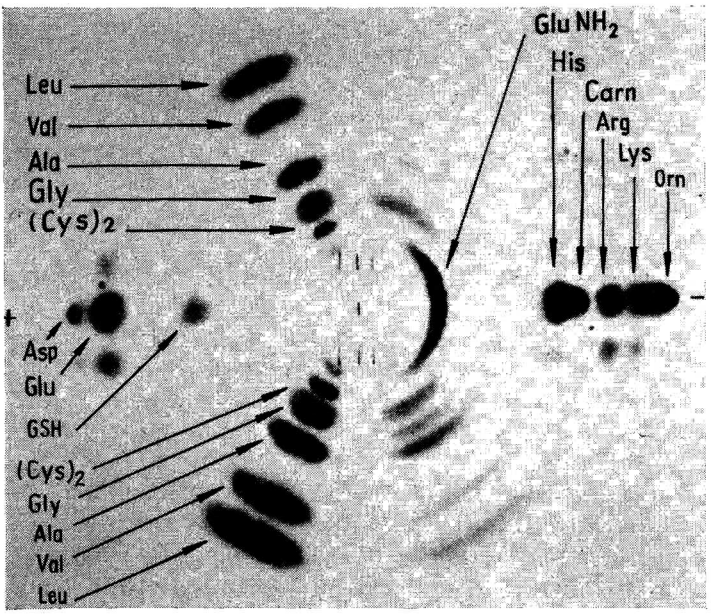

Fig. 1. Aminosäurefraktionierung in zwei Peptid-Totalhydrolysaten. Start zur hochspannungselektrophoretischen Trennung des Peptid-Totalhydrolysates in der oberen und unteren Laufreihe auf dem mittleren Startstrich (in der Mitte der Figur). Mittlere Reihe: Trennung des Modellgemisches saurer und basischer Aminosäuren.

der Mitte der getrennten Substanzen liegen soll, lässt man bei der Elektrophorese an der äusseren Kante des Bogens ein Vergleichsgemisch von Aminosäuren laufen.

Zur Auswertung dieser zweidimensionalen Chromatogramme, von uns als "radial finger prints" bezeichnet, wird der Mittelpunkt der entstandenen Kreissegmente festgelegt. Durch Projektion auf die Ausgangsposition erhält man die ideale Lage der einzelnen Komponenten. Diese "Spektren" sind für Vergleichsmessungen mit Modellgemischen besonders geeignet, da beide Substanzgemische unter streng vergleichbaren Bedingungen getrennt werden. Wie Fig. 1 zeigt, sind die sauren und basischen von den neutralen Aminosäuren in eindeutiger Weise getrennt.

Die Methode ermöglicht aber auch eine erfolgreiche Trennung innerhalb der Gruppe der neutralen Substanzen (Fig. 2). Da die sauren und basischen Aminosäuren gegenüber der neutralen Gruppe durch die Elektrophorese sehr viel weiter vom Startpunkt entfernt werden, erreicht sie bei der radialen Entwicklung das Fliessmittel sehr spät, so dass sie fast keine Veränderung ihrer Lage mehr zeigen, während die neutralen Aminosäuren, mit Ausnahme des Paares Glycin–Serin, getrennt werden.

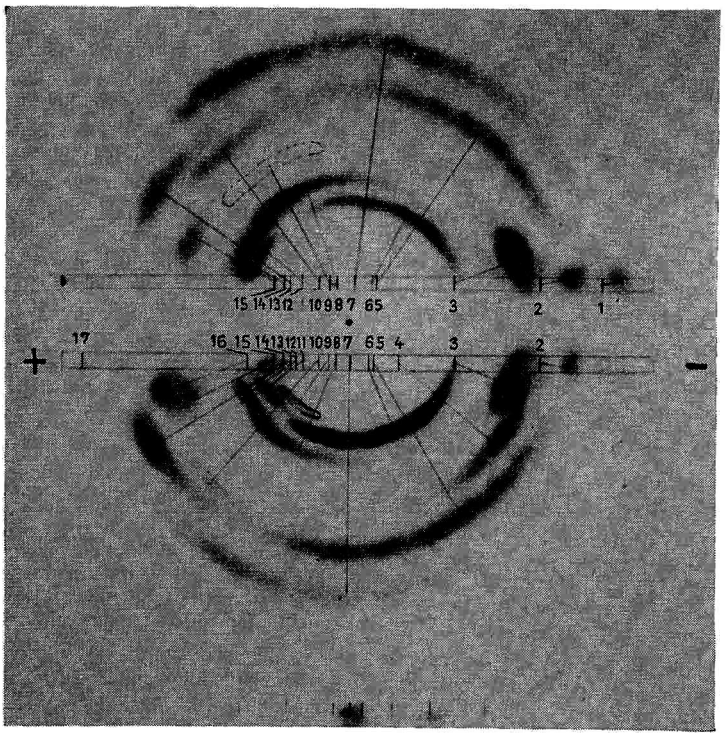

Fig. 2. Fraktionierung der neutralen Aminosäuren in einem Fleischextrakt (bakterieller Nährboden). Oberes Spektrum: Aminosäure-Vergleichsgemisch. Unteres Spektrum: Fleischextrakt. Start (HE): Kathodisch, am Ende der Doppellinie. Start (PC): Im eingezeichneten Punkt (Blattmitte). 1 = β-Ala; 2 = Gly; 3 = Ala; 4 = Peptid; 5 = Val; 6 = Ser; 7 = Leu; 8 = Thr; 9 = Pro (im Vergleichsgemisch) und Peptid (im Fleischextrakt); 10 = Met; 11 = Peptid; 12 = $GluNH_2$; 13 = Phe; 14 = $(Cys)_2$; 15 = Tyr; 16 = Try; 17 = Tau.

Die Leistungsfähigkeit des Verfahrens konnten wir bei der Trennung von ^{35}S-markierten Cysteinderivaten erneut erproben[5].

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1 B. KICKHÖFEN UND O. WESTPHAL, *Z. Naturforsch.*, 7b (1952) 655.
2 V. M. INGRAM, *Nature*, 178 (1956) 792.
3 J. HEILMANN, J. BARROLLIER UND E. WATZKE, *Z. Physiol. Chem.*, 309 (1957) 219.
4. E. L. DURRUM, *J. Colloid Sci.*, 6 (1951) 274.
5 Nicht publizierte Untersuchungen mit D. DÖRR, *Diplomarbeit*, Technische Universität Dresden, 1965.

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* Direktor: Prof. Dr. habil. U. FREIMUTH.

Trennung der Lanthanide durch Dünnschichthochspannungselektrophorese

Im Rahmen von Untersuchungen zur schnellen Trennung der Lanthanide wurden gute Ergebnisse mit der Hochspannungspapierelektrophorese erzielt[1]. Ziel der vorliegenden Arbeit war es, die Trennung der Lanthanide durch Dünnschichthochspannungselektrophorese zu versuchen. Im einzelnen interessierten folgende Fragen:

(1) Lassen sich die Versuchsbedingungen der Papierelektrophorese auf die Dünnschichtelektrophorese übertragen?

(2) Ergibt die Dünnschichtelektrophorese gegenüber der Papierelektrophorese eine Verbesserung des Trenneffektes (gemessen durch das Verhältnis der Laufstrecken)? (Da die Dünnschichtchromatographie der Papierchromatographie grundsätzlich überlegen ist, sollte das der Fall sein).

(3) Wie verhalten sich die Trennzeiten bei der Papierelektrophorese und bei der Dünnschichtelektrophorese?

(4) Ist es möglich, durch Dünnschichtelektrophorese grössere Mengen Substanz zu trennen?

Bisher sind nur wenige Trennungen von Kationen durch Dünnschichtelektrophorese durchgeführt worden[2,3], so dass noch keine umfangreichen experimentellen Erfahrungen vorliegen.

Experimente

Zur Herstellung der Dünnschichten wurden Glassplatten (20 × 50 cm) in der bei STAHL[4] beschriebenen Weise beschichtet. Schichtdicken von 0.5 mm ergaben die besten Trennungen. Bei dickeren Schichten ist die Wärmeabführung im Falle einer Hochspannungselektrophorese ungenügend, so dass es zur Ausbildung eines Temperaturgradienten innerhalb der Schicht kommt. Es wurden Schichten aus Cellulose, acetylierter Cellulose, Aluminiumoxid, Kieselgel sowie einem Gemisch von 30% Hostaflon und 70% Cellulose verwendet (dieses Gemisch konnte nicht mit Wasser angerührt werden; es war notwendig etwa 30% Aceton zuzugeben). Die Platten wurden getrocknet und in Streifen von etwa 4 cm Breite aufgeteilt. Um die Dünnschicht mit dem Elektrolyten für die Elektrophorese anzufeuchten, wurden verschiedene Methoden angewandt:

(a) Die Substanz wurde mit dem Elektrolyten angerührt.

(b) Die Substanz wurde mit Wasser angerührt, getrocknet und mit dem Elektrolyten besprüht.

(c) Die Substanz wurde mit Wasser angerührt, getrocknet und der Elektrolyt anschliessend eingesaugt.

Mit der letzten Methode wurden die besten Trennungen erzielt. Lässt man den Elektrolyten in einer Kammer wie bei einer Dünnschichtchromatographie über die Länge von 50 cm hochsaugen, dann erfordert dies sehr lange Zeit; ausserdem entsteht ein Konzentrationsgradient. Deshalb wurde die in Fig. 1 gezeigte Anordnung verwendet. Die Küvetten und die beiden Platten bildeten ein abgeschlossenes System. Die beiden Lösungsmittelfronten benötigten etwa 3 h, bis sie sich in der Mitte trafen. Das Einsaugen des Elektrolyten wurde erst nach weiteren 2 h unterbrochen, um einen vollständigen Konzentrationsausgleich zu ermöglichen. Als

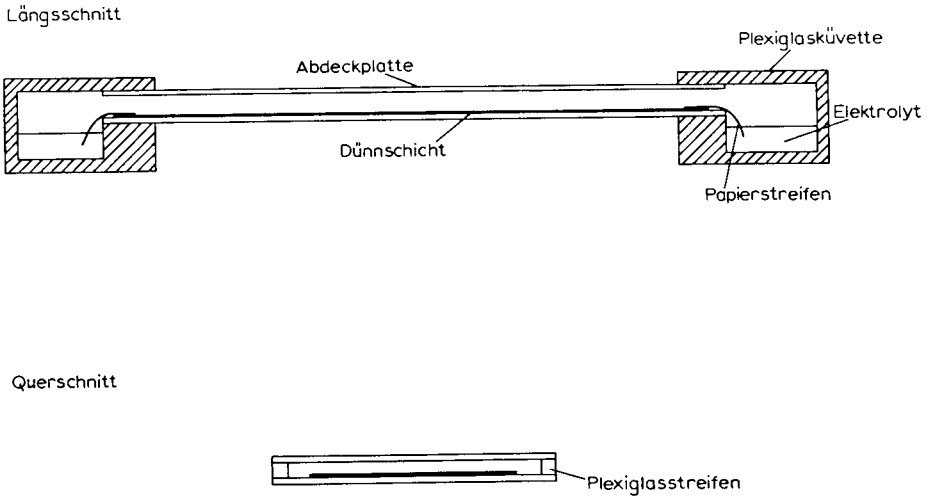

Fig. 1. Anordnung zum Einsaugen des Elektrolyten in die Dünnschicht.

Elektrolyt wurde Weinsäure und α-Hydroxyisobuttersäure (α-HIBA) in verschiedenen Konzentrationen und mit verschiedenen pH-Werten verwendet.

Als Beispiel für die Trennung der Lanthanide wurde die Trennung von Cer, Promethium und Europium untersucht. Es wurden jeweils 5 μl einer Lösung aufgebracht, die ^{144}Ce, ^{147}Pm und ^{152}Eu bzw. ^{154}Eu sowie wechselnde Mengen von inaktivem Cer und Europium als Träger enthielt. Obwohl auf die feuchte Schicht aufgetragen wurde, breitete sich die Substanz nur wenig aus. Für die Elektrophorese wurde der Elektrolyt mit Agar-Agar angedickt und in der schon an anderer Stelle[1] beschriebenen Weise zugeführt.

Die Elektrophorese wurde mit einer Feldstärke von etwa 60 V/cm durchgeführt. Die Kühlung erfolgte mit einer Kühlsole von —10°. Anschliessend wurden die Platten an der Luft getrocknet. Trocknet man bei höherer Temperatur, dann werden die Substanzzonen breiter und es treten Verschiebungen bis zu 2 cm auf. Diesen Effekt haben auch CRIDDLE *et al.*[5] gefunden. Trocknet man bei Zimmertemperatur, so findet nur eine Wanderung von etwa 2 mm statt. Die Auswertung der Dünnschichten erfolgte durch Messung der Aktivität mit einem Methandurchflusszähler.

Ergebnisse

Bei den einzelnen Trennungen wurden die Laufzeit, der Trenneffekt und die Ausbreitung der Substanzzonen gemessen. Fig. 2 zeigt eine Trennung, die unter optimalen Bedingungen ausgeführt wurde. Die in der Einleitung gestellten Fragen können folgendermassen beantwortet werden:

Zu (1) Die Versuchsbedingungen für eine Papierelektrophorese lassen sich auf die Dünnschichtelektrophorese übertragen. Im Gegensatz zur Papierelektrophorese, bei der 0.7 *M*-α-HIBA (pH-Wert 2.2) die besten Trennungen lieferte, ergaben sich für die Dünnschichtelektrophorese als optimale Bedingungen: 1.1 *M*-α-HIBA, pH-Wert 2.4. Von den verwendeten Dünnschichtsubstanzen wurden mit Cellulose die besten Ergebnisse erzielt (vgl. Fig. 2).

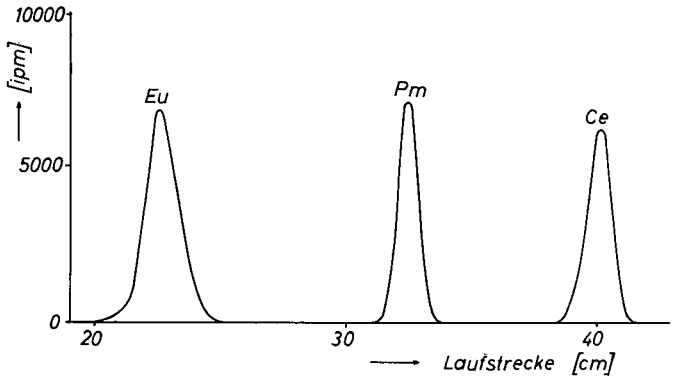

Fig. 2. Trennung von Ce, Pm und Eu (Ce und Eu je 100 g) mit 1.1 *M*-α-HIBA, pH-Wert 2.4, Versuchsdauer 2 h.

Zu (2) Der Trenneffekt ist bei der Dünnschichtelektrophorese etwas besser als bei der Papierelektrophorese. Die verhältnismässig grosse Breite der Substanzzonen ist wahrscheinlich auf die erhöhte Diffusion in der Dünnschicht zurückzuführen.

Zu (3) Die Trennzeiten für Dünnschicht- und Papierelektrophorese sind etwa gleich.

Zu (4) Mit der Dünnschichtelektrophorese lassen sich grössere Substanzmengen trennen als mit der Papierelektrophorese. Während bei der Papierelektrophorese Trennungen von Substanzmengen in der Grössenordnung von 100 μg pro Komponente eine starke Verbreiterung der Substanzzonen bewirken, ist bei der Dünnschichtelektrophorese ein Einfluss der zugesetzten Substanzmengen nicht erkennbar. So ist in Fig. 2 der Unterschied zwischen Cer, Europium und Promethium nicht sehr gross, obwohl sich die Konzentrationen des Pm und des Eu bzw. Ce wie $1:10^5$ verhalten. Die Substanzmenge hat aber einen Einfluss auf die Laufstrecke, wie aus der Lage des Pm in Fig. 2 hervorgeht.

Herrn Prof. Dr. K. H. LIESER danken wir für wertvolle Diskussionen und die Unterstützung unserer Arbeit.

Lehrstuhl für Kernchemie, Eduard-Zintl-Institut, K. BÄCHMANN
Technische Hochschule Darmstadt (Deutschland) H. GÖRISCH

1 K. BÄCHMANN, *Radiochim. Acta*, 4 (1965) 124.
2 F. DOBICI UND G. GRASSINI, *J. Chromatog.*, 10 (1963) 98.
3 A. MOGHISSI, *Anal. Chim. Acta*, 30 (1964) 91.
4 E. STAHL, *Dünnschicht-Chromatographie*, Springer-Verlag, Berlin, 1962.
5 W. J. CRIDDLE, G. J. MOODY UND J. D. R. THOMAS, *J. Chromatog.*, 18 (1965) 530.

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Book Reviews

Official Methods of Analysis of the Association of Official Agricultural Chemists, tenth edition, published by the Association of Official Agricultural Chemists, Washington, 1965, 957 pp., price $ 22.50.

The tenth edition contains about 35 chromatographic methods of which nine use paper chromatography, three gas chromatography and the rest column chromatography. There is also one starch gel electrophoretic separation of proteins.

As these methods have an official character, it is interesting to note the manner in which they are specified. Paper chromatographic techniques are always based on direct comparison with standard substances on the same sheet of paper. R_F values are not given. As the paper, "Whatman No. 1 or equivalent" is recommended without specification of the direction or the temperature of development. These and other fundamental principles in using paper chromatography should be considered by analysts especially in forensic work.

The "Official Methods" can be recommended for reference when working out new chromatographic methods for analytical work.

MICHAEL LEDERER (Rome)

J. Chromatog., 23 (1966) 339

Cholesterol, Bile Acids and Atherosclerosis, by A. VAN BELLE, North-Holland Publishing Company, Amsterdam, 1965, price Dfl. 18.—.

The author quotes BOYD on the importance of lowering the plasma cholesterol concentration in connection with atherosclerosis, and one must agree that from this viewpoint a profound knowledge of bile acid formation, absorption and excretion is very necessary indeed. VAN BELLE offers a very thorough review of bile acid chemistry (62 pages) as well as metabolism (34 pages), based on a comprehensive survey of world literature up to 1964. The steroid chemist would have liked a description of the chemical path to the pregnane and androstane derivatives (degradation of the side-chain) as well as the chemistry of the nor- and bisnor-bile acids, but this may be taken up in the next edition, together with some more information about the chemical variation in the side-chain (for instance cholanols), though it is realized that these aspects have no connection with the main theme, namely cholesterol and atherosclerosis.

The chapter dedicated to the "factors affecting bile acid synthesis and metabolism" is a real pleasure for everybody engaged in the study of atherosclerosis, including the clinician. All modern viewpoints and therapeutic (as well as experimental) trials and avenues for lowering blood cholesterol are discussed. The reader gets a

concise but critical picture of the whole problem. According to VAN BELLE, as stressed in his concluding remarks, cholesterol levels may be lowered "by enhancing the catabolism (of cholesterol) to bile acids and increasing the fecal elimination of these compounds". He outlines the rationale of the pathways through which this could be achieved, but unfortunately cannot offer the solution, *i.e.* the ideal drug.

Cholesterol, Bile Acids and Atherosclerosis is a welcome addition to this inexhaustible and so very complicated subject.

G. HECHT-LUCARI (Darmstadt)

J. Chromatog., 23 (1966) 339–340

Dünnschicht-Chromatographie, von K. RANDERATH, zweite, neubearbeitete und erweiterte Auflage, Verlag Chemie, Weinheim, 1965, XV + 291 Seiten, 96 Abbildungen, Ganzleinen DM 28,—.

Die von STAHL zu einer Standardmethode entwickelte Dünnschichtchromatographie gehört bereits zu den meistgebrauchten Laboratoriumsmethoden. Obschon die Anzahl einschlägiger Arbeiten exponentionell zunimmt — es dürften heute über 3000 Arbeiten erschienen sein — ist zu begrüssen, dass RANDERATH in der zweiten Auflage seines bekannten und beliebten Buches versucht die *gesammte* Dünnschichtchromatographie darzustellen. Ein solches Buch ist zweifelsohne nötig und wertvoll — als erste Einführung. Die Aufgabe, nämlich die Erfassung des gesammten vorliegenden Erfahrungsmaterials, ist freilich im Rahmen eines Buches unmöglich. RANDERARTH's Buch wird daher desto wertvoller sein, je mehr ihm gelungen ist mit der Entwicklung der Methode Schritt zu halten.

Im *allgemeinen Teil* des Buches werden theoretische Grundlagen und die *Technik* der Dünnschichtchromatographie dargestellt. Leider werden dabei theoretisch wichtige Zusammenhänge, wie zum Beispiel die Martin-Beziehung in der Dünnschichtchromatographie, überhaupt nicht erwähnt. Eine Behandlung der Ionenaustauscherchromatographie sollte auch an dieser Stelle zu finden sein. Demgegenüber ist die Darstellung der allgemeinen Technik vorzüglich: auch die neuesten Entwicklungen wurden berücksichtigt. Man wird deshalb den *allgemeinen Teil* des Buches in der Praxis häufig anwenden.

Der spezielle Teil enthält Anwendungsbeispiele zur Dünnschichtchromatographie von *Alkaloiden, Aminen, Aminosäuren und Aminosäurederivaten, Steroiden, Lipiden, Vitaminen, Arzneimitteln, Phenolen, Purinen und Pyrimidinen, Kohlehydraten, natürlichen und synthetischen Farbstoffen* und von *anorganischen Ionen*. Es würde den Rahmen dieser Besprechung sprengen, wenn man alle *wichtige Arbeiten* nennen wollte, welche der Autor unberücksichtigt liess. In manchen Fällen, z.B. Amine und Aminosäuren, ist die Auswahl der Beispiele nicht besonders glücklich. Trotzdem ist der *spezielle Teil* sehr wertvoll, weil der Leser, vor allem der Anfänger, die Möglichkeiten der Methode erkennen kann, und durch zusätzliche Angaben einen Weg zur Literatur findet.

Dieses vorzüglich ausgestattete und preiswerte Buch ist jedem zu empfehlen, der Chromatographie in der Praxis gebraucht.

GYÖRGY PATAKI (Basel)

News

MEETING ON THE GAS CHROMATOGRAPHIC DETERMINATION OF HORMONAL STEROIDS

Rome, 22nd–23rd September, 1966

The Second International Endocrinological Symposium will be held in Rome, Accademia Nazionale dei Lincei, 22nd–23rd September, 1966.

Chairmen of this Meeting are: Prof. L. CALIFANO, President of the Biological Committee, and Prof. M. LEDERER, Director of the Chromatographic Laboratory of the Italian National Research Council, Rome.

The Secretaries are: Drs. F. POLVANI and M. SURACE, Centro di Endocrinologia, C.N.R., Milan, and Dr. M. LUISI, Istituto Patologica Mediga dell'Università, Pisa.

The Meeting will consist of a series of 12 plenary lectures and 3 Round Tables. The Meeting will have three official languages: Italian, English, and French; simultaneous translation is planned.

For registration of short communications please write to The Secretariat: Accademia Nazionale dei Lincei, via della Lungara 230, Rome, Italy.

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TECHNICON EUROPEAN SYMPOSIUM 1966

We have already announced that we are holding a European Symposium on Automation in Analytical Chemistry, in Paris on November 2nd, 3rd and 4th, 1966.

Last year 235 papers were presented at Technicon Symposia in Europe and America by scientific workers from many parts of the world.

They indicate the nature and scope of our Symposia which embrace automated procedures and techniques in clinical chemistry, haematology, immunology, enzymology, chromatography, pollution detection, agricultural and a wide field of industrial chemistry.

This year we hope the areas covered will be even greater.

The purpose of this letter is to invite you to present a paper at our 1966 European Symposium on any original work you have done or are doing.

Abstracts will be placed before the Scientific Committee and it will also be helpful if you can forward a flow diagram and any other information which will assist the Scientific Committee to evaluate the work.

We hope to have the final programme established soon and shall appreciate it very much if you will let us know as soon as possible if you wish to submit a paper.

Finally we can tell you that we propose to publish the Proceedings of the Symposium in book form, but presentation of your paper at our Symposium does not preclude publication in any other Journal.

TECHNICON INSTRUMENTS COMPANY LTD.

J. Chromatog., 23 (1966) 341

New Chemicals

A gas chromatographic support material for use in both qualitative and quantitative analysis of highly polar compounds has been announced by Fluorocarbon Co., Anaheim, Calif., U.S.A. The material Fluoropak 80 combines ease of coating and packing with substantial reduction in tailing for such materials as ammonia, water, and hydrogen chloride. Fluoropak 80 is inert to most chemicals and may be used with bromine and hydrogen sulphide.

Rohm and Haas of Philadelphia have announced the availability of regeneratable, non-ionic cross-linked copolymers of styrene and divinylbenzene suitable for separating water-soluble organic compounds in column or batch operations. First of a new series, XAD-1 and XAD-2 have uniform pore size, uniform pore-size distribution and high internal surface area. Due to the selective nature of the adsorption, these resins offer a variety of uses. They may be employed up to 150°C, resist physical wear and tear, and do not swell or contract appreciably during usage. Since these resins contain no functional groups, the mechanism of separation is based mainly on hydrophobicity. But since the pore size is fairly uniform the resins may also be used to separate compounds on basis of molecular weight.

Waters Associates, Inc., of Framingham, Mass., U.S.A., announce the introduction of Porapak, which is a form of porous polymer bead which may be used in gas chromatography. These beads which were developed by Dr. O. L. HOLLIS of the Dow Chemical Company, Freeport, Texas, have a distinct number of advantages over the more commonly used packing materials used in gas chromatography. The most noteworthy properties are described by HOLLIS in *Anal. Chem.*, 38 (1966) 309, being the paper he presented at the Houston International Gas Chromatography Symposium, October 1965. The advantages claimed for Porapak are: (a) No bleeding from the packing material; (b) no adsorption of polar compounds; (c) since there is no stationary phase in the accepted sense of the word, there is no change in retention time (due to bleeding from the column); (d) stable to 250°C; (e) easy column packing with high efficiency, the polymer beads are rigid structures and are very easy to handle; (f) rapid overload recovery; (g) separates water from organic materials; (h) beads may also be coated in the usual way and have a large surface area.

Polyscience Corporation of 909 Pitner Avenue, Evanston, Ill., U.S.A., announce the availability of Polyscience kits which are kits of the purest chemicals available for identification purposes in gas chromatography. A large variety may be ordered including alcohols, hydrocarbons, amines, ketones. It is intended that these kits be used for calibration, identification and standardisation of instruments such as gas chromatographs, spectrophotometers, thin-film and paper chromatography. Kits containing chlorinated insecticides, phosphate insecticides, herbicides, fungicides and rodenticides are also available.

Bio-Rad Laboratories, 32nd & Griffin Avenue, Richmond, Calif., U.S.A. have released their Price-List R for Winter–Spring January 1st, 1966, concerning materials for ion-exchange, adsorption, and gel-filtration.

Manufacturers Literature

Previews and Reviews for Gas Chromatography, October 1965

The Aerograph Moduline research gas chromatographs are featured along with the ovens and ionization detectors produced by this company. Wilkens Instrument and Research Inc., 2700, Mitchell Drive, Walnut Creek, Calif., U.S.A.

The Programmer, Vol. 1, October 1965

This entire issue is devoted to a discussion of the Series 810 research gas chromatographs. F. & M., Scientific Division, Hewlett-Packard Corporation, Route 41, Avondale, Pa., U.S.A.

Column, Vol. 1, No. 1, Autumn 1965

This is the first volume of a new periodical. It describes the Series F104 chromatograph and accessories. W.G. Pye and Co., Ltd., Cambridge, Great Britain.

The 3rd Gas Chromatography Symposium, October 27th, 1965

This symposium was organised by Wilkens Instrument and Research AG at the International Congrescentrum RAI, Europaplein 8, Amsterdam. The following lectures were given:

P. TRINLER, General Manager, Aerograph, Holland N.V., welcome address.
Ir. C. A. M. G. CRAMERS, Technological University, Eindhoven, The Netherlands. Injection techniques.
Dr. H. M. MCNAIR, Wilkens Instrument & Research Inc., Walnut Creek, Calif., U.S.A. Quantitative analysis of hydrocarbons with automatic electronic integration.
Dr. R. P. W. SCOTT, Unilever Research Laboratories, Sharnbrook, Great Britain. Factors that effect the efficient trapping of GLC column eluent.
Dr. H. STRICKLER, Firmenich Research Laboratories, Geneva, Switzerland. Qualitative identification in GLC.
Prof. Dr. W. SIMON, Swiss Federal Institute of Technology, Zürich, Switzerland. Structural elucidation with a thermal fragmentation–gas chromatography–mass spectrometry combination.
Dr. H. M. MCNAIR, Wilkens Instrument & Research Inc., Walnut Creek, Calif., U.S.A. Résumé of the Third International Symposium on Advances in GLC at Houston.
General discussion and end.

Reprints of certain of the articles are available on request from Wilkens Instrument & Research AG, Pelikanweg 2, P.O. Box 734, 4002 Basel, Switzerland.

L.K.B. instrument journal "Sciences Tools", August 1965

This issue announces and describes the L.K.B. 9000 gas chromatograph–mass spectrometer. In most cases organic compounds which are separated by gas chromatography, require subsequent analysis to determine the structure of the organic compounds so separated. In this connection, the mass spectrometer is a universal detector in both qualitative and quantitative analysis leading to structural analysis in most cases. L.K.B. developed this instrument from the research carried out by RAGNAR RYHAGE, Karolinska Institutet, Stockholm, Sweden, on the use of a mass spectrometer as detector and analyzer for effluents from gas–liquid chromatographs.

The concentration of the components in the gas stream from GLC columns is usually very low–hence direct injection into the ion source of the mass spectrometer

(via the normal pressure-reducing capillary leak inlet system) produces low sensitivities. By using helium as carrier gas followed by a molecular separator (based on the principles announced by E. BECKER, Kernforschungszentrum, Karlsruhe, W. Germany), RYHAGE was able to selectively remove helium from the gas stream and obtain an increase in concentration of about fifty times at least. This separator made it possible to connect the GLC column directly to the ion source. By careful design, the zone broadening has been kept to almost negligible levels.

The L.K.B. 9000 is the first commercial instrument to integrate a gas chromatograph, a molecular separator, and a mass spectrometer. The GLC spectrum is produced on a monitor recorder through continuous recording of the total ion current in the spectrometer. The mass spectrometer can be used at any time during the separation to identify the separated substances, the spectra are recorded at three sensitivities on a multi-channel U.V. recording oscillograph, the operation of which is automatically synchronized to the automatic scan device. The scan which is magnetic type may be set to any preset values using limit switches. The scan time varies from 1–960 seconds in 9 steps. For further details contact L.K.B.-Produkter AB, P.O. Box 76, Stockholm-Bromma 1, Sweden.

Facts and Methods, Vol. 6, No. 6, 1965

In this issue gas chromatography at work is described. The subjects treated include separations of xylidine isomers, cresol isomers (as trimethylsilyl derivatives), plasticizer analysis, production control of dyestuffs, and head-space gas analysis.

Instrument News, Vol. 16, No.2 , 1965

From Perkin-Elmer Corp., Norwalk, Conn., U.S.A. This issue includes a description of their Model 880 Gas Chromatograph. Two intriguing papers are given describing the elucidation of the organic materials responsible for the atmospheric blue haze seen above mountain forest during certain periods of the year, while the other outlines the solution of difficult biomedical analyses by gas chromatography.

Bibliography Section

Paper Chromatography

1. REVIEWS AND BOOKS

FOMIN, A. A.: (Possibility of using paper chromatography in studying food). *Gigiena i Sanit.*, 30, No. 3 (1965) 91–96; *C.A.*, 62 (1965) 16873c.

5. HYDROCARBONS AND HALOGEN DERIVATIVES

ZONOVA, Z. T., KNYAZEVA, M. S., GRIGOR'EV, S. M. AND MAIOROV, N. S.: (Application of paper and thin-layer chromatography to the control of the degree of purity of certain polycyclic hydrocarbons purified by zone refining). *Khim. Pererabotka Smol, Akad. Nauk SSSR, Inst. Goryuch. Iskop.*, (1965) 95–102; *C.A.*, 63 (1965) 9714a — PC and TLC.

6. ALCOHOLS

DATTA, D. D. AND GHOSH, D.: Identification of glycerol in complex pharmaceutical preparations by paper chromatography. *Indian J. Pharm.*, 27 (1965) 265; *C.A.*, 63 (1965) 16129f.

7. PHENOLS

NIKIFOROVA, I. S. AND MEL'KANOVITSKAYA, S. G.: (Allylation of phenols and their ethers. Chromatographic study of the products of allylation of phenols and their ethers on a thin layer of loose alumina). *Uzbeksk. Khim. Zh.*, 9, No. 4 (1965) 23–27; *C.A.*, 63 (1965) 16242c — R_F values for 68 compounds.

8. SUBSTANCES CONTAINING HETEROCYCLIC OXYGEN

JAIN, A. C., MATHUR, S. K. AND SESHADRI, T. R.: Synthesis of 4′,5,6,8-tetramethoxyflavone and simpler flavones derived from *o*-hydroxyacetophenone. *Indian J. Chem.*, 3 (1965) 351–353; *C.A.*, 63 (1965) 18015c.

KRUG, H. AND OLECHNOWICZ-STEPIEN, W.: (Flavonoid compounds of *Ledum palustre*). *Dissertationes Pharm.*, 17 (1965) 213–219; *C.A.*, 63 (1965) 18010d.

REDDY, K. K., RAJADURAI, S. AND NAYUDAMMA, Y.: Dhava (*Anogeissus latifolia*) tannins. III. Polyphenols of bark, sapwood and heartwood of dhava. *Indian J. Chem.*, 3 (1965) 308–310; *C.A.*, 63 (1965) 16625b.

REYES, R. E. AND GONZALEZ, A. G.: (Chemistry of *Rutaceae*. IV. Coumarins from *Ruta pinnata* leaves—structure of a new furocoumarin). *Anales Real Soc. Espan. Fis. Quim. (Madrid), Ser. B*, 61 (1965) 803–810; *C.A.*, 63 (1965) 14837a — PC and TLC.

9. OXO COMPOUNDS

KREICBERGA, D. AND GUDRINIECE, E.: (Chromatographic separation of diketones. IV. Thin-layer chromatography of 2-substituted 2-phenylindane-1,3-diones). *Latvijas PSR Zinatnu Akad. Vestis, Kim. Ser.*, No. 2 (1965) 149–152; *C.A.*, 63 (1965) 15524h.

LEMLI, J. AND CUVEELE, J.: Recherches sur les drogues à principes antraquinoniques. XII. Isolement des sennosides C et D à partir des feuilles de séné. *Pharm. Acta Helv.*, 40 (1965) 667–670.

SREPEL, B.: (Paper chromatography of volatile oils of some *Ruta* species). *Acta Pharm. Jugoslav.*, 13 (1963) 131–141; *C.A.*, 63 (1965) 9739h.

10. CARBOHYDRATES

DWORSCHAK, E.: (Determination of sorbitol in diabetic products). *Enelmiszervizsgalati Koezlemenyek (Lebensmittelanalyt. Mitt.)*, 10 (1964) 12–19; *C.A.*, 63 (1965) 17801d.

ELBEIN, A. D. AND HEATH, E. C.: The biosynthesis of cell wall lipopolysaccharide in *Escherichia coli*. II. Guanosine diphosphate 4-keto-6-deoxy-D-mannose, an intermediate in the biosynthesis of guanosine diphosphate colitose. *J. Biol. Chem.*, 240 (1965) 1926–1931.

GURAY, P.: (Raffinose determination by means of paper chromatography in the laboratory of the Eskisehir (beet) sugar factory). *Seker*, 13 No. 1 (1964) 16–18; *C.A.*, 63 (1965) 15082g.

JANECKE, H. AND WEISSER, W.: Über das Polysaccharid aus *Flores Chamomillae*. *Pharmazie*, 20 (1965) 637–643.

LEEDALE, G. F., MEEUSE, B. J. D. AND PRINGSHEIM, E. G.: Structure and physiology of *Euglena spyrogyra*. III–VI. *Arch. Mikrobiol.*, 50 (1965) 132–155; *C.A.*, 62 (1965) 16662d.

NIKAIDO, H. AND NIKAIDO, K.: Biosynthesis of cell wall polysaccharide in mutant strains of *Salmonella*. IV. Synthesis of S-specific side-chain. *Biochem. Biophys. Res. Commun.*, 19 (1965) 322–327.

SPRINGER, G. F., TAKAHASHI, T., DESAIL, R. AND KOLECKI, B. J.: Cross reactive human blood-group H(O) specific polysaccharide from *Sassafras albidum* and characterization of its hapten. *Biochemistry*, 4 (1965) 2099–2112.

SUZUKI, T. AND HOCHSTER, R. M.: On the extracellular accumulation and isolation of D-ribulose in cultures of *Agrobacterium tumefaciens*. *Biochem. Biophys. Res. Commun.*, 19 (1965) 637–642.

SVOBODA, F. AND ŠRÁMEK, J.: (Chromatographic and electrophoretic studies of the degradation of starch under the influence of various desizing agents). *Textil-Rundschau*, 20, No. 7 (1965) 183–193; *C.A.*, 63 (1965) 18434f.

11. ORGANIC ACIDS AND LIPIDS

11a. Organic acids

GHOSH, D.: Goran tannin. III. *Leather Sci. (Madras, India)*, 12 (1965) 263–267; *C.A.*, 63 (1965) 15121g.

HANSON, K. R.: Chlorogenic acid biosynthesis: chemical synthesis and properties of the mono-O-cinnamoylquinic acids. *Biochemistry*, 4 (1965) 2719–2731.

WEISSBACH, H., REDFIELD, B., BEAVEN, V. AND KATZ, E.: 4-Methyl-3-hydroxyanthranilic acid, an intermediate in actinomycin biosynthesis. *Biochem. Biophys. Res. Commun.*, 19 (1965) 524–530.

11b. Lipids and their constituents

LUBOCHINSKI, B., MEURY, J. AND STOLKOWSKI, J.: Transport du potassium et synthèse des phospholipides chez l'*Escherichia coli*. I. Les phospholipides des souches B163 et B525. *Bull. Soc. Chim. Biol.*, 47 (1965) 1529–1532.

TRIPPEL, A. I.: (Some difficulties in paper chromatographic analysis of fatty acid glycerides). *Sb. Tr. Leningr. Inst. Sov. Torgovli*, No. 23 (1964) 151–153; *C.A.*, 63 (1965) 18497f.

VERESHCHAGIN, A. G. AND NOVITSKAYA, G. V.: The triglyceride composition of linseed oil. *J. Am. Oil Chemists' Soc.*, 42 (1965) 970–974.

13. STEROIDS

NEHER, R., KAHNT, F. W., ROVERI, G. D. AND BOMPIANI, A.: Steroid transformation *in vitro* by testicular tissue from two cases of testicular feminisation. *Acta Endocrinol.*, 49 (1965) 177–192.

VOIGT, K. D., TAMM, J., VOLKWEIN, U. AND SCHEDEWIE, H.: Metabolism of pregnenolone-3-sulphate in the perfused dog liver. *Acta Endocrinol.*, 49 (1965) 427–435 — PC and TLC.

15. TERPENE DERIVATIVES

KUNKAR, A.: (Bitter orange (*Citrus aurantium*). IV. The residue on evaporation and the coumarins in the essential oil). *Riv. Ital. Essenze-Profumi-Piante Offic.-Aromi-Saponi-Cosmet.*, 47 (1965) 239–242; *C.A.*, 63 (1965) 14631c — PC and TLC.

SYPER, L.: Separation of terpenes by paper chromatography. *Dissertationes Pharm.*, 17, No. 11 (1965) 33–44; *C.A.*, 63 (1965) 9035d.

TAKEMOTO, T. AND KOMETANI, K.: (Triterpene glycosides (mubenins) from the seeds of *Stauntonia hexaphylla*). *Ann. Chem.*, 685 (1965) 237–246; *C.A.*, 63 (1965) 10051h.

16. NITRO AND NITROSO COMPOUNDS

WAGNER, G. AND SINGER, D.: (Synthesis of nitrosalicoylureides and nitro-2,4-dioxodihydro-1,3-benzoxazines). *Pharm. Zentralhalle*, 103 (1964) 791–798; *C.A.*, 63 (1965) 9937d.

17. AMINES, AMIDES AND RELATED NITROGEN COMPOUNDS

DALY, J. W., BENIGNI, J., MINNIS, R., KANAOKA, Y. AND WITKOP, B.: Synthesis and metabolism of 6-hydroxycatecholamines. *Biochemistry*, 4 (1965) 2513–2525 — R_F values and colour reactions for 11 6-hydroxycatecholamines.

JAVET, P. AND HINTERMANN, H. E.: (The determination of certain additives in electroplating baths). *J. Suisse Horl.*, No. 3–4 (1965) 37–40; *C.A.*, 63 (1965) 15562e — PC and TLC.
JINDRA, A., KUNZ, K. AND ŠÍPAL, Z.: (The metabolism of mesocaine *in vitro*). *Česk. Farm.*, 14 (1965) 319–322.
SOLOMON, D. H. AND SWIFT, J. D.: Reaction of α,α-diphenyl-β-picrylhydrazyl with acids. *J. Polymer Sci., Pt. A*, 3 (1965) 3107–3116; *C.A.*, 63 (1965) 16238d — PC and TLC.
TOUL, J. AND OKÁČ, A.: (Determination of isomers in dioximes). *Chem. Listy*, 59 (1965) 1468–1472.
ULLRICH, W.: (Paper chromatographic determination of urea in serum). *Wilner Tieraerztl. Monatsschr.*, 51 (1964) 822–830; *C.A.*, 62 (1965) 16612c.

18. AMINO ACIDS

DELAVIER-KLUTCHKO, C. AND FLAVIN, M.: Enzymatic synthesis and cleavage of cystathionine in fungi and bacteria. *J. Biol. Chem.*, 240 (1965) 2537–2549.
EICKHOFF, W. AND HERBERHOLD, C.: Intraoperative Gewinnung menschlicher Halslymphe und papierchromatographische Bestimmung ihres Jodaminosäuregehaltes. *Acta Endocrinol.*, 49 (1965) 466–470.
FUJII, N.: Free amino acids in Formosan termite. II. Determination of free amino acids in egg, worker and soldier of Formosan termite by one-dimensional ascending PC. *Miyazaki Daigaku Nogakubu. Kenkyu Jiho*, 9 (1964) 213–216; *C.A.*, 62 (1965) 16667b.
GRITSCH, J., BRAD, I., GHEORGHIU, V. AND HAIDU, V.: (New data concerning the importance of content and the determination of amino acids in champagne). *Ind. Aliment.*, 15 (1964) 585–586; *C.A.*, 62 (1965) 16919f.
JONES, E. E. AND BROQUIST, H. P.: Saccharopine, an intermediate of the aminoadipic acid pathway of lysine biosynthesis. II. Studies in *Saccharomyces cerevisiae*. *J. Biol. Chem.*, 240 (1965) 2531–2536.
LUK'YANOV, V. B. AND SIMONOV, E. F.: (Determination of norleucine with ninhydrin). *Vestn. Mosk. Univ., Ser. II, Khim.*, 20, No. 4 (1965) 30–33; *C.A.*, 63 (1965) 17154g.
MUDD, S. H., FINKELSTEIN, J. D., IRREVERRE, F. AND LASTER, L.: Threonine dehydratase activity in humans lacking cystathionine synthase. *Biochem. Biophys. Res. Commun.*, 19 (1965) 665–670.
TURULA, K.: Chromatographic and electrophoretic separation of thyroid hormones and some iodine-containing organic compounds. *Acta Endocrinol.*, 48 (1965) 31–39.
WADA, S. AND TOYOTA, T.: Isolation of D(—)-2,3-diaminopropionic acid from digestive fluid of silkworm larvae, *Bombyx mori*. *Biochem. Biophys. Res. Commun.*, 19 (1965) 482–486.

19. PEPTIDES; CHEMICAL STRUCTURE OF PROTEINS AND PEPTIDES

BRICAS, E., VAN HEIJENOORT, J., BARBER, M., WALSTENHOLME, W. A., DAS, B. C. AND LEDERER, E.: Determination of amino acid sequences in oligopeptides by mass spectrometry. IV. Synthetic N-acyl oligopeptide methyl esters. *Biochemistry*, 4 (1965) 2254–2260 — PC and TLC.
GHUYSEN, J. M., TIPPER, D. J., BIRGE, C. H. AND STROMINGER, J. L.: Structure of the cell wall of *Staphylococcus aureus* strain Copenhagen. VI. The soluble glucopeptide and its sequential degradation by peptidases. *Biochemistry*, 4 (1965) 2245–2254.
PUTNAM, F. W. AND EASLEY, C. W.: Structural studies of the immunoglobulins. I. The tryptic peptides of Bence-Joncs proteins. *J. Biol. Chem.*, 240 (1965) 1626–1638.
TOI, K., BYNUM, E., NORRIS, E. AND ITANO, H. A.: Chemical modification of arginine with 1,2-cyclohexanedione. *J. Biol. Chem.*, 240 (1965) 3455–3457.
VITALI, R. A., INAMINE, E. AND JACOB, T. A.: The isolation of γ-L-glutamyl peptides from a fermentation broth. *J. Biol. Chem.*, 240 (1965) 2508–2511.

21. PURINES, PYRIMIDINES, NUCLEOSIDES, NUCLEOTIDES, NUCLEIC ACIDS, BARBITURATES

AJINOMOTO CO., INC.: (Preparation of guanosine). *Neth. Appl.*, 6,409,806 (Cl. C07d), March 1, 1965; *Japan. Appl.*, Aug. 30, 1963, Jan. 27 and April 20, 1964; 21 pp.; *C.A.*, 63 (1965) 10055c.
BANKS, G. R. AND COHEN, D.: The activation of sulphuric acid esters by use of ethoxyacetylene in the synthesis of adenosine-5′-sulphatophosphate. *J. Chem. Soc.*, (1965) 6209–6212.
BERKOWITZ, W. F. AND BENDICH, A.: o,5′-Thymidylyl-L-serine: A model for possible linkers in DNA. *Biochemistry*, 4 (1965) 1979–1983.
BRIMACOMBE, R. L. C., GRIFFIN, B. E., HAINES, J. A., HASLAM, W. J. AND REESE, C. B.: An approach to the methylation of polynucleotides. *Biochemistry*, 4 (1965) 2452–2458 — R_F values for 23 compounds.

JOSSE, J. AND MOFFATT, J. G.: Syntheses of deoxynucleoside 3'-triphosphates. *Biochemistry*, 4 (1965) 2825–2831.
LEDVINA, M. AND BALADOVÁ, D.: (The use of chromatography on a column of Dowex 1 for the identification and isolation of metabolites of barbiturates from the urine). *Česk. Farm.*, 14 (1965) 457–461.
STEVENS, H. M.: Identification and separation of barbiturates by selective oxidation of their side chains. *Proc. Can. Soc. Forensic Sci.*, 4 (1965) 187–196; *C.A.*, 63 (1965) 9745h.
WEIL, J. H.: Effet des modifications chimiques sur l'activité biologique du RNA de transfert. *Bull. Soc. Chim. Biol.*, 47 (1965) 1303–1323.

22. ALKALOIDS

BOHINC, P.: (Chromatography of xanthine alkaloids). *Acta Pharm. Jugoslav.*, 14 (1965) 3–7; *C.A.*, 63 (1965) 9745a.
BROSSI, A. AND BORER, R.: Analysis and purification of a commercial sample of sanguinarine nitrate. *Lloydia*, 28 (1965) 199–202; *C.A.*, 63 (1965) 17801c.
KARAWYA, M. S. AND BALBAA, S. I.: Separation of some solanaceous alkaloids by paper chromatography. *Pharm. Sci. U. Arab. Rep.*, 4 (1963, Publ. 1965) 57–62; *C.A.*, 63 (1965) 9745d.
PFEIFER, S. AND MANN, I.: Über Alkaloide der Gattung *Papaver*. 7. Mitt. *Papaver glaucum* Boiss. et Hauskn. und *Papaver feddei* Schwz. *Pharmazie*, 20 (1965) 643–649 — PC and TLC.

23. OTHER SUBSTANCES CONTAINING HETEROCYCLIC NITROGEN

CHUMAKOV, YU. I. AND FILIPOVICH, M. N.: (Chromatographic separation of quinoline and isoquinoline in their mixtures). *Zh. Analit. Khim.*, 20 (1965) 856–859; *C.A.*, 63 (1965) 17111f — PC and TLC.
MARKACHEVA, T. M. AND KORNILOVA, L. I.: (Chromatographic separation of carbazole from anthracene and phenanthrene during the analysis of coal-tar products). *Zh. Analit. Khim.*, 20 (1965) 879–881; *C.A.*, 63 (1965) 17741a.
MATTU, F. AND MASCIA, C.: (The interpretation of phototropism of 2-nitrobenzylideneisonicotinic hydrazide-^{14}C). *Rend. Seminario Fac. Sci. Univ. Cagliari*, 34 (1964) 207–213; *C.A.*, 63 (1965) 15756h.
STILL, C. C., FUKUYAMA, T. T. AND MOYED, H. S.: Inhibitory oxidation products of indole-3-acetic acid. Mechanism of action and route of detoxification. *J. Biol. Chem.*, 240 (1965) 2612–2618.

24. ORGANIC SULPHUR COMPOUNDS

MISKIEWICZ, W.: (Artificial sweetening agents. I. Chromatographic method for saccharin detection in presence of dulcin). *Roczniki Panstwowego Zakladu Hig.*, 16 (1965) 33–38; *C.A.*, 62 (1965) 16875e.
RITSCHEL-BEURLIN, G.: Zur Chromatographie der Sulfonamide. *Arzneimittel-Forsch.*, 15 (1965) 1247–1249 — PC and TLC.
SCHMANDKE, H.: (Photometric determination of 2-thion-3-benzyl-5β-hydroxyethyl-tetrahydro-1,3,5-thiadiazine (D4733) by the application of paper chromatography). *Nahrung*, 9 (1965) 381; *C.A.*, 63 (1965) 17156g.
ŠIMEK, J.: (Paper chromatographic determination of hydroquinonedisulfonic acid in photographic developers). *Chem. Prumysl*, 15 (1965) 553–555; *C.A.*, 63 (1965) 15765d.

25. ORGANIC PHOSPHORUS COMPOUNDS

JACHYMCZYK, W., MENAGER, L. AND SZABO, L.: Phosphorylated sugars. XI. Syntheses of D-isosaccharinic acid 6-phosphate and 3-deoxy-D-pentonic acid 5-phosphate by alkaline degradation of cyclic monosaccharide phosphates. *Tetrahedron*, 21 (1965) 2049–2058; *C.A.* 63 (1965) 14960e.

27. VITAMINS

BRADY, R. N., LI, L.-F., MCCORMICK, D. AND WRIGHT, L. D.: Bacterial and enzymatic degradation of biotin. *Biochem. Biophys. Res. Commun.*, 19 (1965) 777–782.
CHASSY, B. M. AND MCCORMICK, D. B.: Structural requirements of the flavin moiety of flavin-adenine dinucleotide for intramolecular complex formation. *Biochemistry*, 4 (1965) 2612–2615.
HOSKINS, D. D. AND BJUR, R. A.: The electron-transferring flavoprotein of primate liver mitochondria. Purification of the enzyme and reconstitution of the sarcosine oxidase system. *J. Biol. Chem.*, 240 (1965) 2201–2208.

28. ANTIBIOTICS

BENIGNI, F., CIERI, L., GIACOMELLO, G. AND STEIN, M. L.: (New penicillins). *Farmaco (Pavia), Ed. Sci.*, 20 (1965) 499–505; *C.A.*, 63 (1965) 16327g.

FLOREA, V., AITEANU, E., MEDIANU, M. AND BUCUR, I.: (Methodology for physicochemical control of spiramycin). *Rev. Chim. (Bucharest)*, 15 (1965) 575; *C.A.*, 63 (1965) 16127g.

MAKAREVICH, V. G. AND LAZNIKOVA, T. N.: (Quantitative paper chromatography of tetracyclines). *Med. Prom. SSSR*, 19, No. 6 (1965) 51–53; *C.A.*, 63 (1965) 9746g.

MAKAREVICH, V. G., LAZNIKOVA, T. N. AND BEL'KIND, M. G.: (The quality of tetracycline preparations. I. Chromatographic investigation of the process of isolation of the tetracycline base by the method of extraction with carrier). *Med. Prom. SSSR*, 19, No. 7 (1965) 30–33; *C.A.*, 63 (1965) 9746f.

SUGANO, Y. AND WAKAZAWA, T.: Coloration of streptomycin. III. Paper chromatography of streptobiosamine and streptidine. *Nippon Nogei Kagaku Kaishi*, 37 (1963) 743–746; *C.A.*, 63 (1965) 14640e.

29. INSECTICIDES AND OTHER PESTICIDES

HENNEBERG, N.: (Chromatographic detection of nitro aromatic herbicides in toxicological analysis). *Acta Polon. Pharm.*, 21 (1964) 296–302; *C.A.*, 62 (1965) 16865b.

ZADROZINSKA, J.: (Separation of mixtures and detection of trace amounts of some organophosphorus pesticides in plant material by means of paper chromatography). *Roczniki Panstwoweg. Zakladu Hig.*, 16 (1965) 53–63; *C.A.*, 62 (1965) 16876g.

30. SYNTHETIC AND NATURAL DYES

ARTYM, M. I. AND MORYGANOV, P. V.: (Relation between chromatographic constants of vat dyes and affinity to cellulosic fibers). *Izv. Vysshikh Uchebn. Zavedenii, Tekhnol. Tekstil'n. Prom.*, No. 3, (1965) 102–107; *C.A.*, 63 (1965) 16517c.

HABERMEHL, G.: Die Chemie der Warn- und Mimikrifärbung bei Tieren. I. Mitt. Über den gelben Farbstoff aus der Haut der australischen Unke *Pseudophryne corroboree*. *Z. Naturforsch.*, 20b (1965) 1130.

31. PLASTICS AND THEIR INTERMEDIATES

BIELING, H. AND WENZEL, G.: (Paper chromatographic separation of products appearing during the technical melamine process). *Plaste Kautschuk*, 11 (1964) 409–410; *C.A.*, 63 (1965) 16538h.

LUCIANI, M.: (Identification of some synthetic resins used as sizing and waterproofing agents in paper). *Indicatore Grafico, Suppl.*, No. 9 (1964) 10 pp.; *C.A.*, 63 (1965) 18453d.

32. PHARMACEUTICAL AND FORENSIC APPLICATIONS; METABOLISM OF DRUGS

DA COSTA, A. N.: (A new chromatographic process for Lisozime determination in pharmaceutical products). *Rev. Brasil. Farm.*, 46, No. 2 (1965) 79–81; *C.A.*, 63 (1965) 17801b.

DOS SANTOS, A. C.: (Analytical methods for the examination of compounds in the Brazilian Pharmacopeia). *Rev. Fac. Farm. Santa Maria (Univ. Santa Maria)*, 11, No. 1/2 (1965) 27–37; *C.A.*, 63 (1965) 16129d.

DOUGLAS, J. S. AND NICHOLLS, P. J.: The urinary secretion of aminoglutethimide in man. *J. Pharm. Pharmacol.*, 17 (1965) 115S–117S.

VEČERKOVÁ, J., KÁCL, K., NOVOTNÁ, E. AND NIKOLIČOVÁ, L.: (Systematic identification of synthetic drugs by colour reactions and its use in the analysis of biological material). *Česk. Farm.*, 14 (1965) 444–457.

VEČERKOVÁ, J. AND NOVOTNÁ, E.: (The use of paper chromatography for the toxicological identification of drugs. IV. Demonstration of guajacuran in the urine by paper chromatography). *Česk. Farm.*, 14 (1965) 462–464.

33. INORGANIC SUBSTANCES

AKIYAMA, T. AND SHIOKAWA, K.: Circular paper chromatography of a few metals by the dithiozone complex. *Kyoto Yakka Daigaku Gakuho*, No. 12 (1964) 51–52; *C.A.*, 63 (1965) 17110e.

CHANG, C.-C., YIN, P.-H., TUNG, C.-P. AND SHEN, S.-N.: Ion exchange properties of ammonium salts of 12-heteropolyacids. II. Ion exchange chromatography of the alkali metal ions on ammonium tungstophosphate paper. *Hua Hsueh Hsueh Pao*, 31 (1965) 218–221; *C.A.*, 63 (1965) 15526h.

DUCA, A. AND BUDIU, T.: (Cobalti-molybdates, polarographic and chromatographic study). *Studii Cercetari Chim.*, 14 (1965) 179–193; *C.A.*, 63 (1965) 9428d.

DUCA, A. AND BUDIU, T.: (Cobalti-molybdates, polarographic and chromatographic study). *Rev. Roumaine Chim.*, 10 (1965) 193–207; *C.A.*, 63 (1965) 9428d.

GANCHEV, N. AND GANCHEVA, A.: (Microchemical analysis on paper, microquantitative determination of Fe). *Compt. Rend. Acad. Bulgare Sci.*, 18 (1965) 441–444; *C.A.*, 63 (1965) 9050a.

KELEMEN, I. B.: (Rutin in the paper chromatographic determination of metal ions). *Acta Phys. Chim. Debrecina*, 10 (1964, Pub. 1965) 133–138; *C.A.*, 63 (1965) 14036c.

KELEMEN, I. B. AND SZARVAS, P.: (Paper chromatographic separation and detection of vanadium (V), (IV) and (III)). *Acta Phys. Chim. Debrecina*, 10 (1964, Publ. 1965) 127–131; *C.A.*, 63 (1965) 14041c.

KUO, C.-C. AND CHENG, T.-H.: Chromatographic separation of the alkali metals on ammonium molybdotungstophosphate paper. *Hua Hsueh Tung Pao*, (1965) 247–249; *C.A.*, 63 (1965) 17113f.

NASCUTIU, T. AND GRIGOROVICI, A.: (Separation of tantalum, niobium, and hafnium by the paper chromatographic method). *Studii Cercetari Chim.*, 14 (1965) 441–448; *C.A.*, 63 (1965) 14018g.

NASCUTIU, T. AND GRIGOROVICI, A.: Separation of tantalum, niobium and hafnium by the paper chromatographic method. *Rev. Roumaine Chim.*, 10 (1965) 449–456; *C.A.*, 63 (1965) 14018g.

OSHIMA, T. AND YOSHINO, T.: Determination of inorganic ions with ion exchange paper. II. Separation and determination of chlorine, bromine, and iodine with anion exchange paper. *Kagaku To Kogyo (Osaka)*, 39 (1965) 350–355; *C.A.*, 63 (1965) 15524f.

OSHIMA, T. AND YOSHINO, T.: Determination of inorganic ions with ion exchange paper. III. Separation and determination of thiocyanate and halogen with anion exchange paper. *Kagaku To Kogyo (Osaka)*, 39 (1965) 356–361; *C.A.*, 63 (1965) 15524g.

SOO, A., ONICIU, L. AND GIURGIU, M.: (Radiochromatographic study of basic chromium complexes). *Studia Univ. Babes-Bolyai, Ser. Chem.*, 10 (1965) 79–85; *C.A.*, 63 (1965) 15824f.

WERNER, G.: (Separation of rare earths by liquid ion exchange. II. Separation of thorium, rare earths, and scandium by reversed phase paper chromatography). *Z. Chem.*, 5 (1965) 311–312; *C.A.*, 63 (1965) 17115f.

YOZA, N. AND OHASHI, S.: Oxidation of red phosphorus with hydrogen peroxide and isolation of disodium dihydrogen hypophosphate. *Bull. Chem. Soc. Japan*, 38 (1965) 1408–1409; *C.A.*, 63 (1965) 15845f.

34. RADIOACTIVE AND OTHER ISOTOPIC COMPOUNDS

UCHIDA, T.: Synthesis of ^{3}H- or ^{32}P-labeled O,O-dimethyl S-(methylcarbamoylmethyl) phosphorodithioate and the chromatography of related compounds. *Yakugaku Zasshi*, 85 (1965) 638–641; *C.A.*, 63 (1965) 9799d.

35. MISCELLANEOUS COMPOUNDS AND COMPLEX MIXTURES

CHONG, Y. W. Y. AND PARISH, D. H.: Preliminary examination of the organic N compounds present in cane molasses. *Mauritius Sugar Ind. Res. Inst. Ann. Rept.*, (1964) 112–114; *C.A.*, 63 (1965) 18433e.

ROSEN, O. M., HOFFEE, P. AND HORECKER, B. L.: The mechanism of action of aldolases. VII. Formation of 2-methyl-2-deoxypentose catalyzed by deoxyribose 5-phosphate aldolase. *J. Biol. Chem.*, 240 (1965) 1517–1524.

Thin-layer Chromatography

1. REVIEWS AND BOOKS

FRANGOPOL, M.: (Thin-layer chromatography). *Studii Cercetari Chim.*, 14 (1965) 281–300; *C.A.*, 63 (1965) 9091h.

MORITA, K.: Advance in thin layer chromatography. *Tampakushitsu Kakusan Koso*, 9 (1964) 11–16; *C.A.*, 63 (1965) 14086d.

MUSIL, F.: (Thin-layer chromatography with and without binder). *Pracovni Lekar.*, 17 (1965) 212–216; *C.A.*, 63 (1965) 15517e.

NAKAZAWA, Y.: (Thin-layer chromatography, its principle and procedure). *Taisha*, 2 (1965) 694–699; *C.A.*, 63 (1965) 14017a.

3. TECHNIQUES I

BUMANS, R., VEISS, A. AND OLTE, M.: (Crystalline aluminium hydroxides as adsorbents in thin-layer chromatography). *Latvijas PSR Zinatnu Akad. Vestis, Khim. Ser.*, (1965) 35–42; *C.A.*, 63 (1965) 15526b.

PITRA, J. AND ŠTĚRBA, J.: (Luminescent silica gel). *Chem. Listy*, 59 (1965) 1462–1464.

5. HYDROCARBONS AND HALOGEN DERIVATIVES

BORDONALI, C., PATIMO, C. AND SALVEMINI, F.: (Adsorbents for thin-layer chromatography of polyphenyls). *Comit. Nazl. Energia Nucl.*, RT/CHI (64) 28 (1964) 13 pp.; *C.A.*, 63 (1965) 15525h.

CRUMP, G. B.: The analysis of gas oil fractions by loose-layer chromatography. *Am. Chem. Soc., Div. Petrol. Chem., Preprints*, 8, No. 4 (1963) C87–C94; *C.A.*, 63 (1965) 9713g.

JAEGER, J. AND LUGROVA, O.: (Determination of 3,4-benzopyrene by thin layer chromatography). *Cesk. Hyg.*, 10 (1965) 319–323; *C.A.*, 63 (1965) 17152b.

6. ALCOHOLS

HASHIMOTO, A., HIROTANI, A. AND MUKAI, K.: Higher alcohols. IV. Separation of alcohols by thin-layer chromatography on activated bleaching earth. *Yukagaku*, 14 (1965) 343–347; *C.A.*, 63 (1965) 17874c.

HASHIMOTO, A., SHIRO, K. AND MUKAI, K.: Higher alcohols. III. Separation of the saturated and unsaturated alcohols by the mercuric acetate adduct method. *Yukagaku*, 13 (1964) 586–589; *C.A.*, 63 (1965) 17874b.

7. PHENOLS

VAZQUEZ, A. T. AND MONTES, A. L.: (Chromatography of phenolic compounds from essential oils). *Anales Asoc. Quim. Arg.*, 51 (1963) 231–241; *C.A.*, 63 (1965) 9739c.

8. SUBSTANCES CONTAINING HETEROCYCLIC OXYGEN

MESSERSCHMIDT, W.: Quantitative Bestimmung des Khellins mit Hilfe der präparativen Dünnschichtchromatographie. *Arzneimittel-Forsch.*, 15 (1965) 1339–1340.

VIALARD-GOUDOU, A. AND BLANCHECOTTE, N.: (Formation of flavones by thermal condensation of monosubstituted monophenols and ethyl 3,4,5-trimethoxybenzoylacetate). *Compt. Rend.*, 260 (1965) 6930–6932; *C.A.*, 63 (1965) 9908b.

9. OXO COMPOUNDS

BUZLANOVA, M. M. AND STEPANOVSKAYA, V. F.: (Separation of some ketones by thin-layer chromatography). *Zh. Analit. Khim.*, 20 (1965) 859–862; *C.A.*, 63 (1965) 14019f.

FREUDENBERG, K., LAUTENSCHLAEGER, L. AND TAUSEND, H.: (Guaiacyl vinyl ketone). *Ann. Chem.*, 685 (1965) 139–141; *C.A.*, 63 (1965) 9854e.

10. CARBOHYDRATES

URBACH, G.: Thin-layer chromatography of aliphatic γ- and δ-lactones. *J. Am. Oil Chemists' Soc.*, 42 (1965) 927–930.

WEICKER, H., KUHN, D. UND STEGEMANN, H.: Nachweis eines kleinmolekularen Glykoproteids bei Neoplasien und die Charakterisierung seiner chemischen Besonderheiten. *Klin. Wochschr.*, 43 (1965) 1215–1220.

11. ORGANIC ACIDS AND LIPIDS

11a. Organic acids

ÄNGGARD, E., GRÉEN, K. AND SAMUELSSON, B.: Synthesis of tritium-labeled prostaglandin E_2 and studies on its metabolism in guinea pig lung. *J. Biol. Chem.*, 240 (1965) 1932–1940.

ROOMI, M. W., SUBBARAM, M. R. AND ACHAYA, K. T.: Catalytic hydrogenation of *cis*-9,10-epoxyoctadecanoic and *cis*-13,14-epoxydocosanoic acids. *Indian J. Chem.*, 3 (1965) 311–313; *C.A.*, 63 (1965) 14791a.

11b. Lipids and their constituents

ARVIDSON, G. A. E.: Fractionation of naturally occurring lecithins according to degree of unsaturation by thin-layer chromatography. *J. Lipid Res.*, 6 (1965) 574–577.

BAUMANN, N. A., HAGEN, P. O. AND GOLDFINE, H.: Phospholipids of *Clostridium butyricum*. Studies on plasmalogen composition and biosynthesis. *J. Biol. Chem.*, 240 (1965) 1559–1567 — TLC and PC.

CULP, T. W., HARLOW, R. D., LITCHFIELD, C. AND REISER, R.: Analysis of triglycerides by consecutive chromatographic techniques. II. Ucuhuba kernel fat. *J. Am. Oil Chemists' Soc.*, 42 (1965) 974–978.

EL-NOCKRASHY, A. S. AND OSMAN, F.: A simple technique for the analysis of vegetable and animal phospholipids by thin-layer chromatography. *Planta Med.*, 13 (1965) 326–330; *C.A.*, 63 (1965) 14638d.

JURRIENS, G. AND OELE, J. M.: Determination of hydroxy-acid triglycerides and lactones in butter. *J. Am. Oil Chemists' Soc.*, 42 (1965) 857–861.

JURRIENS, G. AND SCHOUTEN, L.: (Analysis of the triglycerides of corn, sunflower, and rapeseed oils). *Rev. Franc. Corps Gras*, 12 (1965) 505–510; *C.A.*, 63 (1965) 18498a.

KAUFMANN, H. P. AND VISWANATHAN, C. V.: Die Dünnschicht-Chromatographie auf dem Fettgebiet. XVII. Mitt. Die Analyse von Hefelipoiden. *Fette-Seifen-Anstrichmittel*, 67 (1965) 662–666.

LEDEEN, R. AND SALSMAN, K.: Structure of the Tay-Sachs' ganglioside. *Biochemistry*, 4 (1965) 2225–2233 — TLC and PC.

LEDERKREMER, J. M. AND JOHNSON, R. M.: Purification of neutral lipid fractions by thin-layer chromatography on aluminium oxide. *J. Lipid. Res.*, 6 (1965) 572–574.

LEIKOLA, E., NIEMINEN, E. AND SALOMAA, E.: Occurrence of methyl esters in the pancreas. *J. Lipid Res.*, 6 (1965) 490–493.

MILLER, R. W., EARLE, F. R. AND WOLFF, I. A.: Search for new industrial oils. XIII. Oils from 102 species of Cruciferae. *J. Am. Oil Chemists' Soc.*, 42 (1965) 817–821.

NAUDET, M., PASERO, J. AND BIASINI, S.: (Determination of small amounts of partial glycerides in fats). *Rev. Franc. Corps Gras*, 12 (1965) 525–534; *C.A.*, 63 (1965) 18497h.

OETTE, K.: Identification of some lipid peroxides by thin-layer chromatography. *J. Lipid Res.*, 6 (1965) 449–454.

PARKER, F. AND PETERSON, N. F.: Quantitative analysis of phospholipids and phospholipid fatty acids from silica gel thin-layer chromatograms. *J. Lipid Res.*, 6 (1965) 455–460.

SAHASRABUDHE, M. R.: Application of TLC to the quantitative estimation of tissue triglycerides. I. Triglyceride distribution in the livers of calf, pig and rat. *J. Am. Oil Chemists' Soc.*, 42 (1965) 862–864.

SAHASRABUDHE, M. R.: Application of TLC to the quantitative estimation of tissue triglycerides. II. Influence of methyl parathion on the composition of liver triglycerides in the rat. *J. Am. Oil Chemists' Soc.*, 42 (1965) 864–866.

THOMAS III, A. E., SCHAROUN, J. E. AND RALSTON, H.: Quantitative estimation of isomeric monoglycerides by thin-layer chromatography. *J. Am. Oil Chemists' Soc.*, 42 (1965) 789–792.

VANDERWAL, R. J.: Semiquantitative structural analysis of fats by thin-layer chromatography of the allyl esters of the products of von Rudloff oxidation. *J. Am. Oil Chemists' Soc.*, 42 (1965) 754–757.

13. STEROIDS

BENRAAD, TH. J. AND KLOPPENBORG, W. C.: Double isotope assay of aldosterone in urinary extracts with the combined use of thin-layer and paper chromatography. *Clin. Chim. Acta*, 12 (1965) 565–574 — TLC and PC; R_F values for a great number of steroids.

FREIMUTH, V., ZAWTA, B. AND BUECHNER, M.: (Separation of corticosteroids by means of thin-layer chromatography on polyamide layers). *Acta Biol. Med. Ger.*, 13 (1965) 624–628; *C.A.*, 62 (1965) 16575a.

LISSE, K. AND SCHÜRENKÄMPFER, P.: Die Progesteron-Biosynthese in Gewebsschnitten von Rinderovarien im Verlauf des Zyklus. *Acta Endocrinol.*, 50 (1965) 429–438.

NAMBARA, T. AND YANO, M.: Analytical chemical studies on steroids. VI. Chemistry of C-16 oxygenated 5α,14β-androstanes. *Chem. Pharm. Bull.* (*Tokyo*), 13 (1965) 1004–1008; *C.A.*, 63 (1965) 14932h.

SATO, Y.: Basic bile acids. V. Syntheses and properties of basic bile acids and their derivatives. *Bull. Chem. Soc. Japan*, 32 (1965) 1790–1791; *C.A.*, 63 (1965) 18205c.

SCHWERS, J., ERIKSSON, G. AND DICZFALUSY, E.: Metabolism of oestrone and oestradiol in the human foeto-placental unit at midpregnancy. *Acta Endocrinol.*, 49 (1955) 65–82 — TLC and PC.

SULIMOVICI, S., LUNENFELD, B. AND SHELESNYAK, M. C.: A practical method for estimation of urinary pregnanediol and allopregnanediol using thin-layer chromatography. *Acta Endocrinol.*, 49 (1965) 97–106.

ZEITMAN, B. B.: Rapid method for determining free and esterified cholesterol in plasma extracts. *J. Lipid Res.*, 6 (1965) 578–580.

14. STEROID GLYCOSIDES AND SAPONINS

LONGO, R. AND MEINARDI, G.: (Chromatographic determination of *Senna* glycosides). *Boll. Chim. Farm.*, 104 (1965) 503–510; *C.A.*, 63 (1965) 17799g.

15. TERPENE DERIVATIVES

EL-HAMIDI, A. AND RICHTER, G.: Preliminary investigation of Egyptian cumin oil by thin-layer chromatography. *Lloydia*, 28 (1965) 252–256; *C.A.*, 63 (1965) 17791h.

FUJITA, E., FUJITA, T., FUJI, K. AND ITO, N.: Absolute configuration of enmein–transformation of enmein into (—)-kaurane. *Chem. Pharm. Bull.* (*Tokyo*), 13 (1965) 1023–1027; *C.A.*, 63 (1965) 14918f.

MUEHLETHALER, B. AND GIGER, S.: (Testing of turpentine oils by thin-layer chromatography). *Farbe Lack*, 70 (1964) 116–120; *C.A.*, 63 (1965) 18464a.

TEISSEIRE, P.: (Influence of modern physicochemical methods on the analysis of essential oils). *Recherches* (*Paris*), No. 14 (1964) 12–32; *C.A.*, 63 (1965) 14631e.

WALLS, F., SALMON, M., PADILLA, J., JOSEPH-NATHAN, P. AND ROMO, J.: (Structure of perezone). *Bol. Inst. Quim. Univ. Nacl. Auton. Mex.*, 17 (1965) 3–15; *C.A.*, 63 (1965) 18179c.

17. AMINES, AMIDES AND RELATED NITROGEN COMPOUNDS

ALESSANDRO, A. AND DE SIO, F.: (Reagents for detecting sympathomimetic amines by thin-layer chromatography). *Boll. Chim. Farm.*, 104 (1965) 498–502; *C.A.*, 63 (1965) 17798c — R_F values for 26 compounds.

18. AMINO ACIDS

BLANC, P., BERTRAND, P., DE SAQUI-SANNES, G. AND LESCURE, R.: (Thin-layer-chromatographic separation of amino acids). *Chim. Anal.* (*Paris*), 47 (1965) 285–287; *C.A.*, 63 (1965) 14020c.

BÜRGI, W., COLOMBO, J. P. AND RICHTERICH, R.: Dünnschichtchromatographie von säure- und ätherlöslichen DNP-Aminosäuren im Urin. *Klin. Wochschr.*, 43 (1965) 1202–1208.

FIGGE, K.: Beitrag zur quantitativen Dünnschichtchromatographie dinitrophenylierter Aminosäuren aus Körperflüssigkeiten. *Clin. Chim. Acta*, 12 (1965) 605–619.

LINDSTEDT, G. AND LINDSTEDT, S.: Separation of carnitine from related compounds by ion-exchange and thin-layer chromatography. *Recent Res. Carnitine, Its Relation Lipid Metab., Papers Symp., Cambridge, Mass.*, (1964, Publ. 1965) 11–21; *C.A.*, 63 (1965) 16454h.

21. PURINES, PYRIMIDINES, NUCLEOSIDES, NUCLEOTIDES, NUCLEIC ACIDS, BARBITURATES

MORRISON, J. C. AND CHATTEN, L. G.: Estimation of barbiturates by quantitative thin-layer chromatography. *J. Pharm. Pharmacol.*, 17 (1965) 655–658.

22. ALKALOIDS

AHMED, Z. F., RIZK, A. M. AND HAMMOUDA, F. M.: Phytochemical studies of Egyptian *Plantago* species (alkaloids). *J. Pharm. Pharmacol.*, 17 (1965) 39S–40S.

BECKETT, A. H., SHELLARD, E. J., PHILLIPSON, J. D. AND LEE, C. M.: Alkaloids from *Mitragyna speciosa* (Korth). *J. Pharm. Pharmacol.*, 17 (1965) 753–755.

BICAN-FISTER, T. AND BROZ-KAJGANOVIC, V.: (Determination of ephedrine and codeine in mixtures). *Acta Pharm. Jugoslav.*, 14 (1964) 17–22; *C.A.*, 63 (1965) 9745f.

POETHKE, W. AND KINZE, W.: (Detection and determination of strychnine and brucine by thin-layer chromatography). *Pharm. Zentralhalle*, 104 (1965) 489–492; *C.A.*, 63 (1965) 16130g.

ROENSCH, H. AND SCHREIBER, K.: (Solanum alkaloids. LXIII. 15α-Hydroxysoladulcidine and 15α-hydroxytomatidine, two new steroid alkaloids from *Solanum dulcamara*). *Tetrahedron Letters*, (1965) 1947–1952; *C.A.*, 63 (1965) 18207a.

SCHOEPF, C. AND MUELLER, E.: (Absolute configuration of (—) -lobeline and of its reduction products). *Ann. Chem.*, 687 (1965) 241–250; *C.A.*, 63 (1965) 18184g.

SZASZ, G., SZASZ-ZACSKO, M. AND POLANKAY, V.: (A new method for the separation of caffeine, theobromine, and theophylline by thin-layer chromatography). *Acta Pharm. Hung.*, 35 (1965) 207–212; *C.A.*, 63 (1965) 14637f.

23. OTHER SUBSTANCES CONTAINING HETEROCYCLIC NITROGEN

CHIAVARELLI, S., TOEFFLER, H. F., FENNOY, L. V., LANDI-VITTORY, R. AND MAZZEO, P.: (Synthesis of 1,5-diphenylbispidin-9-ones. VIII. 1,5-Diphenyl-3,7-dialkylbispidin-9-ones' Asymmetrically substituted derivatives). *Farmaco (Pavia), Ed. Sci.*, 20 (1965) 408–420; *C.A.*, 63 (1965) 14864a.

FRAM, D. H. AND GREEN, J. P.: The presence and measurement of methylhistamine in urine. *J. Biol. Chem.*, 240 (1965) 2036–2042 — TLC and PC.

KATO, T. AND NIITSUMA, T.: Synthesis of methylpyridine derivatives. XXI. Amination of chloro-2,6-lutidines and their N-oxides via a hetaryne mechanism. *Chem. Pharm. Bull. (Tokyo)*, 13 (1965) 963–968; *C.A.*, 63 (1965) 14804e.

MERCK, A. G., E.: (Synthesis of substituted piperidine derivatives). *Neth. Appl.*, 6,413,199 (Cl. C 07*d*), June 8, 1965; *Ger. Appl.*, Dec. 7, 1963; 42 pp.; *C.A.*, 63 (1965) 18038g.

24. ORGANIC SULPHUR COMPOUNDS

PASTOR, J. AND RAIMONDI, R.: (Separation and identification of sulfonamides by thin-layer chromatography). *Bull. Soc. Pharm.-Marseille*, 13 (1964) 193–195, 197–201; *C.A.*, 63 (1965) 16133d.

REISCH, J., BORNFLETH, H. AND TITTEL, G. L.: (Analysis of drug mixtures. IV. Thin-layer chromatography of some sulfonamides used as antidiabetics). *Pharm. Ztg., Ver. Apotheker-Ztg.*, 109 (1964) 74–75; *C.A.*, 63 (1965) 16129g.

TAKAGI, T. AND FUKUZUMI, K.: Application of thin-layer chromatography to oil chemistry. I. Chromatography of synthetic surfactants by silica plates. *Yukagaku*, 13 (1964) 520–523; *C.A.*, 63 (1965) 18485f.

25. ORGANIC PHOSPHORUS COMPOUNDS

GELDERN, L.: (Identification of zinc O,O-dialkyl dithiophosphates in petroleum products by thin-layer chromatography). *Erdöl Kohle*, 18 (1965) 545–548; *C.A.*, 63 (1965) 9713h.

REUTER, H. AND HANKE, H.: (Thin-layer chromatography of phosphoric acid ethyl esters). *Pharm. Zentralhalle*, 104 (1965) 323–327; *C.A.*, 63 (1965) 14687c.

27. VITAMINS

LUDWIG, E. AND FREIMUTH, U.: (Thin-layer chromatography in food chemistry. III. The separation of some water-soluble vitamins). *Nahrung*, 9 (1965) 41–42; *C.A.*, 62 (1965) 16612b.

30. SYNTHETIC AND NATURAL DYES

PIETSCH, H. P. AND MEYER, R.: (Thin-layer chromatographic separation of artificial organic food dyes with kieselgel D). *Nahrung*, 9 (1965) 154; *C.A.*, 62 (1965) 16875b.

31. PLASTICS AND THEIR INTERMEDIATES

VAN DER NEUT, J. H.: (Analysis of macromolecular materials). *Plastica*, 18 (1965) 307–315; *C.A.*, 63 (1965) 18347h.

32. PHARMACEUTICAL AND FORENSIC APPLICATIONS; METABOLISM OF DRUGS

ANAND, D. R.: (Thin-layer chromatography of neostigmine). *Pharm. Zentralhalle*, 104 (1965) 371–376; *C.A.*, 63 (1965) 9750f.

HÁZNAGY, A., SZENDREI, K. AND TÓTH, L.: Die Dünnschichtchromatographie und ihre Bedeutung in der Pharmakognosie. 2. Mitt. Nachweis von Drogenverfälschungen. *Pharmazie*, 20 (1965) 651–654.

LOPES, E. S.: (Thin-layer chromatography in pharmaceutical analysis). *Rev. Port. Farm.*, 15, No. 2 (1965) 105–127; *C.A.*, 63 (1965) 17800a.

PRIBILLA, O.: Zum chemischen Nachweis von 1,3-Dihydro-7-nitro-5-phenyl-2 H-1,4-benzodiazepin-2-on. *Arzneimittel-Forsch.*, 15 (1965) 1148–1152.

RIEDER, J.: Methoden zur Bestimmung von 1,3-Dihydro-7-nitro-5-phenyl-2H-1,4-leurodiazepin-2-on und seinen Hauptmetaboliten in biologischen Proben und Ergebnisse von Versuchen über die Pharmakokinetik und den Metabolismus dieser Substanz bei Mensch und Ratte. *Arzneimittel-Forsch.*, 15 (1965) 1134–1148.

SAVIDGE, R. A. AND WRAGG, J. S.: A limit test for *p*-chloroacetanilide and other impurities in paracetamol and phenacetin using thin-layer chromatography. *J. Pharm. Pharmacol.*, 17 (1965) 60S–64S.

TSUTSUI, K., SAKAI, T. AND TAKAHASHI, M.: Identification and separation of non-barbituric acid hypnotics by thin-layer chromatography. *Yakuzaigaku*, 25 (1965) 53–56; *C.A.*, 63 (1965) 17802a.

33. INORGANIC SUBSTANCES

HU, C.-R. AND LIU, C.-L.: Application of thin-layer chromatography in inorganic analysis. III. Separation of niobium and tantalum, and noble metals. *Hua Hsueh Hsueh Pao*, 31 (1965) 267–269; *C.A.*, 63 (1965) 15525f.

MUTO, M.: Quantitative analysis of I, Br, Cl, and PO_4 ions by thin-layer precipitation chromatography with silver nitrate. *Nippon Kagaku Zasshi*, 86 (1965) 59–62; *C.A.*, 63 (1965) 14034c.

WU, M.-Y. AND WU, L.-F.: Thin-layer inorganic chromatography. II. Separation of the platinum group and gold. *K'o Hsueh T'ung Pao*, (1964) 939; *C.A.*, 63 (1965) 14020e.

34. RADIOACTIVE AND OTHER ISOTOPIC COMPOUNDS

MIRKINA, N. N. AND KHARLAMOV, V. T.: (Thin-layer chromatograph analysis of ^{35}S-labeled thio-TEPA). *Metody Analiza Radioaktivn. Preparatov, Sb. Statei*, (1965) 155–161; *C.A.*, 63 (1965) 9744h — TLC and PC.

35. MISCELLANEOUS COMPOUNDS AND COMPLEX MIXTURES

COVELLO, M. AND SCHETTINO, O.: (Application of thin-layer chromatography to investigations of antifermentatives in food). *Riv. Ital. Sostanze Grasse*, 41, No. 2 (1964) 75–78; *C.A.*, 62 (1965) 16877f.

VAN DER HEIDE, R. F., MAAGDENBURG, A. C. AND VAN DER NEUT, J. H.: (Identification of antioxidants in plastics). *Chem. Weekblad*, 61 (1965) 440–443; *C.A.*, 63 (1965) 15050b.

WANDEL, M. AND TENGLER, H.: (Chromatographic methods for the identification of plasticizers. Analysis of phthalate esters). *Kunststoffe*, 55 (1965) 655–659; *C.A.*, 63 (1965) 18356b.

WOGGON, H., KORN, O. AND JEHLE, D.: (Requirements for plastics. Determination of antioxidants in polyethylene). *Nahrung*, 9 (1965) 495–505; *C.A.*, 63 (1965) 18348h.

Gas Chromatography

1. REVIEWS AND BOOKS

HORNING, E. C. AND VANDENHEUVEL, W. J. A.: La chromatographie en phase gazeuse. Application aux problèmes biochimiques et biologiques. *Chim. Anal. (Paris)*, 48 (1966) 79–84 — final part of a review; 34 references, many original examples.

KIRK, P. L. AND KINGSTON, C. R.: Present status of gas chromatography in the criminalistics laboratory. *J. Criminal Law, Criminol. Police Sci.*, 56 (1965) 247–256.

2. FUNDAMENTALS, THEORY AND GENERAL

2c. Thermodynamics and theoretical relationships

BARRAL II, E. M., PORTER, R. S. AND JOHNSON, J. F.: Gas chromatography using cholesteryl ester liquid phases. *J. Chromatog.*, 21 (1966) 392–401 — sharp changes in retention of some hydrocarbons are noted at or near mesophase transition temperatures; the elution times increase (3.5 times) in the transition from the cholesteric mesophase to the isotropic liquid.

BEYER, H., FEJES, P., SCHAY, G. AND VARGA, K.: Neuere Untersuchungen über frontale Gaschromatographie unter Berücksichtigung der Strömungsgeschwindigkeite während der Sorption. III. Bestimmung von HTS-Werten mittels frontaler Gaschromatographie. *Acta Chim. Acad. Sci. Hung.*, 47 (1966) 13–22 — a method is developed for the determination of the effective diffusion constants in the gas phase and the mass transport velocity constants from frontal GC data; application to the Van Deemter equation is given.

GIDDINGS, J. C.: The nature of eddy diffusion. *Anal. Chem.*, 38 (1966) 490–491 — a reply to the criticism by A. KLINKENBERG in *Anal. Chem.*, 38 (1966) 489.

KAMBARA, T. AND OHZEKI, K.: Revision of the Van Deemter theory for diffusion and pressure drop in the gas phase. *J. Chromatog.*, 21 (1966) 383–391 — the Van Deemter theory is considered to hold as the limiting law and to be quite legitimate in liquid phase chromatography.

KLINKENBERG, A.: Evidence on the nature of eddy diffusion in gas chromatography from inert (nonsorbing) column data. *Anal. Chem.*, 38 (1966) 489–490 — a discussion of the paper by J. C. GIDDINGS, in *Anal. Chem.*, 35 (1963) 1338.

KLINKENBERG, A.: The nature of eddy diffusion in gas chromatography. *Anal. Chem.*, 38 (1966) 491–492 — a final word in the discussion with J. C. GIDDINGS (*Anal. Chem.*, 38 (1966) 490).

VARGA, K., FEJES, P. AND BEYER, H.: Neuere Untersuchungen über frontale Gaschromatographie, unter Berücksichtigung der Strömungsgeschwindigkeit während der Sorption. IV. Beurteilung chromatographischer Trennsäulen auf Grund frontalchromatographisch gemessener Transportgeschwindigkeits- und Diffusionskonstanten. *Acta Chim. Acad. Sci. Hung.*, 47 (1966) 23–35 — experiments suggest that the sorption rate in the liquid phase is the velocity-determining factor and that in the case of low coating the stationary liquid is not uniformly distributed on the support surface.

2d. General

ROSE, H. C., STERN, R. L. AND KARGER, B. L.: Studies on the mechanism of separation of diastereoisomeric esters by gas–liquid chromatography. Effect of bulk dissymetry and distance between optimal centers. *Anal. Chem.*, 38 (1966) 469–472 — the relative volatilities and free energy differences in gas–liquid partition energy, $\Delta(\Delta G°)$, of diastereoisomeric esters of acetylated lactic acid are given.

VIGDERGAUZ, M. S. AND AFANAS'EV, M. I.: (Calculation of accuracy of chromatographic analysis of substances). *Neftekhimiya*, 6 (1966) 123–130 — the resolution of partially separated peaks is characterised by $\psi = (h_2 - h_{min})/h_2$, where h_2 is the height of the smaller peak and h_{min} is the minimum height of the curve between overlapping peaks.

3. TECHNIQUES I

3a. Detectors

AL'TSHULER, O. V., VINOGRADOVA, O. M. AND ELKIN, A. E.: (Application of a katharometer working with two different carrier gases). *Zavodsk. Lab.*, 32 (1966) 121–122 — He (H_2) is applied to one detector wire and Ar (N_2) to the second one; analysis with suitable sensitivity is realised for gases of different thermal conductivity.

GORSHKOV, V. I., TANTSYREV, G. D. AND TAL'ROZE, V. L.: (Use of commercial mass spectrometers as gas chromatographic detectors). *Zavodsk. Lab.*, 32 (1966) 114–115 — C_3–C_5 hydrocarbons at m/e = 39, 41 and 43; no references except to papers of the authors.

HENNEBERG, D.: High resolution mass spectra of compounds separated by capillary columns. Use of a plate scan technique. *Anal. Chem.*, 38 (1966) 495–496 — scheme of the connection device given.

3b. Column performance and filling studies

BEREZKIN, V. G., KOLBANOVSKIĬ, YU. A. AND KYAZIMOV, E. A.: (Kinetics of radiation-initiated polymerisation of acrylonitrile from gas phase on a mineral support). *Kinetika i Kataliz*, 7, No. 1 (1966) 187 — modification of GC support material INZ-600.

FIDDLER, W. AND DOERR, R. C.: Low temperature discontinuity of retention times with Carbowax 20M. *J. Chromatog.*, 21 (1966) 481–482 — it was found that retention time as a function of column temperature shows a discontinuity; a change in retention occurs at 60°.

SHEFTER, V. E., BARON, N. YU. AND DEMENT'EVA, M. I.: (Influence of thermal modification of support material INZ-600 on its structure and adsorptive properties). *Neftekhimiya*, 6 (1966) 139–144 — heating to 1100° lowers the adsorption forces of support material.

3c. Apparatus, accessories and materials for GC

ALISHOEV, V. P., BEREZKIN, V. G. AND PAKHOMOV, V. P.: (Injection device for sampling of small quantities of liquids into a gas chromatograph). *Zavodsk. Lab.*, 32 (1966) 260 — sampling errors for 0.2 μl 5–7%.

ALLEN, E. R.: Quantitative gas chromatographic sampler for static gaseous reaction systems. *Anal. Chem.*, 38 (1966) 527–528.

DERGE, K.: Die Kompensationstechnik in der Gaschromatographie. *Erdöl Kohle*, 19 (1966) 122–125 — principle of an analogue-baseline-compensator given.

GABRIEL, W. P. AND MORRIS, R. A.: A flame ionization meter for gas chromatography. *J. Sci. Instr.*, 43 (1966) 104–107 — a meter for the measurement of small direct currents is described in detail; it has six linear ranges with full scale deflections of 0.1 nA–10 μA, a logarithmic range of 0.1 nA–10 μA, and three integrating ranges; 230 V a.c.

HÁNA, K., PIETSCH, K. AND ČERMÁK, R.: (Method and equipment for automated process control (by gas chromatography) of followed and quantified chemical analyses). *Czech. Pat.*, 108,998 (Nov. 15, 1963); *French Pat.*, 1,323,169 (Febr. 25, 1964); *Brit. Pat.*, 1,007,527 (Aug. 20, 1965); *Ital. Pat.*, 667,144 (Sept. 15, 1964); and *Swiss Pat.*, 400,614 (Nov. 16, 1965).

HAYER, M., MANDL, M. AND DOUBEK, J.: (Monitoring of furnace atmospheres by gas chromatography). *Rept. Res. Inst. Iron Met., Prague*, No. 10 (1964) 39 pp. — a multicolumn system GC monitor based on *Czech. Pat.* 108,998.

KLAVER, R. I. AND LETOURNEAU, R. L.: A fast mass spectrum digitizer for a gas chromatograph–mass spectrometer combination. *Proc. Am. Petrol. Inst.*, 43 (1963) 255–257.

MANAHAN, C. M. AND PURCHASE, I. F.: Gas-holding apparatus for use in gas chromatography. *Brit. J. Anaesth.*, 37 (1963) 210–211.

VERGNAUD, J. M., MANGE, M., BOURSIN, M., PERRET, M., BUATHIER, B. AND GARIAZZO, B.: Détermination du degré de pureté et de cristallinité des tamis moléculaires. *Chim. Anal. (Paris)*, 48 (1966) 59–65.

4. TECHNIQUES II

4a. Preparative-scale GC

KISHIMOTO, K. AND KINOSHITA, K.: (Application of preparative gas chromatography for microcomponents in special grade benzene). *Japan Analyst*, 14 (1965) 3–6.

PERRY, J. A.: A yield index for comparing columns in preparative gas chromatography. *Chem. Ind. (London)*, (1966) 576–577 — yield index $Y = N\text{–}Q/t$; where N = number of theoretical plates, Q = ml of the injected component, and t = retention time of the component in minutes.

4b. Programmed temperature and programmed pressure GC

LANTZ, C. D.: Temperature programming in gas chromatography. *Instr. Control Systems*, 38, No. 8 (1965) 93–94.

4d. Special microtechniques

NAKAGAKI, M. AND NISHINO, M.: (A theoretical study on gas chromatography accompanied by a chemical reaction). *J. Pharm. Soc. Japan*, 85 (1965) 305–313.

4f. Measurement of physico-chemical and related values

KUGE, Y. AND YOSHIKAWA, Y.: A new method to determine relative surface area by gas chromatography. I. *Bull. Chem. Soc. Japan*, 38 (1965) 948–951.

MARTIRE, D. E. AND POLLARA, L. Z.: Solute activity coefficients at infinite dilutions and specific retention volumes measured by gas liquid chromatography. *J. Chem. Eng. Data*, 10 (1965) 40–43.

TUL'BOVICH, B. I., KLYAEV, V. I., SLISARENKO, F. A. AND SOROKIN, S. I.: (Determination of specific surface by the thermal desorption method). *Zavodsk. Lab.*, 32 (1966) 50–51 — scheme of apparatus given; no reference to many works on the same subject.

5. HYDROCARBONS AND HALOGEN DERIVATIVES

5a. Gaseous hydrocarbons

LU, PEI-CHANG, WANG, CHAI-CHU, WANG, RUI-HUA AND WANG, HSIAO-YU: (Determination of trace amounts of gaseous impurities in butadiene). *Acta Chim. Sinica*, 32 (1966) 26–33 — 2–100 p.p.m. of H_2, N_2, O_2, CH_4 and CO on molecular sieve 5A and CO_2, C_1–C_4 hydrocarbons on ethyl acetoacetate.

RENNHAK, S., DÖRING, C. E., SCHMID, G., SCHNELLER, D., STÜRTZ, H. AND WERNER, E.: Gaschromatographische Spurenuntersuchungen in C_2–C_4-Olefinfraktionen. *Chem. Tech. (Berlin)*, 17 (1965) 688–692.

5b. Other hydrocarbons

ALEKSEEVA, K. V. AND SOLOMATINA, L. C.: (High-speed gas-chromatographic determination of divinylbenzene in its plant products). *Zavodsk. Lab.*, 32 (1966) 138–139 — on polyphenylmethyl silicone PFMS-4 at 140°.

BELIKOVA, N. A., PLATE, A. F. AND BOBYLYOVA, A. A.: (Isomerization of bridged bicyclic hydrocarbons). *Zh. Org. Khim.*, 2 (1966) 46, 53 and 77.

EBEID, F. M. AND ABO-LEMON, F. S.: A study of isomerization of a C_5–C_6 fraction of Land-Belayim gasoline. *J. Chem. U.A.R.*, 8 (1965) 217–237 — quinoline–brucine + liquid paraffin column at 30°.

EBEID, F. M. AND ABOUL-GHEIT, A. K.: Red-sea stabilized straight-run gasoline. IV. A study of toluene production in a 86–105° fraction. *J. Chem. U.A.R.*, 8 (1965) 195–215.

JANÁK, J., REZL, V. AND ZVĚŘINA, Z.: (A contribution to the composition of the distillation residue of butadiene from Lebedev synthesis). *Chem. Prumysl*, 16 (1966) 154–159 — 24 olefins are identified and determined by a combination of fractional distillation, capillary GC, hydrogenation and group-selective reactions.

KRAPLINA, KH. F., KRICHMAR, S. I., PINSKER, A. E., SOKOLOV, V. G. AND STEPANENKO, V. E.: (Chromatographic determination of impurities in benzene and ethylene fractions). *Koks i Khimiya*, No. 2 (1966) 43–46 — on SE-301 at 80°; more detailed information about a substantial part of the problem was given before by J. JANÁK AND K. VOJTOVIČ in *Chem. Prumysl*, 8 (1958) 127.

KRÖGER, C. AND BURGOS, F.: Steinkohlenhydrierung mit α-Pipecolin. II. Die Natur der Hydrierprodukte. *Brennstoff-Chem.*, 47 (1966) 47–52.

LIST, G. R., HOFFMANN, R. L. AND EVANS, C. D.: Gas–solid chromatography of hydrocarbons on activated alumina. *J. Am. Oil. Chemists' Soc.*, 42, No. 3 (1965) 116A.

MEIER ZU KÖCKER, H.: Unverbrennte Pyrolyse-Kohlenwasserstoffe in den Verbrennungsgasen eines Ölvergasungsbrenner. *Brennstoff-Chem.*, 47 (1966) 13–21 — GC of tar oil I–III; a combination with paper chromatography, I.R. and U.V. spectroscopy.

MIYAKE, H. AND MITOOKA, M.: Gas chromatographic analysis of aromatic hydrocarbons from C_6 through C_{10}. *Bull. Japan Petrol. Inst.*, 5 (1963) 58–64.

SZYNAGEL, P. AND JEDRYCHOWSKA, M.: (Chromatographic analysis of 1-olefins in the products of thermal cracking of paraffin and slack wax). *Chem. Anal. (Warsaw)*, 11 (1966) 83–89 — retention data of C_5–C_{17} *n*-paraffins and 1-olefins on β,β′-oxydipropionitrile at 50° and 70° and on Apiezon L at 100° and 150°.

5c. Halogen derivatives of hydrocarbons

GOL'DINOV, A. L., PANSHIN, YU. A., PURETSKAYA, N. A. AND SOBOLEVA, G. I.: (Chromatographic determination of trifluoroethylene and tetrafluoroethylene). *Zavodsk. Lab.*, 32 (1966) 288–289 — trifluoroethylene is sorbed on a molecular sieve 4 A column while tetrafluoroethylene is eluted.

HANDRICK, K.: Herstellung und Verwendung von Chlormethylaromaten. *Erdöl Kohle*, 19 (1966) 172–177.

MOGER, G. G. AND SERGEEV, G. B.: (Gas-chromatographic analysis of the products of low-temperature bromination and hydrobromination of olefins). *Vestn. Mosk. Univ., Ser. II, Khim.*, No. 2 (1963) 14–16.

SOROCHKINA, R. M. AND LAZARIS, A. YA.: (Qualitative and quantitative determination of impurities in vinylidene chloride). *Zh. Analit. Khim.*, 21 (1966) 248–250 — vinyl chloride, *cis*- and *trans*-1,2-dichloroethylene, 1,1,2-trichloroethylene and 1,2-dichloroethane on PEGA at 85°.

6. ALCOHOLS

SINGER, D. D.: The analysis and composition of potable spirits: Determination of C_3, C_4 and C_5 alcohols in whisky and brandy by direct gas chromatography. *Analyst*, 91 (1966) 127–134 — not the total higher alcohol content but the proportions in which the different alcohols occur are characteristic of the type of spirituous beverage.

7. PHENOLS

GOREN-STRULS, S., KLEIJN, H. F. W. AND MOSTAERT, A. E.: Identification and determination of phenols and chlorophenols in very dilute aqueous solutions by gas–liquid chromatography, paper chromatography and spectrophotometry. *Anal. Chim. Acta*, 34 (1966) 322–336 — retention data of 12 phenols on xylenyl phosphate at 130°.

OAKLEY, E. T., MILLHAM, J. O. AND WEISSBECKER, L.: Empirical correlation of two methods for phenols in cigarette smoke. *Anal. Chim. Acta*, 31 (1964) 272–278 — steam-distilled phenols on Castorwax at 175°.

SHAPOSHNIKOV, YU. K., KOSYUKOVA, L. V. AND VODZINSKIĬ, YU. V.: (Gas-chromatographic analysis of monobasic phenols). *Gidrolizn. i Lesokhim. Prom.*, No. 1 (1966) 21 — C_6–C_{10} phenols on PFMS-4.

8. SUBSTANCES CONTAINING HETEROCYCLIC OXYGEN

VANETTEN, R. L. AND BOTTINI, A. T.: Separation of *cis*- and *trans*-alkyl substituted oxiranes and aziridines. *J. Chromatog.*, 21 (1966) 408–412 — retention data of fourteen 2,3-dialkyloxiranes and six 1,2,3-trialkylaziridines on four column packings at 136–175°.

9. OXO COMPOUNDS

CESARI, A., CUSMANO, A. M. AND BONIFORTI, L.: Gas-chromatographic determination of 3-hydroxybutan-2-one (acetoin) and biacetyl in vinegar. *Boll. Lab. Chim. Provinciali (Bologna)*, 15 (1964) 444–450 — on Hallcomid M 18 at 135°.

TER HEIDE, R.: Gaschromatographie ringhomologer Cyclanone. *Z. Anal. Chem.*, 216 (1966) 412–416 — retention indices I on Apiezon L and Carbowax-4000-monostearate at 150°, $\Delta I/10°$ and ΔI at 150° for C_5–C_{17} cyclanones.

11. ORGANIC ACIDS AND LIPIDS

11a. Organic acids

BALANDINA, V. A. AND KLESHCHEVA, E. V.: (Analysis of vinyl butyrate by a gas chromatographic method). *Zh. Anal. Khim.*, 21 (1966) 243–245 — on decyl sebacate with sebacic acid at 135°.

NOBE, B. AND STANLEY, R. L.: Gas chromatographic determination of sodium benzoate in meat. *J. Assoc. Offic. Agr. Chemists*, 48 (1965) 791–793.

PROCHÁZKOVÁ, V., BENEŠ, J. AND VEREŠ, K.: Separation of malonic acid esters by gas chromatography. *J. Chromatog.*, 21 (1966) 402–407 — a structural study; retention data of 20 esters on Reoplex 400 and Apiezon M at 108° and 135°.

VAN DER VEN, B.: Detection of γ- and δ-oxo-acids in butter fat. *Rec. Trav. Chim.*, 83 (1964) 976–982 — esters on Apiezon L at 190° and lactones on silicone oil at 188°.

11b. Lipids and their constituents

CHIOFFI, V. AND MAGON, G.: (Gas-chromatographic analysis of demargarination pastes from olive press residue oil. Comparison with lard composition). *Boll. Lab. Chim. Provinciali (Bologna)*, 15 (1964) 466–481 — on EGS at 210°.

GUNDSTONE, F. D. AND SUBBARAO, R.: The occurrence of linolenic acid and stearolic acid in *Santalum acuminatum* seed oil. *Chem. Ind. (London)*, (1966) 461–462.

LITCHFIELD, C., HARLOW, R. D. AND REISER, R.: Quantitative gas–liquid chromatography of triglycerides. *J. Am. Oil Chemists' Soc.*, 42, No. 3 (1965) 132A.

LOTTI, G.: Analytical characteristics of oils of legumes. *Ric. Sci., Rend., Sez. B*, 4 (1964) 503–510.

METCALFE, L. D., SCHMITZ, A. A. AND PELKA, J. R.: Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Anal. Chem.*, 38 (1966) 514–515 — BF_3–methanol transesterification technique used.

MOORE, J. H. AND WILLIAMS, D. L.: Studies on the composition and structure of the phosphatidylcholine, phosphatidylethanolamine and triglyceride isolated from rabbit liver. *Biochim. Biophys. Acta*, 98 (1965) 137–150.

MORRIS, L. J. AND MARSHALL, M. O.: Occurrence of stearolic acid in *Santaluceae* seed oils. *Chem. Ind. (London)*, (1966) 460–461.

MORRISSON, W. R. AND SMITH, L. M.: Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride–methanol. *J. Lipid Res.*, 5 (1964) 600–608.

MUNTONI, F., TISCORNIA, E. AND DE GIULI, G.: Gas chromatography in cereal chemistry. III. Identification of fats used in the manufacture of seasoned bread. *Boll. Lab. Chim. Provinciali (Bologna)*, 15 (1964) 427–443.

NAKAE, T. AND ELLIOT, J. A.: Volatile fatty acids produced by some lactic acid bacteria. I. Factors influencing production of volatile fatty acids from casein hydrolysate. *J. Dairy Sci.*, 48 (1965) 287–292.

NAKANISHI, T. AND WATANABE, K.: (Studies on fatty acids composition of meat tissue lipids. Part II. Fatty acid composition, from C_1–C_{12} of meat tissue lipids). *J. Agr. Chem. Soc. Japan*, 38 (1964) 525–530.

RACCAGNI, S.: (Gas chromatographic examination of higher fatty alcohols). *Riv. Ital. Sostanze Grasse*, 41 (1964) 459–462 — C_6–C_{18} fatty alcohols on Apiezon L at 220°.

12. ORGANIC PEROXIDES

LADYCHIK, B. YA. AND SARAEVA, V. V.: (Chromatographic determination of dimethylperoxide). *Zavodsk. Lab.*, 32 (1966) 153.

13. STEROIDS

FRAVOLINE, A. AND BEGLIOMINI, A.: (Gas-chromatographic determination of stilboestrol in feeding-stuffs). *Igiene Sanita Pubblica (Rome)*, 20 (1964) 436–443 — on SE-30 at 200°.

LEHNERT, G., MÜCKE, W. AND VALENTIN, H.: Gas-chromatographische Analyse von 11-Deoxy-17-oxosteroiden in dem Urine und Resultate für menschlichen Harn. *Endokrinologie*, 46 (1964) 241–246 — on 2,2-dimethylpropane-1,3-diol succinate at 180°.

LEHNERT, G., VALENTIN, H. AND MÜCKE, W.: Gas chromatographische Analyse von Steroidhormonen in Harn und Plasma. *Med. Welt.*, No. 28 (1964) 1489–1494 and 1499–1500.
NISHIOKA, I., IKEKAWA, N., YAGI, A., KAWASAKI, T. AND TSUKAMOTO, T.: Studies on the plant sterols and triterpenes. II. Separation of stigmasterol, β-sitosterol and campesterol, and about so-called "γ-sitosterol". *Chem. Pharm. Bull. (Tokyo)*, 13 (1965) 379–384.
VAN DER MOLEN, H. J., RUNNEBAUM, B., NISHIZAWA, E. E., KRISTENSEN, E., KIRSCHBAUM, T., WIEST, W. G. AND EIK-NES, K. B.: On the presence of progesterone in blood plasma from normal women. *J. Clin. Endocrinol. Metab.*, 25 (1965) 170–176.

15. TERPENE DERIVATIVES

LISHTVANOVA, L. N. AND GORYAEV, M. I.: (Gas–liquid chromatography of camphor plant products). *Gidrolizn. i Lesokhim. Prom.*, No. 1 (1966) 19 — on lanoline.
NIGAM, I. C. AND LEVI, L.: Essential oils and their constituents. XX. Detection and estimation of menthofuran in *Mentha arvensis* and other mint species by coupled gas-liquid — thin-layer chromatography. *J. Pharm. Sci.*, 53 (1964) 1008–1013 — on SAIB (sucrose diacetate hexaisobutyrate) at 170°.
NIGAM, I. C. AND LEVI, L.: Essential oils and their constituents XXVI. Rearrangement of caryophyllene oxide during gas chromatography. *J. Org. Chem.*, 30 (1965) 653–654.
OHLOFF, G. AND UHDE, G.: Absolute Konfiguration von Terpineol-4. *Helv. Chim. Acta*, 48 (1965) 10–28.

17. AMINES, AMIDES AND RELATED NITROGEN COMPOUNDS

WAHLROOS, Ö. AND SAARIVIRTA, M.: Benzyl cyanide, benzyl thiocyanate and benzyl isothiocyanate: gas-chromatographic data and determination. *Acta Chem. Scand.*, 18 (1964) 2191–2193 — on butanediol succinate polyester at 190°.

18. AMINO ACIDS

HALPERN, B., RICKS, J. AND WESTLEY, J. W.: Biochemical application of gas–liquid chromatography: The stereospecific hydrolytic action of acylase I (hog kidney). *Anal. Biochem.*, 14 (1966) 156–159 — on DEGS/EGSS-X at 110°.
VITT, S. V., SAPOROVSKAYA, M. B. AND BELIKOV, V. M.: (On the analysis of amino acids by gas–liquid chromatography). *Zh. Anal. Khim.*, 21 (1966) 227–231 — retention data of *n*-butyl and fluorobutyl esters of N-trifluoroacetyl acids on Apiezon L at 120°.

23. OTHER SUBSTANCES CONTAINING HETEROCYCLIC NITROGEN

CAMY-PALOU, J., PARROT, J. AND PATY, M. M.: Étude par chromatographie en phase gazeuse d'amides dérivant de composés hétérocycliques azotés saturés. *Bull. Soc. Chim. France*, (1966) 493 — N-acetyl- and N-halogen-acetyl-piperidines on DC 200.

26. METALLO-ORGANIC COMPOUNDS

KRAMER, K.: Gaschromatographische Bestimmung der Bleialkyle in Ottokraftstoffen mit dem Elektroneneinfangdetektor. *Erdöl Kohle*, 19 (1966) 182–185 — Apiezon L at 120°.
LEONHARDT, W. S., MORRISON, R. C. AND KAMIENSKI, C. W.: Gas chromatographic method for quantitative determination of vinyllithium. *Anal. Chem.*, 38 (1966) 466–468 — vinyllithium is hydrolysed to ethylene on a polyol precolumn; on a second GC apparatus acetylene and 1,3-butadiene are separated from evolved ethylene.
MORIE, G. P. AND SWEET, T. R.: Determination of aluminium and iron by solvent extraction and gas chromatography. *Anal. Chim. Acta*, 34 (1966) 314–321 — as trifluoroacetylacetonate on DC-550 on glass micro beads at 128°.

27. VITAMINS

MURRAY, T. K., DAY, K. C. AND KOLICEK, E.: The differentiation and assay of vitamins D_2 and D_3 by gas–liquid chromatography. *Biochem. J.*, 96, No. 2 (1965) 29P–30P.

29. INSECTICIDES AND OTHER PESTICIDES

MATTSON, A. M., KAHRS, R. A. AND SCHNELLER, J.: Use of microcoulometric gas chromatograph for triazine herbicides. *J. Agr. Food Chem.*, 13 (1965) 120–122.

31. PLASTICS AND THEIR INTERMEDIATES

JEDLIŃSKI, Z. AND HIPPE, Z.: (γ-Radiation induces chemical changes in polyvinyl butyral). *Roczniki Chem.*, 40 (1966) 257–264 — pyrolytic degradation products (at 1500°) on E-301 and Desmophen 800.

LIDDICOET, T. H. AND SMITHSON, L. H.: Analysis of surfactants using pyrolysis–gas chromatography. *J. Am. Oil Chemists' Soc.*, 42, No. 3 (1965) 114A.

MASHIKO, Y. I., ACHIZEN, A. AND NAKAO, A.: (Gas chromatographic rapid analysis of polymers). *J. Chem. Soc. Japan, Ind. Chem. Sect.*, 68 (1965) 1206–1209.

MATHIAS, A. AND MELLOR, N.: Analysis of alkylene oxide polymers by nuclear magnetic resonance spectrometry and by gas–liquid chromatography. *Anal. Chem.*, 38 (1966) 472–477 — polymers are split with HBr and the brominated product is analysed by GC.

32. PHARMACEUTICAL AND FORENSIC APPLICATIONS; METABOLISM OF DRUGS

KOEHLER, H. M. AND HEFFERREN, J. J.: Gas liquid chromatography of local anesthetics and related compounds. *J. Oral Therap. Pharmacol.*, 1 (1965) 539.

MAKLEIT, J. K., RETHY, A. AND RICHTER, P.: Thin layer and gas chromatographic analysis of syphilis antigens. *Acta Microbiol. Acad. Sci. Hung.*, 11 (1965) 341–350.

33. INORGANIC SUBSTANCES

33a. Permanent and rare gases

LASA, J.: (Detection of the fixed gases in gas chromatography by radioionization methods). *Chem. Anal. (Warsaw)*, 11 (1966) 151–157 — sensitivity limits for He, Ne, H_2, O_2, N_2, CH_4, CO, and CO_2 given.

MONKMAN, J. L.: Analysis of compressed air by gas chromatography. *Occupational Health Rev.*, 16 (1964) 17–21.

ZIELIŃSKI, E.: (Determination of argon in presence of oxygen by gas chromatography). *Chem. Anal. (Warsaw)*, 11 (1966) 67–70 — on a mixture of molecular sieves 4A and 5A; very incomplete references about this question.

33b. Volatile inorganic compounds

LEMMICH, E. AND GJALDBÆK, J. C.: Analysis of anaesthetics by gas chromatography. III. Nitrous oxide. *Dansk. Tidsskr. Farm.*, 38 (1964) 193–203 — CO, O_2, and N_2 on molecular sieve 5A at 30°, H_2O on PEG 1500 at 75° and CO_2 on dimethyl sulphoxide at —11°.

VIRIN, M. I., LYUDMER, G. V. AND DZHAGATSPANYAN, R. V.: (Gas-chromatographic determination of impurities in hydrogen chloride). *Zavodsk. Lab.*, 32 (1966) 152 — on tricresyl phosphate at 80°.

34. RADIOACTIVE AND OTHER ISOTOPIC COMPOUNDS

SCHULZE, H. W. AND BOTHE, H. K.: Gas-chromatographische Analyse radioaktiv markierter Stoffe. *Atomkernenergie*, 9 (1964) 363–369 — ^3H- and ^{14}C-labelled organic compounds are detected by a Geiger-Müller flow tube.

35. MISCELLANEOUS COMPOUNDS AND COMPLEX MIXTURES

KINGSTON, B. H.: The interpretation and value of gas liquid chromatography (GLC) data. *Am. Perfumer Cosmet.*, 79, No. 11 (1964) 50–52.

KOCH, J. AND SCHILLER, H.: Identifizierung von Substanzen des Apfelaromas. *Z. Lebensm. Untersuch. Forsch.*, 125 (1964) 364–368 — on DEGS at 100°.

LARSON, B. T.: Gas chromatography of organic volatiles in human breath and saliva. *Acta Chem. Scand.*, 19 (1965) 159–164.

NISHIMOTO, T. AND UYETA, M.: Gas-chromatographic analysis of food additives. I. *J. Food Hyg. Soc. Japan*, 5 (1964) 287–293 — 13 antioxidants on DEGS, 2,2-dimethylpropane-1,3-diol succinate polyester, Tween 80, and SE-30 and QF-1.

Errata

J. Chromatog., 21 (1966) 119–121

Page 119. In eqn. (1) "$r = 1$" should read "$r - 1$".
The correct equation is as follows:

$$P_{n,r} = Q_{n,r} \cdot \prod_{j=0}^{r-1} R_j.$$

Page 121, Table III. The data "Strychnine 0.77 0.021 32.3 $1.00 \cdot 10^{-8}$" should be moved one line up.

J. Chromatog., 21 (1966) 381

Section 24, OBOLENTSEV, R. D. *et al.*, 2nd and 3rd lines:
For "*Khimiya*, (1964) 243–252" read "*Khim. Seraorgan. Soedin., Soderzhashch. v Neft. i Nefteprod., Akad. Nauk SSSR, Bashkirsk. Filial*, 7 (1964) 243–252".

J. Chromatog., 22 (1966) 139–142

Page 139, first paragraph, 3rd line: "The latter concluded..." should read "GRUNZE concluded...".

Page 142, Fig. 3. The peak labelled "D" was not identified as tetrametaphosphate but may reasonably be assumed to correspond to tetrapolyphosphate.

TERPENOIDS

LXXXV. EVALUATION OF POLYESTERS AS STATIONARY PHASES IN GLC COLUMNS*

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In previous communications[1] on the evaluation of the polyesters as stationary phases we have reported the results of the investigation on polyesters derived from different 1,ω-dicarboxylic acids, HOOC—$(CH_2)_n$—COOH, ranging from malonic acid (n = 1) to 1,16-hexadecanedicarboxylic acid (n = 16) with diethylene glycol.

It was observed that the number of methylene groups in the acidic fragment of the polyester has a profound influence on the retention time, on the basis of which a clear arithematical relationship was developed. Retention time was found to be an integral characteristic of the number of methylene groups between the two groups of the acidic fragment of the polyester.

It was then felt desirable to determine whether a similar relationship would hold good if a change was brought about in the glycol fragment of the polyester, keeping the acid fragment unchanged. The present work deals with this investigation and other related matters.

A series of polyesters were synthesised from adipic acid (constant acid fragment) and 1,ω-glycols, HO—CH_2—$(CH_2)_n$—CH_2—OH, ranging from ethylene glycol to 1,15-pentadecanediol.

In this series of polyesters, it was observed that a change in the number of methylene groups in the glycolic fragment also similarly influenced the degree of separation of compounds in GLC analyses. In order to have a clear idea of the behaviour of these polyesters, three more series of polyesters were synthesised.

(I) Ethylene glycol (constant) and succinic acid, adipic acid, sebacic acid.

(II) 1,5-Pentanediol (constant) and succinic acid, adipic acid and sebacic acid.

(III) Hexadecane-1,16-dicarboxylic acid (constant) and 1,4-butanediol, 1,10-decanediol, and 1,15-pentadecanediol.

Several terpenic and a few non-terpenic compounds were analysed on GLC columns using these polyesters as stationary phases with very interesting results which are dealt with in the discussion.

EXPERIMENTAL

The modified Griffin & George Model MK-II VPC apparatus has been used as described in our earlier communication[1].

* Communication No. 873 from the National Chemical Laboratory, Poona-8, India.

Preparation and general properties of the polyesters

The preparation was carried out according to the previously described procedure[1], with minor modifications*. The polyester derived from ethylene glycol and adipic acid is a viscous liquid, while that derived from 1,4-butanediol and adipic acid is a semi-solid; the others obtained from 1,5-pentanediol onwards up to 1,15-pentadecanediol and adipic acid are low-melting solids. Polyesters derived from hexadecane-1,16-dicarboxylic acid, as well as sebacic acid, are all low-melting solids.

The approximate molecular weight determinations of some of the polyesters were carried out by end group titrations in alcoholic solution. The results are shown in Table I.

TABLE I

MOLECULAR WEIGHTS OF THE POLYESTERS AS DETERMINED BY THE END GROUP TITRATIONS

Components of the polyester		*Molecular weights*
Acid	*Glycol*	
Adipic acid	Ethyleneglycol	433, 420
Adipic acid	1,3-Propanediol	442, 466
Adipic acid	1,4-Butanediol	1021, 978
Adipic acid	1,5-Pentanediol	—
Adipic acid	1,6-Hexanediol	7563, 6730
Adipic acid	1,9-Nonanediol	insoluble
Adipic acid	1,10-Decanediol	insoluble
Adipic acid	1,13-Tridecanediol	insoluble
Adipic acid	1,15-Pentadecanediol	insoluble
Succinic acid	Ethyleneglycol	333, 286
Adipic acid	Ethyleneglycol	433, 420
Sebacic acid	Ethyleneglycol	1138, 1008
Hexadecane-1,16-dicarboxylic acid	1,4-Butanediol	insoluble
Hexadecane-1,16-dicarboxylic acid	1,10-Decanediol	insoluble
Hexadecane-1,16-dicarboxylic acid	1,15-Pentadecanediol	insoluble
Succinic acid	1,5-Pentanediol	insoluble
Adipic acid	1,5-Pentanediol	insoluble
Sebacic acid	1,5-Pentanediol	insoluble

The following terpenic and non-terpenic samples were employed for the comparative evaluation of the polyesters:

(1) α-Pinene, (2) β-pinene, (3) Δ^3-carene, (4) limonene, (5) tricyclene, (6) camphene, (7) 1:8-cineole, (8) *p*-cymene, (9) cyclohexane, (10) methyl alcohol and (11) ethyl alcohol.

Monoterpenic oxygenated compounds. (1) α-Terpineol, (2) linalool, (3) *l*-borneol, (4) isoborneol, (5) menthol, (6) isopulegol, (7) camphor, (8) carvone, (9) bornyl acetate.

Sesquiterpenic hydrocarbons. (1) Tetrahydroelemene, (2) β-selinene, (3) caryophyllene, (4) humulene, (5) longifolene, (6) longicyclene, (7) cyclopentadecane.

* The temperature for the ester formation in the present case is 145–150° as against 135–140° employed previously, as this slightly higher temperature was necessary to ensure homogeneity of the reaction mass.

All the samples of the dicarboxylic acids were obtained from the laboratory stock and the 1,ω-glycols, wherever necessary, were synthesised by the known procedures available in the literature[2], mainly by the reduction of the respective diesters with lithium aluminium hydride.

As usual, their purity was ascertained by m.p./b.p., elemental analyses and also GLC analyses in the case of volatile samples.

Impregnation and filling

These were performed as described previously[1].

RESULTS AND DISCUSSION

The weight of the column liquid was 1.980 g in all the cases. Retention times of the above mentioned compounds were recorded as before[1].

A column temperature of 80° was used for monoterpenic hydrocarbons and 163–164° was used for sesquiterpenic hydrocarbons and monoterpenic oxygenated compounds. Results are recorded in Tables II and III.

The arithmetical relationship

$$\frac{t_{R^x} - t_{R^y}}{x - y} = C$$

observed in our previous series of polyesters, was applied in the case of the 1,ω-glycols and adipic acid polyester series. The results are recorded in Tables IV A and B. It was clear that the linear relationship was valid for the present series as well. The arithmetical pattern of the relationship of the retention time has been further indicated in Table V, in which the average ratio of the retention time for six monoterpenes and six sesquiterpenes on different polyesters have been shown against the expected values. It can be seen that the maximum deviation is about 9 %. When the retention times of these monoterpenic and sesquiterpenic hydrocarbons are plotted against the number of intervening methylene groups in the alcoholic fragment of the polyester, fairly straight lines are obtained (Figs. 1a and b).

TABLE II

RETENTION TIME IN MINUTES AT 80°; ACIDIC COMPONENT (ADIPIC ACID) CONSTANT

Compound	*Number of methylene groups in the glycolic fragment of the polyester*					
	2	*3*	*4*	*5*	*6*	*9*
α-Pinene	2.95	4.10	5.47	6.35	8.30	11.9
β-Pinene	5.05	7.23	9.50	10.75	14.35	19.5
Δ^3-Carene	6.70	9.08	12.10	13.86	18.10	25.5
Limonene	9.13	12.70	17.00	19.26	25.3	35.0
Tricyclene	2.833	3.93	5.10	5.75	8.20	10.6
Camphene	4.280	5.80	7.50	8.66	11.10	15.3
p-Cymene	15.25	20.000	26.0	28.40	37.0	41.3
Cyclohexane	0.366	0.460	0.66	0.666	1.00	1.40
Methyl alcohol	1.366	—	1.42	1.50	1.15	1.10
Ethyl alcohol	1.783	—	2.10	2.00	1.70	1.60
1:8-Cineole	13.70	—	22.1	24.0	27.6	33.9

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TABLE III

RETENTION TIME IN MINUTES AT 163–164°: ACIDIC COMPONENT (ADIPIC ACID) CONSTANT

Compound	*Silicone*	*Apiezon*	*Number of methylene groups in the glycolic fragment of the polyester*								
			2	*3*	*4*	*5*	*6*	*9*	*10*	*13*	*15*
α-Terpineol	4.30	4.80	7.55	9.20	9.20	10.60	—	12.00	—	12.40	11.75
Linalool	2.53	3.70	3.40	4.00	4.15	4.95	4.50	4.60	4.75	4.90	4.60
Borneol	3.95	9.20	6.75	8.75	8.60	9.70	10.40	10.93	11.06	11.45	10.75
Isoborneol	3.70	3.47	5.90	7.33	7.30	8.95	—	9.75	10.25	10.00	9.75
l-Menthol	3.86	9.33	5.20	7.00	7.15	8.17	8.80	9.66	9.86	9.75	10.00
Camphor	3.35	7.75	4.70	5.40	5.50	6.10	6.80	6.85	7.10	7.35	7.00
Carvone	5.37	14.10	10.00	11.57	11.50	12.80	13.60	15.10	14.80	13.80	13.50
Isopulegol	3.66	10.00	4.80	5.86	6.0	6.87	7.93	9.10	8.30	8.13	9.40
Bornyl acetate	6.10	13.60	5.00	5.80	6.20	7.00	8.25	9.77	9.83	9.81	9.70
Tetrahydroelemene	10.90	27.35	3.40	4.05	4.90	6.60	8.10	11.50	12.70	15.13	14.65
β-Selinene	16.13	45.50	8.0	9.63	11.35	15.00	17.80	23.90	26.13	29.56	28.75
Longifolene	11.65	33.00	4.66	5.53	6.93	8.70	10.80	15.00	16.00	19.16	18.60
Caryophyllene	12.66	34.00	5.40	6.00	7.43	9.60	10.90	15.90	16.90	19.26	19.10
Humulene	14.30	30.50	6.93	8.17	9.90	12.25	15.00	21.13	21.85	25.41	26.00
Longicyclene	10.33	32.80	3.50	4.40	5.26	6.75	9.00	11.80	13.00	14.15	15.40
Cyclopentadecane	27.60	—	7.25	10.00	12.40	18.00	22.00	31.56	33.83	43.50	—

TABLE IVA

EVALUATION OF C AT 80°

$t_R^x - t_R^y$	$x - y$	C	$t_R^x - t_R^y$	$x - y$	C
α-Pinene			*β-Pinene*		
8.95	9 — 2 = 7	1.26	14.45	9 — 2 = 7	2.06
7.80	9 — 3 = 6	1.30	12.27	9 — 3 = 6	2.05
6.43	9 — 4 = 5	1.29	10.00	9 — 4 = 5	2.00
Δ³-Carene			*Limonene*		
18.80	9 — 2 = 7	2.70	25.87	9 — 2 = 7	3.70
16.42	9 — 3 = 6	2.74	22.30	9 — 3 = 6	3.71
13.40	9 — 4 = 5	2.68	18.00	9 — 4 = 5	3.60
Tricyclene			*Camphene*		
7.77	9 — 2 = 7	1.11	11.02	9 — 2 = 7	1.57
6.67	9 — 3 = 6	1.11	9.50	9 — 3 = 6	1.58
5.50	9 — 4 = 5	1.10	7.80	9 — 4 = 5	1.56
Cyclohexane					
1.034	9 — 2 = 7	0.148			
0.940	9 — 3 = 6	0.157			
0.740	9 — 4 = 5	0.148			

TABLE IVB

EVALUATION OF C AT 163–164°

$t_R^x - t_R^y$	$x - y$	C	$t_R^x - t_R^y$	$x - y$	C
Tetrahydroelemene			*β-Selinene*		
9.30	10 — 2 = 8	1.16	18.15	10 — 2 = 8	2.27
8.65	10 — 3 = 7	1.23	16.52	10 — 3 = 7	2.36
7.80	10 — 4 = 6	1.30	14.80	10 — 4 = 6	2.46
6.11	10 — 5 = 5	1.22	11.14	10 — 5 = 5	2.23
Longifolene			*Caryophyllene*		
12.36	10 — 2 = 8	1.54	11.50	10 — 2 = 8	1.45
10.47	10 — 3 = 7	1.49	10.90	10 — 3 = 7	1.56
9.07	10 — 4 = 6	1.51	9.47	10 — 4 = 6	1.58
7.30	10 — 5 = 5	1.46	7.30	10 — 5 = 5	1.46
Humulene			*Longicyclene*		
14.92	10 — 2 = 8	1.87	9.50	10 — 2 = 8	1.33
13.68	10 — 3 = 7	1.95	8.60	10 — 3 = 7	1.23
11.95	10 — 4 = 6	1.99	7.74	10 — 4 = 6	1.29
9.63	10 — 5 = 5	1.92	6.25	10 — 5 = 5	1.25

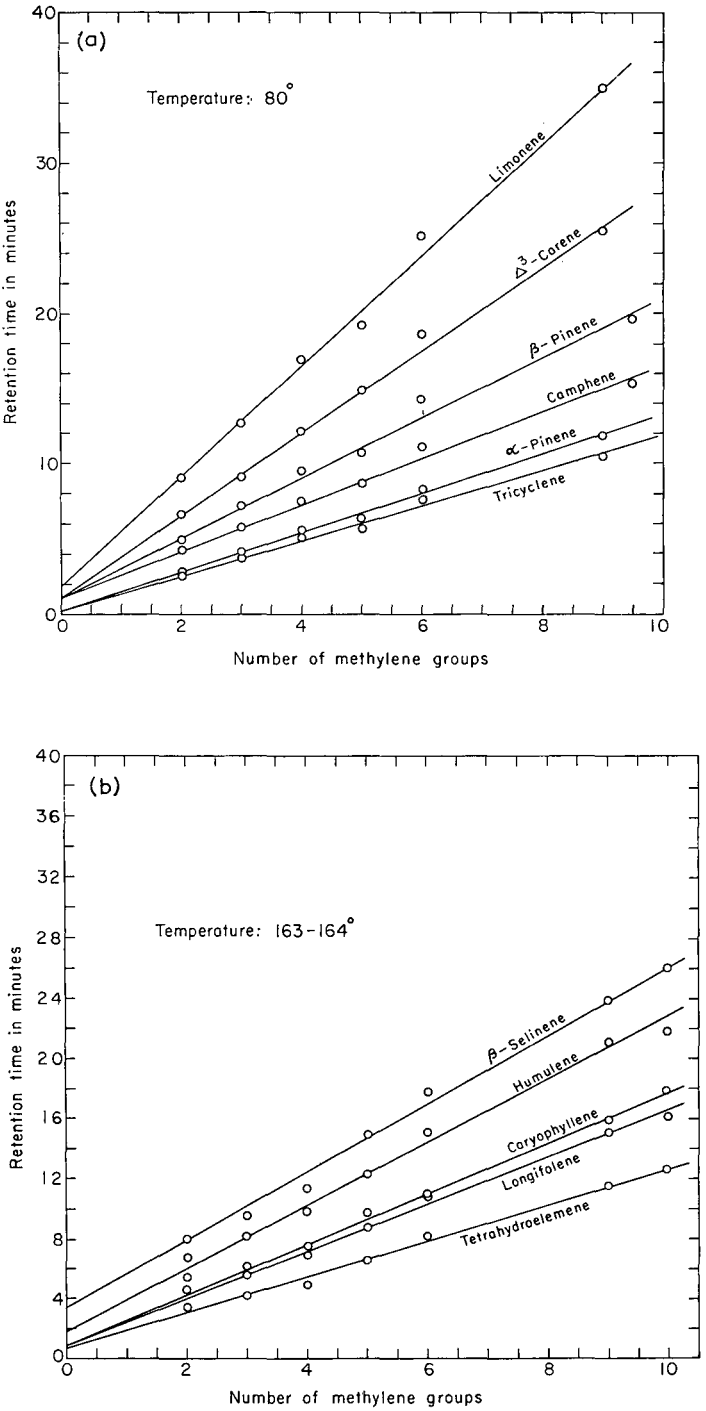

Fig. 1. Number of methylene groups in the glycolic fragment of the polyester (keeping adipic acid constant) *vs.* retention time. (a) Monoterpenes at 80°; (b) sesquiterpenes at 163–164°.

TABLE V

RELATIONSHIP BETWEEN THE RATIO OF THE NUMBER OF METHYLENE GROUPS AND THE RATIO OF THE RETENTION TIMES IN THE RESPECTIVE SUBSTRATES

Ratio of the number of methylene groups	*Average ratio of observed retention times at 163°*	*Calculated ratio*	*Error (%)*	*Average ratio of observed retention times at 80°*	*Calculated ratio*	*Error (%)*
10/4	2.36	2.50	5.6	—	—	—
10/6	1.50	1.66	9.6	—	—	—
9/4	2.19	2.25	3	2.09	2.26	7
9/6	1.42	1.50	5	1.37	1.50	8
6/3	1.91	2.0	5	1.99	2.0	<1
6/4	1.58	1.5	5	1.51	1.50	<1
4/3	1.21	1.33	9	1.31	1.33	2
3/2	—	—	—	1.39	1.50	7

In this series it appears that the linearity breaks down near the 1,10-decanediol–adipic acid polyester. This is similar to previous observations in the case of the diethylene glycol–1,ω-dicarboxylic acid polyesters series, where the linearity breaks at the brassylic acid polyester, which contains eleven methylene groups in the acid chain.

In order to confirm the deviation of the constant C and its root cause, some more polyesters were tested under exactly the same conditions as before.

Ethylene glycol (constant) and succinic acid, adipic acid, sebacic acid polyesters

The retention time data recorded on the above mentioned columns are tabulated in Table VI.

TABLE VI

RETENTION TIME IN MINUTES: GLYCOLIC FRAGMENT (ETHYLENE GLYCOL) CONSTANT

Compound	*Number of methylene groups in the acidic fragment of the polyester*		
	8	*4*	*2*
α-Terpineol	3.80	7.55	1.066
Linalool	4.70	3.40	2.00
l-Borneol	10.70	6.75	4.40
Isoborneol	—	5.90	0.700
l-Menthol	—	5.20	3.20
Camphor	6.80	4.70	3.10
Carvone	14.10	10.0	6.85
Isopulegol	8.30	4.80	3.0
Bornyl acetate	8.60	5.0	2.83
Tetrahydroelemene	9.00	3.40	1.83
β-Selinene	19.83	8.0	3.90
Longifolene	11.66	4.66	2.20
Caryophyllene	12.10	5.40	2.50
Humulene	16.30	6.93	3.40
Longicyclene	9.60	—	2.20
Cyclopentadecane		7.25	3.50

Calculation of the average ratio of the retention times of sesquiterpenic hydrocarbons on sebacic acid polyester and succinic acid polyester shows that the ratio is 5.083 instead of 4, and in the case of adipic acid polyester and succinic acid polyester, it is 2.42 instead of 2.0. However, it is interesting to note that in both cases the values are almost exactly 25 % higher when compared to the expected values, and the ratio of the observed and the expected values is nearly constant, though not in the form of a multiple of unity. This can be attributed to the difference in the nature of the glycols (diethylene glycol in the previous series and ethylene glycol in this series). This probably indicates that only diethylene glycol, having ethereal oxygen is responsible for the exact unitary nature of the arithmetical relationship in the polyester series prepared from 1,ω-dicarboxylic acids and diethylene glycol. This, however, is merely incidental.

1,5-Pentanediol (constant) and succinic acid, adipic acid, sebacic acid

The retention time data recorded on the above mentioned columns are tabulated in Table VII.

In this case, calculation of the average ratio of the retention times of the same samples of hydrocarbons on sebacic acid polyester and adipic acid polyester shows that the ratio is 1.45 instead of 2 and in the case of adipic acid polyester and succinic acid polyester it is 1.48 instead of 2. These values are nearly 26 % lower than the calculated value of 2.

TABLE VII

RETENTION TIME IN MINUTES AT 163–164°: GLYCOLIC FRAGMENT (1,5-PENTANEDIOL) CONSTANT

Compound	*Number of methylene groups in the acidic fragment of the polyester*		
	8	*4*	*2*
α-Terpineol	11.45	10.60	8.75
Linalool	5.23	4.95	3.90
l-Borneol	10.45	9.70	8.17
Isoborneol	8.66	8.95	7.17
l-Menthol	8.66	8.17	6.70
Camphor	6.40	6.10	5.40
Carvone	12.60	12.80	11.40
Isopulegol	8.50	6.87	5.60
Bornyl acetate	8.00	7.00	5.70
Tetrahydroelemene	9.56	6.66	4.40
β-Selinene	20.80	15.00	10.50
Longifolene	12.70	8.70	5.70
Caryophyllene	14.20	9.60	6.70
Humulene	18.00	12.25	8.70
Longicyclene	10.00	6.75	4.33
Cyclopentadecane	—	18.00	10.90

This transition from higher values in the case of the ethylene glycol and 1,ω-dicarboxylic acid series to lower values in the case of pentanediol polyester series is most probably connected with structural features associated with the chain length and conformational features of the glycol part. This aspect is receiving our special attention and will form the subject of a separate communication.

Polyester from long-chain dicarboxylic acids and long-chain glycols

After careful observation of the retention data in the case of above mentioned polyester series, it was felt necessary to investigate the behaviour of the polyesters synthesised from very-long-chain dicarboxylic acids and long-chain glycols. Therefore polyesters were prepared from hexadecane-1,16-dicarboxylic acid (constant) and 1,4-butanediol, 1,10-decanediol and 1,15-pentadecanediol. Retention time data are presented in Table VIII.

TABLE VIII

RETENTION TIME IN MINUTES: ACIDIC FRAGMENT (HEXADECANE-1,16-DICARBOXYLIC ACID) CONSTANT

Compound	*Number of methylene groups in the glycolic fragment of the polyester*		
	4	*10*	*15*
α-Terpineol	17.60	4.00	4.10
Linalool	7.66	4.60	4.83
l-Borneol	15.60	10.70	10.90
Isoborneol	13.50	2.50	2.60
l-Menthol	14.20	10.00	9.80
Camphor	9.20	6.90	7.30
Carvone	18.10	12.70	13.83
Isopulegol	11.50	8.10	7.93
Bornyl acetate	13.60	10.20	10.30
Tetrahydroelemene	17.81	16.40	19.00
β-Selinene	34.50	32.50	37.50
Longifolene	22.00	22.30	24.10
Caryophyllene	23.10	20.50	22.83
Humulene	29.20	27.00	30.10
Longicylene	18.00	18.50	19.50
Cyclopentadecane	—	—	—

Surprisingly it was found that there are some cases of a saturation point being reached in the retention times of hydrocarbons on these polyesters. While going from a polyester derived from 1,4-butanediol and hexadecane-1,16-dicarboxylic acid to a polyester derived from 1,15-pentadecanediol and the same dicarboxylic acid, it was observed that the retention times of hydrocarbons recorded on both these polyesters are almost the same, being only slightly higher in the latter case.

Comparative assessment of related factors

Relative retention time data* show that polyesters synthesised from the long chain, 1,ω-glycols and adipic acid exhibit very high solubilities for sesquiterpenic hydrocarbons. These results can also be conveniently used for the separation of monoterpenic oxygenated compounds and sesquiterpenic hydrocarbons.

The values of α the relative volatility, N the number of theoretical plates and s the separation factor have been calculated for three typical pairs of monoterpenic hydrocarbons according to PURNELL[3] and are presented in Table IX.

Thus a mixture of isopulegol and longifolene would require nearly 50,000 plates

* These can be easily calculated from the data presented in Table II and III, taking the value of camphor as unity.

TABLE IX

Components of the polyester	*Compound pair*	α	N	S
Adipic acid and ethylene glycol	Borneol and caryophyllene	1.250	1009	900
	Bornyl acetate and caryophyllene	1.08	7570	6560
	Isopulegol and longifolene	1.03	49840	42430
Adipic acid and 1,15-pentadecanediol	Borneol and caryophyllene	1.776	196.4	188.6
	Bornyl acetate and caryophyllene	1.969	154.8	148.6
	Isopulegol and longifolene	1.979	153.3	147.1
Hexadecane-1,16-dicarboxylic acid and 1,15-pentadecanediol	Borneol and caryophyllene	2.095	136.5	131.7
	Bornyl acetate and caryophyllene	2.217	123.7	119.5
	Isopulegol and longifolene	3.039	82.68	79.97
Silicone elastomer	Tetrahydroelemene and β-selinene	1.480	—	342.2
	Caryophyllene and humulene	1.129	—	2758
	Longifolene and longicyclene	1.128	—	2797
Adipic acid and 1,15-pentadecanediol	Tetrahydroelemene and β-selinene	1.963	—	149.5
	Caryophyllene and humulene	1.361	—	512.1
	Longifolene and longicyclene	1.208	—	1213

if it is to be resolved on a column of polyester synthesised from adipic acid and ethylene glycol, but the same mixtures can be resolved by only 83 plates if a column of polyester synthesised from hexadecane-1,16-dicarboxylic acid and 1,15-pentadecanediol is used. The case is similar with other pairs presented in Table IX.

This type of stationary phase, prepared from either long-chain 1,ω-glycols or long-chain dicarboxylic acids, seems to give a very nice and distinctive cut between the oxygenated compounds and hydrocarbons. At the same time these phases will also resolve the individual components. From Table IX, it is clear that the separation of tetrahydroelemene and β-selinene would require nearly 342 plates on a silicone column, but the same mixture can be separated by only 150 plates if it is chromatographed on a polyester synthesised from adipic acid and 1,15-pentadecanediol; and similarly with other pairs of hydrocarbons.

Calculation of N, the number of theoretical plates, in the case of capillary columns used to give very high values, but the same degree of separation was also attainable by packed columns. This anomaly was clearly explained by PURNELL[3]. The general equation given by him is as follows:

$$N = S\left[1 + 2\left(\frac{V_d}{V_R}\right) + \left(\frac{V_d}{V_R}\right)^2\right]$$

In this equation full allowance is made for the dead volume of the column, which becomes significant when the retention volume is very low compared to the dead volume.

In the present series of polyesters, it can be seen from the retention time data that, while going from ethylene glycol–adipic acid polyester to the last polyester,

the retention times of hydrocarbons are increased several fold. Similarly the retention times of oxygenated compounds are also increased by nearly a factor of two; consequently the effect of dead volume should be pronounced in the case of lower polyesters and negligible in the case of higher polyesters.

The values of N, S and α calculated for typical pairs of compounds (Table IX) indicate that there is not much difference in the values of N and S in the case of long-chain polyesters and this point would strongly favour the use of these polyesters as stationary phases in capillary columns.

When a graph of relative retention times (Fig. 2) (with respect to camphor) on 1,3-propanediol–adipic acid polyester is plotted against the relative retention times on 1,10-decanediol–adipic acid polyester, two distinct straight lines are obtained.

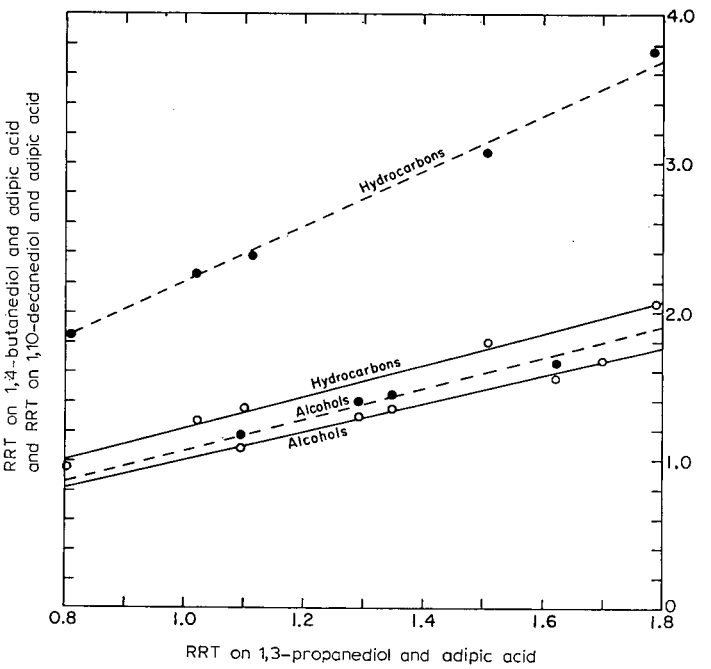

Fig. 2. O—O = 1,3-propanediol and adipic acid *vs.* 1,4-butanediol and adipic acid; ●—● = 1,3-propanediol and adipic acid *vs.* 1,10-decanediol and adipic acid.

One line represents the hydrocarbons and the other one represents the alcohols. As the number of methylene groups in the alcoholic fragment of the polyester goes on increasing, these lines diverge further apart. This clearly indicates that these two groups of compounds will be separated on the polyesters synthesised from long-chain glycols. If we examine relative retention time data obtained on ethylene glycol–adipic acid polyester and 1,15-pentadecanediol–adipic acid polyester, we find that there is no apparent loss in the resolution of the individual components of the oxygenated compounds, but at the same time the hydrocarbon group is well separated also with no loss in the resolution of the individual components of the hydrocarbon group.

SUMMARY

Polyesters from different 1,ω-glycols, ranging from ethylene glycol to 1,15-pentadecanediol, with adipic acid were prepared and evaluated as substrates for gas–liquid chromatography. Several terpenic and a few non-terpenic compounds were analysed. It was observed that the retention time is a characteristic of the number of methylene groups in the glycolic fragment of the polyester and, as before, follows a linear relationship, which starts deviating at 1,10-decanediol. The effect on retention times by using ethylene glycol and changing the acid fragment was also studied. Similarly, the effect on retention times by using 1,5-pentanediol (which has the same number of polyvalent atoms in the chain unit as diethylene glycol) and changing the acidic fragment was also examined. From the above mentioned observations, it was clear that the retention times of hydrocarbon-type compounds may be enormously increased by using a polyester derived from very-long-chain dicarboxylic acid and long-chain 1,ω-glycol. However, the retention times of hydrocarbons reach a certain saturation point above which their solubilities do not increase appreciably. Finally, the effect of dead volume of the column was discussed with respect to the case of long-chain as well as short-chain polyesters. Some of the stationary phases described in the paper are likely to be very useful for capillary columns and for preparative gas–liquid chromatography.

REFERENCES

1 B. V. BAPAT, B. B. GHATGE AND S. C. BHATTACHARYYA, *J. Chromatog.*, 18 (1965) 308; *ibid.*, 23 (1966) 227.
2 W. F. HUBER, *J. Am. Chem. Soc.*, 73 (1951) 2730.
3 J. H. PURNELL, *J. Chem. Soc.*, (1960) 1268.

RETENTION TIME RELATIONSHIPS IN THE GAS CHROMATOGRAPHY OF THE METHYL ESTERS OF FATTY ACIDS

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INTRODUCTION

The success of polyester substrates[1–4] in the separation of the methyl esters of fatty acids has introduced the problem of identifying the large number of chromatographic peaks obtained. The retention times of fatty esters have been examined on a polar and non-polar hydrocarbon column[5], on aged and new polyester columns[6], and on two different polyester phases[7]. The procedures produce a variation in retention times but are of limited value in a systematic evaluation.

ACKMAN has reported several correlations between the structure of unsaturated methylene-interrupted fatty esters and retention data. The correlations show that:

(1) Esters of monounsaturated fatty acids with the same end carbon chain length exhibit a linear relationship between the logarithm of their retention times, log V_R, and the total chain length[8].

(2) Normal methylene-interrupted polyunsaturated acid esters with the same number of double bonds and the end carbon chain length constant also exhibit a linear relationship between log V_R and the total chain length. The slope of the plots is the same as that for the monounsaturated acids provided the end carbon chain length is in all cases constant[8].

(3) Separation factors can be derived by dividing retention data of one methyl fatty ester by the lesser value of another comparable ester. The three types of separation factors reported[9] consider esters of the same total chain length with varying unsaturation and (a) the same and (b) different carbon chain lengths, and (c) esters of the same total chain length, same number of double bonds and different end carbon chains.

These methods apart from being somewhat involved in their application do not give an overall picture of the relative effects of the various structural parameters on the retention behaviour.

A single correlation has been reported by HAKEN AND SOUTER[10,11] where all the data is represented nomographically using three structural parameters as the co-ordinate scales. The data of the saturated esters has been considered; these fall on the end carbon chain axis at a point corresponding to a large value and approxi-

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mating the total chain length. If this interpretation is valid, it implies that the saturated and unsaturated esters provide a mutually compatible set of data.

Retention data and structural features of sterols and their derivatives have been studied and the procedure of separation factors has been developed and extended by CLAYTON[12]. For each isolated double bond introduced into the sterol ether molecule the retention time of the saturated parent compound varies by a constant factor depending on the position of the unsaturation.

The relationship is shown by the equation:

$$V_{R(s+x)} = V_s \times k_x$$

where $V_{R(s+x)}$ is the retention time of the compound with a double bond in position x, V_s is the retention time of the parent compound and k_x is a constant characteristic of the position of the unsaturation.

With two non-interacting double bonds at positions x and y, the retention time $V_{R(s+x+y)}$ is given by the equation:

$$V_{R(s+x+y)} = V_R \times k_x \times k_y$$

where k_y is the retention factor characteristic of the second double bond.

The retention time of a polysubstituted steroid with non-interacting substituent groups a, b, c, ...etc., might be expressed by the general equation:

$$V_{R(n+a+b+c\cdots)} = V_R \times k_a \times k_b \times k_c \times \ldots \text{etc.}$$

where $V_{R(n+a+b+c)}$ is the retention time of the total structure, V_R is the retention time of the unsubstituted nucleus and k_a, k_b, k_c, ...etc. are constants characteristic of the substituent a, b, c, ...etc. and their individual structural position.

It is shown here that the methylene-unit separation factor reported by JAMES AND MARTIN[13] with the methyl esters of the n-saturated fatty acids is of more general application. Methyl esters of different chain length, constant number of double bonds and constant end carbon chain exhibit a constancy throughout the series of esters studied, while a relationship similar to that developed by CLAYTON[12] is reported where retention times of the fatty esters of different chain length and degree of unsaturation may be calculated, the end carbon chain length in a particular case being constant.

DATA

The retention data are shown in Table I and are those used by ACKMAN[8,9] and ACKMAN AND BURGHER[14]. The chromatography was conducted substantially by FARQUHAR and his coworkers[15] using an ionisation detector with a ethylene glycol adipate column (20% w/w on acid and alkali washed Celite 545; 100–120 mesh) operated at 197°.

A number of provisional identifications (noted in Table I) were made by FARQUHAR and this data where used are compatible with the correlations of ACKMAN[8,9] and of HAKEN AND SOUTER[11].

TABLE I

SEPARATION FACTORS OF ESTERS OF FATTY ACIDS WITH VARYING CHAIN LENGTH, SAME NUMBER OF DOUBLE BONDS AND SAME END CHAIN LENGTH WITH EACH SERIES

Fatty acid chain length and No. of double bonds	*Position of double bonds*	*End carbon chain length*	$\frac{V_{R\,(x+2,y)}}{V_{R\,(x,y)}}$	
14:0	—	—	0.302	1.82
12:0	—	—	0.165	
16:0	—	—	0.550	1.82
14:0	—	—	0.302	
18:0	—	—	1.00	1.82
16:0	—	—	0.550	
20:0	—	—	1.82	1.82
18:0	—	—	1.00	
22:0	—	—	3.30	1.81
20:0	—	—	1.82	
18:3	9, 12, 15	3	1.72	1.81
16:3	7, 10, 13	3	0.95	
20:3	11, 14, 17[a]	3	3.10	
18:3	9, 12, 15	3	1.72	1.80
22:3	13, 16, 19	3	5.65	
20:3	11, 14, 17[a]	3	3.10	1.82
20:4	8, 11, 14, 17	3	3.51	1.78
18:4	6, 9, 12, 15	3	1.97	
22:4	10, 13, 16, 19	3	6.40	1.82
20:4	8, 11, 14, 17	3	3.51	
22:5	7, 10, 13, 16, 19	3	7.00	1.81
20:5	5, 8, 11, 14, 17	3	3.85	
18:2	11, 14	4	1.45	1.80
16:2	9, 12	4	0.810	
18:2	9, 12	6	1.34	1.80
16:2	7, 10	6	0.745	
20:2	11, 14	6	2.45	1.82
18:2	9, 12	6	1.34	
22:2	13, 16	6	4.35	1.80
20:2	11, 14	6	2.45	
18:3	6, 9, 12	6	1.54	1.80
16:3	4, 7, 10	6	0.86	
20:3	8, 11, 14	6	2.76	1.80
18:3	6, 9, 12	6	1.54	
22:3	10, 13, 16[a]	6	5.00	1.81
20:3	8, 11, 14	6	2.76	
22:4	7, 10, 13, 16	6	5.50	1.80
20:4	5, 8, 11, 14	6	3.04	
18:2	8, 11	7	1.33	1.80
16:2	6, 9	7	0.74	
20:2	10, 13	7	2.39	1.80
18:2	8, 11	7	1.33	
18:1	9	9	1.12	1.80
16:1	7	9	0.625	
20:1	11	9	2.02	1.80
18:1	9	9	1.12	
22:1	13	9	3.68	1.80
20:1	11	9	2.02	
20:2	8, 11	9	2.32	1.80
18:2	6, 9	9	1.29	
22:2	10, 13[a]	9	4.20	1.81
20:2	8, 11	9	2.32	
			Average	1.81

[a] Provisional identification.

DISCUSSION AND RESULTS

Table I shows separation factors obtained by considering fatty esters of different total chain length but with the same number of double bonds, and with the same end carbon chain length. The first five separation factors (*i.e.* saturated esters) are analogous to the methylene separation factors reported by JAMES AND MARTIN[13] and occur with all homologous series that produce a linear relationship of log V_R with total chain length. It is apparent, however, that a constant value is obtained with all of the series of esters independent of unsaturation providing the end carbon chain length is the same in any series. The data available allow only a comparison of esters with the chain length increasing by an ethylene unit, but it would be expected that the same situation would occur if the chain length were increased by a methylene group throughout the whole series of esters.

Separation factors described by ACKMAN[9] as type 1, where pairs of esters are considered with the same chain length and the same end carbon chain, but with different numbers of double bonds are shown in Table II. While it is apparent that a slightly lower value is obtained with increasing unsaturation or decreasing end carbon chain length a relatively constant value is obtained for all of the pairs of esters examined. For simplicity the esters considered are shown in all the tables in terms of x and y, the total carbon chain length and the number of methylene-interrupted double bonds present respectively.

In Table III retention data of esters of the type $(x + 2, y + 1)$ are shown calculated from data of esters which may be shown as (x, y) by multiplication of the average separation factors shown in Tables I and II according to the relationship:

$$V_{R\,(x+2,\,y+1)} = V_{R\,(x,y)} \times V_{R\,(x+2,\,y)} \times V_{R\,(x,y+1)}$$
$$= V_{R\,(x,y)} \times 1.81 \times 1.12$$

Table IV similarly shows data calculated for esters of the type $(x + 4, y + 1)$ by the formula:

$$V_{R\,(x+4,\,y+1)} = V_{R\,(x,y)} \times V_{R\,(x+2,\,y)} \times V_{R\,(x+2,\,y)} \times V_{R\,(x,\,y+1)}$$
$$= V_{R\,(x,y)} \times 1.81 \times 1.81 \times 1.12$$

The deviations from the experimentally determined values are shown in Table III and IV. CLAYTON[12] has shown that with bifunctional steroids agreement within $\pm$ 4.0 % is obtained and with certain sapogenins calculated from the work of VANDENHEUVEL AND HORNING[16] agreement of $\pm$2.0 % is possible. The variations experienced in this work are comparable with the earlier work; and as the values reported are in general lower than those determined experimentally, it would be possible to decrease the average variation by a slight modification of the separation factors.

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TABLE II

SEPARATION FACTORS OF ESTERS OF FATTY ACIDS WITH THE SAME CHAIN LENGTH, VARYING UNSATURATION, AND THE SAME END CARBON CHAIN LENGTH WITH EACH SERIES

Fatty acid chain length and No. of double bonds	*Position of double bonds*	*End carbon chain length*	$\frac{V_{R\,(x,y+1)}}{V_{R\,(x,y)}}$	
16:1	9	7	0.634	1.15
16:0	—	—	0.550	
16:1	7	9	0.625	1.13
16:0	—	—	0.550	
18:1	9	9	1.12	1.12
18:0	—	—	1.00	
20:1	11	9	2.02	1.11
20:0	—	—	1.82	
22:1	13	9	3.68	1.10
22:0	—	—	3.30	
16:3	6, 9, 12	4	0.904	1.11
16:2	9, 12	4	0.810	
18:4	6, 9, 12, 15	3	1.97	1.14
18:3	9, 12, 15	3	1.72	
20:5	5, 8, 11, 14, 17	3	3.85	1.10
20:4	8, 11, 14, 17	3	3.51	
22:4	10, 13, 16, 19	3	6.40	1.13
22:3	13, 16, 19	3	5.65	
20:4	8, 11, 14, 17	3	3.51	1.13
20:3	11, 14, 17	3	3.10	
22:5	4, 7, 10, 13, 16	3	7.00	1.09
22:4	7, 10, 13, 16	3	6.40	
16:3	4, 7, 10	6	0.86	1.15
16:2	7, 10	6	0.745	
18:3	6, 9, 12	6	1.54	1.13
18:2	9, 12	6	1.34	
20:3	8, 11, 14	6	2.76	1.12
20:2	11, 14	6	2.45	
20:4	5, 8, 11, 14	6	3.04	1.10
20:3	8, 11, 14	6	2.76	
22:3	10, 13, 16	6	5.00	1.13
22:2	13, 16	6	4.35	
22:5	4, 7, 10, 13, 16	6	6.09	1.11
22:4	7, 10, 13, 16	6	5.50	
22:4	7, 10, 13, 16	6	5.50	1.10
22:3	10, 13, 16	6	5.00	
16:2	9	7	0.74	1.16
16:1	9	7	0.634	
18:2	6, 9	9	1.29	1.14
18:1	9	9	1.120	
20:2	8, 11	9	2.32	1.14
20:1	11	9	2.02	
22:2	10, 13	9	4.20	1.14
22:1	13	9	3.68	
			Average	1.12

TABLE III

CALCULATION OF RETENTION DATA OF ESTERS OF THE TYPE $(x + 2, y + 1)$ FROM THAT OF ESTERS OF THE TYPE (x,y)

$V_{R(x,y)}$			$V_{R(x+2,\,y+1)}$					
Fatty acid	*End carbon chain length*	$V_{R(x,y)}$	*Fatty acid*	*End carbon chain length*	*Determined*	*Calculated*	*Error*	%
16:0	—	0.55	18 1	9	1.12	1.11	—0.01	—0.90
18:0	—	1.00	20:1	9	2.02	2.02	0	0
20:1	—	1.82	22:1	9	3.68	3.67	—0.01	—0.27
16:3	3	0.950	18:4	3	1.97	1.92	—0.05	—2.40
18:3	3	1.72	20:4	3	3.51	3.47	—0.04	—1.13
20:4	3	3.51	22:5	3	7.00	7.02	—0.02	—0.28
20:5	3	3.85	22:6	3	7.75	7.70	—0.05	—0.64
16:2	6	0.745	18:3	6	1.54	1.50	—0.04	—2.60
18:2	6	1.34	20:3	6	2.76	2.69	—0.07	—2.53
18:3	6	1.54	20:4	6	3.04	3.09	0.05	+1.64
20:3	6	2.76	22:4	6	5.50	5.52	0.02	+0.38
20:4	6	3.04	22:5	6	6.09	6.09	0	0
16:1	7	0.634	18:2	7	1.33	1.29	—0.04	—3.00
16:1	9	0.625	18:2	9	1.29	1.26	—0.03	—2.32
18:1	9	1.12	20:2	9	2.32	2.25	—0.07	—3.88
							Average	1.20

TABLE IV

CALCULATION OF RETENTION DATA OF ESTERS OF THE TYPE $(x + 4, y + 1)$ FROM THAT OF ESTERS OF THE TYPE (x,y)

$V_{R\,(x,y)}$			$V_{R\,(x+4,\,y+1)}$					
Fatty acid	*End carbon chain length*	$V_{R\,(x,y)}$	*Fatty acid*	*End carbon chain length*	*Determined*	*Calculated*	*Error*	%
16:3	3	0.950	20:4	3	3.51	3.41	—0.10	—2.85
16:4	3	1.08	20:5	3	3.85	3.89	+0.02	+0.52
18:3	3	1.72	22:4	3	6.40	6.31	—0.09	—1.40
18:4	3	1.97	22:5	3	7.00	7.09	+0.09	—1.28
16:2	6	0.745	20:3	6	2.76	2.69	—0.07	—2.53
16:3	6	0.86	20:4	6	3.04	3.10	+0.06	+1.89
18:2	6	1.34	22:3	6	5.00	4.85	—0.15	—3.00
18:3	6	1.54	22:4	6	5.50	5.54	+0.04	+0.73
16:0	—	0.55	20:1	9	2.02	1.98	—0.04	—1.98
16:1	9	0.625	20:2	9	2.32	2.26	—0.06	—2.58
18:0	—	1.00	22:1	9	3.68	3.61	—0.07	—1.90
18:1	9	1.12	22:2	9	4.20	4.12	—0.08	—1.90
							Average	1.35

SUMMARY

The influence of structural parameters on the retention behaviour of the methyl esters of methylene-interrupted fatty acids, their monounsaturated and saturated homologs may be shown by a simple mathematical relationship.

REFERENCES

1 C. H. ORR AND J. E. CALLEN, *J. Am. Chem. Soc.*, 80 (1958) 249.
2 C. H. ORR AND J. E. CALLEN, *Ann. N.Y. Acad. Sci.*, 72 (1959) 649.
3 S. R. LIPSKY AND R. A. LANDOWNE, *Ann. N.Y. Acad. Sci.*, 72 (1959) 666.
4 S. R. LIPSKY AND R. A. LANDOWNE, *Biochim. Biophys. Acta*, 27 (1958) 666.
5 A. T. JAMES, *J. Chromatog.*, 2 (1959) 552.
6 P. MAGEDMAN, S. F. HERB, R. A. BARFORD AND R. W. RIEMENSCHNEIDER, *J. Am. Oil Chemists' Soc.*, 39 (1962) 137.
7 R. G. ACKMAN, R. D. BURGHER AND P. M. JANGAARD, *Can. J. Biochem. Physiol.*, 41 (1963) 1627.
8 R. G. ACKMAN, *J. Am. Oil Chemists' Soc.*, 40 (1963) 558.
9 R. G. ACKMAN, *J. Am. Oil Chemists' Soc.*, 40 (1963) 564.
10 J. K. HAKEN, *Proc. Intern. Symp. Advan. Gas Chrom., 3rd., Houston, October, 1965.*
11 J. K. HAKEN AND P. SOUTER, *J. Gas Chromatog.*, 4 (1966).
12 R. B. CLAYTON, *Biochemistry*, 1 (1962) 357.
13 A. T. JAMES AND A. J. MARTIN, *Biochem. J.*, 63 (1956) 144.
14 R. G. ACKMAN AND R. D. BURGHER, *J. Am. Oil Chemists' Soc.*, 42 (1965) 38.
15 J. W. FARQUHAR, W. INSULL, P. ROSEN, W. STAFFEL AND E. H. AHRENS, *Nutr. Rev.*, 17 (1959) Suppl. 1.
16 W. J. A. VANDENHEUVEL AND E. C. HORNING, *J. Org. Chem.*, 26 (1961) 634.

CORRELATION BETWEEN THE STRUCTURE OF THE STATIONARY PHASE AND ACTIVITY COEFFICIENTS IN GAS–LIQUID CHROMATOGRAPHY: ESTERS OF BENZENEDICARBOXYLIC ACIDS AND SOME OTHER SIMILAR PHASES

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In the practical application of gas–liquid chromatography, the selection of the best liquid phase for a particular analytical purpose is fundamental. With regard to this, the investigation of the thermodynamics of solution of organic vapours in high-boiling solvents is a question of special interest in gas–liquid chromatography.

The data published up to now[1–5] have usually been concerned with stationary phases which are very different in their chemical nature and therefore do not give any opportunity for a systematic study of the influence of the structure on their thermodynamic properties.

An exception to this is a paper by CHOVIN AND DUCROS[6]. It is a thermodynamic study of the tartrates of *n*-butyl, *n*-amyl, *n*-hexyl and *n*-heptyl alcohols as phases in gas–liquid chromatography.

In our previous paper[7], we pointed out the influence of the length and structure of the alcohol chain of phthalic acid esters on the activity coefficients of some aromatic hydrocarbons. It was found that the values of the activity coefficients decrease linearly with the increasing number of carbon atoms in normal aliphatic alcohols.

The present study is an attempt to establish more completely the influence of the nature of the alcohol chain and the position of the carboxylic acid groups in the benzene ring of benzenedicarboxylic acids, as well as the influence of some other structural changes on the values of activity coefficients. This would provide data which might be used as a reasonable basis for a quantitative approach to liquid phase selection.

The gas-chromatographic data are related to the activity coefficients by:

$$\gamma^\circ = \frac{RT}{MP^\circ V_g{}^t} \tag{1}$$

where γ° is the activity coefficient of the solute in the solvent at infinite dilution, R is $6.236 \cdot 10^4$ ml.mm per mole per degree, T is the column temperature in °K, M is the molecular weight of the stationary phase, P° is the vapour pressure of the pure solute in mm Hg and $V_g{}^t$ is the retention volume in ml (measured from the air peak, then corrected for compressibility) per gram of solvent at the column temperature.

On the other hand, according to thermodynamics, the value of the activity

coefficient depends on the value of the excess partial molar enthalpy and entropy at infinite dilution by:

$$\ln \gamma^\circ = \frac{\Delta H_e^\circ}{RT} - \frac{\Delta S_e^\circ}{R} \qquad (2)$$

which may be employed for their estimation.

EXPERIMENTAL

All measurements were carried out, as in our previous work, with a "Fractovap" model B apparatus, produced by "Carlo Erba", Italy.

The stainless steel chromatographic column was 2 m long and had an internal diameter of 6 mm. Ground unglazed tile (particle size diameter 0.2–0.4 mm) was used as solid support. The amount of stationary phase was 17.6 % w/w of the inert support.

Pure dry nitrogen was used as carrier gas. The outlet gas velocity in all experiments was 120 ml/min. In the calculations to follow it was corrected for the water vapour pressure in the soap flowmeter at its temperature.

All the phases were investigated at 120°, 130° and 140°.

The hydrocarbons employed as solutes were again benzene, toluene, ethylbenzene and *o*-, *m*- and *p*-xylenes. The samples were injected into the preheater (180°) with a Hamilton microsyringe. The sample size was 0.001 ml.

The vapour pressures of the pure hydrocarbons under investigation at the temperatures of the experiments were calculated from reference data[8].

All the esters used for stationary phases (diphenyl phthalate[9], dicyclohexyl phthalate[10], dibenzyl phthalate[11], di-(β-phenylethyl) phthalate[12], di-*n*-hexyl isophthalate[12], di-*n*-hexyl terephthalate[12], catechol dibenzoate[13], catechol dibutyrate[13], and di-*n*-butyl phthalyl-bis-glycolate[14]) were synthesized by the authors.

RESULTS AND DISCUSSION

The retention volumes of the aromatic hydrocarbons per gram of solvent at various column temperatures, and the corresponding activity coefficients are shown in Tables I and II.

It is evident from Table I, that the elution order of the hydrocarbons investigated on all the phases is in accordance with the order of the boiling points of the solutes.

The values of the activity coefficients are less than unity, or slightly greater than unity, but always less than two.

The excess partial molar enthalpy and entropy found from the data of the activity coefficients at the temperatures of the experiments are positive in all cases. However, a more exact quantitative interpretation of the absolute values of ΔH_e° and ΔS_e° is impossible because of the experimental errors and insufficient number of measurements for a particular value.

If the data of the retention volumes of the six aromatic hydrocarbons on dicyclohexyl phthalate and diphenyl phthalate are compared with those on di-*n*-

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TABLE I

RETENTION VOLUMES, V_g^t, OF AROMATIC HYDROCARBONS

Hydrocarbons	*Temperature* (°C)	*Phases*								
		Diphenyl phthalate	*Dicyclo-hexyl phthalate*	*Dibenzyl phthalate*	*Di-(β-phenyl-ethyl) phthalate*	*Di-n-hexyl isophthalate*	*Di-n-hexyl terephthalate*	*Catechol dibenzoate*	*Catechol dibutyrate*	*Di-n-butyl phthalyl bis-glycolate*
Benzene	120	28.5	39.4	34.0	30.6	46.4	45.0	31.4	45.8	38.9
	130	25.4	33.8	28.2	26.4	38.8	38.8	27.2	39.3	32.4
	140	22.6	28.8	23.9	22.5	32.9	33.7	23.8	34.0	27.4
Toluene	120	55.5	77.5	64.9	59.3	96.5	92.9	61.6	86.1	70.9
	130	47.2	62.8	53.3	48.0	78.2	77.6	52.1	70.5	58.5
	140	40.5	51.2	43.6	39.3	63.2	63.5	43.3	57.0	48.3
Ethylbenzene	120	93.5	139.8	109.9	99.6	171.0	167.0	105.1	148.3	120.0
	130	79.0	110.0	88.5	80.0	135.0	136.0	85.1	119.9	96.5
	140	68.5	86.5	72.5	64.7	106.7	108.6	69.0	95.2	77.4
o-Xylene	120	130.6	176.8	147.5	137.0	227.8	229.0	145.0	193.0	158.9
	130	108.0	139.9	117.6	109.0	177.5	181.0	115.2	154.1	125.0
	140	87.7	109.0	95.5	86.6	139.5	141.2	91.2	123.0	99.4
m-Xylene	120	101.1	147.9	120.7	110.8	194.0	191.5	118.2	158.1	129.0
	130	84.5	119.0	96.4	88.0	152.5	153.2	95.5	127.3	102.2
	140	71.2	94.5	78.3	70.6	120.6	120.7	77.0	102.5	82.7
p-Xylene	120	99.9	144.0	118.0	107.6	191.1	189.9	112.8	153.5	128.3
	130	83.5	113.2	94.3	85.5	148.5	149.8	90.4	125.3	101.5
	140	70.4	89.5	76.1	68.4	116.6	119.2	73.8	101.0	80.2

hexyl phthalate[7], it is evident that their values are lowest on diphenyl phthalate and highest on di-*n*-hexyl phthalate.

The values of the activity coefficients for the same phases are lowest on di-*n*-hexyl phthalate and highest on diphenyl phthalate. Moreover, the deviations from Raoult's law on di-*n*-hexyl phthalate are negative, while on diphenyl phthalate they are positive. The data for dicyclohexylphthalate have an intermediate position among them.

From the data for these three phases it follows that the hydrocarbons are retained best on di-*n*-hexyl phthalate and worst on diphenyl phthalate. According to eqn. (2) the interaction of the aromatic hydrocarbons with di-*n*-hexyl phthalate and of benzene and toluene with dicyclohexyl phthalate, when $\gamma^\circ < 1$, $\Delta H_e^\circ > 0$, $\Delta S_e^\circ > 0$, assumes the predominance of the entropy term. This assumption is supported by the fact that in the above mentioned cases the temperature has practically no effect on the activity coefficient.

The positive enthalpy indicates that the molecular interactions between the solute and the solvent are less than the interactions among the molecules of the pure solute. The positive value for the entropy shows that the molecules of the solute are able to come into contact with a greater number of molecules of the solvent than with their own molecules.

The interaction of ethylbenzene and the xylenes with dicyclohexyl phthalate and all of the hydrocarbons with diphenyl phthalate, when $\gamma^\circ > 1$, $\Delta H_e^\circ > 0$, $\Delta S_e^\circ > 0$, assumes that the influence of the enthalpy term upon the value of the activity coefficient is greater than that of the entropy term. The predominance of this term, however, over the entropy term, is probably not very great.

It is evident, from the data for diphenyl phthalate, dibenzyl phthalate and di-(β-phenylethyl) phthalate, that the retention volumes of the hydrocarbons are smallest on diphenyl phthalate and greatest on dibenzyl phthalate. The corresponding values of the activity coefficients are practically equal when the dibenzyl- and di-(β-phenylethyl) phthalate phases are concerned and higher for the diphenyl phthalate phase. With the exception of benzene, which on dibenzyl- and on di-(β-phenylethyl) phthalates has $\gamma^\circ < 1$, all hydrocarbons on the three phases give positive deviations from Raoult's law. Therefore the hydrocarbons are retained on dibenzyl phthalate and di-(β-phenylethyl) phthalate better than on diphenyl phthalate. Di-(β-phenylethyl) phthalate has one methylene group more than dibenzyl phthalate but this fact has not such a noticeable influence on the activity coefficient as is the case with diphenyl and dibenzyl phthalates.

Comparison of the data for di-*n*-hexyl phthalate with those for di-*n*-hexyl isophthalate and di-*n*-hexyl terephthalate shows that the activity coefficients are practically independent of the position of the ester groups in the benzene ring of the phthalate.

It is the authors' opinion that it would be interesting to be able to determine how the position of the functional groups and atoms influences the activity coefficient.

If the data for di-*n*-propyl phthalate[7] are compared with the corresponding data for its isomer, catechol dibutyrate, it can be seen that the retention volumes for the former phase are greater than the retention volumes for the latter. In di-*n*-propyl phthalate, the aliphatic radicals are attached to the carboxyl oxygen, while in the case of catechol dibutyrate they are attached to the carboxyl carbon atom. If the radicals

TABLE II

ACTIVITY COEFFICIENTS, γ°, OF AROMATIC HYDROCARBONS

Hydrocarbons	*Temperature* (°C)	*Phases*								
		Diphenyl phthalate	*Dicyclo-hexyl phthalate*	*Dibenzyl phthalate*	*Di-(β-phenyl-ethyl) phthalate*	*Di-n-hexyl isophthalate*	*Di-n-hexyl terephthalate*	*Catechol dibenzoate*	*Catechol dibutyrate*	*Di-n-butyl phthalyl bis-glycolate*
Benzene	120	1.20	0.84	0.93	0.95	0.70	0.73	1.09	0.95	0.71
	130	1.10	0.79	0.91	0.90	0.68	0.68	1.04	0.90	0.67
	140	1.01	0.77	0.88	0.86	0.66	0.65	0.98	0.86	0.67
Toluene	120	1.41	0.97	1.11	1.12	0.77	0.80	1.27	1.16	0.89
	130	1.31	0.95	1.07	1.10	0.75	0.76	1.19	1.12	0.86
	140	1.22	0.93	1.04	1.07	0.75	0.74	1.14	1.10	0.83
Ethylbenzene	120	1.71	1.10	1.34	1.36	0.89	0.91	1.52	1.37	1.08
	130	1.56	1.08	1.28	1.31	0.87	0.86	1.45	1.31	1.03
	140	1.46	1.07	1.22	1.27	0.86	0.84	1.40	1.29	1.00
o-Xylene	120	1.56	1.11	1.27	1.26	0.85	0.85	1.40	1.34	1.03
	130	1.43	1.07	1.21	1.21	0.83	0.82	1.34	1.28	1.00
	140	1.37	1.06	1.15	1.18	0.82	0.81	1.31	1.24	0.97
m-Xylene	120	1.72	1.14	1.33	1.34	0.86	0.87	1.48	1.40	1.09
	130	1.58	1.08	1.28	1.29	0.83	0.83	1.40	1.34	1.06
	140	1.47	1.07	1.23	1.26	0.83	0.83	1.36	1.30	1.02
p-Xylene	120	1.70	1.14	1.33	1.34	0.85	0.86	1.51	1.41	1.07
	130	1.57	1.11	1.28	1.30	0.84	0.83	1.45	1.33	1.04
	140	1.45	1.10	1.23	1.27	0.83	0.81	1.38	1.28	1.03

are aromatic instead of aliphatic (*e.g.*, the phases are catechol dibenzoate and diphenyl phthalate), the effect is reversed, the retention volumes on diphenyl phthalate being less than those on catechol dibenzoate.

The activity coefficients have lowest values with di-*n*-propyl phthalate and highest with diphenyl phthalate. The two catechol diesters have an intermediate position.

Investigation of the influence of the number of carboxyl groups in the side chains of the phthalic ester was performed on di-*n*-butyl phthalyl-bis-glycolate.

The values of the retention volumes on di-*n*-butyl phthalyl-bis-glycolate are less than those on di-*n*-butyl phthalate. The activity coefficients on these two phases are practically equal.

SUMMARY

The present work is an attempt to investigate the correlation between the stationary phases and the activity coefficients in gas–liquid chromatography. Nine esters of benzenedicarboxylic acids or their isomers were chosen as liquid stationary phases and aromatic hydrocarbons were used as solutes.

It was found that the most energetic interactions between solute and solvent take place with the phthalates, isophthalates and terephthalates of the aliphatic alcohols.

It is thought that the data obtained in this work might serve as a good basis for liquid phase selection.

REFERENCES

1 S. H. LANGER AND J. H. PURNELL, *J. Phys. Chem.*, 67 (1963) 263.
2 P. E. PORTER, C. H. DEAL AND F. H. STROSS, *J. Am. Chem. Soc.*, 78 (1956) 2999.
3 C. J. HARDY, *J. Chromatog.*, 2 (1959) 490.
4 A. KWANTES AND G. V. A. RIJNDERS, in D. H. DESTY (Editor), *Gas Chromatography 1958*, Butterworths, London, 1958, pp. 125–135.
5 G. Z. PIEROTTI, C. H. DEAL, E. L. DERR AND P. E. PORTER, *J. Am. Chem. Soc.*, 78 (1956) 2989.
6 P. CHOVIN AND M. DUCROS, *Publ. Group. Avan. Methodes Spectrog., 1962*, 203–216; *C.A.*, 56 (1963) 6966g.
7 N. PETSEV AND CHR. DIMITROV, *J. Chromatog.*, 20 (1965) 15.
8 *Lange's Handbook of Chemistry*, 9th Ed., McGraw Hill, New York, 1956, pp. 1424–1438.
9 F. BLICKE AND O. WEINKAUFF, *J. Am. Chem. Soc.*, 54 (1932) 331.
10 H. WALDMANN AND H. SCHUBERT, *Ber.*, 84 (1951) 139.
11 T. J. THOMPSON AND G. J. LEUCK, *J. Am. Chem. Soc.*, 44 (1922) 2895.
12 *Organic Syntheses*, Russian Edition, Vol. 4, State Publishing House for Foreign Literature, Moscow, 1949, p. 447.
13 O. DÖBNER, *Ann.*, 210 (1881) 261.
14 M. SENFF, *Ann.*, 208 (1881) 273.

LINEAR ELUTION ADSORPTION CHROMATOGRAPHY

XII. FUNCTIONAL GROUP ADSORPTION ENERGIES ON THE METAL OXIDE ADSORBENTS

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INTRODUCTION

Solute adsorption energies $S°$ determine the retention volumes and separation order of sample components in linear elution adsorption chromatography (LEAC) (*e.g.* Part XI[1]). These solute adsorption energies are in turn defined by the adsorption energies $Q°_i$ of individual solute groups or substituents, although the interactions between different groups in the same molecule must also be taken into account[1]. The adsorption energies of different groups in the *eluent* molecule also determine its strength $\varepsilon°$ (ref. 2). The first tabulation of relative adsorption strengths (on alumina) of different functional groups was that of BROCKMANN[3]. Numerous quantitative data for functional group adsorption energies have since been reported for alumina, Florisil, and silica[4–10]. $Q°_i$ values for a given group i (*e.g.* bromo, nitro, amino) are considered to vary with the adsorbent type (*e.g.* alumina *versus* silica) and with whether i is attached to aliphatic or aromatic substituents, or to both. If relationships between $Q°_i$ values for different adsorbents, and for aliphatic *versus* aromatic substituents could be established, it would greatly reduce the data required for prediction of separation in different chromatographic systems. It would also simplify the classification of the fundamental differences in the separation capabilities of different adsorbents. Finally it would further clarify the fundamental molecular processes which determine solute adsorption energies. The present paper will show the existence of such relationships and discuss some of their implications.

EXPERIMENTAL

Retention volume data for elution from alumina (Alcoa, F-20) and silica (Davison code 12) were obtained as previously (*e.g.* refs. 5, 7), in order to supplement previous $Q°_i$ values for these two adsorbents. The linear equivalent retention volume $\underline{R}°$ for pentane elution of benzothiophene from 7.5 % H_2O–SiO_2 (Davison code 12) was 6.1 ml/g, compared to a value of 6.3 for naphthalene. From this it was calculated that $S°$ for the adsorption of benzothiophene on silica is 2.00, and $Q°_i$ for the sulfur group is therefore about 0.02 less than $Q°_i$ for two aromatic carbons, or $Q°_i$ (—S—) equals 0.48 in benzothiophene. Log $\underline{R}°$ for elution of N-methylcarbazole from 22 %

H_2O-SiO_2 by pentane was equal 0.48, from which $S°$ was calculated equal 3.30, and $Q°_i$ equal 0.96 for the —$N(CH_3)$— group in this molecule.

The following data were obtained for elution from 3.8 % H_2O–Al_2O_3 (Alcoa F-20). Log $\underline{R}°$ for elution of benzal-methylamine by methylene chloride was —0.17. $S°$ for this solute was calculated equal 6.3. Benzanilide eluted by 52 % v methylene chloride–pentane and by methylene chloride gave log $\underline{R}°$ values equal 0.97 and 0.18, respectively. From these values $S°$ was calculated equal 8.59. Pentane elution of *n*-butanethiol and diethyl disulfide gave log $\underline{R}°$ values equal 0.04 and —0.04, respectively. $S°$ values for these two solutes were then calculated, equal 2.86 and 2.74, respectively. Log $\underline{R}°$ for benzoic acid eluted by 35 % v isopropanol–pentane was larger than 2.00, from which $S°$ was calculated as greater than 12.4.

DISCUSSION

Aliphatic versus aromatic substituents

Previous studies[4,8] have established that $Q°_i$ for a group i adsorbed on a given adsorbent varies widely, depending on whether i is substituted on a saturated hydrocarbon chain (*e.g.* R—S—R, $Q°_s$ equal 2.65 on alumina), on an aromatic ring (*e.g.* ϕ—S—R, $Q°_s$ equal 1.32), or is part of an aromatic ring (*e.g.* thiophene, $Q°_s$ equal 0.76). Various explanations have been offered for these differences in $Q°_i$ values[4,5]. Thus substituents which possess unsaturation or p-electrons can conjugate with the aromatic ring, which in turn should affect the availability of these electrons for interaction with adsorption sites. Similarly an aromatic substituent on a group i (*i.e.* phenyl) should sterically interfere with the adsorption of i to a greater extent than an alkyl group such as methyl. Both of these explanations predict a lessening of $Q°_i$ when an aliphatic substituent is replaced by an aromatic substituent. In support of these theories it is generally noted that $Q°_i$ is substantially lower for aromatic groups i than for aliphatic groups. Two additional factors determine the apparent difference between aromatic and aliphatic group $Q°_i$ values; ring delocalization[2] and ring electronic activation[1,6], when an aromatic ring is attached to i. Strongly adsorbing groups i tend to reduce the adsorption energies of attached aromatic rings by delocalization of the ring, and electron withdrawing groups reduce the adsorption energy of attached rings. Neither effect can significantly influence the adsorption energies of attached aliphatic groups, since these are very weakly adsorbed. Since ring delocalization and electronic activation are normally ignored in calculating aromatic $Q°_i$ values, this effect is incorporated in the resulting ("nominal") $Q°_i$ values.

Table I summarizes a number of $Q°_i$ values for groups with both aliphatic and aromatic substituents. These data permit a detailed examination of the reasons for differences in $Q°_i$ values between aromatic and aliphatic groups. Three classes of groups i may be distinguished: (I) groups with no "normal" double bonds (the semi-polar bonds of the nitro group are not considered normal double bonds); (II) groups with ordinary double bonds; (III) hydrogen bonding groups (*e.g.* —OH). Table I includes all available data where both aliphatic and aromatic $Q°_i$ values have been measured for the same group i, exlusive of basic groups on silica and acidic groups on alumina. These latter values are excluded because of preferential adsorption of acids on alumina and bases on silica (see following section). The aromatic $Q°_i$ values can be corrected for ring delocalization and electronic activation as described previously[1,2]. Fig. 1 shows

TABLE I

CORRELATION OF Q°_i VALUES FOR ALIPHATIC AND AROMATIC FUNCTIONAL GROUPS

Group	*Adsorbent*	*Ref.**	Q°_i		*Ring deloc.*** Δ_L	*Ring activ.**** Δ_σ	*Net* Q°_i *(arom.)*§	Q°_i *(aliph.) minus net* Q°_i *(arom.)*
			Aliph.	*Arom.*				
Class I								
—Cl	Al_2O_3	a, b	1.82	0.20	0.00	0.17	0.37	1.45
—Br	Al_2O_3	a, c	2.00	0.33	0.00	0.18	0.51	1.49
—I	Al_2O_3	a, c	2.00	0.51	0.00	0.18	0.69	1.31
—S—R	Al_2O_3	c	2.65	1.32	0.20	0.02	1.54	1.11
—O—R	Al_2O_3	c	3.50	1.77	0.39	—0.03	2.13	1.37
—NO_2	Al_2O_3	c, d	5.40	2.77	0.75	0.25	3.77	1.63
	Average							1.39 ± 0.17 (S.D.)
—Cl	SiO_2	e, f	1.32	—0.20	0.00	0.14	—0.06	1.38
—Br	SiO_2	e, f	1.32	—0.17	0.00	0.14	—0.03	1.35
—I	SiO_2	e, f	1.20	—0.15	0.00	0.14	—0.01	1.21
—S—R	SiO_2	e, f	2.94	1.29	0.15	0.02	1.46	1.48
	Average							1.36 ± 0.11 (S.D.)
Class II								
—O_2C—R	Al_2O_3	c	5.00	3.48	0.94	0.20	4.62	0.38
—CO—R	Al_2O_3	c	5.00	3.74	1.00	0.11	4.85	0.15
—CO_2—R	Al_2O_3	c	5.00	3.32	0.92	0.13	4.37	0.63
—C≡N	Al_2O_3	c	5.00	3.25	0.89	0.25	4.39	0.61
—CO_2—ϕ	Al_2O_3	c	5.18	4.02	0.84	0.15	5.01	0.17
—CO—ϕ	Al_2O_3	c, g	5.60	4.36	0.92	0.11	5.39	0.21
—N=C—ϕ	Al_2O_3	h, i	6.30	4.14	0.95	~0.14	5.23	1.07
—CO—NH—ϕ	Al_2O_3	h, i	8.20	7.04	1.27	0.08	9.39	—0.19
	Average							0.38 ± 0.38 (S.D.)
Class III								
—OH	SiO_2	j	5.60	4.20	0.85	—0.04	5.01	0.59
	Average							0.59

* (a) ref. 5; (b) unreported data; n-C_{16}—Cl gave Q°_i equal 1.82 for —Cl on 0.7% H_2O–Al_2O_3; (c) ref. 6; (d) ref. 2; (e) ref. 1; (f) unreported data, see Part XIII; (g) ref. 9; (h) ref. 10; (i) experimental section; (j) ref. 4 (see ref. 7).

** From Fig. 1.

*** $\Delta_\sigma = 0.31(1.86 - \Delta_L)\bar{\sigma}$ for Al_2O_3; $\Delta_\sigma = 0.31(1.50 - \Delta_L)\bar{\sigma}$ for SiO_2; values of $\bar{\sigma}$ from JAFFEE[12].

§ Equation 1.

the ring delocalization energy Δ_L *versus* the nominal Q°_i value of the aromatic substituent (the latter is calculated from S° for the i-substituted benzene, assuming no delocalization or electronic activation of the aromatic ring). These Δ_L values were derived earlier[2], assuming normal delocalization (compare Fig. 7, ref. 2). Similar changes in ring adsorption energy Δ_σ due to electronic activation by i should be approximately proportional to the average HAMMETT sigma function[11] for *meta* and *para* substituents on a benzene ring ($\bar{\sigma}$) and to the adsorption energy of the ring after

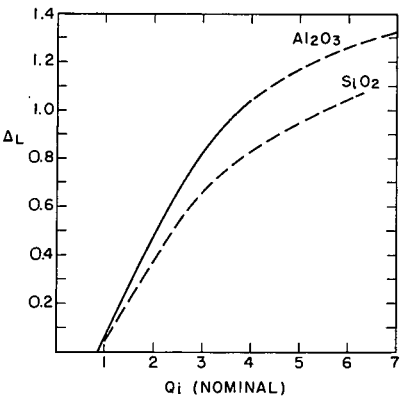


Fig. 1. Ring delocalization energy in solutes of type C_6H_5-i. ——— Al_2O_3 (ref. 2); – – – SiO_2; equal values for Al_2O_3 corrected for smaller adsorption energy of the benzene ring on silica ($\times$ 1.50/1.86).

delocalization by i (*i.e.* 1.86 — Δ_L for alumina, 1.50 — Δ_L for silica). From the increase in ring adsorption energy of the alkylbenzenes as a result of substitution by alkyl groups[7] we estimated that Δ_σ equals —0.31 (1.86 — Δ_L) $\bar{\sigma}$ for alumina and —0.31 (1.50 — Δ_L) $\bar{\sigma}$ for silica. Finally the net (actual) adsorption energy of an aromatic group i, corrected for ring activation and delocalization, is given by Eqn. (1):

$$Q°_i \text{ (net)} = Q°_i \text{ (nominal)} + \Delta_L - \Delta_\sigma \qquad (1)$$

Values of the net adsorption energies of the various aromatic groups of Table I are tabulated there. The differences in adsorption energies of aliphatic and aromatic (net) groups are also given in Table I, and these are seen to be constant within each of the three classes: 1.38 $\pm$ 0.15 for class I; 0.38 $\pm$ 0.38 for class II; 0.59 for class III. Within each class these differences show no dependence on the group adsorption energy.

Simple steric interference by an attached phenyl group to the adsorption of i should lead to a difference between net $Q°_i$ values for aliphatic and aromatic groups which is proportional to the size of $Q°_i$. This is true of the *ortho-i*-toluenes[1] and of various di-aza-aromatics[9], and is also theoretically reasonable. The complete absence of any such correlation for the data of Table I therefore discounts the relative importance of simple steric interference to adsorption by an attached phenyl group. The extent of conjugation of the group i with the aromatic ring is expected to vary widely among the groups of Table I; however, relative conjugation should be generally more important for groups with double bonds (*i.e.* class II) than for groups without. This should lead to greater loss in adsorption energy for the unsaturated groups (class II). The opposite of this prediction is actually noted, so that simple conjugation as an explanation for these $Q°_i$ value differences also appears unsatisfactory. We believe that the actual origin of these differences in $Q°_i$ values arises from a difference in preferred orientations of the various groups with the adsorbent surface. It is proposed that unsaturated groups such as cyano or aceto (class II) prefer to lie in the plane of the adsorbent surface, with polarization (or bond formation) of the group π-electrons in the perpendicular direction by the adsorbent surface field. Since the aromatic ring (*i.e.*

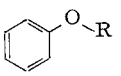
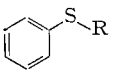
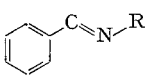
benzene) attached to such groups also prefers to lie in the plane of the adsorbent surface[13], and since in *i*-phenyl compounds of this type it is possible for both groups to lie in the surface plane simultaneously, little loss in adsorption energy of *i* would result upon its attachment to a benzene ring. This is in fact observed; the average difference between aliphatic and net aromatic group adsorption energies for class II groups is small (within the S.D. from zero, in fact). In the case of saturated groups *i* such as the halogens and ethers (class I), it is suggested that the preferred orientation of the C—*i* bond is tilted to the plane of the adsorbent surface so as to permit a closer approach of *i* to specific sites, or a better alignment to the C—*i* bond with specific surface atoms (dipole interaction). This preferred orientation is of course not possible when *i* is attached to an aromatic ring. These different adsorbed configurations of the oxygen atom in aromatic and aliphatic ethers (class I) have in fact been invoked previously as an explanation for certain eluent anomalies (see Fig. 5, ref. 2).

The adsorption energy of the hydroxyl group (class III) may arise in part from hydrogen bonding by the alcohol proton to the adsorbent surface. Such hydrogen bonding would be less restricted by attachment of an aromatic ring to the hydroxyl group, relative to class I groups. This could account for the smaller difference in net adsorption energies of the aliphatic *versus* aromatic hydroxyl group.

The same trends in $Q°_i$ values upon substituting aromatic for aliphatic substituents on *i* are observed for further substitution of aromatic rings. This is illustrated in Table II, where corrected net $Q°_i$ values are compared for a few compound pairs of

TABLE II

FURTHER CORRELATION OF NET $Q°_i$ VALUES WITH THE POSITION OF *i* IN THE MOLECULE

Group	*Structure*	*Net* $Q°_i$*	*Net* $Q°_i$ *(aliph.) minus net* $Q°_i$*(arom.)*
—O—		2.13	—
			1.09
		1.04	
—S—		1.54	
			0.77
		0.77	
—N=		6.30**	
			0.47
		5.83	

* Ref. 9.

** Assumes ø-ring twisted 90° from plane of —C=N—R, because of reduced hindrance to adsorption of —N= (ref. 10).

this type*. We see in Table II that groups of class I (—O—, —S—) suffer a further substantial adsorption energy (0.77–1.09) loss in going from a mixed aliphatic, aromatic molecule to a fully aromatic molecule, while the one group of class II (—N=) shows a smaller adsorption energy difference for this same change (0.47, which is close to the average value of Table I for other class II groups).

The constancy of $Q°_i$ value differences between aromatic and aliphatic groups within each class of Table I permits us to accurately estimate aliphatic or aromatic $Q°_i$ values from the opposite type using Eqn. (1) and the average values of the adsorption energy difference: 1.4 (class I); 0.4 (class II); 0.6 (class III). The fact that this correlation fits data for both alumina and silica equally well suggests that it is also applicable for other metal oxide adsorbents.

$Q°_i$ values on alumina versus silica

It has been shown previously[7,8] that the $Q°_i$ values for a particular group adsorbed on alumina, silica, and Florisil are generally, but not always, similar. Table III summarizes $Q°_i$ values for 27 different groups adsorbed on both alumina and silica. The alumina $Q°_i$ values are plotted *versus* the silica values in Fig. 2. It is apparent that a number of groups vary widely in their relative adsorption energies on the two adsorbents. The larger differences are classifiable into two categories: acidic groups

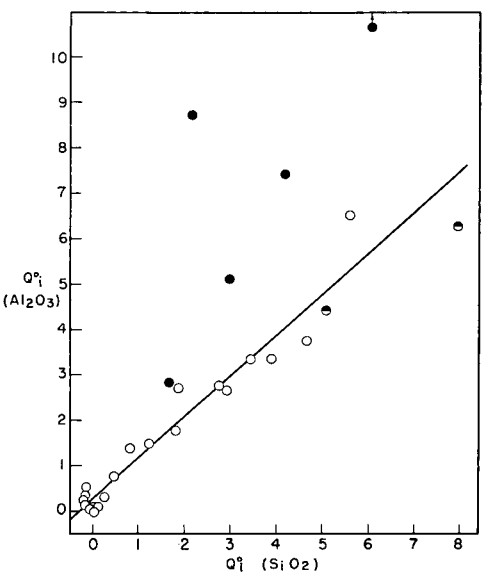


Fig. 2. Correlation of $Q°_i$ values on alumina *versus* those on silica. (●) acidic groups; (◓) basic groups; (○) neutral groups.

(closed circles) all show higher adsorption energies on alumina, while basic groups (half closed circles) show higher adsorption energies on silica. These deviations are in fact consistent with the commonly accepted descriptions[14] of alumina as "basic" and silica

* Additional data of this same type are available for molecules with more than one ring (*e.g.* carbazole, fluorenone), but other complications (*e.g.* steric interference to adsorption) are present in these cases, preventing a simple interpretation of $Q°_i$ values.

TABLE III

CORRELATION OF $Q°_i$ VALUES FOR ALUMINA AND SILICA

Group	*Ref.**	$Q°_i$		$\Delta Q°_i$	pK_A *of group***	pK_B *of group***
		Silica	*Alumina*			
Ar)—Cl	a, b	—0.20	0.20			
Ar)—Br	a, b	—0.17	0.33			
Ar)—F	a, b	—0.15	0.11			
Ar)—I	a, b	—0.15	0.51			
R)—CH_2—	c	—0.05	0.02			
Ar)—CH_2—	c	0.01	0.07			
R)—CH_3	c	0.05	—0.03			
Ar)—CH_3	c	0.11	0.06			
Ar)—CH=(Ar	a, b	0.25	0.30			
Ar)—S—(Ar	b, d	0.48	0.76			
Ar)—SH	d, h	0.67	8.70	7.85	7	
Ar)—N(CH_3)—(Ar	d, e	0.96	1.37			
Ar)—S—R	a, f	1.29	1.32			
R)—SH	g, d	~1.70	2.80	1.02	11	
Ar)—O—R	a, f	1.83	1.77			
R)—S_2—(R	g, d	~1.90	2.70			
Ar)—NO_2	a, f	2.77	2.75			
R)—S—(R	a, b	2.94	2.65			
Ar)—NH—(Ar	a, e	3.00	5.10	2.15	12	
Ar)—CO_2—(R	a, f	3.45	3.32			
Ar)—CHO	a, f, i	3.90	3.35			
Ar)—CO—(R	a, f	4.69	3.74			
Ar)—OH	a, h, i	4.20	7.40	3.37	9.9	
Ar)—NH_2	a, f	5.10	4.41	0.48		9.3
R)—OH	a, h, i	5.60	6.50			
Ar)—COOH	a, d, i	6.10	> 10.6	> 4.9	4.2	
R)—NH_2	a, i, j	8.00	6.4 ± 0.4	1.2		3.2

* (a) ref. 7; (b) ref. 5; (c) ref. 16; (d) experimental section; (e) ref. 10; (f) ref. 6; (g) approximate values estimated from relative adsorption strength values of ref. 17; (h) ref. 2; (i) ref. 4; (j) alumina value estimated from Table I, assuming difference in aliphatic and aromatic $Q°_i$ values is between the values for class I and class III.

** Ref. 15.

as "acidic". If acidic and basic groups are ignored, it is found that the alumina and silica $Q°_i$ values can be correlated with a standard deviation of only ±0.43 units by means of Eqn. (2):

$$Q°_i \text{ (alumina)} = 0.90\ Q°_i \text{ (silica)} + 0.25 \qquad (2)$$

This relationship can be inverted to permit silica group absorption energies to be estimated from values for alumina:

$$Q°_i \text{ (silica)} = 1.11\ Q°_i \text{ alumina)} - 0.28 \qquad (2a)$$

It has been shown previously[8] that $Q°_i$ values for adsorption on silica and Florisil are equal, when the standard activity for Florisil ($\alpha = 1.00$) is chosen for 1 % H_2O-

Florisil (see ref. 16). Consequently $Q°_i$ values for aromatic and aliphatic groups adsorbed on Florisil may be derived from corresponding data for either alumina or silica. Similarly, aliphatic $Q°_i$ values for Florisil may be calculated from aromatic values just as in the case of alumina and silica.

The excess adsorption energies of acidic groups on alumina can be correlated with the acid strength or pK_A value of the group. Table III summarizes values of these group excess adsorption energies $\Delta Q°_i$ on alumina for acidic groups; $\Delta Q°_i$ equals experimental $Q°_i$ value minus value calculated from Eqn. (2). Similar group excess adsorption energies are also listed in Table III for the adsorption of bases on silica, using Eqn. (2a). Fig. 3 shows a plot of $\Delta Q°_i$ *versus* pK_A for acidic groups on alumina. In addition to the $\Delta Q°_i$ values of Table III similar values for the substituted phenols (dark circles) from a previous study[2] are included. These latter values are calculated relative to phenol (on alumina). It is clear from the data of Fig. 3 that the preferential adsorption ($\Delta Q°_i$ value) of acidic compounds on alumina is greater, the more acidic is the compound. If we assume that the surface of alumina behaves as a base of definite pH, which is capable of ionizing acid adsorbates SH according to the scheme $SH \rightarrow S^- + H^+$, and if we assume that the equilibrium constant for this reaction is the same as in water, then the relative concentrations of SH and of S^- on the alumina surface may be calculated as a function of pK_A and the H^+ concentration on the alumina surface (H^+):

$$\frac{(SH)}{(SH) + (S^-)} = \frac{10^{pK_A}(H^+)}{10^{pK_A}(H^+) + 1} \equiv f_{SH}.$$

We will assume that the acid SH consists of a single functional group i, for purposes of simplicity, although this does not detract from the generality of the present argument. In the absence of ionization of SH (*i.e.* normal adsorption, as on silica) it may be shown that for an adsorbent of activity α, $\alpha Q°_i = \log (SH)_a/(SH)_s V_a$. $(SH)_a$ and $(SH)_s$ refer to undissociated SH in adsorbed and solution phases, respectively, and V_a is the adsorbent surface volume (see ref. 5). With dissociation of SH on the alumina surface, the group adsorption energy $\alpha Q°_i$ is equal to $\log [(SH)_a + (S^-)_a]/(SH)_s V_a$. The excess adsorption energy of i, $\Delta Q°_i$, is then given as $1/\alpha$ times the difference in these two values of $\alpha Q°_i$ (*i.e.* the value for dissociation minus the value for no dissociation), which is equal to $-(1/\alpha) \log f_{SH}$. The term $\Delta Q°_i$ is then given as:

$$\Delta Q°_i = -(1/\alpha) \log \left[\frac{10^{pK_A}(H^+)}{10^{pK_A}(H^+) + 1}\right] \qquad (3)$$

For small values of pK_A, Eqn. (3) simplifies to:

$$\Delta Q°_i = \frac{pK_A - pH}{\alpha} \qquad (3a)$$

where pH refers to the pH of the alumina surface, and pK_A is the acidity of the adsorbate. The data of Fig. 3 suggest a value of 12 for the pH of the alumina surface, and the dashed curve of Fig. 3 is calculated from Eqn. (3) with pH equal 12. The calculated curve provides a reasonable fit to the experimental data of Fig. 3.

The similar correlation of the excess adsorption energies of bases adsorbed on

silica with pK_B is shown in Fig. 4. The $\Delta Q°_i$ value for aniline (—0.48) is essentially within the standard deviation of Fig. 2, Eqn. (2a), so we cannot decide *a priori* whether aniline actually exhibits a greater than normal affinity for the silica surface. The theoretical curve, analogous to Eqn. (3), drawn through the point in Fig. 4 for a primary aliphatic amine assumes a pH for the silica surface equal 4. From this it appears that bases with pK_B greater than 5 (*e.g.* aniline) do *not* preferentially adsorb on the silica surface.

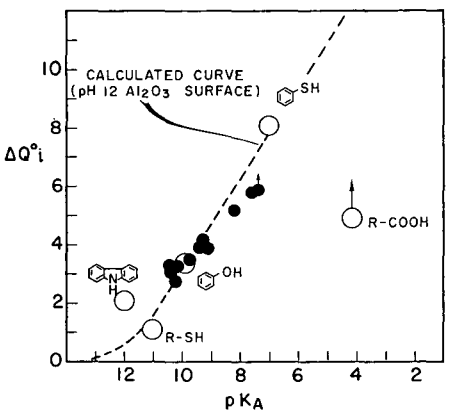


Fig. 3. Excess adsorption energies of acidic groups on alumina *versus* pK_A. (●) substituted phenol values[2].

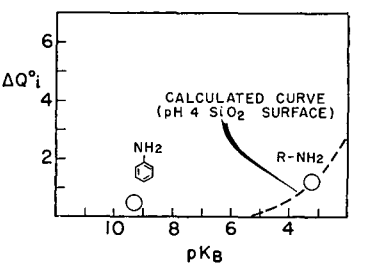


Fig. 4. Excess adsorption energies of basic groups on silica *versus* pK_B.

The above pH values for the alumina and silica surfaces are in agreement with other measures of the relative acidity or basicity of these surfaces. Thus the ionization constant of silicic acid H_3SiO_3 is equal to 10^{-10} (ref. 18) and the pH of a 1 *M* solution is approximately 5, in good agreement with the pH value of the silica surface (*i.e.* 4) inferred from Fig. 4. Similarly the basicity of alumina is assumed due to surface oxide ions, whose basicity should be similar to aluminate ion. The ionization constant of aluminic acid H_3AlO_3 is $4 \cdot 10^{-13}$ (ref. 18), and the pH of a 1 *M* solution of aluminate ion is approximately 12.4, again in good agreement with the pH of the alumina surface (*i.e.* 12) inferred from Fig. 3.

Bases have been claimed to preferentially adsorb on Florisil, but presently available data do not permit an estimate of the importance of this effect.

Fundamental basis of group adsorption energies on the metal oxides

Apart from the special case of adsorption of acidic groups on basic adsorbents, and *vice versa*, it is apparent that group adsorption energies stand in the same relative order on alumina, silica, Florisil, and (probably) other metal oxides and hydroxides. This suggests a similar mechanism of adsorption for isolated polar functional groups on all polar adsorbents. The previous correlational relationships permit us to reduce a large number of group $Q°_i$ values for different adsorbents and substituent types to a single basis (or standard state) for examination of the major molecular forces responsible for the adsorption of each group on a metal oxide surface. Table IV provides such

a summary of standard state $Q°_i$ values $(Q°_i)_s$, for the adsorption of aliphatic groups on alumina. These $(Q°_i)_s$ values are corrected for the preferential adsorption of acids on alumina (*i.e.* $\Delta Q°_i$ is subtracted out). The average $(Q°_i)_s$ values of Table IV show a standard deviation from the individual values of only ± 0.3 units, implying an accuracy in $(Q°_i)_s$ of about ± 5–10 %.

TABLE IV

STANDARD STATE GROUP ADSORPTION ENERGIES

Group[a]	$(Q°_i)_s$[b]					μ_i[c]	E_B[d]
	Silica		*Alumina*		*avg.*		
	aliph.	*arom.*	*aliph.*	*arom.*			
C=C		0.81		0.62	0.71	~0.0	
—F		1.65		1.64	1.64	1.46	
—Cl		1.65	1.82	1.78	1.75	1.58	
—Br		1.69	2.00	1.95	1.88	1.54	
—I		1.69	2.00	2.10	1.93	1.30	
—SH	1.78	2.23			2.00	1.30	
—S—S—	1.95		2.70		2.32		
—S—	2.90	3.09	2.65	2.95	2.90	1.30	0.04
—O—		3.71	3.50	3.54	3.58	1.25	0.65
—N< [e]				4.40	(4.40)		
—CHO		5.21		4.73	4.97	2.76	
$-NO_2$		5.16	5.40	5.18	5.25	3.98	
—C≡N			5.00	4.77	4.88	3.90	0.53
$-CO_2-$		4.76	5.00	5.00 4.75	4.87	1.83	0.64
—CO—		6.08	5.00	5.23	5.44	2.89	0.71
—OH	5.60	5.72	6.50		5.92	1.60	0.78
—C=N—				6.30	6.30	0.50	0.88
$-NH_2$		7.20		6.84	7.02	1.53	1.19
—SO—			6.70		6.70	4.03	0.97
—COOH		7.42			7.42	1.64	
$-CONH_2$				9.34	9.34		

[a] Substituted by aromatic or alphatic substituents as noted.

[b] Aliphatic substituent on alumina basis; original $Q°_i$ values converted first to alumina basis, then to aliphatic basis.

[c] Dipole moments of compounds ø-*i* in solution; data of refs. 19 and 20.

[d] "Hard" base parameters; data of ref. 21.

[e] Probable steric hindrance in original compound (N,N-dimethylaniline).

Many different types of bonding between polar adsorbents and adsorbates have been proposed to explain the differing adsorption energies of various organic compounds: electrostatic interaction of adsorbate dipoles with the electric field of the adsorbent, hydrogen bonding between adsorbate and adsorbent, charge transfer complexation of adsorbent and adsorbate, etc. CHESSICK *et al.*[22] observed that the adsorption energies of various polar groups on rutile, corrected for dis-

persion forces*, are proportional to their dipole moments. This suggests a major role for simple electrostatic forces in adsorption. The extension of this proposition to the group adsorption energies of Table IV is tested in Fig. 5, where $(Q°_i)_S$ is plotted *versus* group dipole moment μ. It is clear, at least for many of the groups of Fig. 5, that group dipole moment by itself cannot account for the total group

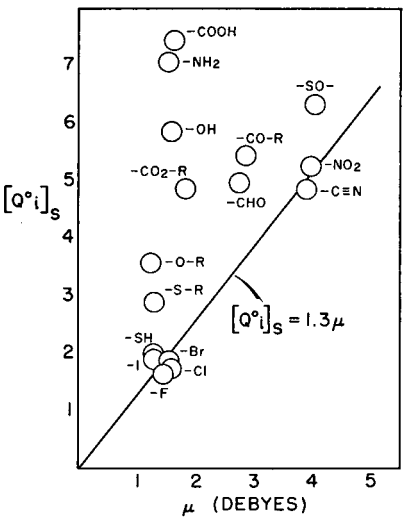


Fig. 5. Standard state group adsorption energies *versus* group dipole moment.

adsorption energy. The maximum possible contribution of group dipole moment to adsorption energy is given by the solid line of Fig. 5 through the lower points. Since this line accurately correlates 7 out of the 16 points of Fig. 5, it is tempting to assume that these 7 groups adsorb by aligning their dipoles with the adsorbent surface field, with most of the group adsorption energy resulting from this interaction.

The various groups that lie above the lower line of Fig. 5 are all recognizable as being more basic than the 7 points on the line (in the sense of being better able to hydrogen bond with a proton donor, for example). This suggests an alternative adsorption mechanism, relative to dipole interaction, involving weak bond formation between a basic adsorbate group i and an acidic adsorbent group. Such an acid–base interaction is not to be confused with complete ionization of the group by proton addition or subtraction, as in the adsorption of acids on alumina, and bases on silica. Weak acid–base interactions without proton transfer have recently been treated quantitatively by DRAGO AND WAYLAND[21]. These authors propose that the heat of such reactions can be expressed by a four parameter equation which recognizes both "hard" and "soft" interactions between the acid and base involved:

$$-\Delta H = E_A E_B + C_A C_B \qquad (4)$$

$-\Delta H$ refers to the heat of reaction of the acid and base, E_A and C_B are parameters

* The group adsorption energies of Table IV are essentially on this basis, since they represent the adsorption energy of the group minus that of an equivalent part of a pentane molecule; dispersion forces should roughly cancel in this subtraction.

which are proportional to the relative acidity of "hard" and "soft" acids, and E_B and C_B are proportional to the relative basicities of "hard" and "soft" bases, respectively. Actually every acid and base simultaneously possesses both "hard" and "soft" character, "hard" referring primarily to electrostatic interactions as in hydrogen bonds and "soft" referring to covalent interactions as in iodine complexes with bases. Since heats and free energies of adsorption from solution have been noted to be approximately equal[16], $(Q°_i)$ could be substituted for $-\Delta H$ in Eqn. (4) for those cases where similar acid–base interactions describe the adsorption of i. To a first approximation Eqn. (4) for adsorption can be split into two limiting relationships for those cases which can be cleanly classified as either "hard" or "soft" interactions:

$$\text{(hard)}\ (Q°_i)_s = E_A E_B \tag{5}$$

$$\text{(soft}\ \ (Q°_i)_s = C_A C_B \tag{5a}$$

This is a necessary simplification in attempting to treat adsorption reactions, since both "hard" and "soft" acid sites may exist separately on the adsorbent surface, with their relative importance as adsorption sites varying with whether a "hard" or "soft" adsorbate group is involved.

Comparison of the $(Q°_i)_s$ values of Table IV with Eqns. (5) and (5a) suggests that acid–base interactions, if they contribute significantly to group adsorption energy, are predominantly "hard" in character; *i.e.* Eqn. (5) applies. The latter relationship is tested in Fig. 6 for those groups whose E_B values have been reported[21]. A fairly

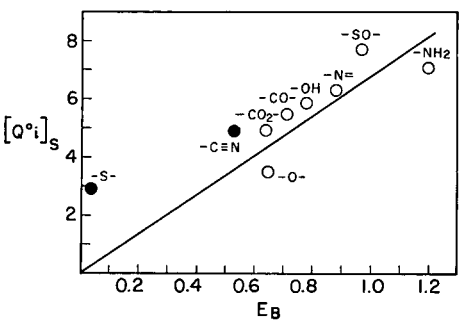


Fig. 6. Standard state group adsorption energies *versus* group basicity.

reasonable correlation is noted ($\pm$ 0.8 unit in $Q°_i$), with the exception of the sulfide group (—S—). Possibly the sulfide group adsorbs as a "soft" base on a different adsorbent site, since this group is predominantly "soft" in character relative to the other groups of Fig. 6. The point for the cyano group is also somewhat high, and this may reflect adsorption of this group by a dipole–adsorbent surface field interaction (*i.e.* purely electrostatic), rather than by an acid–base interaction. However, both of these processes are predominantly electrostatic in origin, and it may be meaningless to attempt to differentiate them. Tentatively we assume that three different adsorption mechanisms are available to a polar group: (*i*) dipole–adsorbent surface field interactions, with $(Q°_i)_s$ equal 1.3 μ; (*ii*) adsorption as a "hard" base on a "hard" acid site, with $(Q°_i)_s$ equal 6.8 E_B; (*iii*) adsorption as a "soft" base on a "soft" acid site, with

TABLE V

GROUP ADSORPTION ENERGIES FOR ALUMINA, SILICA AND FLORISIL

Group	$Q°_i$								
	Alumina			*Silica*			*Florisil**		
	X, Y=Ar	*X=Al Y=Ar*	*X, Y=Al*	*X, Y=Ar*	*X=Al Y=Ar*	*X, Y=Al*	*X, Y=Ar*	*X=Al Y=Ar*	*X, Y=Al*
X—CH_3 methyl	0.06	—	—0.03	0.11	—	0.07	0.10	—	—0.01
X—CH_2—Y methylene	0.12	0.07	0.02	0.07	0.01	—0.05	0.19	0.10	0.01
X—Cl chloro	0.20	—	1.82	—0.20	—	1.32	—0.20	—	1.74
X—F fluoro	0.11	—	1.64	—0.15	—	1.30	—0.15	—	1.54
X—B bromo	0.33	—	2.00	—0.17	—	1.32	—0.17	—	1.94
X—I iodo	0.51	—	2.00	—0.15	—	1.28	—0.15	—	1.94
X—SH mercapto	8.70	—	2.80	0.67	—	1.70	0.67	—	1.70
X—S—S—Y disulfide	?	~ 1.1	2.70	?	0.94	1.90	?	0.94	1.90
X—S—Y sulfide	0.76	1.32	2.65	0.48	1.29	2.94	?	1.30	2.94
X—O—Y ether	1.04	1.77	3.50	0.87	1.83	3.61	0.87	1.81	3.61
X—N—Y tertiary amine	?	2.48	4.40	?	2.52	~ 5.8	?	?	?
X—CHO aldehyde	3.35	—	4.73	3.48	—	4.97	3.35	—	4.97
X—NO_2 nitro	2.75	—	5.40	2.77	—	5.71	3.07	—	5.71
X—C≡ nitrile	3.25	—	5.00	3.33	—	5.27	3.33	—	5.27
X—CO_2—Y ester	4.02	3.40	5.00	4.18	3.45	5.27	4.18	3.45	5.27
X—CO—Y keto	4.36	3.74	5.00	4.56	4.69	5.27	4.56	4.32	5.27
X—OH hydroxyl	7.40	—	6.50	4.20	—	5.60	4.20	—	5.60
X—C=N—Y imine	4.14	4.46	6.00	?	?	?	?	?	?
X—NH_2 amino	4.41	—	6.24	5.10	—	8.00	?	?	?
X—SO—Y sulfoxide	?	4.0	6.70	?	4.2	7.2	?	4.2	7.2
X—COOH carboxylic acid	19	—	21	6.1	—	7.6	6.1	—	7.6
X—$CONH_2$ amide	6.2	—	8.9	6.6	—	9.6	6.6	—	9.6
—C= aromatic carbon	0.31	0.31	0.31	0.25	0.25	0.25	0.18	0 18	0.18

* Assumes $\alpha = 1.00$ for 1% H_2O– Florisil (see ref. 16).

$(Q°_i)_s$ equal 0.37 C_B (from the adsorption energy of the sulfide group). That mechanism which gives the largest value of $(Q°_i)_s$ is assumed to predominate, so that $(Q°_i)_s$ can be predicted when the values of μ, E_B, and C_B for a group are available.

GILES[23] has suggested that hydrogens in such groups as —OH, —SH, >NH, and —CHO may adsorb on alumina by hydrogen bonding to the adsorbent. If true this should manifest itself by anomalously high $(Q°_i)_s$ values for such groups. As seen in Fig. 5 (—SH) and Fig. 6 (—OH, —NH_2), no such enhancement of the adsorption energies of these groups is evident. With the exception of groups with pK_A values less than 12, we conclude that hydrogen bonding of group protons to the adsorbent is unimportant in contributing to group adsorption energy.

Regardless of the actual fundamental interpretation of the data of Table IV these $(Q°_i)_s$ values can be used to predict nominal group adsorption energies for a wide range of groups on each of the present three adsorbents. Table V summarizes these predicted and measured values, the experimental values being given where available.

ACKNOWLEDGEMENT

The author is grateful to his associate, Dr. J. K. FOGO, for editing the original manuscript.

SUMMARY

Functional group adsorption energies $Q°_i$ for adsorption of a sample on alumina, silica, Florisil, and (presumably) other metal oxide adsorbents can now be correlated in terms of a single theoretical model. Given a value of $Q°_i$ for the adsorption of a polar group i on any of these three adsorbents it is possible to predict reliable $Q°_i$ values for the same group on the other two adsorbents. Furthermore, given $Q°_i$ values for the group i substituted either by an aromatic or an aliphatic substituent, it is possible to calculate $Q°_i$ for the opposite case. This in turn permits the calculation of numerous solute adsorption energies and eluent strength values that were previously unavailable. A number of group adsorption energies are now available in a standard state for interpretation in terms of the fundamental molecular processes which give rise to selective adsorption. The present correlations throw additional light on the general mechanisms of adsorption of polar compounds on the metal oxide adsorbents. Alumina preferentially adsorbs acids with pK_A values less than 13, relative to silica and Florisil, while silica appears to preferentially adsorb bases with pK_B values less than 5.

REFERENCES

1 L. R. SNYDER, *J. Chromatog.*, 20 (1965) 463.
2 L. R. SNYDER, *J. Chromatog.*, 16 (1964) 55.
3 H. BROCKMANN, *Discussions Faraday Soc.*, 7 (1949) 58.
4 A. H. SPORER AND K. H. TRUEBLOOD, *J. Chromatog.*, 2 (1959) 499.
5 L. R. SNYDER, *J. Chromatog.*, 6 (1961) 22.
6 L. R. SNYDER, *J. Chromatog.*, 8 (1962) 319.
7 L. R. SNYDER, *J. Chromatog.*, 11 (1963) 195.
8 L. R. SNYDER, *J. Chromatog.*, 12 (1963) 488.
9 L. R. SNYDER, *J. Chromatog.*, 17 (1965) 73.
10 L. R. SNYDER, *J. Phys. Chem.*, 67 (1963) 2344.
11 L. P. HAMMETT, *Physical Organic Chemistry*, McGraw-Hill, New York, 1940, Chap. VII.

12 H. H. JAFFEE, *Chem. Rev.*, 53 (1953) 191.
13 L. R. SNYDER, *J. Phys. Chem.*, 67 (1963) 234.
14 D. WALDI, in E. STAHL (Editor), *Thin-Layer Chromatography, A Laboratory Handbook*, Academic Press, New York, 1965, p. 30.
15 L. R. SNYDER AND B. E. BUELL, *J. Chem. Eng. Data*, in press.
16 L. R. SNYDER, *Advan. Anal. Chem. Instr.*, 3 (1964) 251.
17 D. HARESNAPE, F. A. FIDLER AND R. A. LOWRY, *Ind. Eng. Chem.*, 41 (1949) 2691.
18 W. M. LATIMER AND J. H. HILDEBRAND, *Reference Book of Inorganic Chemistry*, revised Ed., Macmillan, New York, 1940, Appendix XI.
19 C. P. SMYTH, *Dielectric Behavior and Structure*, McGraw-Hill, New York, 1955, Chap. VIII.
20 M. J. ARONEY, L. R. FISHER AND R. J. W. LEFEVRE, *J. Chem. Soc.*, (1963) 4450.
21 R. S. DRAGO AND B. B. WAYLAND, *J. Am. Chem. Soc.*, 87 (1965) 3571.
22 J. J. CHESSICK, A. C. ZETTLEMOYER, F. H. HEALEY AND G. J. YOUNG, *Can. J. Chem.*, 33 (1955) 251.
23 C. H. GILES, in E. HEFTMAN (Editor), *Chromatography*, Reinhold, New York, 1961, Chap. 4.

CARBOBENZOXY DERIVATIVES OF AMINO ACIDS AND PEPTIDES: INSTANT THIN-LAYER CHROMATOGRAPHY AS HYDROBROMIDES*

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INTRODUCTION

In the course of radioactive synthesis of glycylprolylhydroxyproline, a substance which has been isolated from human urine and which may be a factor in the metabolism of collagen, it became necessary to devise a system for determining the purity of the ^{14}C-labelled N-carbobenzoxy derivatives. It was difficult to use conventional methods because of the small amounts of material employed for the radioactive synthesis and because of the inability of some of the more common procedures to effect adequate separation of our constituents. To have pure starting materials for subsequent steps of the synthesis, it was necessary to ascertain the purity of the ^{14}C intermediates (in one instance carbobenzoxyglycine and carbobenzoxyglycylproline). The melting points of the respective synthetic products, 120° *vs.* 155°, should have been sufficient; however, it was difficult to be certain of the melting point of a single crystal with the conventional Mel-Temp apparatus***, although we think we were successful. Another possible solution to the problem was the use of infrared spectroscopy on a microscale. This was ruled out because microequipment for our spectrophotometer was not available and especially because of possible contamination of the equipment with radioactivity. Other comparatively expensive instrumental techniques such as mass spectrometry were also ruled out since such apparatus was not available.

Separation from relative solubility of the crystals offered another possibility, although a measurement of purity such as melting points was still necessary. It was found that complete separation of carbobenzoxyglycine from carbobenzoxyglycylproline was possible by extraction with anhydrous ether when the crystals of either one of these substances were of certain dimensions; that is particle size appeared to be the governing factor. When the carbobenzoxyglycylproline crystals were large enough, a given amount of solvent would remove only the carbobenzoxyglycine.

Preliminary experiments with column chromatography on silica gel indicated that this may be suitable for separating the components. With each pure substance, it appeared that complete removal of the carbobenzoxyglycine from the column was possible before carbobenzoxyglycylproline was eluted. One experiment was carried

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*** Laboratory Devices, Cambridge, Mass., U.S.A.

out on a mixture of equal parts of carbobenzoxyglycine and carbobenzoxyglycylproline with increasing amounts of acetone and decreasing amounts of methylene chloride. Carbobenzoxyglycine was eluted with 11 % acetone, whereas carbobenzoxyglycylproline was eluted with 16 % acetone. The lowered melting point and extended range of the 16–22 % acetone fraction attested to the presence of two compounds. It is probable that utilization of 11 % acetone for a longer time would have provided essentially complete separation.

Paper and thin-layer chromatography nevertheless seemed to be a more convenient solution to the problem. Several workers have published methods for the chromatography of N-carbobenzoxy compounds on both paper and thin layers[1–5]. None that we tried with our compounds proved successful. Preliminary experiments with carbobenzoxyglycine and carbobenzoxyglycylproline on Whatman 1 and 3 MM paper were run in the following solvent systems: *s*-collidine–$H_2O(NH_3)$ (125:44); *n*-BuOH–HOAc–H_2O (4:1:2); EtOH–H_2O (7:3); EtOH–H_2O (1:1); acetone–methylene chloride (11:89); acetone–methylene chloride (2:98). Detection was with Cl_2–starch–KI[6]. In all cases where spots were obtained the R_F's were essentially the same for carbobenzoxyglycine and carbobenzoxyglycylproline. In some of these studies with paper, complications resulted because of reaction of the chlorine with developing systems, such as *s*-collidine, which could only be removed adequately from Whatman 3 MM paper by aeration for approximately three days. In other experiments phenol interfered with the chlorine method of detection.

As a consequence of the difficulties encountered in the separation on paper, we considered the advantages of the recently introduced Gelman Type S Instant Thin-Layer Chromatography plates (ITLC)[7]. These supports feel and handle like paper, are constructed of potassium silicate and glass fibers, and resemble standard silica gel preparations except that they are more alkaline (pH 10.5). In 1959, NEUMANN, LEVIN, BERGER, AND KATCHALSKI described the detection of N-carbobenzoxy derivatives with gaseous hydrogen bromide and ninhydrin after chromatography on Whatman No. 1 paper with *n*-BuOH–HOAc–H_2O (25:6:25) and *n*-PrOH–H_2O–conc. NH_4OH (100:50:1). In one experiment with the ITLC plates in EtOH–H_2O (7:3) and with this method of detection we did not separate carbobenzoxyglycine and carbobenzoxyglycylproline. It seemed to us that treatment with hydrogen bromide gas before chromatography would decarbobenzoxylate the compounds to hydrobromides directly on the plates and might lead to better separation. This was tried in another experiment with the solvent system *n*-BuOH–HOAc–H_2O (4:1:2), and with 0.3 % ninhydrin in water-saturated *n*-butanol for detection. Two definite spots resulted, which seemed to confirm our prediction. The phenol-water system appeared to be a better one to try with this technique since it yields scattered R_F's with many amino acids and peptides. A method based on these principles, which has proved most helpful in our synthesis, is described in this paper.

EXPERIMENTAL*

Standard method

Preparation of ITLC plates. A plate 10 × 20 cm proved most suitable for the method; we therefore cut the 20 × 20 cm commercial plates in half. Two spots (about 1/4 inch in diameter) of 20 μg of material are placed approximately 2 inches

apart and 1 inch from the bottom. One of these is the substance to be tested; the other is carbobenzoxyglycine, which is included in each determination as a reference. It is convenient to use 0.01 ml of solutions containing 2 mg/ml in acetone.

Treatment with HBr. Treatment with hydrogen bromide gas is performed in a well-vented fume hood. Two plates are placed in a 250 mm glass desiccator with sleeve valve in the bottom of which there is a small inverted glass funnel to keep the plates separated, with all surfaces exposed to the gas. The desiccator cover and the sleeve valve are well greased with silicone grease. The cover is adjusted so that there is an opening of about 1 mm for excess gas to escape during filling, rubber tubing from a small tank (lecture bottle) is attached to the inlet tube, and gaseous hydrogen bromide is rapidly introduced for 20 sec. The tank valve, desiccator cover, and desiccator valve are then closed immediately. To prevent any considerable leakage of gas, the rubber tubing is removed from the desiccator, and the inlet tube is stuffed with a plug of Kimwipe**. The plates are kept in the gas for 17 min, during which time they tend to curl up and accumulate orange spots. The desiccator cover is then drawn about one-third open to allow rapid escape of gas.

Washing with ether. After 10 min each plate is placed in separate shallow glass dishes 300 × 85 mm, and 300 ml of fresh anhydrous ether are added to each. The plates are washed with the ether for 5 min by carefully rocking the dishes 3 or 4 times/min. They are removed to dry beakers, and the ether is allowed to evaporate for 10 min. The waste ether wash contains considerable orange color. After evaporation, the beakers containing the plates are placed in an oven, and drying is continued for 15 min with circulating air at room temperature.

Chromatography. Rectangular glass museum jars, 250 × 250 × 155 mm, with covers that are well greased with silicone grease are used for ascending chromatography. Phenol–water (4:1, w/w), 177.5 ml, usually prepared by adding 20 ml of

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* *Materials.* (1) Gelman Instant Thin-Layer Chromatography Type S plates (ITLC), Gelman Instrument Co., Ann. Arbor, Mich. (2) Liquefied phenol, 90.7%, certified reagent, Fisher. (3) Anhydrous ether, ammonium hydroxide, and pyridine, analytical reagents, Mallinckrodt. (4) Anhydrous hydrogen bromide gas, Matheson. (5) 1-Butanol, acetic acid, and *tert.*-butanol, certified reagents, Fisher. (6) Ninhydrin, Dougherty Chemical Co., N.Y., recrystallized once from hot water. (7) Glycine (HGlyOH), A grade, Calbiochem. (8) L-Proline (L-HProOH), L-hydroxyproline (L-HyproOH), carbobenzoxyglycyl-L-leucine (ZGly-L-LeuOH), carbobenzoxyglycyl-L-tryptophan (ZGly-L-TryOH), carbobenzoxy-L-prolyl-L-leucylglycine (Z-L-Pro-L-LeuGlyOH), and carbobenzoxyglycylsarcosine (ZGlySarcosine), M.A., Mann Research Laboratories, Inc., N.Y. (9) Carbobenzoxy-L-proline (Z-L-ProOH), carbobenzoxy-L-hydroxyproline (Z-L-HyproOH), carbobenzoxy-L-tryptophan (Z-L-TryOH), carbobenzoxyglycyl-L-serine (ZGly-L-SerOH), carbobenzoxy-L-methionine (Z-L-MetOH), and dicarbobenzoxy-L-cystine [diZ-L-$(CySOH)_2$], C.P., Mann Research Laboratories, Inc., N.Y. (10) Carbobenzoxy-L-alanine (Z-L-AlaOH), carbobenzoxy-L-phenylalanine (Z-L-PheOH), carbobenzoxy-L-leucine (Z-L-LeuOH), carbobenzoxyglycyl-L-phenylalanine (ZGly-L-PheOH), and carbobenzoxy-L-leucylglycine (Z-L-LeuGlyOH), Cyclo Chemical Corp. (11) L-Tryptophan (L-HTryOH), General Biochemicals, Inc. (12) Carbobenzoxyglycine (ZGlyOH), m.p. 118°–120°, prepared according to procedure described by GREENSTEIN AND WINITZ[8]. (13) Carbobenzoxyglycyl-L-proline (ZGly-L-ProOH), m.p. 152°–156°, prepared according to RYDON AND SMITH[9]. (14) Carbobenzoxyglycyl-L-prolyl-L-hydroxyproline benzyl ester (ZGly-L-Pro-L-HyproOBz)-I, purchased commercially. (15) Carbobenzoxyglycyl-L-prolyl-L-hydroxyproline benzyl ester (ZGly-L-Pro-L-HyproOBz)-II, synthesized according to POROSHIN *et al.*[10] (16) Glycyl-L-prolyl-L-hydroxyproline benzyl ester hydrobromide (HBr·HGly-L-Pro-L-HyproOBz), synthesized from another sample of ZGly-L-Pro-L-HyproOBz by the usual procedure for decarbobenzoxylation with dry hyrogen bromide gas in liquid acetic acid.

** Kimberly Clark Corp., Neenah, Wisc., U.S.A.

water to 157.5 ml of liquefied phenol and shaking, is poured into the jars. A 50-ml beaker containing 1 ml of 2 *N* ammonia and 29 ml of water is placed in the bottom, and one prepared plate is adjusted so that the top is inclined and rests on the wall. The jar is covered immediately, and chromatography is allowed to proceed at room temperature until the solvent front has travelled at least 10 cm. After development, the plate is removed and inverted, and the solvent front is marked with pencil. It is then transferred to a dry beaker and placed in the oven for drying overnight at room temperature with circulating air (usually 16 h).

After drying, the plate is sprayed with 0.3 % ninhydrin in water-saturated 1-butanol, prepared by shaking 100 ml of 1-butanol with 30 ml of water and draining off the bottom layer. The plate is sprayed throughout until the change in color (darkening) indicates complete wetting. After spraying, the plate, resting in a beaker, is brought to 110° in the oven with circulating air. It is then examined for initial coloration, returned to the oven, and kept at 110° for 15 min. This process produces various colors according to the materials tested and leaves the background essentially colorless or white.

Method with equilibration

This method is the same as the standard method until chromatography. The only difference is that the walls of the museum jar are neatly lined with Whatman No. 1 filter paper while still dry. The solvent mixture is then poured in and shaken around in the jar to wet the lower portions of the filter paper lining. A beaker of the dilute ammonia is placed in the bottom, and the prepared plate is adjusted in the chamber with its bottom resting on an inverted dish to keep the plate from coming in contact with solvent. The covered jar is allowed to equilibrate in this way for 1 h. Then the cover is opened rapidly, the dish is removed, and the plate is placed in contact with the solvent. After replacing the cover, chromatography is allowed to proceed as usual. The remainder of the method is the same as the standard method.

Method modified for other systems

This is the same as the standard method except that the plates, after chromatography, are dried at room temperature in the circulating oven for only 1 h instead of overnight.

RESULTS AND DISCUSSION

Most of the chromatographic work reported in this study was done without equilibration. In 35 determinations of carbobenzoxyglycine, 11 of carbobenzoxyglycylproline, 6 of carbobenzoxyhydroxyproline, and 7 of carbobenzoxytryptophan without equilibration, the values of R_F were found to vary considerably. However, some that were obtained with equilibration showed more uniformity; this is consistent with the literature for thin layer chromatography[11–13]. Part of Table I shows the results by the standard method for four pairs of amino acids and their corresponding carbobenzoxy derivatives. Each pair was chromatographed in the same tank and at the same time. Each has almost identical values, indicating that the same, and only one compound, probably results on hydrogen bromide treatment of the amino acid and of its respective carbobenzoxy derivative. (Omission of hydrogen bromide before

chromatography of the free amino acids in the phenol–water system on the Gelman Type S plates produces considerable blurring that masks subsequent detection with ninhydrin.) The rest of Table I contains results of individual determinations (two or three are averages of duplicates) of 13 other N-carbobenzoxy-protected amino acids and peptides. Most of the carbobenzoxy compounds flow very nearly as fast as the solvent front in the phenol–water system; *i.e.* their R_F values are about 0.9 or higher.

TABLE I

R_F VALUES OF CARBOBENZOXY COMPOUNDS AND SOME FREE AMINO ACIDS

Compound	R_F[a]	*Solvent front (mm)*	*Development time (min)*
ZGlyOH	0.395	139	120
HGlyOH	0.42	139	120
ZProOH	0.91	144	128
HProOH	0.98	137	128
ZHyproOH	0.89	137	120
HHyproOH	0.84	137	120
ZTryOH	0.97	148	128
HTryOH	0.94	144	128
ZMetOH	0.97	118	205
Di-Z(CySOH)$_2$	0.27	106	205
ZPheOH	0.965	115	210
ZALaOH	0.68	127	210
ZLeuOH	0.97	124	132
ZGlyProOH	0.93[b]	—	—
ZGlyLeuOH	0.93	134	126
ZGlySarcosine	0.89	110	136
ZGlySerOH	0.56	104	136
ZGlyTryOH	0.94	139	125
ZLeuGlyOH	0.96	137	154
ZGlyPheOH	0.97	129	132
ZProLeuGlyOH	0.93	123	236

[a] R_F values without equilibration.
[b] Average for this compound.

The carbobenzoxy compounds probably are quantitatively converted into the hydrobromides by the hydrogen bromide technique, and, therefore, other solvent systems, more suitable for the separation of specific hydrobromides from mixtures of carbobenzoxy compounds, can be substituted for the phenol–water system of the standard method. Table II shows the results of determinations on two samples of N-carbobenzoxyglycyl-L-prolyl-L-hydroxyproline benzyl ester (ZGly-L-Pro-L-HyproOBz) and one of glycyl-L-prolyl-L-hydroxyproline benzyl ester hydrobromide (HBr·HGly-L-Pro-L-HyproOBz) by the standard method and by the method modified for other systems. On the assumption that a second treatment with hydrogen bromide does not alter the ester hydrobromide, all three compounds should have the same R_F in a given solvent system. The values are the same in all five systems used. The presence of additional ninhydrin spots with different R_F's indicates that impurities are present in one of the carbobenzoxy esters and in the hydrobromide.

It should be mentioned that when the technique of hydrogen bromide treatment before chromatography was tried on filter paper (Whatman No. 1, 3 MM, 3 MC), a

TABLE II

CHROMATOGRAPHY OF GLYCYLPROLYL HYDROXYPROLINE BENZYL ESTERS

Compound	*Developing system*	R_F	*Solvent front (mm)*	*Development time (min)*
ZGly-L-Pro-L-HyproOBz (I)	phenol–$H_2O(NH_3)$(4:1)	0.96	149	120
HBr·HGly-L-Pro-L-HyproOBz	phenol–$H_2O(NH_3)$(4:1)	0.97	146	120
ZGly-L-Pro-L-HyproOBz (I)	*n*-BuOH–HOAc–H_2O(4:1:2)	0.71	113	150
		0.345		
HBr·HGly-L-Pro-L-HyproOBz	*n*-BuOH–HOAc–H_2O(4:1:2)	0.71	110	150
ZGly-L-Pro-L-HyproOBz (I)	*n*-BuOH–HOAc–H_2O (4:1:2)	0.66	139	—
		0.29		
HBr·HGly-L-Pro-L-HyproOBz	*n*-BuOH–HOAc–H_2O(4:1:2)	0.66	139	—
ZGly-L-Pro-L-HyproOBz (I)	*n*-BuOH–HOAc–H_2O(4:1:1)	0.69	137	—
HBr·HGly-L-Pro-L-HyproOBz	*n*-BuOH–HOAc–H_2O(4:1:1)	0.70	140	—
ZGly-L-Pro-L-HyproOBz (I)	*t*-BuOH–HOAc–H_2O(4:2:1)	0.90	140	165
HBr·HGly-L-Pro-L-HyproOBz	*t*-BuOH–HOAc–H_2O(4:2:1)	0.82	147	165
		0.53		
ZGly-L-Pro-L-HyproOBz (II)	*n*-BuOH–ϕOH–HOAC–H_2O(25:10:10:10)	0.71	135	142
HBr·HGly-L-Pro-L-HyproOBz	*n*-BuOH–ϕOH–HOAc–H_2O(25:10:10:10)	0.69	130	142
		0.13		
ZGly-L-Pro-L-HyproOBz (II)	*n*-BuOH–ϕOH–HOAC–H_2O(25:10:10:10)	0.68	130	142
HBr·HGly-L-Pro-L-HyproOBz	*n*-BuOH–ϕOH–HOAc–H_2O(25:10:10:10)	0.64	133	142

marked blurring was encountered with all of our solvent systems. This was quite the same as the blurring produced by omission of hydrogen bromide before chromatography in the phenol–water system with the Gelman plates. This effect on filter paper was unexpected, since the conditions of Katchalski *et al.*[5] and ours were apparently similar. Although we were unable to avoid blurring completely with ninhydrin in acetone and pyridine for detection[5] and incorporation of bases with the ether wash after hydrogen bromide treatment, these modifications did yield acceptable chromatograms about half of the time. This difficulty can probably be completely avoided with further study. The technique of hydrogen bromide treatment before chromatography can then be extended to filter paper.

SUMMARY

A method is described for the chromatography of carbobenzoxy derivatives of amino acids and peptides on instant thin-layer chromatography plates as the hydrobromides by treatment with HBr gas on the plates before chromatography. It is shown that the method produces the same and only one compound from each of four pairs of free amino acids and their corresponding carbobenzoxy derivatives. Data are presented for the chromatography of 18 carbobenzoxy derivatives by the method. The results and the method, as well as modifications for equilibration and for solvent systems other than the phenol–water system, are described. The method appears to be suitable for the detection of impurities in this type of compound and therefore is useful in the synthesis of peptides.

REFERENCES

1 P. SCHELLENBERG, *Angew. Chem.*, 74 (1962) 118.
2 G. PATAKI, *J. Chromatog.*, 12 (1963) 541; 16 (1964) 553.
3 F. CRAMER AND E. EHRHARDT, *J. Chromatog.*, 7 (1962) 405.
4 H. N. RYDON AND P. W. G. SMITH, *Nature*, 169 (1953) 922.
5 H. NEUMANN, Y. LEVIN, A. BERGER AND E. KATCHALSKI, *Biochem. J.*, 73 (1959) 33.
6 R. A. BOISSONNAS AND S. GUTTMAN, *Helv. Chim. Acta*, 43 (1960) 200.
7 GELMAN INSTRUMENT COMPANY, *Tech. Bull.*, No. 201.
8 J. GREENSTEIN AND M. WINITZ, *Chemistry of the Amino Acids*, Wiley, New York, 1961, p. 895.
9 H. N. RYDON AND P. W. G. SMITH, *J. Chem. Soc.*, (1956) 3642.
10 K. T. POROSHIN, T. D. KOZARENKO, V. A. SHIBNEV AND V. G. DEBABOV, *Biochemistry USSR*, 25 (1960) 531.
11 K. RANDERATH, *Thin-Layer Chromatography*, Academic Press, New York, 1963, p. 59.
12 M. BRENNER AND A. NIEDERWIESER, *Experientia*, 16 (1960) 378.
13 M. S. J. DALLAS, *J. Chromatog.*, 17 (1965) 267.

A STUDY OF THE CONVERSION OF PHENOTHIAZINE DERIVATIVES TO THE CORRESPONDING SULFOXIDES ON THIN-LAYER PLATES

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Previous experimental work[1] reported from this laboratory on the phenothiazine derivatives has dealt mainly with color, crystal and spectrophotometric tests as a means of identification. In a recent preliminary report[2] at a meeting of the American Academy of Forensic Science attention was drawn to the value of the sulfoxides as a further means of identification of these drugs and a tentative method for preparing them quickly and accurately was described. The present paper extends the data of the preliminary report and deals with a complete study of 40 phenothiazine derivatives and their respective sulfoxides employing ultraviolet spectrophotometry, thin-layer and gas chromatography.

MATERIALS

Reference compounds

The phenothiazine derivatives and a few sulfoxides were obtained from the various manufacturers. The remaining sulfoxides were prepared according to the method of SCHMALZ AND BURGER[3]. The identity of the synthesized products was established by means of ultraviolet and infrared spectrophotometry, color tests, thin-layer and where applicable gas chromatography.

Apparatus

The ultraviolet spectrophotometric data were obtained on a Beckman DK-2A Ratio Recording Spectrophotometer, the infrared data on a Beckman IR-4 Infrared Spectrophotometer and the gas chromatographic work was done on a Microtek GC-2500 R gas chromatograph. A short-wave ultraviolet lamp, model SL 2537 manufactured by Ultraviolet Products Inc., South Pasadena, Calif., was used to locate the spots on the thin-layer chromatography plates.

Thin-layer chromatography

Plates: 20.5 × 20.5 cm.

Absorbants: Silica Gel GF-254 and Silica Gel G according to STAHL, E. Merck, Darmstadt, Germany; Adsorbasil P-2, Applied Science Laboratories Inc., State College, Pa., U.S.A.; Aluminum Oxide G, according to STAHL, E. Merck, Darmstadt, Germany.

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Solvent: ammonium acetate 1.5 g, dist. water 10.0 ml, methanol up to 50.0 ml.
Locating agents: U.V. light (short wave).
Iodoplatinic acid.

Gas chromatography

Column: type: stainless steel and glass; length 60 cm; internal diameter 7 mm; coating 3 % SE-30; support Gas Chrom Q, 80–100 mesh, Applied Science Laboratories, Inc., State College, Pa., U.S.A.

Carrier gas: helium; inlet pressure 30 p.s.i.; outlet pressure atmospheric.

Temperature: column 210° and 250°; inlet 300°; outlet 290°; detector 290°.

Detector: flame ionization.

Recorder: Minneapolis-Honeywell.

EXPERIMENTAL

Each of the following drugs in form of their salts were used in the experiments described below: chlorpromazine, thioridazine, trifluoperazine, fluphenazine, triflupromazine, promazine, promethazine.

During routine work on the identification of phenothiazines it was observed that when a developed thin-layer plate was left in the open air for some days, the ultraviolet spectrum of an eluted spot no longer resembled that of the original product, but was closer to its sulfoxide. Gas–liquid and thin-layer chromatography confirmed, that indeed the sulfoxide had been formed, and because of this, certain conditions influencing the oxidation of phenothiazines on thin-layer plates were further investigated.

Plates were prepared for ten different compounds. Each plate contained eight spots of the drug assigned to it. The plates were developed in the solvent cited above and were allowed to stand in the open air in day light at approximately 20°. Each day one spot was eluted with distilled water and the ultraviolet spectrum recorded. After an interval of 48 to 72 h, depending on the derivative under study, marked changes in the adsorption spectrum had taken place; the spectrum began to resemble that of the corresponding sulfoxide. At the end of 8 days the spectrum was that of the pure sulfoxide (see Table I).

While a change in color of the spot was noted a few minutes after the dry plate was exposed to daylight, it was not accompanied by a change in the ultraviolet spectra. Gas–liquid and thin-layer chromatography confirmed that only the sulfoxide was found.

It was found that the oxidation was dependent mainly on the availability of oxygen. If the experiments were performed without circulating air, no oxidation occurred. The same was observed when the plates, spotted in the usual manner, were kept in a tightly closed vessel in an atmosphere of nitrogen for up to two weeks.

Apart from this it was evident that neither daylight nor ultraviolet light was essential in the oxidation process; no change in the ultraviolet spectra was observed after four days of irradiation of the plates with ultraviolet light in a dark room without circulating air.

It was observed, however, that deep brownish colors had developed in the spots. A control plate placed in the same room, but excluded from ultraviolet irradia-

TABLE I

SPECTROPHOTOMETRIC DATA AT THE END OF EIGHT DAYS IN DAYLIGHT AND OPEN AIR (*ca.* 20°) OBTAINED FROM THE ELUTED SPOTS

Generic name	*Maxima of the standard drugs*	*Maxima of the sulfoxides eluted from TLC plates*
Chlorpromazine	254–305	238–274–298–340
Thioridazine	262–313	235–260–276–304
Trifluoperazine	257–308	244–275–304–336
Thioproperazine	234–264–315	249–275–304–342
Levopromazine	251–303	240–275–296–329
Perphenazine	255–307	233–275–300–340
Fluphenazine	258–308	233–255–274–302–340
Triflupromazine	257–308	233–255–274–300–340
Promazine	252–303	233–272–295–340
Promethazine	249–299	234–260–295–330

tion had acquired faint colors only and again no change in the ultraviolet spectra was noted.

Reports in the literature have indicated that the adsorbant gels may cause a chemical change in the compounds adsorbed on it. Our experience with several absorbants is, that per se they do not have any effect on the formation of the sulfoxides and it was further established that the thickness of the gels was not a decisive factor. It is therefore our opinion that the oxidation reaction appears to be associated with a relatively large increase in the surface area of the phenothiazine drugs adsorbed on the plates. Such an increase is brought about by the development of the plate after spotting with drug.

In order to check the effect of temperature on the oxidation, a series of plates were spotted and placed in an oven with circulating air at 60°. Conversion to the sulfoxide became measurable after about 2 h and was complete after 3–4 days. Contrary to our expectation, only traces of oxidized material were detectable by ultraviolet measurements at the end of three days at 120° but further experimentation in this area is in progress. No oxidation took place on a plate left at 0° for five weeks.

In all these experiments it was observed, that when spotted plates were maintained in daylight at approximately 20°, with or without oxygen, a color characristic of the compound employed developed in the spots within 15 min. In darkness the development of color was delayed. When ultraviolet irradiation was applied to spots at 20° in a dark room a color developed about as rapidly as it did in daylight. The shades were pastel at first, but with longer time of irradiation they assumed a different hue in contrast to those developed in daylight when the shade of the color increased. The temperature at which the plates were stored, played an important role in the color development. After six weeks at 0° only very faint colors were evident, but with temperatures well above 20° the colors appeared very rapidly and were more intense.

Experiments employing hydrogen peroxide as oxidizing agent

In our preliminary report[3] it was shown that spots of phenothiazine developed on thin-layer plates could be oxidized to the sulfoxide rapidly by use of a 3 % solution

of hydrogen peroxide. At that time the optimum concentration of peroxide had not been determined. Further investigations have shown that the concentration of hydrogen peroxide is not critical but that best results are obtained when the concentration is between 10 % and 20 %. The oxidation product obtained in this manner on the plate was confined to one spot only as indicated in Fig. 1 above the spot treated with 10 % to 20 % hydrogen peroxide and the R_F value corresponds to that of the pure sulfoxide. The optimum hydrogen peroxide concentration was determined as follows.

In Fig. 1 the original compound chlorpromazine HCl was spotted in such a manner as to obtain a large spot without tailing and the chromatogram allowed to develop in direction 1. The spot was outlined under ultraviolet light and one strength of peroxide was applied to the upper part and another concentration to the lower part of the spot. On line with, and next to the spot two reference spots were applied, one

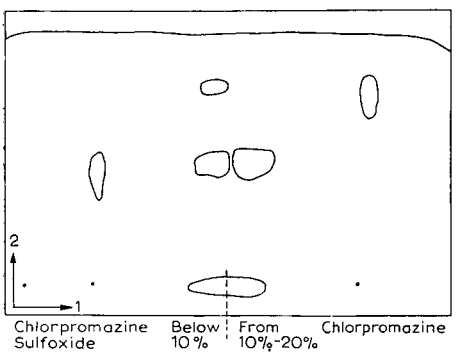

Fig. 1. Effect of the concentration of hydrogen peroxide on the complete oxidation of chlorpromazine.

of chlorpromazine and one of chlorpromazine sulfoxide. The plate was now allowed to develop in direction 2 perpendicular to the first one. This experiment indicated that in order to obtain a complete conversion to the sulfoxides for the quantity employed a concentration of at least 10 % to 20 % hydrogen peroxide was essential. Below that concentration only portions of the drug were oxidized and two spots were obtained upon rechromatographing. There was some indication that when 30 % hydrogen peroxide was employed together with hot air to dry the spot a second compound was formed, travelling behind the sulfoxide on the plate. This compound might be the corresponding sulfone. The sulfoxides were identified by their ultraviolet spectra, color tests, thin-layer chromatography and where applicable by gas–liquid chromatography.

Gas chromatography

Gas chromatography represents the most satisfactory means to date of separating and identifying the phenothiazines and their respective sulfoxides. Excellent separations of these were achieved utilizing the SE-30 column at different temperatures (Tables II and III). It should be noted, however, that only very broad peaks could be obtained for the sulfoxides of the four phenothiazines with the highest molecular weight. The retention times for these four varied from 40 to 60 min. All

TABLE II

RETENTION TIMES OF PHENOTHIAZINE DERIVATIVES

Compound	*Retention time*		
	Flow rate 80 ml/min (200°)	*Flow rate 90 ml/min (210°)*	*Flow rate 120 ml/min (250°)*
Trifluprom azine	6.8	2.4	—
Promethazine	7.8	2.8	—
Promazine	9.1	3.1	—
Chlorpromazine	—	4.5	—
Levopromazine	—	5.7	—
Trifluoperazine	—	8.6	—
Fluphenazine	—	28.7	4.3
Thioridazine	—	29.8	5.8
Perphenazine	—	—	9.6
Thioproperazine	—	—	17.6

compounds were injected in the free state as well as in salt form without any differences appearing in the chromatogram.

Proposed method for the identification of phenothiazine derivatives

The phenothiazine derivative is spotted in the lower two corners of a thin-layer plate (20 × 20 cm) approximately 3 cm from the edges. The plate is placed in the developing tank, with the spots at the bottom, until the solvent has travelled to about 3–4 cm from the top, after which time it is taken out and allowed to dry. The spots are now outlined under the short wave ultraviolet light (cited above) and the right hand one is just wetted with 10 % to 20 % hydrogen peroxide solution by adding it dropwise in small drops. The spot is dried in a hot air stream (60°) after which the plate is placed in the tank again with the peroxide treated spot at the bottom and allowed to develop in a direction perpendicular to the initial one. In this way

TABLE III

RETENTION TIMES OF PHENOTHIAZINE SULFOXIDES

Sulfoxide of	*Retention time*	
	Flow rate 90 ml/min (210°)	*Flow rate 120 ml/min (250°)*
Trifluprom azine	4.2	—
Promethazine	6.6	—
Promazine	7.8	—
Chlorpromazine	10.2	2.5
Levopromazine	11.8	3.1
Trifluoperazine	16.8	3.6
Fluphenazine	—	—
Thioridazine	—	—
Perphenazine	—	—
Thioproperazine	—	—

TABLE IV

R_F VALUES AND SPECTROPHOTOMETRIC DATA OF PHENOTHIAZINE SULFOXIDES

Generic name	*R_F value*		*Maxima of the sulfoxides obtained in the manner described*
	Original drug	*Sulfoxide*	
1 Acetophenazine	0.69	0.16	251–274S–310
2 Acetopromazine or acetylpromazine	0.58	0.39	251–272S–310–343
3 Aminopromazine or proquamazine	0.60	0.28	232–266–295–333
4 Carphenazine	0.71	0.16	246–277S–310
5 Chlorpromazine	0.62	0.48	238–273–298–340
6 Chlorproethazine	0.69	0.49	238–250S–273–298–340
7 Chlorprothixene	0.66	0.45	255–302
8 Cyamepromazine	0.61	0.39	243–274S–304–340
9 Diethazine	0.69	0.44	233–268–293–338
10 Dimethoxanate	0.56	0.41	240–274–295
11 Ethopropazine or prophenamine	0.70	0.47	233–267–292–336
12 Fluphenazine	0.75	0.59	232–273–304–343
13 Isopromethazine	0.63	0.39	233–267–291–336
14 Isothipendyl	0.64	0.41	238–273–336
15 Levopromazine	0.87	0.59	250–276S–296–333
16 Mepazine	0.58	0.39	231–272–299–342
17 Methdilazine	0.64	0.43	232–272–298–342
18 Methopromazine or methoxypromazine	0.67	0.35	244–274S–294–330
19 Methylpromazine	0.62	0.40	238–272–299–340
20 Perphenazine or chlorpiprozine	0.65	0.45	240–250S–274–342
21 Phenothiazine or fenethazine	0.69	0.37	232–266–294–334
22 Pipamazine	0.83	0.53	239–274–300–342
23 Prochlorperazine	0.55	0.15	238–274–300–340
24 Promazine	0.51	0.37	231–271–299–342
25 Promethazine	0.66	0.38	232–270–297–340
26 Propiomazine	0.77	0.54	246–265S–304–360
27 Prothipendyl	0.69	0.48	238–276–340
28 Pyrathiazine or pyrrolazate	0.64	0.43	232–269–295–336
29 Thiazinamium	0.53	0.33	232–269–294–336
30 Thiethylperazine	0.47	0.28	238–272–301–350
31 Thiopropazate	0.77	0.25	238–274–300–340
32 Thioproperazine	0.43	0.26	245–262S–275–304–342
33 Thioridazine	0.71	0.46	237–273–302–340
34 Transergan	0.53	0.33	225–266–291–330
35 Trifluoperazine	0.63	0.41	233–273–302–343
36 Triflupromazine	0.69	0.50	233–274–301–343
37 Trimeprazine or alimemàzine	0.71	0.50	232–297–340
38 No. 6710 Rhône-Poulenc	0.63	0.46	251–273–298–332
39 No. 9260 Rhône-Poulenc	0.85	0.59	240–274–305
40 No. 7261 Smith Kline & French	0.78	0.27	233–272–302–340

two R_F values can be obtained, one for the phenothiazine derivative and one for the sulfoxide. The spots can now be removed from the plate and eluted with distilled water. After the ultraviolet spectra are obtained from the supernatant the original phenothiazine and its sulfoxide can be compared with reference compounds by use of thin-layer and gas–liquid chromatography. It should be noted that even after the most careful elution the gel remaining in the centrifuge tube still contains some sulfoxide which may be demonstrated by color tests[1].

In Table IV is shown the ultraviolet spectrophotometric data on the 40 phenothiazine sulfoxides investigated together with the R_F values and those of the parent compound.

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SUMMARY

The difficulty of obtaining the sulfoxides of the numerous phenothiazine derivatives now in use in medicine has limited their value in forensic chemistry as a satisfactory means of identifying these important drugs. In this paper is described a relatively simple and efficient method for oxidizing the phenothiazines to their respective sulfoxides in pure state. It also describes how the sulfoxides may be obtained quickly and satisfactory by elution from thin-layer chromatography plates so that the necessary chemical, spectrophotometric and chromatographic tests may be carried out. Extensive data on 40 phenothiazine derivatives and their sulfoxides studied to date are presented.

REFERENCES

1 G. H. W. LUCAS AND C. FABIERKIEWICZ, *J. Forensic Sci.*, 8, No. 3 (1963) 462.
2 C. KORCZAK-FABIERKIEWICZ, J. KOFOED AND G. H. W. LUCAS, *J. Forensic Sci.*, 10, No. 3 (1965) 308.
3 A. C. SCHMALZ AND A. BURGER, *J. Am. Chem. Soc.*, 76 (1954) 5456.

STUDIES IN THE RELATIONSHIP BETWEEN MOLECULAR STRUCTURE AND CHROMATOGRAPHIC BEHAVIOUR

PART VI. THE BEHAVIOUR OF SOME ALKYL ETC. PHENOLS CHROMATOGRAPHED BY REVERSED-PHASE THIN-LAYER PARTITION CHROMATOGRAPHY*

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INTRODUCTION

In earlier papers[1,2] we have reviewed the use of reversed-phase partition paper chromatography in the separation of phenols and, in particular, the use of such systems in a study of the relationship between molecular structure and chromatographic behaviour.

GREEN and co-workers[3–6] used papers impregnated with ethyl oleate in a study of the MARTIN[7] additivity principle, relating molecular structure to chromatographic behaviour.

Using the same impregnant, ethyl oleate, in reversed-phase thin-layer partition chromatography, we have found that much less was required to impregnate cellulose powder than to impregnate cellulose in the form of paper strips[1].

The chromatographic parameters, R_F and R_M, obtained by chromatographing a series of nitrophenols on thin layers of cellulose impregnated with ethyl oleate[2], under carefully controlled conditions, using polar mobile phases were used to explain the behaviour of these compounds, relative to the physico-chemical processes involved. It was suggested that the mechanism of the chromatographic process was essentially partition of the nitrophenols between the non-polar stationary phase and the polar mobile phase. The phenols, at first dissolved in the non-polar phase, may be dissolved by the mobile phase as a result of:

(i) Solvation of the phenolic group by the proton acceptor (water or ethanol).

(ii) Solvation of the nitro group with the mobile phase.

The non-polar or hydrophobic part of the molecule will be dissolved by the non-polar stationary phase, by the normal processes of dissolution. This factor will be of relatively greater importance in the chromatographic behaviour of the alkylated phenols than it is for the polar nitrophenols.

The overall amount of solvation of the phenolic group will be affected by:

(i) The ability of the phenol group to be solvated. This may be influenced by steric factors.

* For Parts II, III, IV and V of this series, see refs. 8, 1, 2 and 9.

(ii) Altering the polarity of the phenolic grouping.

(iii) Self association of the phenol.

To minimise the polarity effects, homologous series of alkylated phenols were chosen. Such low concentrations of the phenols were present that the relatively large number of hydroxyl groups in the mobile phase caused self association to be improbable.

EXPERIMENTAL

Cellulose (15 g), slurried with 70 ml of a 0.75 % (v/v) solution of ethyl oleate in diethyl ether, was used to coat glass plates using a Shandon thin-layer applicator, as previously described[2].

Aqueous ethanol (25 % v/v and 37.5 % v/v) were the mobile phases[2]. The application of the phenols (1 μl of 0.25 % solutions in suitable solvents) to the layers was done with our multiple-spotting device[8], and the chromatograms were eluted by an ascending technique in our double saturation chamber[8], at a constant temperature of 25° ± 0.5°, for a fixed period of time, until the solvent front had travelled a distance of 14.5 ± 0.5 cm. The phenols were detected as yellow spots on a purple background by spraying the layers with alkaline permanganate.

RESULTS

The results are shown in the various sections of Table I. Where the R_F/R_M values of a given phenol are quoted in more than one section of the Table, this is done to enable comparisons to be made. The results are the mean of 4 runs on plates carrying an internal standard. The values for the internal standard did not differ by more than ± 0.01 R_F units from a pre-determined mean. The results for the individual phenols were also reproducible to ± 0.01 R_F units.

DISCUSSION

For convenience the compounds are divided into arbitrary groups similar to those considered for the adsorption chromatography of these compounds on alumina surfaces[9].

(a) *Methylated phenols*

The R_F/R_M values of these phenols in the eluent systems, aqueous ethanol (25 % v/v) (to be referred to as System 1) and aqueous ethanol (37.5 % v/v) (to be referred to as System 2) are given in Table Ia. From this and from Fig. 1 it can be seen that the migration of the phenols is dependent upon the position of the methyl groups relative to the phenolic group, and that, as was the case for adsorption chromatography[9], they may be divided into three groups according to the number of *ortho*-substituents present in the molecule.

It can be seen from Table Ia and from Fig. 1 that the addition of methyl groups to one or both *ortho*-positions has a smaller effect on the R_F values than was the case in adsorption chromatography[9]. Indeed the addition of the second *ortho*-methyl group has a smaller effect than the addition of the first. This is in accord with

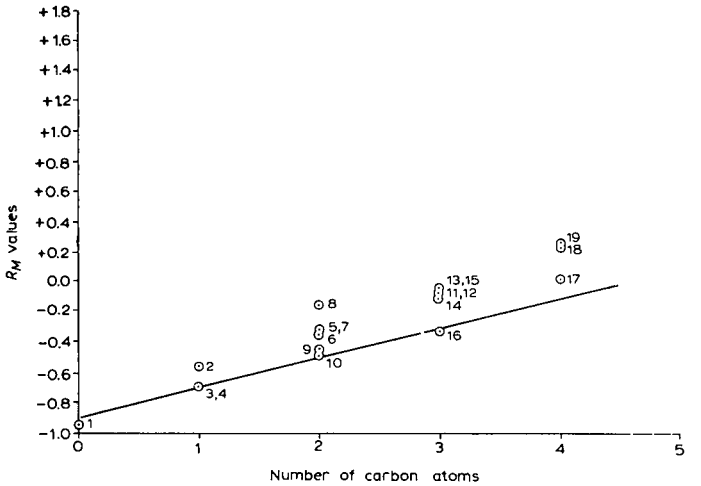

Fig. 1. R_M values (System 2) for methylated phenols *vs.* numbér of carbon atoms in the side chains.

the observation of GREEN *et al.*[4] from their work on ethyl oleate impregnated paper.

A probable explanation is that in the adsorption system the approach of the phenolic oxygen to the hydroxylated alumina is sterically hindered. In the partition system, however, the steric hindrance is to solvation of the phenolic group by the aqueous eluents. HEINEN[10] has suggested that the acid dissociation constant of 2,6-dimethylphenol shows no steric hindrance of solvation of the phenolic group by water, but that the same molecule does exhibit steric hindrance to hydrogen bonding with acetone. He suggests that this is because of the different sizes of the solvating molecules, the approach of the small water molecule is unhindered while that of the larger acetone molecule is. In the partition system, predominantly aqueous phases are the mobile phases and migration of the phenols is supposed to take place as a consequence of solvation of the phenolic group by this mobile phase. This, by token of HEINEN's suggestion[10], is little affected by steric hindrance.

In each of the three groups, the addition of one or more methyl groups to the ring results in a decrease in R_F values relative to that of the parent phenol. In Group 1 (no *ortho*-substituents), Fig. 1 clearly shows that the MAŔTIN[7] relation is strictly obeyed, as was found by GREEN *et al.*[4].

For Groups 2 and 3 (1-*ortho*- and 2-*ortho*-substituents respectively), it is clear from Fig. 1 that the MARTIN[7] relation is approximately true. On balance, for nuclear methylated phenols, the MARTIN[7] relation is valid, subject to positional effects as suggested by BARK AND GRAHAM[11] for nuclear methylated phenoxyacetic acids.

(b) 3- and/or 4-straight chain monoalkyl-substituted phenols

GREEN AND MARCINKIEWICZ[3] have pointed out that for R_F values to be meaningful in a study of the MARTIN[7] relation, they must lie in the range 0.20 to 0.80. Bearing in mind this limitation, R_F values outside this range are used to show trends rather than for a strict evaluation of the MARTIN[7] relation.

From Table Ib and Fig. 2 it is obvious that within the imposed limits, the MARTIN[7] relation is strictly valid for *para*-straight chain alkyl-phenols. This confirms

TABLE I

REVERSED PHASE THIN-LAYER CHROMATOGRAPHY USING AS THE STATIONARY PHASE CELLULOSE (15 g) IMPREGNATED WITH ETHYL OLEATE (70 ml) OF ETHYL OLEATE IN DIETHYL ETHER (0.75 % v/v)

Key	*Phenol*	*System 1*		*System 2*	
		R_F	R_M	R_F	R_M
(*a*) *Methylated phenols*					
1	Phenol	0.795	—0.588	0.900	—0.955
2	2-Methyl-	0.625	—0.221	0.785	—0.562
3	3-Methyl-	0.675	—0.318	0.830	—0.688
4	4-Methyl-	0.660	—0.288	0.830	—0.688
5	2,3-Dimethyl-	0.455	+0.079	0.680	—0.327
6	2,4-Dimethyl-	0.425	+0.131	0.690	—0.348
7	2,5-Dimethyl-	0.475	+0.043	0.680	—0.327
8	2,6-Dimethyl-	0.400	+0.176	0.635	—0.240
9	3,4-Dimethyl-	0.530	—0.048	0.765	—0.513
10	3,5-Dimethyl-	0.515	—0.026	0.740	—0.455
11	2,3,4-Trimethyl-	0.290	+0.389	0.575	—0.131
12	2,3,5-Trimethyl-	0.290	+0.389	0.550	—0.077
13	2,3,6-Trimethyl-	0.280	+0.410	0.540	—0.070
14	2,4,5-Trimethyl-	0.300	+0.368	0.570	—0.123
15	2,4,6-Trimethyl-	0.280	+0.410	0.540	—0.070
16	3,4,5-Trimethyl-	0.430	+0.123	0.680	—0.327
17	2,3,4,5-Tetramethyl-	0.240	+0.501	0.500	0.000
18	2,3,4,6-Tetramethyl-	0.180	+0.659	0.370	+0.231
19	2,3,5,6-Tetramethyl-	0.150	+0.750	0.360	+0.250
(*b*) *3- and/or 4-straight chain monoalkyl-substituted phenols*					
1	Phenol	0.795	—0.588	0.900	—0.955
3	3-Methyl-	0.675	—0.318	0.830	—0.688
4	4-Methyl-	0.660	—0.288	0.830	—0.688
20	3-Ethyl-	0.485	+0.027	0.700	—0.368
21	4-Ethyl-	0.455	+0.079	0.690	—0.348
22	4-*n*-Propyl-	0.240	+0.501	0.550	—0.077
23	4-*n*-Butyl-	0.110	+0.908	0.375	+0.223
24	4-*n*-Amyl-	0.060	+1.195	0.210	+0.550
25	4-*n*-Nonyl-	0.000	—	0.060	+1.195
(*c*) *3- and/or 4-branched chain monoalkyl-substituted phenols*					
22	4-*n*-Propyl-	0.240	+0.501	0.550	—0.077
26	4-Isopropyl-	0.250	+0.477	0.585	—0.149
23	4-*n*-Butyl-	0.110	+0.908	0.375	+0.223
27	4-*sec.*-Butyl-	0.140	+0.789	0.440	+0.105
28	3-*tert.*-Butyl-	0.215	+0.526	0.480	+0.035
29	4-*tert.*-Butyl-	0.230	+0.867	0.500	0.000
24	4-*n*-Amyl-	0.060	+1.195	0.210	+0.550
30	4-*sec.*-Amyl-	0.055	+1.234	0.180	+0.659
31	4-*tert.*-Amyl-	0.150	+0.750	0.355	+0.259
32	4-(3-Methylbutyl)-	0.075	+1.091	0.390	+0.195
33	4-*tert.*-Octyl-	0.040	+1.380	0.060	+1.195
(*d*) *4-Monosubstituted phenols containing other structural groups*					
1	Phenol	0.795	—0.588	0.900	—0.955
22	4-*n*-Propyl-	0.240	+0.501	0.550	—0.077
34	4-Allyl-	0.350	+0.269	0.670	—0.307
23	4-*n*-Butyl-	0.110	+0.908	0.375	+0.223
35	4-Crotyl-	0.170	+0.689	0.500	0.000
36	4-Cyclopentyl-	0.130	+0.826	0.370	+0.231

(*continued on p. 421*)

TABLE I (*continued*)

Key	*Phenol*	*System 1*		*System 2*	
		R_F	R_M	R_F	R_M
37	4-Cyclopent-2-enyl-	0.190	+0.630	0.470	+0.051
38	4-Cyclohexyl-	0.070	+1.124	0.220	+0.550
39	4-Phenyl-	0.110	+0.908	0.445	+0.096
40	4-Benzyl-	0.105	+0.931	0.420	+0.140
41	4-Cumyl-	0.080	+1.061	0.220	+0.550
(*e*) *Polyalkyl-substituted phenols containing no ortho-substituent*					
22	4-*n*-Propyl-	0.240	+0.501	0.550	—0.077
16	3,4,5-Trimethyl-	0.430	+0.123	0.680	—0.327
42	3-Methyl-5-ethyl-	0.300	+0.368	0.610	—0.195
43	3-Methyl-4-isopropyl-	0.245	+0.489	0.550	—0.077
44	3-Methyl-5-isopropyl-	0.175	+0.673	0.485	+0.027
45	3-Methyl-5-*sec.*-butyl-	0.070	+1.124	0.310	+0.348
46	3,5-Di-*tert.*-butyl-	0.000	—	0.110	+0.908
(*f*) *Substituted alkyl-phenols containing one ortho-group*					
1	Phenol	0.795	—0.588	0.900	—0.955
2	2-Methyl-	0.625	—0.221	0.785	—0.562
47	2-Ethyl-	0.400	+0.176	0.610	—0.195
48	2-*n*-Propyl-	0.200	+0.602	0.460	+0.070
49	2-*sec.*-Butyl-	0.115	+0.886	0.340	+0.288
50	2-*tert.*-Butyl-	0.080	+1.061	0.240	+0.501
51	2-*n*-Octyl-	0.000	—	0.040	+1.380
52	2-Allyl-	0.280	+0.410	0.575	—0.131
53	2-Phenyl-	0.130	+0.826	0.415	+0.149
54	2-Cyclohexyl-	0.060	+1.195	0.195	+0.616
55	2-Methyl-4-*tert.*-butyl-	0.085	+1.032	0.320	+0.327
56	2-Methyl-4-octyl-	0.020	+1.690	0.060	+1.195
57	2-*tert.*-Butyl-3-methyl-	0.040	+1.380	0.120	+0.865
58	2-*tert.*-Butyl-4-methyl-	0.050	+1.279	0.150	+0.750
59	2-Octyl-4-methyl-	0.000	—	0.020	+1.690
(*g*) *Substituted alkyl-phenols containing two ortho-groups*					
1	Phenol	0.795	—0.588	0.900	—0.955
8	2,6-Dimethyl-	0.400	+0.176	0.635	—0.240
60	2,6-Dimethyl-4-*n*-propyl-	0.080	+1.061	0.240	+0.501
61	2,6-Dimethyl-4-allyl-	0.095	+0.979	0.310	+0.348
62	2,6-Di-*tert.*-butyl-	0.030	+1.510	0.050	+1.279
63	2-Methyl-4,6-di-*tert.*-butyl	0.000	—	0.050	+1.279
64	2,6-Di-*tert.*-butyl-4-methyl-	0.000	—	0.020	+1.690
(*h*) *Alkoxy-phenols*					
1	Phenol	0.795	—0.588	0.900	—0.955
65	2-Methoxy-	0.810	—0.629	0.855	—0.780
66	3-Methoxy-	0.685	—0.334	0.835	—0.703
67	4-Methoxy-	0.680	—0.348	0.835	—0.703
68	3,5-Dimethoxy-	0.170	—0.389	0.870	—0.827
69	4-Ethoxy-	0.660	—0.288	0.825	—0.674
70	4-Cyclopentyloxy-	0.315	+0.338	0.620	—0.213
71	4-Heptoxy-	0.000	—	0.120	+0.865
72	4-Dodecyloxy-	0.000	—	0.020	+1.690
73	4-Tetradecyloxy-	0.000	—	0.020	+1.690
74	4-Hexadecyloxy-	0.000	—	0.020	+1.690
75	4-Phenoxy-	0.300	+0.368	0.515	—0.026
76	3,5-Dicarbomethoxy-	0.000	—	0.000	—

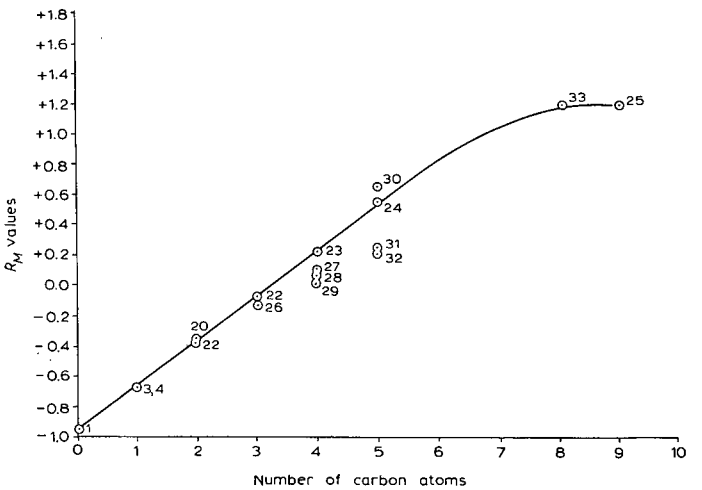

Fig. 2. R_M values for 3- and 4-alkyl-substituted phenols *vs.* number of carbon atoms in the side chains.

the results of GREEN *et al.*[4], for the same phenols in ethyl oleate/aqueous ethanol paper partition chromatography.

Table II shows the R_F values of the C_2 and C_3 alkyl-substituted phenols together with those of GREEN *et al.*[4] It can be seen that the order of R_F values is the same in both cases. They enable an appraisal of the chromatographic system to be made.

TABLE II

R_F VALUES OF C_2 AND C_3 ALKYL-SUBSTITUTED PHENOLS

Phenol	*R_F value*	
	System 2	*From ref. 4*
3,4-Dimethyl-	0.785	0.735
3,5-Dimethyl-	0.740	0.735
3-Ethyl-	0.700	—
4-Ethyl-	0.690	0.700
3,4,5-Trimethyl-	0.680	0.585
4-*n*-Propyl-	0.550	0.450

It is supposed that the hydrocarbon part of the molecule will lie flat in the interface of the two phases, with the phenolic group acting as a solvent hook. Solvation of the phenolic group will remove the molecule from the stationary phase to the mobile phase, so carrying it in the direction of eluent flow. Thus any reduction in solvation will reduce the R_F values. This will be effected in two ways:

(i) By electronic interaction increasing the electron density at the phenolic hydrogen. This will reduce its tendency to protonate the eluent and so give lower R_F values.

(ii) By a steric factor which will enhance the solubility of the hydrocarbon part of the molecule in the stationary phase.

In the case of (i), the inductive and hyperconjugative release of electrons by the methyl group will increase the electron density at the hydrogen atom and so reduce the R_F values. An increase in the numbers of methyl groups will enhance this effect, still further reducing the R_F values.

It would be expected that the electron release will decrease with an increase in the alkyl chain length, and that the R_F values of the polymethylated phenol should be lower than that of a mono-alkylated phenol containing the same number of carbon atoms. This is not so, thus while electronic effects may have some importance, they alone cannot account for the order of R_F values. GREEN *et al.*[4] attempted to account for the differences in terms of differences in the electronic effects of side chain hydrogen atoms held on the α, β, γ, etc. carbon atoms, and calculated ΔR_M parameters for each of these. They did not consider the second possibility. This, however, is done here.

The steric factor (ii) is best considered in terms of the addition of the substituents relative to the direction of flow of the mobile phase. Taking 4-methylphenol as the parent of the series, the addition of methyl groups must be to the 3- and/or 5-positions, *i.e.* the increase in molecular size is at an angle to the direction of solvent flow. The addition of (CH_2)-groups to the 4-methyl group increases the chain length, and extends the molecular axis in the direction of eluent flow. Because each molecule may be regarded as being solvated at the phenolic group only, the force needed to remove the more compact molecule from the stationary phase is probably less than that needed to remove the more extensive molecule. The R_F values of the polymethylated isomers are higher than those of the corresponding long chain isomers. By analogy, it is easier to pick a coil of rope clear of the ground than it is to pick up the same piece of rope when uncoiled.

(c) 3- and/or 4-branched chain monoalkyl-substituted phenols

Branched chain isomers with no *ortho*-substituents (Table Ic) generally have R_F/R_M values which differ from those of the straight chain isomer. GREEN *et al.*[4] have attempted to show that where such differences occurred that they were caused by electronic differences resulting from the different contributions of the α, β, γ, etc. hydrogen atoms. For the large difference between the *tert.*-butyl- and *n*-butylphenols, they suggested a resonance contribution from a non-bonded methyl group. This effectively meant that there was a weakening of the carbon–hydrogen bond for each of the 3-methyl groups, and that these hydrogen atoms could take part in hydrogen bonding with the mobile phase and so increase the R_F values. It is alternatively suggested here that the effect of chain branching is to increase the size of the substituent and so force the hydrocarbon part of the molecule out of the two-dimensional stationary phase into the mobile phase and so increase the R_F values. The results in Table Ic show that, generally, the increase in the bulk of the substituent relative to the straight chain isomer does increase the R_F values.

(d) 4-Monosubstituted phenols containing other structural groups

The presence of a double bond in the molecule has the expected result of increasing the polarity of the molecule and hence the R_F values (Table Id), the increase

being attributed to the formation of a hydrogen bond between the hydrogen atoms of the eluent system and the electrons of the double bond.

The presence of an alicyclic substituent gives the molecule an R_F value equivalent to that of a saturated straight chain hydrocarbon of one less carbon atom. The values for the three aromatic substituted phenols may be explained in part by assuming some eluent–double bond interaction to give increased R_F values. That this is not as large as is expected from the number of such bonds may be due to the increased solubility of these compounds in the stationary phase. The difference between *p*-benzyl- and *p*-cumylphenol may be caused by the branching of the isopropyl part of the molecule between the rings forcing part of the molecule further into the stationary phase.

(e) Polyalkyl-phenols containing no ortho-substituent

The results for polyalkyl-substituted phenols (Table Ie), confirm the previously expressed view regarding the easier removal of a polysubstituted phenol from the stationary phase compared with an isomeric 4-alkyl straight chain substituted compound. Fig. 3 emphasises this, but shows that the MARTIN[7] relation is still approximately valid.

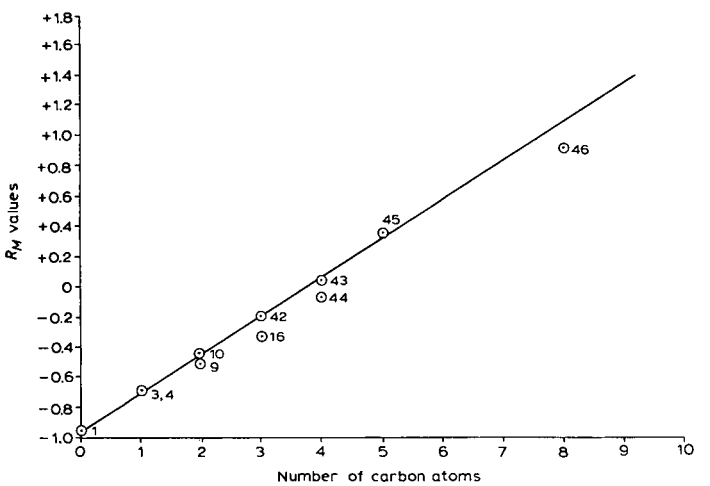

Fig. 3. R_M values (System 2) for polyalkyl-phenols containing no *ortho* groups *vs.* number of carbon atoms in the side chains.

(f) Substituted alkyl-phenols containing one ortho-group

Here again the results (Table If) indicate the approximate validity of the MARTIN[7] relation. Too few straight chain compounds were available for study to establish the strict validity of the relation for these compounds, though the results for the first three members of the series indicate the probability of this. The effects of other structural features follow the pattern expected of them from the behaviour of groups containing the same structural features when substituted in the 3- and/or 4-positions. The expected increase in R_F value of the 2-allyl group, compared with 2-*n*-propyl is seen. The values of 2-cyclohexyl- and 2-phenylphenol show the same order with respect to each other as did their 4-substituted isomers. The effects of

chain branching and polysubstitution compared with straight chain substitution are also shown in Fig. 4.

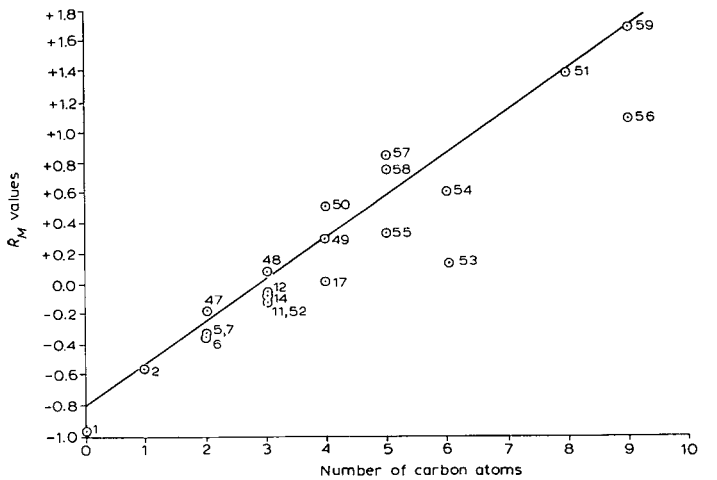

Fig. 4. R_M values (System 2) for alkyl-phenols containing one *ortho*-substituent *vs.* number of carbon atoms in the side chains.

(*g*) *Substituted alkyl-phenols containing two ortho-groups*

The effect of di-*ortho*-substitution with bulky groups is shown in the results for the 2,6-dimethyl- and the 2,6-di-*tert.*-butylphenols (Table Ig). The increased polarity of the molecule caused by the presence of the double bond is seen in the results for the pair 2,6-dimethyl-4-*n*-propyl- and 2,6-dimethyl-4-allylphenol. The isomeric pair, 2-methyl-4,6-di-*tert.*-butyl- and 2,6-di-*tert.*-butyl-4-methylphenol is just separable in System 2; this is accounted for by the replacement of an *o*-methyl group

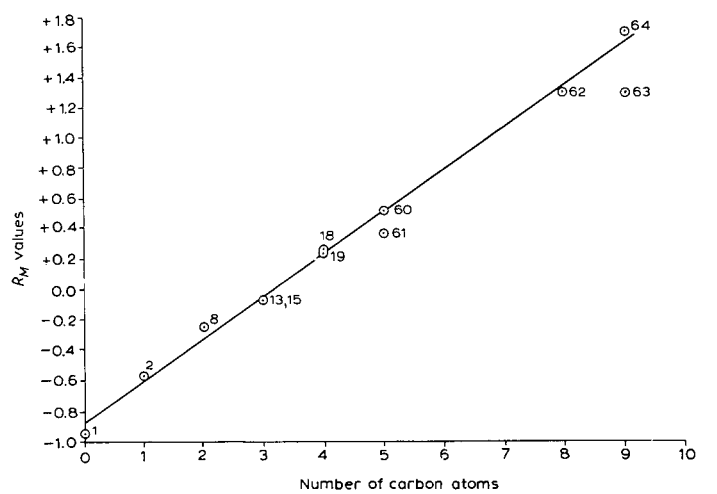

Fig. 5. R_M values (System 2) for alkyl-phenols containing two *ortho*-substituents *vs.* number of carbon atoms in the side chain.

by an *o-tert.*-butyl group. The approximate validity of the MARTIN[7] relation is illustrated by Fig. 5.

(*h*) *Alkoxy-phenols*

The aliphatic ethers (Table Ih) follow the expected pattern of a reduction in R_F values with increasing chain length of the alkyl part of the ether. Some evidence of an *ortho*-effect is seen in the results for the isomeric methoxyl derivatives. The effects of the presence of an alicyclic ring, and an aromatic one are seen in the results for 4-cyclopentyloxyl-, and 4-phenoxylphenol, and are of the expected order. The increase in R_F values for the 3,5-dimethoxyl compound may be a result of solvation of the ether oxygen. The value for 3,5-dicarbomethoxyphenol may be caused by a molecular weight factor.

CONCLUSION

We consider the mechanism of the separation for alkyl-phenols chromatographed by reversed-phase thin-layer chromatography between a non-polar stationary phase and a polar mobile phase to be essentially a partition process. The phenols at first dissolved in the non-polar phase, are removed as a result of solvation of the phenolic group by the polar mobile phase.

Where solvation of the phenolic group is hindered by the presence of substituents in one or both *ortho*-positions, lower R_F values result, the greater the bulk of the substituent the greater the lowering of R_F values.

For phenols substituted in the 3- and/or 4-positions, a single substituent containing a given number of carbon atoms has a greater effect on the R_F values than two substituents containing, in total, the same number of carbon atoms as the single side chain.

The MARTIN additivity principle is shown to be approximately valid, subject to modifications involving *ortho*-effects, chain branching and for polysubstituted phenols, positional effects.

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We also thank Professor L. HUNTER, The University, Leicester, for discussion.

SUMMARY

The alkyl etc. phenols have been chromatographed under controlled conditions, by reversed-phase thin-layer chromatography on cellulose impregnated with ethyl oleate as the stationary phase, and aqueous ethanol as the mobile phase.

The results indicate that the mechanism of separation is essentially one of partition between the two phases, the phenols being removed from the stationary phase by solvation of the phenolic group by the polar mobile phase. Steric effects are

considered to be of greater importance than electronic effects in influencing the degree of solvation of the phenolic group.

The MARTIN additivity principle is considered to be approximately valid, subject to modifications as a result of the influence of chain branching, steric effects, and for polysubstituted phenols, positional effects.

REFERENCES

1 L. S. BARK AND R. J. T. GRAHAM, *Talanta*, in the press.
2 L. S. BARK AND R. J. T. GRAHAM, *Proc. S.A.C. Conf., Nottingham, 1965*, W. Heffer and Son, Cambridge, 1966, p. 112.
3 J. GREEN AND S. MARCINKIEWICZ, *J. Chromatog.*, 10 (1963) 35.
4 J. GREEN, S. MARCINKIEWICZ AND D. MCHALE, *J. Chromatog.*, 10 (1963) 42.
5 J. GREEN, S. MARCINKIEWICZ AND D. MCHALE, *J. Chromatog.*, 10 (1963) 158.
6 S. MARCINKIEWICZ AND J. GREEN, *J. Chromatog.*, 10 (1963) 184.
7 A. J. P. MARTIN, *Biochem. Soc. Symp.*, 3 (1950) 4.
8 L. S. BARK, R. J. T. GRAHAM AND D. MCCORMICK, *Talanta*, 12 (1965) 122.
9 L. S. BARK AND R. J. T. GRAHAM, *J. Chromatog.*, 23 (1966) 120.
10 W. HEINEN, *Doctoral Thesis*, University of Utrecht, 1964.
11 L. S. BARK AND R. J. T. GRAHAM, *Analyst*, 85 (1960) 663.

THIN-LAYER CHROMATOGRAPHY OF AZINES AND OF AROMATIC NITROGEN HETEROCYCLES ON ALUMINA

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In a series of previous papers we reported studies on the chromatographic adsorption of aromatic hydrocarbons and aromatic nitrogen heterocycles on alumina using column liquid–solid[1,2], column gas–solid[3], and alumina-impregnated glass paper[4] methods. These studies were directed toward the goals of (a) correlation of chromatographic adsorbability with chemical structure of the adsorbate and (b) elucidation of the stereochemistry and mechanism of the adsorption process. Simultaneous investigations of the same phenomena have been conducted by SNYDER[5,6] and by GILES AND MCKAY[7]. Recently SAWICKI *et al.*[8,9] reported thin-layer chromatography (TLC) of some aromatic azacyclic compounds on alumina. Meanwhile, we have extended our studies to TLC of diformalazines, stilbazoles, pyridylethenes, and many other aromatic nitrogen heterocycles. The present paper is a report of our TLC results and a correlation thereof with the data for similar nitrogen compounds obtained by others.

EXPERIMENTAL

Unless otherwise noted chemicals used were obtained from the commercial sources Aldrich Chemical Co., Milwaukee, Wis., U.S.A.; Distillation Products Industries, Rochester, N.Y., U.S.A.; H.M. Chemical Co., Santa Monica, Calif., U.S.A.; and Fluka A.G., Buchs, Switzerland. Liquids were either used directly or, if discolored, were distilled before use. Melting points of solids were checked against those reported in the literature. Where appreciable discrepancies occurred or where the melting range exceeded 2° the compounds were recrystallized until satisfactory purity was attained. Benzo(*b*)- and benzo(*j*)phenanthridines[10], 6,13-diazadibenz(*a,h*)anthracene[10], and the various thienopyridines[11] were prepared in our laboratory in separate studies. Synthesized especially for use in the present investigation were the compounds 9-ethylphenanthridine[12], 9-phenylacridine[13], 4,6-dimethylquinoline[14], 4,8-dimethylquinoline[15], and all of the diformalazines in Table II except Nos. 43, 44 and 53[16].

trans-3-Stilbazole. The mixture which resulted from slow addition of 10.6 g of benzaldehyde to a solution of 3.2 ml of hydrazine (95 %) in 20 ml of methanol was allowed to stand for 2 days, treated with a solution of 10.7 g of 3-pyridinecarboxaldehyde in 20 ml of methanol, and heated on a steam bath for 30 min. From the cold solution was obtained 15 g of crude, yellow *benzal 3-pyridal azine*, m.p. 80–90°. An intimate mixture of the powdered azine and 1 g of sodium hydroxide was pyrolyzed

* NSF Undergraduate Research Participant, summers 1964 and 1965.

at 250° for 1 h[17]. The resultant tar was refluxed with 50 ml of 6 *N* hydrochloric acid for 30 min. The aqueous layer was basified and extracted with benzene. Chromatography of the benzene-soluble portion on Alcoa F-20 alumina (using cyclohexane and then benzene as eluents) gave 0.5 g of waxy solid, m.p. 76–78° (ref. 18: 77–79°) after recrystallization from cyclohexane; n.m.r. absorptions (in $CDCl_3$) at $\delta = 7$–7.8 (8-protons, styryl group plus β-H on pyridine ring), 8.0 (γ-proton on pyridine ring), and 8.5–8.7 p.p.m. (two α-protons on pyridine ring).

Chromatographic procedure and handling of data

Standard glass plates (20 × 20 cm) were pretreated by the successive steps of rinsing with dilute hydrochloric acid, washing with sodium polymetaphosphate (Calgon) and water, rinsing with distilled water and then acetone, rubbing dry with lens-cleaning paper (Kimwipes), wetting with absolute ethanol, rubbing dry as before, and finally polishing with a small amount of dry adsorbent. By means of a Desaga spreader and template the plates (in batches of five at one time) were coated to a thickness of 0.25 mm with a slurry made by grinding for 60 sec in a glass mortar a mixture of two parts (by wt.) of distilled water and one part of aluminum oxide G (according to STAHL, containing *ca.* 15 % $CaSO_4$ binder). Coated plates were dried in air for 1 h, activated at 100–115° for 23–26 h, and cooled and stored (until use) in a dry box containing anhydrous $CaSO_4$. In separate tests, samples (1–2 g) of aluminum oxide G (directly from the container) were weighed into tared, predried (at 110°) Erlenmeyer flasks, treated with two parts (by wt.) of water, and then heated at 110° for 24 h. The samples weighed 1.8 % less at the end than they did directly from the container. It is thus apparent that none of the added water is retained on the plates although some of it may be redistributed (*e.g.* in forming $CaSO_4 \cdot 2H_2O$, d.p. 128°)[19]. Just before use of a plate, a zone of alumina about 0.7 cm wide was removed from each edge and faint pencil markings were made across the coating (perpendicular to the direction in which the coating was deposited) in order to establish the starting line for the spots and (10.0 ± 0.4 cm beyond) the termination line for the solvent front.

Stock solutions (either $2 \cdot 10^{-2}$ *M* or, for insufficiently soluble substances, saturated) of the adsorbates were prepared in absolute ethanol, benzene or chloroform. A number of 15 to 22 spots, each made from a 1-μl aliquot of stock solution, were spaced evenly along the starting line. Plates were equilibrated in an atmosphere of the mobile phase (spectral or reagent grade solvent, see Tables I–III) for 30 min, run at room temperature (19–28°) until the solvent front reached the termination line (15–30 min), placed in air until they appeared dry, and then sprayed with fortified Dragendorff's reagent (see Appendix) in order to reveal the spots. *trans*-Stilbene spots (not revealed in this way) were found by spraying with aqueous $KMnO_4$ solution. Centers of most intense color in the spots were marked and used in calculations of R_F values.

In general, the same group of compounds (including quinoline as a standard of reference) was run on every plate in a particular batch. The 3 or 4 best plates from the batch were selected for determination of the median R_F value and the mean deviation from the median, d_m, for each adsorbate. For every adsorbate (or nearly every adsorbate) d_m was $\leqslant 0.02$. Data for single batches are presented in Tables II and III. Table I contains a composite of data obtained from several different batches of plates, but with the same solvent.

Since there was some variation in median R_F values for quinoline (range 0.30–0.34) from one batch to another, reported median R_F values in Table I have been adjusted to a standard batch by means of eqn. (1) (see Appendix), where

$$\delta R_A = \delta R_Q \left[\frac{R_A(1 - R_A)}{R_Q(1 - R_Q)} \right] \tag{1}$$

R_Q is the standard median R_F value (0.33) for quinoline Q, R_A is the observed R_F value for the adsorbate A, and δR_F values are the corresponding corrections (observed

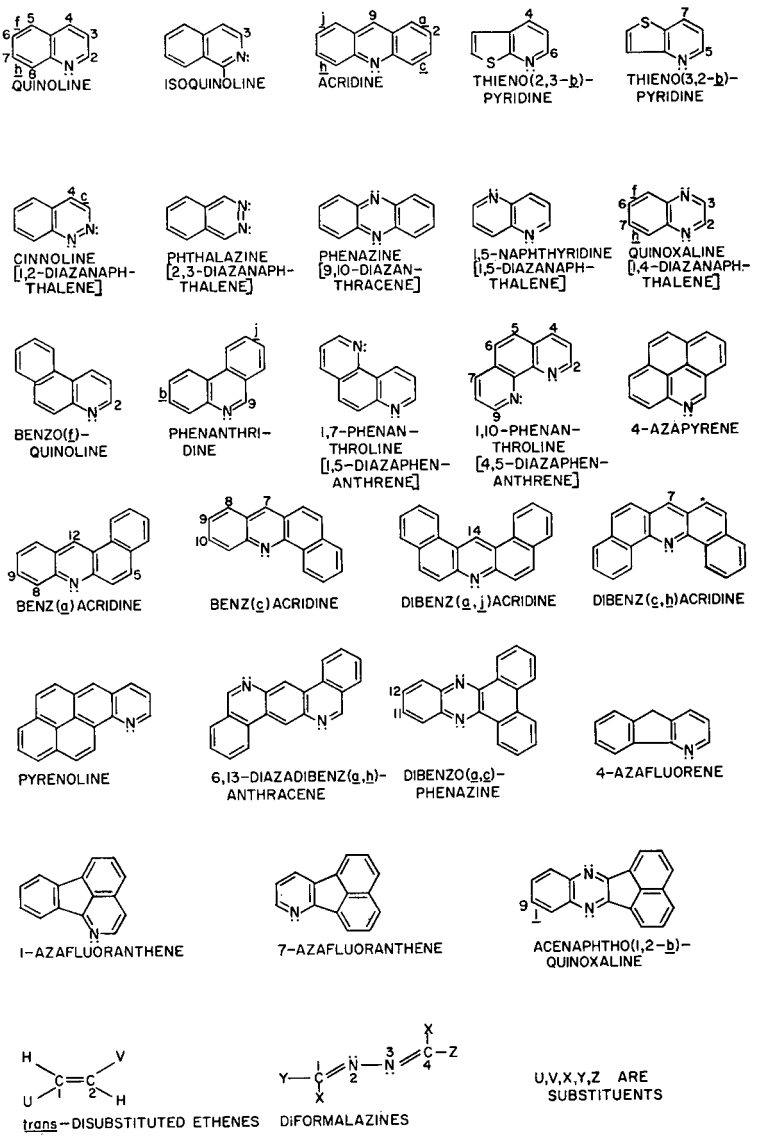


Fig. 1.

for Q, calculated for A) to be made in R_F. In practice, it was found that $\delta R_A = \delta R_Q$ for $R_A > 0.10$. For $R_A \leqslant 0.10$ no correction was made.

Observed colors of spots (after spraying) are also reported in Tables I–III. Core structural formulas, including pertinent numbering and lettering schemes involved in naming the adsorbates used in our studies, as well as in the TLC studies on alumina made by SAWICKI *et al.*[8,9], are presented in Fig. 1.

DISCUSSION

As may be noted from the data presented in Tables I–III thin-layer chromatography on alumina is a facile means of gaining not only highly reproducible R_F values for a particular adsorbate but also a wide range of R_F values for differentiation amongst the relative adsorbabilities of various nitrogen-containing compounds, at least of the azine and aromatic azacyclic types. In test runs we have found, on the other hand, that aromatic hydrocarbons are too weakly adsorbed on these plates to give useful differentiation in adsorbabilities. This gross difference in adsorbability between the hydrocarbons and their nitrogen analogs (*cf. trans*-stilbene, No. 1, with the stilbazoles, Nos. 13, 33 and 35 in the present case) has been noted previously on columns[4], on alumina-impregnated pyrex paper[4], and on thin layers of alumina[8].

For practical application in chromatography on alumina we present herewith a series of general rules which one may use for predicting relative adsorbabilities (especially as found in this study) of aromatic azacycles (azarenes) and allied compounds bearing nitrogen atoms of *only* the sp^2-hybrid electronic type, *i.e.* the type represented by the —$\ddot{N}$= symbolism, where atoms bonded to the nitrogen are either C or N and do not include H, and the dots represent a pair of non-bonding or n-electrons.

Rule I. Generalized adsorbability. A nitrogen compound is more strongly adsorbed than its hydrocarbon analog, where —CH= replaces —$\ddot{N}$=.

Rule II. The steric substituent effect. Introduction of an alkyl group, an aryl group, or a fused benzo ring into a pyridinoid molecule in such fashion as to cause steric hindrance to coordination by the n-electrons of the nitrogen atom with a Lewis acid of large spatial requirement decreases adsorbability (increases R_F).

Rule III. The inductive methyl effect. Introduction of a methyl group into a sterically non-hindering position either increases adsorbability (decreases R_F) or leaves it essentially unaltered.

Rule I has already been considered. The steric substituent effect has been noted previously[3,5a] for alkyl substituents in the 2- and 6-positions of pyridine as well as for the 2- and 8-positions of quinoline. It is further exemplified in the present work by the case of phenanthridine > 9-ethylphenanthridine (*cf.* Nos. 14 and 29) in adsorbability and in the results of SAWICKI *et al.*[8] that benzo(*f*)quinoline > 2-methylbenzo(*f*)-quinoline. Mixed or counteracting effects of methyl groups are illustrated for a number of parent systems investigated here. Thus, in the 1,10-phenanthroline series the steric effect is marked in the 2,9-dimethyl derivative, while the inductive effect is shown by the 5,6- and 4,7-isomers (*cf.* Nos. 36 and 39–41). This result is reminiscent of the observation that steric interference to chelate formation in solution of the 2,9-derivative with Fe^{2+} is so great that not even a color is formed on admixture of the reagents[20,21], in sharp contrast to the facile formation of red tris-(1,10-phenanthroline)-iron(II) complex ion under the same conditions. Similar steric hindrance is shown to

TABLE I.

TLC DATA USING BENZENE–CHLOROFORM (1:1, v/v, SPECTRAL GRADES) AS MOBILE PHASE

No.	*Compound*	*Median* R_F	*Color* of spot*
1	*trans*-Stilbene	0.78	—
2	11,12-DiMe-dibenzo(*a*,*c*)phenazine	0.71	Y
3	11-Me-dibenzo(*a*,*c*)phenazine	0.70	Br
4	Dibenzo(*a*,*c*)phenazine	0.70	Bu
5	Benz(*c*)acridine	0.68	O-Ba
6	5,8-DiMe-benz(*a*)acridine	0.67	RO-Br
7	Dibenzo(*f*,*h*)quinoxaline	0.64	Bu
8	6,7-DiMe-2,3-diϕquinoxaline	0.63	YO
9	2,3-Diϕquinoxaline	0.61	O
10	Benzo(*h*)quinoline	0.59	O
11	Benzo(*l*)acenaphtho(1,2-*b*)quinoxaline	0.52	Br
12	2-ϕPyridine	0.49**	Pi
13	*trans*-2-Stilbazole	0.49	Pe
14	9-Et-phenanthridine	0.48	Y
15	Phenazine	0.47	BuBr
16	Acenaphtho(1,2-*b*)quinoxaline	0.45	Pu
17	9-Me-acenaphtho(1,2-*b*)quinoxaline	0.44	Bu
18	2,3-DiMe-quinoxaline	0.41	O
19	2,3,6,7-TetraMe-quinoxaline	0.41	Pe
20	9-ϕAcridine	0.41	O
21	Quinoxaline	0.40	Pi
22	Acridine	0.39	O
23	2-Me-acridine	0.37	O
24	6,7-DiMe-quinoxaline	0.36	Pi
25	*trans*-4-Styrylquinoline	0.35	YO
26	4-Azafluorene	0.34	RO
27	Quinoline	0.33	O
28	Benzo(*f*)quinoline	0.33	O
29	Phenanthridine	0.32	O-Y
30	Benzo(*b*)phenanthridine	0.30	O-Br
31	Benzo(*j*)phenanthridine	0.30	O-Br
32	1,7-Phenanthroline	0.25	PiO
33	*trans*-3-Stilbazole	0.24	Pi
34	4-ϕPyridine	0.21**	O
35	*trans*-4-Stilbazole	0.18	O
36	2,9-DiMe-1,10-phenanthroline	0.17	O
37	2,2′-Bipyridyl	0.16	A
38	6,13-Diazadibenz(*a*,*h*)anthracene	0.13	YBr
***	*trans*-Dipyridylethenes	0.06	—
***	Bis-1,4-pyridyldiformalazines	0.03	—
39	1,10-Phenanthroline****	0.01	O
40	5,6-DiMe-1,10-phenanthroline****	0.01	O
41	4,7-DiMe-1,10-phenanthroline****	0.00	O

* A = apricot; Ba = black; Bu = blue; Br = brown; G = gray; O = orange; Pe = peach; Pi = pink; Pu = purple; R = red; Y = yellow. For colors written with a hyphen, *e.g.* for compound No. 5, the second color occurs in the center of the spot and the first color in the outer regions of the spot.

** Interpolated from other data.

*** For comparative data on isomers see Table II.

**** Distinguishable by use of pyridine as mobile phase, R_F values found: No. 27: 0.74; No. 39: 0.14; No. 40: 0.09; No. 41: 0.07.

TABLE II

TLC DATA USING 2-BUTANONE ALONE AND IN ADMIXTURE WITH BENZENE–CHLOROFORM (1:1:1, by vol., REAGENT GRADES), AS MOBILE PHASES

No.	*Compound**	*Mobile phase*			
		2-Butanone		*Ternary*	
		Median R_F	*Color** of spot*	*Median R_F*	*Color** of spot*
42	1,4-Bis-(1-naphthyl)-DFA	0.69	Br		
43	1,4-DiphenylDFA***	0.69	O		
44	1,4-Dimethyl-1,4-diphenylDFA	0.69	Pe		
45	1,4-Bis-(*trans*-styryl)-DFA	0.68	YO		
22	Acridine	0.66	O	0.77	O
46	Bis-(9-fluorenylidene)-azine****	0.66	Y		
27	Quinoline	0.65	PeO	0.71	Pe
47	*trans*-1,2-Bis-(2-pyridyl)-ethene			0.65	Pi-G
48	Benzo(*c*)cinnoline	0.63	O		
49	1,4-Bis-(2-pyridyl)-DFA	0.62	O	0.61	Pe
50	*trans*-1-(2-Pyridyl)-2-(3-pyridyl)-ethene			0.53	TPe
51	1,5-Naphthyridine	0.55	O		
52	1,4-Bis-(4-pyridyl)-DFA	0.53	TO	0.46	TGa
53	1,4-Bis-(3-pyridyl)-DFA	0.52	O-Pu	0.40	Pe
54	Cinnoline	0.52	O		
55	*trans*-1,2-Bis-(4-pyridyl)-ethene			0.40	BrPe
56	4-Methylcinnoline	0.50	O		
57	Phthalazine	0.24	O		
39	1,10-Phenanthroline	0.02	O	0.13	O

* DFA = diformalazine.
** See first footnote of Table I.
*** Common name: benzalazine. Using benzene–cyclohexane (1:1, v/v) as mobile phase one finds R_F values of *trans*-stilbene (No. 1): 0.67; *trans*-azobenzene: 0.63; benzalazine: 0.36.
**** A tetrasubstituted diformalazine.

chelation in solution by Zn^{2+} and Cd^{2+} but not by Cu(I) or H^+ (for hydrogen bridge formation, pK_a values are 4.92 for No. 39, 5.94 for No. 41, and 6.15 for No. 36)[20,21]. For both TLC[8] and alumina-impregnated pyrex paper[4] it was found that benz(*a*)-acridine (BaA) $\cong$ benzo(*f*)quinoline in adsorbability. The grossly decreased adsorbability of 5,8-dimethylBaA in TLC (*cf.* Nos. 6 and 28) may be ascribed to an overwhelming steric effect by the 8-methyl group coupled with a possible minor inductive effect of the 5-methyl group. Such relationship is directly apparent in the TLC adsorbability series[8] of 9,12-dimethylBaA = 12-methylBaA = BaA $\gg$ 8,12-dimethylBaA. The methylated quinoline series (see Table III) represents a prime example of the operation of rules II and III. Thus, the order of decreasing adsorbability (for the positions of substitution) of 4,6- = 4- > 6- = 2,4- = 7- > unmethylated = 2,6- $\geqslant$ 2- > 4,8- > 8- > 2,8- may be rationalized on the basis that steric hindrance by an 8-methyl group (ΔR_F from quinoline = 0.14) > that by a 2-methyl group (ΔR_F = 0.03), while the inductive effect of a methyl group in some other position decreases in the order 4- > 6- $\cong$ 7- (*vide infra*). As a result the steric effect of a 2-methyl group is almost exactly counterbalanced by the inductive effect of a 4- or a 6-methyl group. The adsorbability order as found here might be compared with the order of decreasing gas chromatographic retention

TABLE III

TLC DATA FOR QUINOLINES AND RELATED COMPOUNDS USING BENZENE–CHLOROFORM (1:1, v/v, REAGENT GRADES) AS MOBILE PHASE

No.	*Compound**	*Median* R_F	*Color*** *of spot*
58	2,8-DiMeQ	0.66	YO
59	2,2′-Biquinolyl	0.65	OBr
60	6-Methieno(2,3-*b*)Py	0.59	O-Y
61	8-MeQ	0.56	YO
62	4,8-DiMeQ	0.53	O
63	4-Methieno(2,3-*b*)Py	0.52	O-Br
64	5-Methieno(3,2-*b*)Py	0.48	O
65	2-MeQ	0.45	Pi
66	2,6-DiMeQ	0.43	YO
27	Q	0.42	O
67	Thieno(3,2-*b*)Py	0.42	O
68	7-MeQ	0.39	YO
69	2,4-DiMeQ	0.39	PiO
70	6-MeQ	0.39	YO
71	7-Methieno(3,2-*b*)Py	0.38	O
72	3-Me-isoQ	0.37	PiO
73	1-Me-isoQ	0.35	O
74	4-MeQ	0.34	O
75	4,6-DiMeQ	0.33	O
76	IsoQ	0.32	O
51	1,5-Naphthyridine	0.29	PiO
54	Cinnoline	0.12	PiO

* Q = quinoline; Py = pyridine.
** See first footnote of Table I.

times on alumina[3] of unmethylated $\gg$ 2,4- $>$ 2,6- $\gg$ 2,8- $>$ 8-. The change in order of the 8- and the 2,8- derivatives in gas chromatography may reflect the lower volatility of the dimethyl compound, while the very strong retention of quinoline itself may arise from the presence of much stronger acidic sites on the gas chromatographic adsorbent. For the isoquinoline system steric hindrance to adsorption in TLC (as adjudged by ΔR_F) by a 1- or a 3-methyl group approximates that of a 2-methyl group in the quinoline series. In the thieno(3,2-*b*)pyridine system, where the N and S atoms are oriented in opposite directions, R_F values are closely similar to those for the analogous quinoline compounds (Table III, *cf.* Nos. 27 and 67, 64 and 65, 71 and 74). On the other hand, in the isomeric thieno(2,3-*b*)pyridine system, where the N and S atoms are oriented in the same direction, R_F values are considerably larger than for the analogous quinoline compounds (*cf.* Nos. 60 and 65, $\Delta R_F = 0.14$; Nos. 63 and 74, $\Delta R_F = 0.18$). By interpolation in Table III one can estimate an R_F value for thieno(2,3-*b*)pyridine itself of 0.54–0.57, a value which implies that the sulfur atom in this compound has nearly the same negative effect on adsorbability as does the methyl group in 8-methylquinoline. Scale drawings of these two molecules (see Fig. 2) using accepted atomic dimensions[22] show, however, that steric hindrance to the π-electrons on the nitrogen atom due to the presence of the sulfur atom should be far less than that due to the presence of the —C(CH_3)=CH— grouping, which it replaces. Tentatively, then, we ascribe the high R_F value in the (2,3-*b*)-system to an electronic effect of the sulfur atom to decrease electron-availability at the nitrogen atom and/or to an

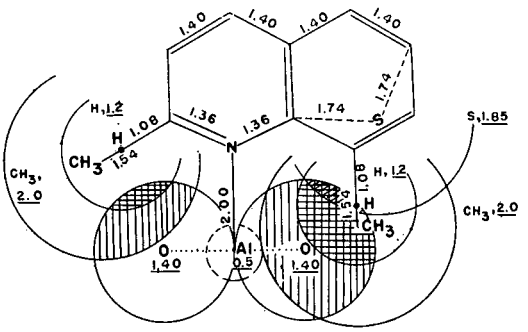


Fig. 2. A composite drawing (to scale) to show relative steric hindrances to edgewise adsorption of quinoline, 2-methylquinoline, 8-methylquinoline and thieno(2,3-*b*)quinoline on a surface of alumina. Numbers written along bonds represent covalent bond lengths in Å. Underlined numbers represent ionic or Van der Waals' radii (in Å) of the corresponding atoms or groups, indicated geometrically by the corresponding full or partial circles[22]. Shaded areas indicate regions of overlap with oxide ions in the outermost aluminum-containing surface layer (*i.e.* perhaps the second layer of the alumina surface) (see Appendix).

electrostatic repulsion by the sulfur atom to the alumina surface. If edgewise adsorption on the nitrogen atom were to occur the latter effect would not be expected in the (3,2-*b*)-series. Further studies on these thienopyridine systems are underway in our laboratory.

Steric hindrance by a phenyl group in a position α to a nitrogen atom is shown in the series (of decreasing absorbabilities) 4-phenylpyridine $\gg$ 2-phenylpyridine (*cf.* Nos. 12 and 34). Such hindrance, combined with minor steric and inductive effects by methyl substituents, is found in the quinoxaline system where one has the order (for substituents) 6,7-dimethyl- > unsubstituted = 2,3,6,7-tetramethyl- = 2,3-dimethyl- $\gg$ 2,3-diphenyl- $\cong$ 6,7-dimethyl-2,3-diphenyl- (*cf.* Nos. 8, 9, 18, 19, 21 and 24). Other series rationalized by steric hindrance of a substituent include the *trans*-stilbazoles (*i.e.* styrylpyridines) in the order 4- > 3- $\gg$ 2- (*cf.* Nos. 13, 33 and 35); the *trans*-bis-pyridylethenes where 4,4'- > 2,3'- > 2,2'- (*cf.* Nos. 47, 50 and 55); and, perhaps, the relationships quinoline > 2,2'-biquinolyl (*cf.* Nos. 27 and 59) and 3,3'- = 4,4'- > 2,2'- in the 1,4-bis-pyridyldiformalazine series (*cf.* Nos. 49, 52, and 53). The very large steric effect of an interfering benzo group has been noted previously[4,8] in the case of benz(*a*)acridine $\gg$ benz(*c*)acridine. Analogous relationships are found in the cases of benzo(*f*)quinoline ($\cong$ benzo(*c*)quinoline[8]) $\gg$ benzo(*h*)quinoline (*cf.* Nos. 10 and 28); phenazine $\gg$ dibenzo(*a*,*c*)phenazine (*cf.* Nos. 4 and 15); quinoxaline > acenaphtho(1,2-*b*)quinoxaline > benzo(*l*)acenaphtho(1,2-*b*)quinoxaline (*cf.* Nos. 11, 16 and 21), and (for two benzo groups) 14-phenyldibenz(*a*,*j*)acridine $\gg$ 7-phenyldibenz(*c*,*h*)acridine[8]. It is interesting to note that in Table I compounds Nos. 2–14, *i.e.* the thirteen nitrogen-containing compounds of highest R_F values, all exhibit some kind of steric substituent effect of the alkyl, aryl or benzo type(s)[36].

Many examples of the inductive methyl effect have already been discussed in the preceding paragraphs. Additional cases, involving rule III only, are found for the parent systems of dibenzo(*a*,*c*)phenazine (*cf.* Nos. 2 and 3 with 4), 2,3-diphenylquinoxaline (*cf.* Nos. 8 and 9), acenaphtho(1,2-*b*)quinoxaline (*cf.* Nos. 16 and 17), acridine[4] (*cf.* Nos. 22 and 23), and cinnoline (*cf.* Nos. 54 and 56). There does not appear to be a simple additive inductive effect of methyl groups (in sterically non-hindering positions)

in the benz(*c*)acridine (BcA) system studied by SAWICKI *et al.*[8] who find 8,10-dimethyl BcA = 7-methylBcA = BcA > 7,10-dimethylBcA = 7,9-dimethylBcA.

Inductive and steric effects by alkyl substituents on aromatic hydrocarbons have been reported previously[2]. However, very few cases of steric hindrance by a methyl group were found amongst the arenes in strong contrast to the azacyclic systems. In the 2-alkylnaphthalene and 9-alkylanthracene series the order ethylarene $\geqslant$ arene was observed, again in contradistinction to the present case of phenanthridine > 9-ethylphenanthridine. As yet, no series of alkylazarenes where alkyl varies from methyl to *tert.*-butyl and is located in a sterically non-hindering position (insofar as availability of the *n*-electrons is concerned) has been investigated. Such series, which we plan to study, should help to elucidate the question of whether or not flatwise adsorption of azarenes is important (*vide infra*). Of the phenylarenes and binaphthyls studied the biaryl was always more strongly adsorbed than the parent arene. Here, the phenylazarenes are all less strongly adsorbed than the parent azarenes, although for the pairs acridine $\geqslant$ 9-phenylacridine (*cf.* Nos. 20 and 22) and dibenz(*a,j*)acridine > 14-phenyldibenz(*a,j*)acridine[8] (no steric hindrance to *n*-electrons, *cf.* 9-phenylanthracene $\geqslant$ anthracene[2]) the two compounds differ only slightly in adsorbability. Note also that quinoline $\geqslant$ 2,2'-biquinolyl while 1,1'-binaphthyl (the binaphthyl isomer which is sterically the most hindered to coplanarity and to flatwise adsorption) $\geqslant$ naphthalene[2].

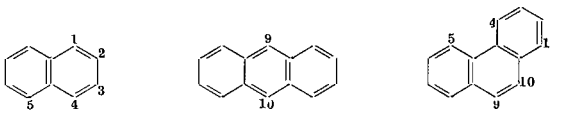
It was proposed previously that a subtle steric substituent effect may occur for a hydrogen atom in a position *peri* to an aromatic nitrogen atom. An examination of our TLC data would appear to corroborate this proposal. Thus we have the orders benzo(*b*)phenanthridine (4 rings) = benzo(*j*)phenanthridine (4 rings) $\geqslant$ phenanthridine (3 rings) = benzo(*f*)quinoline (3 rings) = quinoline (2 rings, all with one *peri*-hydrogen) > acridine (3 rings, with two *peri*-hydrogens) (*cf.* No. 22 with Nos. 27–31); quinoxaline (2 rings, one hydrogen *peri* to each nitrogen) > phenazine (3 rings, two hydrogens *peri* to each nitrogen) (*cf.* Nos. 15 and 21); isoquinoline (no *peri*-hydrogen) > quinoline (Table III, *cf.* Nos. 27 and 76); and possibly phthalazine (2 rings, no *peri*-hydrogens) > cinnoline (2 rings, a hydrogen *peri* to one of the nitrogens) > benzo(*c*)-cinnoline (3 rings, one hydrogen *peri* to each nitrogen) (*cf.* Nos. 48, 54 and 57). It is interesting to note in this regard that acenaphtho(1,2-*b*)quinoxaline $\cong$ phenazine (*i.e.* benzo(*b*)quinoxaline) (*cf.* Nos. 15 and 16).

It is apparent from these data that (in our studies) the size of the π-electron system is of little or no pertinence as compared to the *peri*-hydrogen effect. On the other hand, a marked difference is found in some of the data of SAWICKI *et al.*[8], whereby the size of the π-electron system appears to be of considerable importance in determining relative adsorbabilities of azarenes while *peri*-hydrogen effects are of lesser pertinence. Thus, they find the orders dibenz(*a,j*)acridine (5 rings) > benz(*a*)acridine (4 rings) > acridine (3 rings, all with two *peri*-hydrogens); 4-azapyrene (4 rings) $\geqslant$ benzo(*f*)quinoline (3 rings) $\geqslant$ phenanthridine (3 rings, all with one *peri*-hydrogen); pyrenoline (5 rings with 10 double bonds, no *peri*-hydrogen) > dibenz(*a,h*)acridine (5 rings with 11 double bonds, one *peri*-hydrogen) $\geqslant$ benzo(*h*)quinoline (3 rings, no *peri*-hydrogen, each with a sterically hindering benzo group); but phenanthridine $\geqslant$ acridine. Their observation that 7-azafluoranthene > 1-azafluoranthene seems to be an exception to all of these generalizations.

In Table II are reported R_F values for a number of diformalazines which are believed to exist in the *trans*-form (see Fig. 1). The polar eluent 2-butanone which was

used serves to give limited separation of these diformalazines, though it is clear that these compounds are less strongly adsorbed than are such *cis*-azines as cinnoline and phthalazine. The diformalazines Nos. 49, 52 and 53, wherein Y = Z = pyridyl, are more strongly adsorbed than are those where the substituents are alkyl or aryl.

Various available diazarenes have been studied for comparison with adsorbability of monoazarenes. Particular attention is here given to the cases where the parent analogous arene is naphthalene, anthracene or phenanthrene (see Table IV). Let us assume that the R_F value of the diazarene may be represented as a



simple function of the R_F values of two corresponding monoazarenes—which we shall call "component monoazarenes". Thus, for example, cinnoline (1,2-diazanaphthalene) is treated as a composite of quinoline (1-azanaphthalene) and isoquinoline (2-azanaphthalene), while phthalazine (2,3-diazanaphthalene) is treated as a composite of two molecules of isoquinoline. In Table IV observed R_F values for various diazarenes are compared with calculated R_F values as based on three hypothetical models for adsorption (see Appendix).

In models I and II electronic effects of one nitrogen atom on the other are considered negligible. It is assumed, moreover, that adsorption occurs primarily (if not entirely) at the nitrogen atoms and that the diazarene can move with the solvent only when the adsorbate molecule is completely free (*i.e.* unadsorbed) at both nitrogen atoms simultaneously. Model I allows simultaneous adsorption at both nitrogens, but does not require it. Model II allows adsorption at only one nitrogen atom at a time. Model III, on the other hand, is based on the assumption that the enhanced adsorbability expected for a diazarene molecule on the basis of its having two anchoring sites is either exactly counterbalanced by or is overwhelmed by electronic interaction between the nitrogen atoms. This interaction serves to decrease electron availability at each nitrogen. As expected, calculated R_F values for the three models fall in the order model III > model II > model I. One adsorbate, 1,2-diazanaphthalene (No. 54), gives results consistent with model I; 1,5-diazanaphthalene and 1,5-diazaphenanthrene (Nos. 51 and 32) give results consistent with model II; and eight compounds (Nos. 4, 7, 15, 18, 19, 21, 24 and 48) fit model III.

Three compounds, 2,3-diazanaphthalene (No. 57), 2,2'-bipyridyl (No. 37), and particularly 4,5-diazaphenanthrene (No. 39), are more strongly adsorbed than one predicts from model I. Since the two nitrogen atoms in all three of these compounds are equivalent the result implies that effectively one has enhanced adsorption at both nitrogens in these two compounds, *i.e.* the probability of anchoring by the second nitrogen atom is enhanced whenever anchoring by the first nitrogen atom occurs (and *vice versa*).

Before considering our interpretation of the data in Table IV it is best to summarize the general observations and suggestions of SNYDER[5b] for adsorbability of diazarenes on columns of alumina, since the data and interpretations are, in general, closely similar to ours. First he proposes that the very strong adsorption of 1,10-

TABLE IV

COMPARISON OF OBSERVED AND CALCULATED R_F VALUES FOR SOME DIAZARENES

No.	*Systematic name*	*R_F value observed*		*R_F value calculated*			
		Table	*R_F*	*Monoazarene standard(s) (No.)*	*Using model I*	*Using model II*	*Using model III*
	Naphthalene analogs						
54	1,2-Diaza-	III	0.12	27, 76	0.13	0.21	0.37
21	1,4-Diaza-	I	0.40	27	0.11	0.20	0.33
18	2,3-DiMe-1,4-diaza-	I	0.41	65	(0.13)	(0.22)	(0.36)*
24	6,7DiMe-1,4-diaza-	I	0.36	68, 70	(0.09)	(0.18)	(0.30)*
19	2,3,6,7-TetraMe-1,4-diaza-	I	0.41	66	(0.12)	(0.20)	(0.34)*
51	1,5-Diaza-	II	0.55	27	0.42	0.48	0.65
		III	0.29	27	0.18	0.27	0.42
57	2,3-Diaza-	II	0.24	76	(0.32)	(0.40)	(0.57)*
	Anthracene analog						
15	9,10-Diaza-	I	0.47	22	0.15	0.24	0.39
	Phenanthrene analogs						
32	1,5-Diaza-	I	0.25	10, 28	0.19	0.26	0.46
39	4,5-Diaza-	I	0.01	10	0.35	0.42	0.59
48	9,10-Diaza-	II	0.63	29	(0.41)	(0.47)	(0.64)**
	Other molecules						
4	9,14-Diazadibenz(*a,c*)-anthracene	I	0.70	5	0.46	0.51	0.68
7	1,4-Diazatriphenylene	I	0.64	10	0.35	0.42	0.59
37	2,2′-Bipyridyl	I	0.16	12	0.24	0.32	0.49
38	6,13-Diazadibenz(*a,h*)-anthracene	I	0.13	30, 31	0.09	0.18	0.30
55	*trans*-1,2-Bis-(4-pyridyl)-ethene	I	0.06	35	0.03	0.10	0.18

* Estimated from data in Table III.

** Estimated from data in Table I. Parenthesized values must be considered susceptible to gross error (see text).

phenanthroline (No. 39) "can be attributed to very favorable positioning of the two nitrogen atoms for simultaneous interaction with a single adsorbent site", that 2,2′-bipyridyl (No. 37) is adsorbed in the *s-cis* conformation analogous to No. 39, and that the two nitrogen atoms in *ortho*-diazarenes likewise simultaneously interact with a single site on the adsorbent. Second, electronic interactions in molecules of the pyrazinoid (1,4-diazinoid) type will decrease adsorbability. Third, "the greater the distance between the two nitrogens, as when the nitrogens are in different rings, the greater will be the adsorption energy".

To these considerations of SNYDER we add some further comments. First, the action of No. 39 as a bidentate ligand in the formation of stable complex-ions with various metallic ions (particularly Fe^{2+}) is well known[23,24]. We propose that the strong adsorption of No. 39 results from its bidentate ligation with a single Lewis-acid adsorption site on the surface of the adsorbent in a similar manner, *i.e.* perhaps by edgewise or tilted adsorption (see Fig. 3). 2,2′-Bipyridyl (No. 37) also shows enhanced adsorbability (as compared to 2-phenylpyridine). Although dipole moment measure-

ments indicate that No. 37 exists predominantly in the *s-trans* conformation in solution[25] the action of this compound as a bidentate ligand for metallic ions can occur only when it attains the *s-cis* conformation[26]. In general, for a series of bivalent metal ions M^{2+} the stability[23,27] of the ion $[M(bipy)_x]^{2+}$ is less than that of the corresponding ion $[M(phen)_x]^{2+}$ (for $x = 1$, 2 or 3), where the ligand is constrained to rigid planarity.

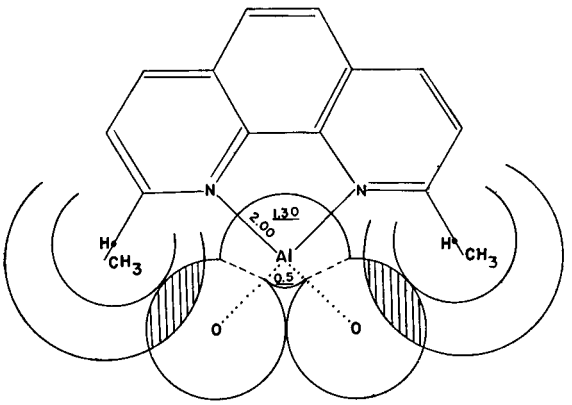


Fig. 3. A composite drawing (to scale) to show relative steric hindrances to edgewise adsorption of 1,10-phenanthroline and 2,9-dimethyl-1,10-phenanthroline, serving as bidentate ligands to Al, on a surface of alumina. Covalent bond lengths, ionic radii, Van der Waals' radii, and overlapping are analogous to those in Fig. 2[22]. The Al atom is here shown as having an ionic radius of 0.5 Å toward oxide ion and a covalent radius of 1.30 Å toward the N atoms (see Appendix).

The enhanced adsorbability of No. 37 plus the observation that No. 37 shows an R_F value intermediate between that of 1,10-phenanthroline (No. 39) and 1,7-phenanthroline (No. 32, approximately analogous to No. 37 in the *s-trans* conformation) is consistent with a preferred adsorption of No. 37 in the *s-cis* conformation and with ion–ligand complexation as the surface process for adsorption. The enhanced adsorbability of compound No. 57, on the other hand, we believe is better ascribed to a rapid shifting back and forth of the anchoring site between 2- and 3-positions while the molecule remains in the surface layer of the adsorbent, *i.e.* during a single overall period of adsorption. Such a process may be envisioned as involving two separate adsorption sites on the surface, to which the nitrogen atoms are anchored alternately with little or no rotation of the molecule necessary in between times, or (more likely, *vide infra*) as involving a single adsorption site and appreciable rotation (rocking) of the molecule in the surface layer so as to bring the anchoring sites alternately into juxtaposition with the adsorption site. For 1,2-diazanaphthalene, where the nitrogen atoms are not equivalent, enhanced adsorbability (over expectations as per model I) is not apparent from the R_F values; while in 9,10-diazaphenanthrene (which fits model III), where the nitrogen atoms are equivalent but both are sterically hindered, such shifting of the anchoring site during a single period of adsorption would appear to be excluded (*cf.* Nos. 28 and 48 in Table V). Because the extrapolation of data between two solvent systems of grossly different polarity is involved in the calculation of the hypothetical R_F values for No. 48, however, the inclusion of this compound in any one of the model groups must be considered doubtful until further experimental data are available[5d].

TABLE V

COMPARATIVE DATA FOR SELECTED AZARENES

No.	*Compound*	R_F[a]	q_N[b]	pK_a[c]	$-\Delta pK_a$[d]
10	Benzo(*h*)quinoline	0.59	1.266	4.25	0
15	Phenazine	0.47	1.171	1.23	4.37
21	Quinoxaline	0.40	1.171	0.56	4.38
22	Acridine	0.39	1.332	5.60	0
27	Quinoline	0.33	1.276	4.94	0
28	Benzo(*f*)quinoline	0.33	1.273	5.15	0
29	Phenanthridine	0.32	1.276	4.52	0
48	Benzo(*c*)cinnoline	(0.31)	1.104	2.20	2.32
76	Isoquinoline	(0.29)	1.251	5.40	0
51	1,5-Naphthyridine	(0.28)	1.256	2.91	2.03
54	Cinnoline	(0.27)	1.145[e] 1.117[f]	2.42	2.75
32	1,7-Phenanthroline	0.25	1.273[e] 1.266[g]	4.0	0.7
57	Phthalazine	(0.15)	1.197	3.47	2.03
39	1,10-Phenanthroline	0.01	1.256	(4.8)	−0.5
—	Pyridine	—	1.245	5.23	0
—	Pyridazine	—	1.151	2.33	2.90
—	Pyrimidine	—	1.247	1.30	3.93
—	Pyrazine	—	1.168	(0.7)	4.5

[a] R_F values are taken from Table I or (where parenthesized) are estimated (assuming consistency with the Non-Crossing Rule) on the same scale by use of data from Tables II and III.

[b] q_N values are electron densities calculated from simple Hückel MO theory using the parameters $h_{\dot{N}} = 0.5$, $k_{\dot{C}-\dot{N}} = 0.8$ and $k_{\dot{N}-\dot{N}} = 0.7$ (ref. 28).

[c] pK_a values[29] are for aqueous solution at 20°. The parenthesized values are estimated from data at 25° or 27°.

[d] ΔpK_a is the difference between the pK_a of a diazarene and the average pK_a of the component monoazarenes (as defined for models I–III).

[e] For 1-position.

[f] For 2-position.

[g] For 7-position.

While 1,5-diazanaphthalene (No. 51) and 1,5-diazaphenanthrene (No. 32) give R_F values consistent with model II, 6,13-diazadibenz(*a,h*)anthracene (No. 38) and *trans*-1,2-bis-(4-pyridyl)-ethene (No. 55) fall in between model groups I and II. In contrast to the molecules considered in the foregoing paragraph, these four molecules have *n*-electrons which are oriented more or less in opposite directions in space. Measurements on Stuart-Briegleb molecular models were made to ascertain the effective linear distances between the two pairs of *n*-electrons in each. For this purpose measurements were actually made on the analogous arenes and the effective *n–n* distance was taken equal to that between centers of the truncated hydrogen atoms at the corresponding positions. Values found were 6.0, 6.4, 7.4 and 11.9 Å, respectively. Although more examples are needed to check on this point it may be that the N atoms are too close together in Nos. 51 and 32 to reach two adsorption sites on the alumina simultaneously while some molecules of Nos. 38 and 55 may, in fact, reach and be simultaneously adsorbed on two adjacent adsorption sites.

Seven of the eight compounds which fall in model group III (*i.e.* all but No. 48 which was discussed earlier) are of the 1,4-diazinoid type for which SNYDER[5b] has presented an adequate explanation in terms of electronic interactions between

nitrogen atoms. This effect can also be experimentally observed (in a few cases) in terms of the relatively low basicities of such diazarenes as compared to the basicities of their component monoazarenes. Thus, $-\Delta pK_a \cong 4.4$ for No. 15, 21 and pyrazine in Table V, but is smaller for non-1,4-diazinoid compounds. On the other hand, neither values of pK_a itself nor of the calculated electron density on the nitrogen atom (q_N) show this effect clearly.

SNYDER[5c] has developed an equation to relate the linear equivalent retention volume of a solute ($\underline{R}°$) to the volume of the adsorbed phase per g of adsorbent (V_a), the adsorbent activity (α), the eluting power of the solvent ($\varepsilon°$), and various parameters dependent on the solute—including the volume of the adsorbate in the surface layer of the adsorbent ($\sum_i \delta_i$). This equation has been rigorously tested for aromatic hydrocarbons and to a lesser extent for 2,6-dimethylpyridine, cases where flatwise adsorption seems well established. However, the adaptability of this equation to the clarification of the geometry of adsorption of relatively non-hindered azarenes is more equivocal and seems not to have been rigorously tested. Hence, we still consider the matter of the geometry of adsorption of azarenes, in general, open to question and possibly confused by semantics or an inadequate picture of alumina surface geometry. We continue to favor the concept of preferential edgewise or tilted adsorption of azarenes (by means of the *n*-electron pair on the nitrogen atom) as being a more fruitful, heuristic model for practical qualitative usage than the flatwise model advocated by SNYDER[6c]. Actually, we proposed earlier[4] that some azarenes may be adsorbed edgewise and others (particularly wherein the *n*-electrons were sterically hindered from forming a coordination bond to the surface but where the π-system was not sterically hindered by sidewise projections) may be adsorbed flatwise. One can also visualize that some molecules of an azarene are held edgewise at the same time as others are held flatwise. Particularly, in such case, the ratio (molecules held flatwise/molecules held edgewise) may be highly dependent on such factors as activity of the alumina, eluting solvent used[5d], and size of the π-electronic system, as well as the availability of *n*-electrons. It should be noted, however, that the terms "flatwise" and "edgewise" (or "tilted") *need not be mutually exclusive or contradictory* for it is conceivable that *a molecule may be adsorbed both ways simultaneously, e.g.* at the inside corner of a step or discontinuity on the surface (with the π-system flatwise to one planar surface and the nitrogen atom anchored either edgewise to a perpendicular planar surface or tilted to an oblique surface), in a crevice (with the N atom anchored at the bottom), at a spike on the surface (analogous to the case of a step), and the like. In fact, the combined edgewise–flatwise or tilted–flatwise π *n*-model would seem to fit the presently available experimental results for azarenes very well in many cases.

SNYDER[6c] suggested that adsorption of an azarene to alumina involves *n*-electron transfer from the substrate to an adsorbent site. We find no serious objection to this suggestion for a molecule which is adsorbed edgewise (or tilted) or, perhaps, even adsorbed in a combined π–*n*-fashion. If, on the other hand, the azarene is adsorbed only flatwise one might question why an *n*-electron is transferred rather than a π-electron, particularly since recent evidence favors the concept that the lowest ionization potential of a non-adsorbed azarene involves loss of an electron from the π-system[30].

APPENDIX

Derivation of eqn. (1)

For chromatographic adsorption of compound A on one batch of plates assume the relationship (2) (for an equilibrium process)[31a], where

$$\frac{\Delta\mu_A}{RT} = -\ln k\left(\frac{1}{R_A} - 1\right) \tag{2}$$

μ_A is the chemical potential of A, k is a constant (assumed to be equal for all batches), and R_A is the observed R_F value for A. Then by differentiation of eqn. (2) one obtains eqn. (3) for a small change

$$\frac{\delta\Delta\mu_A}{RT} = \frac{\delta R_A}{R_A(1 - R_A)} \tag{3}$$

in R_A. Assuming the left side of eqn. (3) is a constant dependent only on the difference in activities of batches of plates and not on the adsorbate, and equating the right side of eqn. (3) to the corresponding expression for the adsorbate Q one immediately obtains eqn. (1).

R_F value for a molecule with two anchoring sites

Assume that for chromatographic adsorption one has available a set of compounds of molecular structures represented by M_i, M_j and M_{ij}, where the subscript i indicates the presence of an anchoring or localization site of structural type i; the subscript j, one of structural type j; and the subscript ij, one of each type. In the present work such a set would be represented by quinoline–isoquinoline–cinnoline, respectively, for i not equivalent to j, or by acridine–phenazine, respectively, for i equivalent to j. Let the observed R_F values for the various compounds be indicated as R_i, R_j and R_{ij}, respectively.

Model I. For M_{ij}, sites i and j are assumed to be non-overlapping, electronically non-interacting, and completely independent in regard to adsorption, *i.e.* adsorption at j in any particular molecule occurs irrespective of whether or not adsorption at i is extant, and *vice versa*. Since R_i and R_j are the time-probabilities that sites i and j are separately non-adsorbed, then R_{ij} (the time-probability that both sites are simultaneously non-adsorbed in M_{ij}) is given by eqn. (4). In the special case where i is equivalent to j

$$R_{ij} = R_iR_j \tag{4}$$

one has eqn. (5):

$$R_{ii} = (R_i)^2 \tag{5}$$

Model II. For M_{ij}, sites i and j are assumed to be non-overlapping and electronically non-interacting, but simultaneous adsorption of any particular molecule on both sites is forbidden. Assume that for M_i (and analogously for M_j) one has a virtual equilibrium of type (6), with a corresponding distribution coefficient α_i given by

$$M_i \text{ (solution)} \rightleftharpoons M_i \text{ (adsorbed)} \tag{6}$$

eqn. (7), where a is a constant having the same value for M_i, M_j and M_{ij}, and brackets

$$\frac{[M_i]_{ads}}{[M_i]_{soln}} = \alpha_i = a\left(\frac{1}{R_i} - 1\right) \tag{7}$$

represent thermodynamic activities of the enclosed species. Now for M_{ij} one has relationship (8). In model II one has the identities shown in eqn. (9). Substitution of

$$[M_{ij}]_{ads} = [M_{ij}]_{ads\ on\ i} + [M_{ij}]_{ads\ on\ j} = \alpha_{ij}[M_{ij}]_{soln} \tag{8}$$

$$\frac{[M_{ij}]_{ads\ on\ i}}{[M_{ij}]_{soln}} \equiv \frac{[M_i]_{ads}}{[M_i]_{soln}} \qquad \frac{[M_{ij}]_{ads\ on\ j}}{[M_{ij}]_{soln}} \equiv \frac{[M_j]_{ads}}{[M_j]_{soln}} \tag{9}$$

eqn. (9) and then eqn. (7) into eqn. (8), plus simplification and rearrangement of terms, lead to eqn. (10):

$$R_{ij} = \frac{R_i R_j}{R_i + R_j - R_i R_j} \tag{10}$$

For the special case where i is equivalent to j one obtains eqn. (11):

$$R_{ii} = \frac{R_i}{2 - R_i} \tag{11}$$

Model III. For M_{ij}, sites i and j are assumed to interact electronically to such an extent and in such manner that the statistical effect of having two anchoring sites on one molecule is exactly counterbalanced or even overwhelmed. Somewhat arbitrarily we take:

$$R_{ij} \geqq \frac{R_i + R_j}{2} \text{ for } i \text{ not equivalent to } j$$

and

$$R_{ii} \geqq R_i \text{ for } i \text{ equivalent to } j.$$

Fortified Dragendorff's reagent

A more concentrated reagent than that reported by LEDERER[31b] was used in order to reveal all spots other than that of *trans*-stilbene. It was prepared as follows.

Solution A: 3.85 g bismuth subnitrate, 55 ml distilled water and 25 ml glacial acetic acid.

Solution B: 8.0 g potassium iodide and 20 ml distilled water.

A mixture of A and B (stock solution) was stored in a brown bottle until needed (stable for several months). Shortly before use solution C was prepared by mixing 7 ml stock solution, 50 ml distilled water, and 10 ml glacial acetic acid and then adding concentrated hydrochloric acid dropwise until the precipitate which was present had dissolved. Solution C (stable for several days) was used directly for spraying the chromatograms.

Details of Figs. 2 and 3

Fig. 2 is drawn on the assumption that the adsorbate is anchored edgewise to the surface by means of an octahedral covalent bond N–Al of length 2.00 Å (1.30 Å for

the estimated[32] octahedral covalent radius of Al plus 0.70 Å for the covalent radius of N). The Al atom is here depicted as located in the center of a square planar arrangement of four O^{2-} ions (only two of which are shown) and directly above a fifth O^{2-} ion (not shown). The contact radius of the Al with the O^{2-} ions is taken as that of Al^{3+}. The general structure of the alumina is presumed to be a defect spinel[33] arrangement of γ-alumina[34], modified at the surface to approach the structure of $Al(OH)_3$ (O–O distance 2.78 Å)[35].

Fig. 3 uses the same types of bonding and spatial geometry as in Fig. 2, except that the Al atom is assumed (a) to have available two unfilled *cis* octahedral bond orbitals directed outward from the surface and (b) to be resting centrally on the two O^{2-} ions shown. The other two O^{2-} ions would be located in the outermost surface layer directly in front of and behind the Al atom in the diagram shown (*i.e.* above and below the plane of the paper).

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SUMMARY

R_F values for a variety of aromatic nitrogen heterocycles, diformalazines, stilbazoles and pyridylethenes were determined by means of thin-layer chromatography on alumina. In general, it was found that adsorbability of an azarene is greater than that of its hydrocarbon analog, is subject to steric hindrance (increased R_F) by groups which interfere with coordination by the n-electrons of the nitrogen atom, and may be subject to an inductive effect (decreased or no change in R_F) by a sterically non-hindering methyl group. Observed R_F values for diazarenes are compared with calculated R_F values as based on three hypothetical models for adsorption and measured R_F values for "component monoazarenes." The geometry of adsorption of these nitrogen compounds is discussed. It is concluded that the concept of edgewise or tilted adsorption of an azarene by means of the n-electrons of the nitrogen atom is qualitatively suitable and/or preferable in many cases. The possibility of simultaneous edgewise (or tilted) and flatwise adsorption on a non-planar alumina surface is considered particularly attractive.

REFERENCES

1 L. H. KLEMM, D. REED and C. D. LIND, *J. Org. Chem.*, 22 (1957) 739.
2 L. H. KLEMM, D. REED, L. A. MILLER AND B. T. HO, *J. Org. Chem.*, 24 (1959) 1468.
3 L. H. KLEMM AND S. K. AIREE, *J. Chromatog.*, 13 (1964) 40.
4 L. H. KLEMM, E. P. ANTONIADES, G. CAPP, E. CHIANG AND E. Y. K. MAK, *J. Chromatog.*, 6 (1961) 420.
5 L. R. SNYDER, *J. Chromatog.*, (a) 6 (1961) 22; (b) 8 (1962) 178; (c) 8 (1962) 319; (d) 16 (1964) 55; (e) 17 (1965) 73.
6 L. R. SNYDER, *J. Phys. Chem.*, 67 (1963) (a) 234; (b) 240; (c) 2344.
7 C. H. GILES AND R. B. MCKAY, *J. Chem. Soc.*, (1961) 58.
8 E. SAWICKI, T. W. STANLEY, J. D. PFAFF AND W. C. ELBERT, *Anal. Chim. Acta*, 31 (1964) 359.
9 E. SAWICKI, W. C. ELBERT AND T. W. STANLEY, *J. Chromatog.*, 17 (1965) 120.

10 L. H. KLEMM AND A. WEISERT, *J. Heterocyclic Chem.*, 2 (1965) 15, 140.
11 L. H. KLEMM AND D. REED, *J. Org. Chem.*, 25 (1960) 1816;
L. H. KLEMM AND C. E. KLOPFENSTEIN, unpublished results.
12 A. PICTET AND A. HUBERT, *Ber.*, 29 (1896) 1182.
13 A. BERNTHSEN, *Ann.*, 224 (1884) 1.
14 K. N. CAMPBELL AND I. J. SCHAFFNER, *J. Am. Chem. Soc.*, 67 (1945) 86.
15 R. H. F. MANSKE, L. MARION AND F. LEGER, *Can. J. Res.*, 20B (1942) 133.
16 F. J. ALLAN AND G. G. ALLAN, *J. Org. Chem.*, 23 (1958) 639;
H. H. SZMANT AND C. M. HARMUTH, *J. Am. Chem. Soc.*, 81 (1959) 962;
T. CURTIUS AND R. JAY, *J. Prakt. Chem.*, [2] 39 (1889) 27;
J. H. WEISBURGER AND P. H. GRANTHAM, *J. Org. Chem.*, 21 (1956) 1160.
17 H. E. ZIMMERMAN AND S. SOMASEKHARA, *J. Am. Chem. Soc.*, 82 (1960) 5865.
18 F. H. CLARKE, G. A. FELOCK, G. B. SILVERMAN AND C. M. WATNICK, *J. Org. Chem.*, 27 (1962) 533.
19 *Handbook of Chemistry and Physics*, 44th Ed., Chemical Rubber, Cleveland, 1963, p. 554.
20 M. YASUDA, K. SONE AND K. YAMASAKI, *J. Phys. Chem.*, 60 (1956) 1667.
21 J. GILLIS, *Anal. Chim. Acta*, 8 (1953) 97.
22 L. PAULING, *The Nature of the Chemical Bond*, 2nd Ed., Cornell University, Ithaca, N.Y., 1945.
23 A. E. MARTELL AND M. CALVIN, *Chemistry of the Metal Chelate Compounds*, Prentice-Hall, New York, 1952, p. 523.
24 F. P. DWYER AND D. P. MELLOR, *Chelating Agents and Metal Chelates*, Academic Press, New York, 1964, p. 112.
25 S. WALKER, in A. R. KATRITZKY, *Physical Methods in Heterocyclic Chemistry*, Vol. I, Academic Press, New York, 1963, p. 197.
26 W. W. BRANDT, F. P. DWYER AND E. C. GYARFAS, *Chem. Rev.*, 54 (1954) 959.
27 H. IRVING AND D. H. MELLOR, *J. Chem. Soc.*, (1962) 5222;
K. YAMASAKI AND M. YASUDA, *J. Am. Chem. Soc.*, 78 (1956) 1324;
R. R. MILLER AND W. W. BRANDT, *J. Am. Chem. Soc.*, 77 (1955) 1384;
R. L. GUSTAFSON AND A. E. MARTELL, *J. Am. Chem. Soc.*, 81 (1959) 525.
28 A. STREITWIESER, *Molecular Orbital Theory for Organic Chemists*, Wiley, New York, 1961, Chaps. 2, 5.
29 A. ALBERT, in A. R. KATRITZKY (Editor), *Physical Methods in Heterocyclic Chemistry*, Vol. I, Academic Press, New York, 1963, p. 65.
30 V. G. KRISHNA AND M. CHOWDHURY, *J. Phys. Chem.*, 67 (1963) 1067;
M. R. BASILA AND D. J. CLANCY, *J. Phys. Chem.*, 67 (1963) 1551.
31 E. LEDERER, *Chromatographie en Chimie Organique et Biologique*, Vol. I, Masson, Paris, 1959, (a) pp. 154, 162; (b) p. 511.
32 H. IRVING, E. J. BUTLER AND M. F. RING, *J. Chem. Soc.*, (1949) 1489.
33 P. P. EWALD AND C. HERMANN, *Strukturbericht*, Vol. I, Akademische Verlagsgesellschaft, Leipzig, 1931, p. 350.
34 C. GOTTFRIED AND F. SCHOSSBERGER, *Strukturbericht*, Vol. III, Akademische Verlagsgesellschaft, Leipzig, 1937, p. 338;
A. F. WELLS, *Structural Inorganic Chemistry*, 3rd Ed., Oxford University Press, London, 1962, pp. 492, 559;
J. B. PERI, *J. Phys. Chem.*, 69 (1965) 220.
35 R. C. EVANS, *An Introduction to Crystal Chemistry*, 2nd Ed., Cambridge University Press, 1964, p. 274.
36 E. SAWICKI, J. E. MEEKER AND M. J. MORGAN, *J. Chromatog.*, 17 (1965) 252.

PAPER CHROMATOGRAPHY OF NITROFURAN DERIVATIVES

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Paper chromatography is playing a rather important role in the analysis of drugs, because it can be used simultaneously for their identification, and for testing their purity and physiological activity (in the cases of isomeric forms with differences in physiological activity).

We carried out investigations on the paper chromatography of a number of nitrofuran derivatives, synthesized by ZHELYASKOV AND ZIKOLOVA[1], which have shown a high antibacterial, fungicidal and antiprotozoal activity[2,3].

The compounds under investigation are Schiff bases of 5-nitrofurfural with a series of aromatic amines. Compounds were chosen which, besides a high biological activity, showed related chemical structure and isomeric forms. The latter was done in order to find conditions for carrying out chromatography and solvent systems with the best separation for this group of compounds. In addition we also included three well known nitrofuran derivatives: 5-nitro-2-furaldehyde semicarbazone (furacin, nitrofurazone), N-(5-nitro-2-furfurylidene)-1-aminohydantoin (nitrofurantoin, furadantin) and N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone (furoxone, furazolidone).

Some valuable data are given in the literature on the paper chromatography of furacin, its determination in foods[6] and the detection of its decomposition products[4]. A communication has also appeared more recently on the paper chromatographic separation of furacin, furadantin and furoxone[5]. Use is chiefly made of hydrophilic solvent systems such as *n*-butyl alcohol–acetic acid–water (4:1:5)[4,5]; *n*-butyl alcohol–ammonia 10%–water (12:1:7)[5]; a less hydrophilic system, chloroform–isopropyl alcohol–potassium benzoate[6]; and from amongst the anhydrous systems, the system dimethylformamide/dioxan–methanol (9:1)[5] was examined without success. U.V. light was used for visualisation of the spots.

The present paper includes results from chromatographic studies on nitrofuran derivatives in nine solvent systems (Table I), selected with regard to the acid, neutral and basic characteristics of the compounds investigated and to their rather differing solubility and polarity. For the more lipophilic compounds, the systems S_3, S_4, S_5 and S_6 from Table I were examined as they were shown to be best fitted for compounds of this type[7]. We have also used systems with a stationary phase of dimethylformamide. Each of the systems under investigation has shown some advantages or disadvantages with respect to single compounds. But, in general, we arrived at the conclusion, that the anhydrous systems with a stationary phase of dimethyl-

* Director: Prof. L. ZHELYASKOV.

TABLE I

SOLVENTS

No.	*Immobile phase*	*Mobile phase*	*Time of chromatography (h)*
S_1	—	*n*-Butanol–acetic acid–water (4:1:5)	11
S_2	—	*n*-Butanol–2.5% NH_3 (1:1)	11
S_3	—	Butyl acetate–H_2O (1:1)	3
S_4	—	Tetrachloromethane–acetic acid (50:1)	$5^1/_2$
S_5	—	Benzene–methanol–H_2O (2:1:1)	3
S_6	—	Petroleum ether (b.p. 60–70°)–methanol–water (2:1:1)	3
S_7	Dimethylformamide	Chloroform	2
S_8	Dimethylformamide	Benzene	2
S_9	Dimethylformamide	Benzene–cyclohexane (4:1)	2

formamide and a mobile phase of chloroform, benzene or, better still, benzene–cyclohexane (4:1) are best fitted for identifying and separating of compounds from this group, as well as for detecting admixtures and products of decomposition. We have established that this also refers to furacin, furadantin and furoxone, which are more satisfactorily, reliably and quickly identified and examined for purity in the system dimethylformamide/benzene–cyclohexane (4:1) than in all the other systems applied up to now. The spots are visualized not only by observation in U.V. light, but also by spraying them with a solution of *p*-dimethylaminobenzaldehyde, after which yellow, orange or red spots are obtained.

EXPERIMENTAL

Materials

The Schiff bases of 5-nitrofurfural, given in Table II, were synthesized by ZHELYASKOV AND ZIKOLOVA[1], and characterised by their m.p. and nitrogen content. Furacin, furadantin and furoxone were supplied by Boehringer, Mannheim.

The initial amines from which the Schiff bases were prepared, had p.a. purity; they were also used as reference substances.

The solvents necessary for preparing the solvent systems, described in Table I, were freshly distilled and mixed 24 h before use.

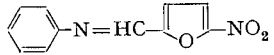
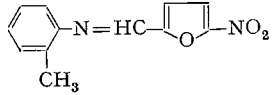
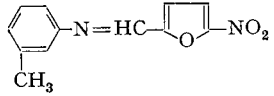
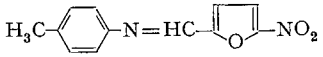
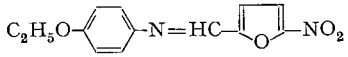
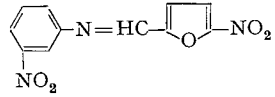
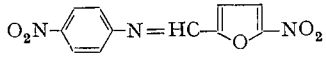
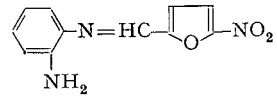
Chromatography

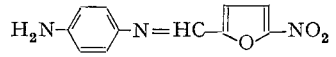
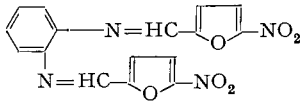
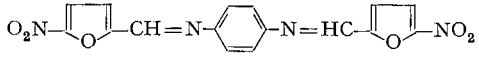
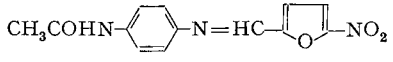
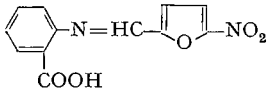
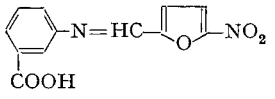
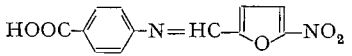
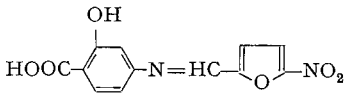
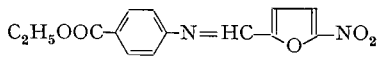
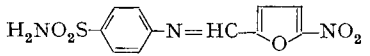
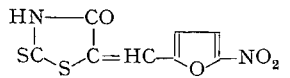
The substances examined were dissolved in alcohol by heating, at a concentration 1 mg/ml and 20–40 γ (0.02–0.04 ml) were applied to Schleicher & Schüll 2043 bMgl paper (13 × 38 cm). The development was carried out by the descending technique at a temperature of 20–22°. The paper strips with the samples were left to equilibrate with the systems S_1 to S_6 overnight in the chromatographic chambers, after which the mobile solvent was poured into the glass troughs. With solvent systems S_7–S_9, the paper strips, to which the samples had been applied, were im-

TABLE II

R_F VALUES OF THE INVESTIGATED SUBSTANCES

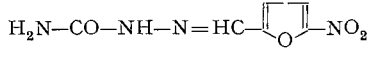
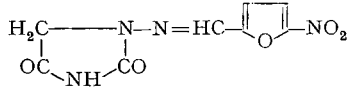
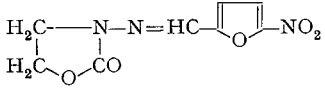
The R_F values are given for the center of the spots. The oblong spots are marked ↕, and the mark ↕ without a figure shows that the spot tails the full length of the chromatogram. The mark ↑ or ↓ indicates that the R_F value is given for the center of a spot, from which are tails either upwards or downwards, respectively.
Fc = furacin; Fd = furadantin; Fs = furoxone.

No.	*Structural formula*	*Solvent systems*									*Colour of the spots*		
		S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	*Daylight*	*U.V.*	*1% p-Dimethylaminobenzaldehyde*
1		0.91	0.90	0.95	0.55↑	0.96	0.80	0.66	0.93	0.94	pale yellow	orange	pale yellow
2		0.90	0.91	0.92	0.61	0.97	0.77	0.65	0.90	0.93	pale yellow	pale brown	pale yellow
3		0.90	0.91	0.92	0.60	0.97	0.76	0.65	0.90	0.92	pale yellow	pale brown	pale yellow
4		0.90	0.91	0.92	0.60	0.97	0.76	0.65	0.90	0.92	pale yellow	pale brown	pale yellow
5		0.88	0.90	0.96	0.50↕	0.98	0.72	0.58	0.88	0.89	yellow	yellow	yellow
6		0.89	0.88	0.91	0.24↕	0.97	0.32↕	0.55	0.90	0.92	pale yellow	dark	yellow
7		0.89	0.87	0.94↓	0.00↑		↕	0.00	0.56	0.90	0.92	red	dark orange	red
8		0.90	0.78		↕	0.12↕	0.72↕	0.04	0.56	0.83	0.80	pale yellow	bright yellow	pale yellow

9		0.52	0.50↑↓	↑↓	0.00	↑↓	0.04	0.50	0.79	0.72	red	dark	red
10		0.90	0.84	0.00	0.67↑	0.78↑↓	0.02	0.61	0.87	0.87	pale yellow	yellow	—
11		0.50↑	0.00	0.00	0.00	0.78↑↓	0.04	↑↓	0.88	0.88	—	—	red
12		0.62	0.70↓	↑↓	0.00	0.05	0.02	0.53	0.81	0.73	pale yellow	—	orange
13		0.87	0.24	0.85	0.48↑↓	0.18	0.02	0.50	0.54	0.62	—	bright blue	pale yellow
14		0.79	0.10	0.52	0.02↑	0.05	0.00	0.22	0.18	0.20	—	green	lemon yellow
15		0.83	0.07	0.62↓	0.02↑	0.03	0.00	0.34	0.28	0.25	—	pale yellow	orange
16		0.84	0.10	0.55↑↓	0.00	0.00	0.00	0.04↑	0.05↑	0.05↑	pale yellow	pale yellow	yellow
17		0.91	0.91	0.92↓	0.30↑↓	0.99	0.60↑↓	0.50	0.89	0.91	—	—	yellow
18		0.64	0.43	0.26↑↓	0.00	0.00	0.00	0.22	0.08	0.06	—	yellowish green	yellow
19		0.90	0.63	0.92	0.28↑↓	0.65	0.00	0.43	0.67	0.64	yellow	dark	yellow

(*continued on p. 450*)

TABLE II (*continued*)

No.	Structural formula	Solvent systems									Colour of the spots		
		S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	*Day-light*	*U.V.*	*1% p-Dimethylamino-benzaldehyde*
Fc		0.70	0.56	0.00 ↑	0.00 ↑	0.00	0.00	0.32	0.40	0.48	pale yellow	pale yellow	—
Fd		0.58	0.13	0.00 ↑	0.00	0.00	0.00	0.43	0.55	0.65	pale yellow	pale yellow	—
Fs		0.72	0.58	0.00 ↑	0.00	0.00	0.00	0.52	0.65	0.72	pale yellow	pale yellow	—

pregnated with a 30 % methanolic solution of dimethylformamide, after which they were dried between two filter papers and allowed to air dry for 15 min, and equilibrate in the chambers for another half hour. When the front of the mobile solvent had moved nearly 28 cm from the start line, the chromatograms were withdrawn and left to dry in the air, after which the spots visible in day light were marked, then those visible in U.V. light and finally the paper was sprayed with a 1 % solution of *p*-dimethylaminobenzaldehyde in 8 % hydrochloric acid. The colours obtained and the R_F values, which represent the average values from 5 to 10 experiments, are given in Table II.

Detection of decomposition products of the compounds obtained as a result of the influence of water, acids and bases

Some aqueous solutions, with a concentration of 2 mg/ml, were prepared from the Schiff bases of 5-nitrofurfural and *o*-, *m*- and *p*-aminobenzoic acids and aminosalicylic acid, which in contrast to the other compounds insoluble in water (furacin, furadantin and furoxone are not taken into consideration) show a certain, though rather low solubility in water. At first the solutions were a light yellow colour which grew darker after 2–3 days. Those which had an insoluble residue were mixed with a little alcohol until a clear solution was obtained, before carrying out the chromatography. The latter was carried out according to the method described in system S_9, after three, seven and more days, and compared with fresh solutions of the compounds, and fresh and aged solutions of the amine.

The effect of acids and bases was followed by adding 0.05 ml 1 *N* HCl or KOH to 1 ml of the alcoholic solutions of the compounds (concentration 1 mg/ml), to give a solution approx. 0.05 *N* with respect to the acid or base. These solutions were left for half an hour at room temperature and refluxed on a steam bath for another half hour, after which they were applied to the paper, and chromatographed in the system S_9. Alcoholic solutions of the substances, as well as alcoholic solutions of the initial amines treated in the same way, were used as reference substances.

RESULTS AND DISCUSSION

Colour of the spots

Most of the compounds under investigation are coloured and appear on the chromatogram as yellow spots. Red is the typical colour of the *p*-nitroaniline and *p*-aminoaniline derivatives. In U.V. light, some of the compounds give bright fluorescent spots and others dark spots. The different fluorescence of the isomeric compounds (derivatives of *o*-, *m*-, and *p*-aminobenzoic acid and *o*- and *p*-phenylenediamine) and the colours obtained with *p*-dimethylaminobenzaldehyde are also characteristic. The spot appears as a bright blue fluorescence when the carboxylic group is in the *ortho* position, pale green when it is in the *meta* position and in the *para* position there is no fluorescence. On the other hand, the most intense colouring (orange) with *p*-dimethylaminobenzaldehyde appears with the *para* derivative. The *meta* derivative gives a lemon-yellow colour and the *ortho* a light yellow one.

When there is an amine group in the *ortho* position (*o*-phenylenediamine) the spot has a bright yellow fluorescence which diminishes if this amine group is condensed with another 5-nitrofurfural molecule. When the amine group appears in the *para* posi-

tion, the compound has a red colour in daylight, but in U.V. light appears dark. These differences thus make it possible to identify these isomers with certainty even in systems where their R_F values are rather close.

Furacin, furadantin and furoxone are pale yellow in daylight and in U.V. light. Unlike the other compounds examined they give no colour with *p*-dimethylaminobenzaldehyde. With an alkaline solution furacin forms a bright red spot, by which it may be differentiated from all the other nitrofuran derivatives investigated.

Shape of the spots

In most of the systems examined the spots are well formed, round or slightly oval. But a number of the compounds in the third, fourth, fifth and sixth solvent systems form an extended spot or remain at the start line. Obviously, in these systems the distribution equilibrium is reached rather slowly, and in some cases is not attained because the substances remain at the start line.

In system nine sharp round spots are obtained, and even compounds with small differences in R_F value are very well separated.

R_F values. Influence of functional groups

The simplest of all the compounds under investigation is 5-nitrofurfurylidene-aniline (therefore it will serve as comparative base for the chromatographic behaviour of all the other compounds). It is characterized, in general, by its high R_F values in all the systems utilized, which is an indication of its lipophility. Its R_F values are comparatively lower in systems S_4 and S_7, which contain tetrachloromethane and chloroform in the mobile phase. In general all the compounds under investigation showed lower R_F values in these two systems.

Introduction of a methyl group in the *ortho*, *meta* or *para* position of the benzene nucleus of 5-nitrofurfurylidene-aniline produces no changes in the chromatographic behaviour of the compounds. An ethoxy group in the *para* position causes a slight lowering of the R_F values, but in general the behaviour in the different solvent systems does not change. The introduction of a nitro group into the *meta* or *para* position also causes very little change in the R_F value in the systems, where well formed spots are obtained. Some changes are observed with the presence of an amine group in the benzene nucleus, particularly when it is in the *para* position (the R_F values are lower). There are characteristic differences between the *ortho* and *para* amino derivatives in the acid system S_1. The *para* amino derivative in this system has a low R_F value because of an increase in dissociation and hence an increase of the solubility in the immobile aqueous phase. This typical behaviour of the amine group is inhibited in *ortho* position. There is a similar effect in systems with a stationary phase of dimethylformamide. The higher R_F values of the *ortho* derivative, compared to those of the *para* derivative, may be explained by the reduced possibility of formation of intermolecular hydrogen bridges between the amine group in the *ortho* position and the immobile phase. Less soluble compounds are obtained by reacting the amine groups with a second 5-nitrofurfurylidene radical; these are difficult subjects for chromatography. By acetylating the amine group in the *para* position the behaviour in the acid system S_1 is mainly changed. The basic properties of the compounds are reduced.

The R_F values are shown to be dropping most sharply with the presence of a

carboxyl group in the benzene nucleus. This shows an increase of the solubility in the more polar immobile phase. Typical of the acid properties are the high R_F values in the acid systems S_1 and low R_F values in the alkaline system S_2. This is explained by inhibition of the dissociation in the first system, and by its potentiation in the second. The position of the carboxyl group is also of importance, the highest R_F values being shown by the compound with the carboxyl group in the *ortho* position, which may be explained by the formation of an intramolecular hydrogen bridge. The compounds possessing carboxyl groups in the *meta* and *para* positions have related R_F values, but are not identical. In the presence of both hydroxylic and carboxylic groups together, the increase of the polarity becomes still more apparent, especially in the systems with dimethylformamide. Obviously, the possibility for intermolecular hydrogen bridge formation with the immobile phase are still more enhanced. The typical behaviour of the carboxylic group vanishes after its esterification and the R_F values increase again.

The $-SO_2NH_2$ group strongly increases the polarity of the compound, especially in the systems with stationary dimethylformamide, where it gives lower R_F values than with the carboxylic group.

The rhodanine derivative (No. 19 in Table II) behaves as an acid, with a higher R_F value in the acid system S_1 and a lower one in the alkaline S_2. It behaves like furacin, furadantin and furoxone, which is due to the presence of a carbonyl group. In general their R_F values are lower, and in the systems S_3, S_4, S_5 and S_6 they do not move at all. In the dimethylformamide systems all three compounds show R_F differences large enough for separations.

Detection of admixtures and decomposition products

The nitrofuran derivatives under investigation, as Schiff bases, may contain admixtures of the amines from which they were prepared or from hydrolysis. The second component, 5-nitrofurfural, cannot be detected directly because of its volatility. That is why our attention was directed towards examining the amines, especially those which are non-volatile. The conversion of the volatile components into non-volatile ones would complicate their detection in admixtures and in fact was not a subject of our investigation.

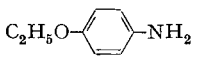
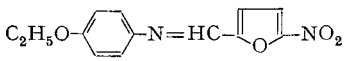
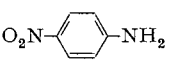
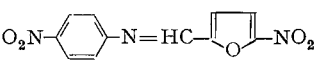
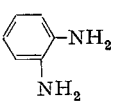
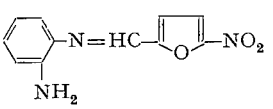
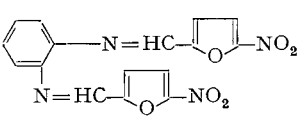
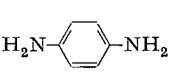
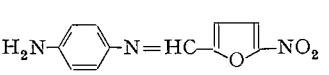
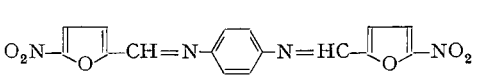
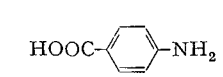
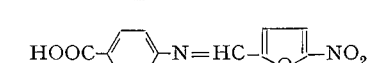
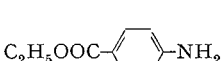
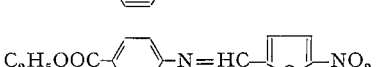
Most of the initial amines, chromatographed at the same time as reference compounds, have shown lower R_F values than the corresponding derivatives and have been detected more easily. It is of interest that the R_F values were changed differently by blocking the amine group of the various amines with 5-nitrofuraldehyde. In *ortho*-, *meta*- and *para*-aminobenzoic acids no changes were shown at all. It seems that the carboxylic group has a dominating influence upon the R_F values and veils the influence of the amine group. It is evident that the ΔR_M value in this case depends not only on the character of the functional group introduced, but also on the type and position of the other substituents in the benzene nucleus. Table III gives the ΔR_M values obtained as a result of the condensation of the amine group in the different aromatic amines used with 5-nitrofuraldehyde.

Stability of the compounds

The chromatograms of the alcoholic solutions in mg/ml concentration have shown no changes over a period of 5–6 days. The aqueous solutions and suspensions

TABLE III

R_F VALUES OF AROMATIC AMINES AND THE CORRESPONDING SCHIFF BASES WITH 5-NITROFURALDEHYDE IN THE SYSTEM DIMETHYLFORMAMIDE/BENZENE–CYCLOHEXANE (4:1)

Structural formula	R_F	R_M	ΔR_M
	0.75	−0.477	
	0.89	−0.908	−0.431
	0.75	−0.477	
	0.92	−1.061	−0.584
	0.44	+0.105	
	0.80	−0.602	−0.707
	0.87	−0.826	−0.224
	0.14	+0.788	
	0.72	−0.410	−1.198
	0.88	−0.865	−0.455
	0.25	+0.477	
	0.25	+0.477	0.0
	0.70	−0.368	
	0.91	−1.005	−0.637

of the *ortho*, *meta* and *para* derivatives of the aminobenzoic acids yielded two spots after 5–6 days (Fig. 1). One of these spots corresponds to the compounds alone, but the second spots have various differing R_F values and do not give a colour with *p*-dimethylaminobenzaldehyde. The second spots are not due to traces of the initial amines, because in these compounds all the initial amines have the same R_F values as their derivatives. They cannot be identified as nitrofurfural because, in such a case, all three compounds would have shown the same R_F value, whereas in this case all three are different. No amine group is present because they do not give a colour with *p*-dimethylaminobenzaldehyde. Probably, the carboxylic groups have also undergone some changes, because the R_F values sharply increase in the *meta* and *para* compounds. The examinations carried out in the microbiological laboratory of the institute have shown that these changes considerably reduce the biological activity of the preparations.

All nitrofurfurylidene derivatives decompose under the influence of 0.05 *N* acids and bases in alcoholic solution. The decomposition in acid medium is lower and the chromatogram shows two spots, one of which corresponds to the compound under investigation and the other to the amine obtained as a result of the decomposition. Decomposition proceeds faster in alkaline medium; the spot of the compound under investigation (especially after heating the solution) disappears and other spots appear on the chromatogram; some of these were not identifiable. Figs. 2 and 3 are given as

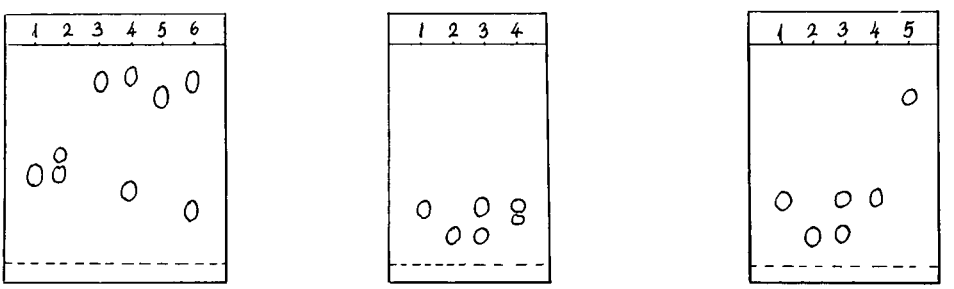


Fig. 1. System: dimethylformamide/benzene–cyclohexane (4:1). 1 = (5-nitrofurfurylidene)-*o*-aminobenzoic acid; 2 = the same compound after aging in an aqueous solution for 6 days; 3 = (5-nitrofurfurylidene)-*m*-aminobenzoic acid; 4 = the same compound after aging in an aqueous solution for 6 days; 5 = (5-nitrofurfurylidene)-*p*-aminobenzoic acid; 6 = the same compound after aging in an aqueous solution for 6 days.

Fig. 2. System: dimethylformamide/benzene–cyclohexane (4:1). 1 = *p*-phenetidine; 2 = (5-nitrofurfurylidene)-*p*-phenetidine; 3 = compound 2 in alcohol 0.05 *N* with respect to HCl, after heating for 30 min; 4 = compound 2 in alcohol, 0.05 *N* with respect to KOH, after heating for 30 min.

Fig. 3. System: dimethylformamide/benzene–cyclohexane (4:1). 1 = *p*-aminobenzoic acid ethyl ester; 2 = (5-nitrofurfurylidene)-*p*-aminobenzoic acid ethyl ester; 3 = compound 2 in alcohol, 0.05 *N* with respect to HCl, at room temperature after 30 min; 4 = compound 2 in alcohol, 0.05 *N* with respect to KOH, at room temperature after 30 min; 5 = compound 2 in alcohol, 0.05 *N* with respect to KOH, after heating for 30 min.

an illustration of the changes occurring with the phenetidine and anesthesine derivatives. The anesthesine derivative completely decomposes in alkaline medium to anesthesine and the latter is converted on heating to *p*-aminobenzoic acid (R_F 0.25).

ACKNOWLEDGEMENTS

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SUMMARY

Some investigations have been carried out on the paper chromatography of a group of new nitrofuran derivatives, along with three known preparations of the same group, *viz.* furacin, furadantin and furoxone. Nine solvent systems were employed, and it was established that for the identification and separation of this group of compounds, together with the detection of isomeric forms and admixtures, systems with a stationary dimethylformamide phase and a mobile phase of chloroform, benzene or better still benzene–cyclohexane (4:1) were best. The decomposition of the nitrofuraldehyde derivatives due to the influence of water, acids and bases was investigated. The influence of functional groups on the R_F values is discussed. Is was shown that the ΔR_M value obtained by blocking the aromatic amine group with 5-nitrofuraldehyde varies in each case and depends on the type and position of the other substituents in the benzene nucleus.

REFERENCES

1 L. ZHELYASKOV AND SV. ZIKOLOVA, *Farm. (Bulg.)*, 10, No. 3 (1960) 11.
2 P. KOZHUKHAROV AND T. KHARIZANOVA, *Pharmazie*, 15 (1960) 492.
3 P. KOZHUKHAROV AND T. KHARIZANOVA, *Pharmazie*, 17 (1962) 134.
4 H. WUNDERLICH, *Pharmazie*, 13 (1958) 202.
5 J. BREINLICH, *Deut. Apotheker-Ztg.*, 104 (1964) 535.
6 E. DIXON, *J. Sci. Food Agr.*, 6 (1955) 556; *C.A.*, 50 (1956) 5056g.
7 Z. PROCHAŽKA, V. ŠANDA AND K. MACEK, *Collection Czech. Chem. Commun.*, 24 (1959) 2928.

THE USE OF TRI-ISO-OCTYLAMINE AS AN ANION EXCHANGER FOR PARTITION CHROMATOGRAPHY

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INTRODUCTION

The application of reversed phase partition chromatography to inorganic separations has recently received considerable attention, and selective column materials have been prepared by immobilising extractants of the type commonly employed for solvent extraction, upon a solid carrier. The resulting solid phase, when packed into a column, can often combine the selectivity of the reagent with the technical advantages of chromatographic operation, and by varying the composition of the stationary and mobile phases, separation of a very wide range of elements can be achieved[1]. Particular interest has been centred on the separation of the rare earths from each other by elution with aqueous acid from columns retaining di-(2-ethylhexyl) hydrogen phosphate, and a number of separation schemes have been reported[2–6]. However, the scope of the technique can quite clearly be extended by varying the complexing agent providing the stationary phase, and we report here experiments which have been carried out to examine the behaviour of an anion exchange material prepared by retaining a high molecular weight amine on a polymeric support.

The use of high molecular weight amines as the stationary phase in partition chromatography has already been described using paper[7,8], cellulose[9], sintered polymers[10] and a fluorinated polymer[11] as carriers. For the work reported here Corvic R51/83, a poly-(vinyl chloride/vinyl acetate) copolymer, already used to retain organic extractants for partition chromatography[12] was used as carrier; tri-iso-octylamine (TIOA) was chosen as the extracting amine since in spite of being an isomeric mixture, it has found useful application to analytical separations by virtue of the good separation factors that can be achieved for many mixtures, its cheapness, low aqueous solubility and high solubility in organic diluents[13]. Zinc tracer was used initially to assess the behaviour of the solid phase but subsequently a number of elements were extracted from hydrochloric acid by TIOA–Corvic.

EXPERIMENTAL

Preparation of Corvic retaining TIOA

In previous work an organic reagent was added to Corvic in a volume of organic solvent which was less than the maximum the solid could take up[2], and distribution of the reagent throughout the solid was obtained by stirring the mixture vigorously

during both the addition of the solution to the polymer, and the subsequent evaporation of the volatile organic solvent. This method was used for the amine, together with a second method which consisted of dissolving the reagent in more organic solvent than the polymer could sorb, and adding this larger volume of solution to the carrier. Again a current of air was employed to evaporate off organic solvents before use. Distribution ratios, found by batch extraction techniques for material made up by both these methods, showed that lower, but constant values were, obtained when excess of solvent was used. Material made up with excess solvent was prepared from 20 g of Corvic R51/83 (100–150 mesh) and 1 g TIOA dissolved initially in 10 ml of toluene and 20 ml of chloroform, while for solvent deficient conditions the quantities of Corvic and TIOA were the same but only 8 ml of toluene and 12 ml of chloroform were used.

Since relatively small volumes of amine were employed for some of the work reported in this paper, it was felt that the reagent would be more uniformly distributed throughout the solid if excess of solvent was used, and therefore this method was finally employed, although evaporation of the organic solvent took longer. The usual technique was to dissolve the required amount of TIOA in 10 ml of toluene and 20 ml of chloroform, add the solution to 20 g Corvic, and finally evaporate off the solvents in an air stream, with continuous stirring.

For some experiments Corvic was prepared retaining the amine salt rather than the free amine. After the amine had been added to the Corvic as described above and the organic solvent evaporated off, the loaded powder was stirred with concentrated hydrochloric acid for 15 minutes. The solid phase was then freed from acid by filtration on a glass sinter and finally stored in a vacuum desiccator until required.

Experimental techniques

Batch extractions have already been used to obtain distribution data for a reversed phase system[14]. A number of prelimiary experiments indicated that batch extractions were also feasible with TIOA retained on Corvic, and therefore this method of obtaining distribution ratios was adopted. Usual quantities of the two phases employed for the extractions were 1 g of the solid and 20 ml of the aqueous phase. The temperature of the extraction could be thermostatically controlled if required.

Radiotracers were generally obtained by irradiating "Specpure" chemicals in a thermal neutron flux of 10^{12} n/sq.cm/sec but in a few cases radioactive materials were obtained from The Radiochemical Centre, Amersham, Bucks. The purity of the tracers was assessed before use by γ-ray spectroscopy and half-life determinations, and interference from impurities, avoided by careful choice of irradiation and decay times, by the use of γ-ray spectroscopy or by chemical separations.

Chromatographic separations were carried out at room temperatures on columns 18 cm long $\times$ 0.6 cm diameter. Radiotracers were used to follow the elution, and the effluent from the column was collected in fractions by means of an automatic fraction collector actuated either by a drop counter or by timing pulses. The radioactivity of individual fractions was measured with a well-type scintillation counter and the activity over the peak identified by means of a 3 in. $\times$ 3 in. NaI (Tl) crystal connected to a 512-channel analyser system.

RESULTS AND DISCUSSION

High molecular weight amines have been extensively used for the extraction of metal ions in liquid–liquid systems[15], and in many cases the extraction behaviour of the amine has been found to bear a resemblance to that of solid anion exchange resins.

A free base high molecular weight tertiary amine can extract acid from aqueous solution to form an amine salt, *e.g.*

$$(R_3N)_o + H^+ + X^- \rightleftharpoons (R_3NH^+X^-)_o \quad (1)$$

where R_3N represents a tertiary amine, and X^- the anion of a simple acid or complex metal acid, and the subscript o denotes species present in the non-aqueous phase. Metal extraction from an aqueous acid phase by this amine salt can therefore take place according to the equation:

$$n(R_3NH^+X^-)_o + Y^{n-} \rightleftharpoons ((R_3NH^+)_nY^{n-})_o + n\ X^- \quad (2)$$

where Y^{n-} is the anion containing the element to be extracted. Clearly equation (2) is similar to the general exchange equilibrium for an anion exchange resin:

$$aB^{-b} + bA_r^{-a} \rightleftharpoons aB_r^{-b} + bA^{-a}$$

where A and B are the exchanging anions and the subscript r denotes species present in the resin phase. Thus a column material for partition chromatography consisting of an amine retained on an inert carrier could be expected to bear at least a superficial resemblance to an anion exchange resin, although the dissimilar nature of the non-aqueous phases in the two cases will result in differences being apparent between the partition and resin systems. Further as the structure of the amine and the nature of the organic diluent are found to affect distribution ratios in liquid–liquid systems, it should be possible to prepare column materials with different exchange characteristics by varying the composition of the immobilised phase. However, as large quantities of organic diluent retained on the solid phase will increase the likelihood of the retained phase stripping from the carrier during chromatographic elution, or batch extraction, the quantity of organic diluent on the solid was kept as small as possible in these experiments by exposing the solid to a current of air before use. Previous experience with partition systems, involving a solid extractant (*e.g.* dithizone) retained on a carrier indicated that the presence of organic solvent on the carrier improved the rate of reaction between a solute dissolved in an aqueous phase and the immobilised extractant, but with the liquid amine retention of large quantities of solvent proved unnecessary.

The extraction of zinc from aqueous hydrochloric acid by TIOA

The extraction of many elements from aqueous solution by high molecular weight amines has been studied and summarised elsewhere[15] but it has been customary to use the amine dissolved in an organic diluent. For the experiments reported here organic diluents were evaporated off the column material before use, and in order to compare the shape of the extraction curve provided by TIOA retained on

Corvic, with that found when TIOA was dissolved in a diluent, zinc was extracted from aqueous hydrochloric acid by 0.1 % w/v TIOA in toluene and by 5 % w/w TIOA on Corvic. The results are presented in Fig. 1 and in both cases show curves with maxima, maximum extraction occurring at an aqueous acidity 3.5 *M* for the liquid–liquid system, and at rather lower acidity when the amine was retained on Corvic.

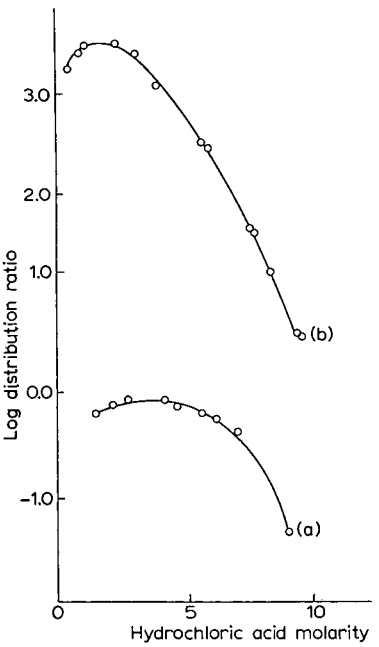

Fig. 1. Extraction of zinc from hydrochloric acid by (a) = 0.1 % w/v TIOA in toluene and (b) = 5 % w/w TIOA–Corvic.

The extraction of zinc from aqueous hydrochloric acid into a solution of methyl dioctylamine in trichlorethylene has already been reported and the results compared with those found for the extraction of zinc from hydrochloric acid by Dowex 1[16]. Experimental extraction curves showing maxima at aqueous acid strengths of 2.3 *M* were obtained, and possible mechanisms leading to this shape of extraction curve were discussed.

The wide range of distribution ratios that can be achieved with zinc in the TIOA–Corvic system (Fig. 1) permit the element to be either extracted by or eluted from a column of the material if the correct aqueous acidity is chosen while the actual value of the distribution ratio at any acidity may be altered by varying the reagent loading of the solid phase. Results for 2.5, 5, 10, 20 and 25 % w/w TIOA–Corvic provided a family of extraction curves of generally similar shape, but with the most heavily loaded material exhibiting strongest extraction of zinc.

The amine hydrochloride retained on Corvic gave similar extraction curves to the free base amine, and in addition showed rather better stability when the column material was kept for long periods, but over a period of two or three weeks, the normal time required to use a batch of TIOA–Corvic, there was insufficient deterioration

of the free base amine to warrant the longer preparation procedure required for the amine salt.

Extraction isotherm

As a concentration wave of solute passes down a chromatographic column during elution analysis, the solute loading of the solid phase at any particular point in the column will first increase, pass through a maximum and then decrease again. In order to reduce the distortion of peak shape caused by different concentrations of

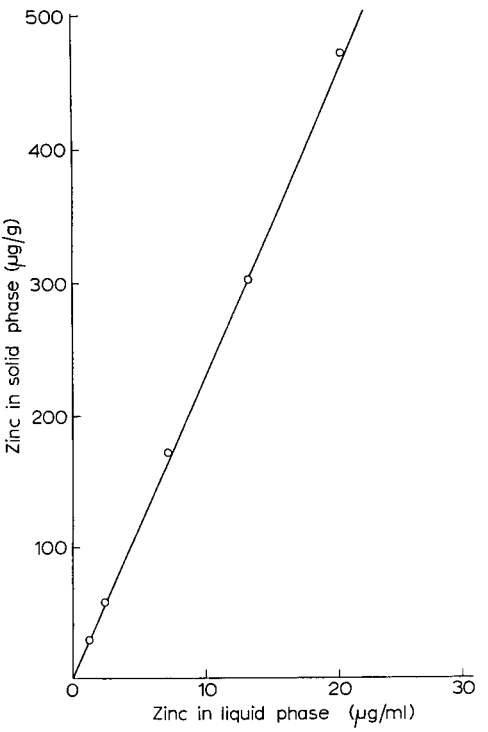

Fig. 2. Extraction isotherm for the extraction of zinc from hydrochloric acid by 5 % w/w TIOA–Corvic.

solute travelling at different velocities, the distribution ratio of solute should remain constant over a wide range of solute loadings. An extraction isotherm obtained for the extraction of zinc from aqueous hydrochloric acid by 5 % w/w TIOA–Corvic is given in Fig. 2 and shows that a good linear dependency is maintained between the concentration of zinc on the polymer and in the hydrochloric acid solution over a wide range of zinc concentrations. In all cases equilibrium was approached from one direction only, that of a metal-rich aqueous phase, and no equilibrations were carried out when net mass-transfer was from the solid to the liquid phase.

Extraction behaviour of individual elements

Schemes for chromatographic separation can be conveniently designed from batch extraction data if this provides a reliable basis for calculating separation fac-

tors, and extensive information is available for the extraction of many elements from hydrochloric acid into solutions of high-molecular weight amines[15]. For the specific case of TIOA the extraction of more than 60 elements has been recorded[17]. However, when amines have been employed for solvent extraction, they have usually been dissolved in organic diluent, whereas in these experiments as much solvent as possible was removed from the Corvic before use; therefore in view of the importance of solvent in amine extractions, batch extractions were carried out to examine the distribution of a number of elements between aqueous hydrochloric acid and 5 % w/w TIOA–Corvic. Results are presented in Fig. 3 in the manner originally used for anion exchange resins[18], and indicated a general pattern of extraction which is similar to that found for TIOA in solution, although the actual distribution ratios are dependent upon the TIOA loading of the solid phase.

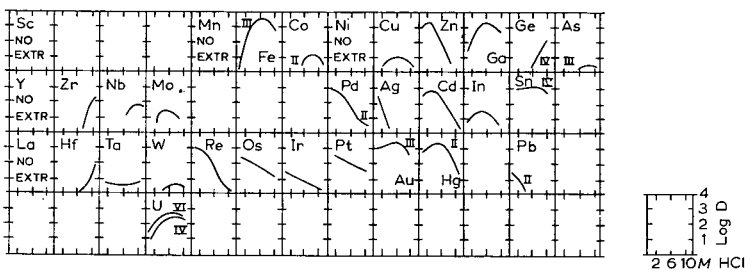

Fig. 3. Extraction of a number of elements from aqueous hydrochloric acid by TIOA–Corvic. D = distribution ratio.

The use of diagrams of the form of Fig. 3 for devising separation schemes has been discussed by KRAUS AND NELSON[18] and will not be reconsidered here. However, the most satisfactory separations will be achieved when there is a large difference in the relative mobilities of the migrating species and when extremely careful control of column conditions is unnecessary. For many applications the most convenient separations are those in which separate solutes are removed from the column with different eluting solutions. Unfortunately even when separation factors are large and distribution ratios can be controlled to give high or low extraction, satisfactory separations may still be impossible to achieve if peak shapes are poor, or if solutes exhibit serious tailing. Although linear isotherms obtained from batch experiments, suggest that good peaks should be obtained at equilibrium, slow mass transfer across the phase boundary might result in distorted peak shapes. Consequently columns of TIOA–Corvic were made up and used for a number of separations, suitable conditions being devised with the aid of data presented in Fig. 3.

Separations

The relatively simple separation of arsenic, germanium and indium was used to evaluate the behaviour of TIOA-Corvic during actual separation and the result of the elution is shown in Fig. 4. The effluent was collected in one-minute fractions, and the acid strengths that are given on the graphs refer to the acidity of the influent whilst a given effluent fraction was being collected. It can be seen that the peak shapes obtained by elution of the solutes from a TIOA–Corvic column are good and

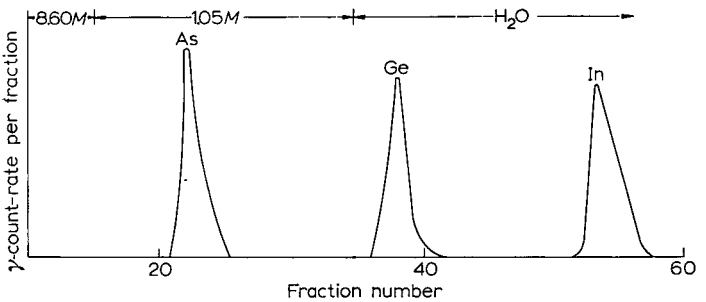

Fig. 4. Separation of As, Ge and In by elution with hydrochloric acid from columns of TIOA–Corvic.

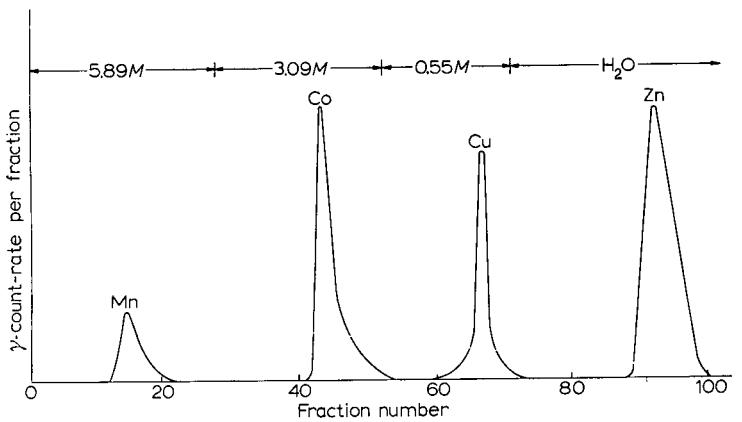

Fig. 5. Separation of Mn, Co, Cu and Zn by elution with hydrochloric acid from columns of TIOA–Corvic.

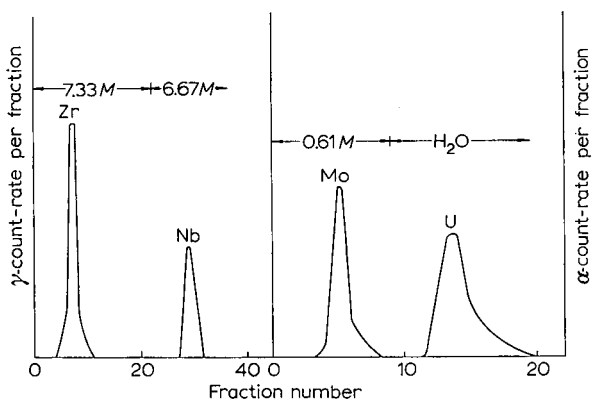

Fig. 6. Separation of Zr from Nb and Mo from U on columns of TIOA–Corvic. Nb eluted with 6.67 *N* sulphuric acid.

show relatively little tailing. The separation of manganese, cobalt, copper and zinc is shown in Fig. 5; as for the separation of the elements on a column of anion exchange resin by elution with hydrochloric acid[19] manganese is eluted first, in this case with 5.89 *M* hydrochloric acid, cobalt is eluted next with 3.09 *M* hydrochloric acid whilst zinc, which is most firmly extracted by TIOA–Corvic is removed from the column with water after the copper has been eluted with 0.55 *M* hydrochloric acid.

Further examples, typical of the separation that can be achieved with TIOA–Corvic are shown in Fig. 6 for two pairs of elements. Again sharp peaks are obtained (a characteristic already noted for other amine columns[9]) which gives good solute disengagement and permits TIOA–Corvic to be used for the satisfactory separation of a wide range of elements.

ACKNOWLEDGEMENT

The authors wish to thank Mr. D. R. A. CUFF for assistance with some of this work.

SUMMARY

A column material for partition chromatography has been prepared by retaining tri-iso-octylamine on an inert carrier. The behaviour of the material has been examined by batch extraction techniques, and a number of separations have been carried out.

REFERENCES

1 E. CERRAI, *Chromatog. Rev.*, 6 (1964) 129.
2 T. B. PIERCE AND P. F. PECK, *Nature*, 195 (1962) 597.
3 E. CERRAI AND C. TESTA, *J. Inorg. Nucl. Chem.*, 25 (1963) 1045.
4 J. W. WINCHESTER, *J. Chromatog.*, 10 (1963) 502.
5 J. BOSHOLM AND H. GROSSE-RUYKEN, *J. Prakt. Chem.*, 26 (1964) 83.
6 R. J. SOCHACKA AND S. SIEKIERSKI, *J. Chromatog.*, 16 (1964) 376.
7 C. TESTA, *J. Chromatog.*, 5 (1961) 236.
8 E. CERRAI AND C. TESTA, *J. Chromatog.*, 5 (1961) 442.
9 E. CERRAI AND C. TESTA, *J. Chromatog.*, 6 (1961) 443.
10 T. B. PIERCE AND P. F. PECK, *Analyst*, 89 (1964) 662.
11 J. MIKULSKI AND I. STRONSKI, *J. Chromatog.*, 17 (1965) 197.
12 T. B. PIERCE AND R. S. HOBBS, *J. Chromatog.*, 12 (1963) 74, 81.
13 C. F. COLEMAN, K. B. BROWN, J. G. MOORE, D. J. CROUSE, *Ind. Eng. Chem.*, 50 (1958) 1756.
14 T. B. PIERCE AND R. S. HOBBS, *U.K.A.E.A. Report R-4358* (1963).
15 F. L. MOORE, *Liquid-Liquid Extraction with High Molecular Weight Amines*, U.S.A.E.C. Nuclear Science Series NAS-NS 3101.
16 U. SCHINDEWOLF, *Z. Elektrochem.*, 62 (1958) 335.
17 T. ISHIMORI, H. M. SAMMOUR, K. KIMURA, H. MURAKAMI AND T. IZUMI, *J. At. Energy Soc., Japan*, 3 (1961) 698.
18 K. A. KRAUS AND F. NELSON, *Proc. Intern. Conf. Peaceful Uses At. Energy, Geneva, 1955*, Vol. 7, p. 113.
19 K. A. KRAUS AND F. NELSON, *J. Am. Chem. Soc.*, 75 (1953) 1460.

Short Communication

Thin-layer chromatography of nucleic acid bases, nucleosides, nucleotides and related compounds

I. Quantitative analysis by direct fluorometry*

Thin-layer chromatography (TLC) is now widely used for the separation of many hydrophilic substances (for reviews see refs. 1–3). For the quantitative analysis of compounds resolved on chromatoplates, the most practical procedure is the direct scanning of spots[1–3]. In this communication we would like to describe our preliminary results concerning direct fluorometry of nucleic acid bases, nucleosides and nucleotides.

Methods

TLC was carried out on purified cellulose MN 300 layers[4], with *n*-propanol–25 % ammonia–water (6:3:1, v/v) in the first, and isopropanol–saturated ammonium sulphate–water (2:79:19, v/v) in the second dimension, using Shandon multiplates-chromatotanks (Shandon Ltd., London) without chamber saturation[4]. This solvent systems has been well established in the chromatographic laboratory of Robapharm for the paper chromatography of nucleo-derivatives.

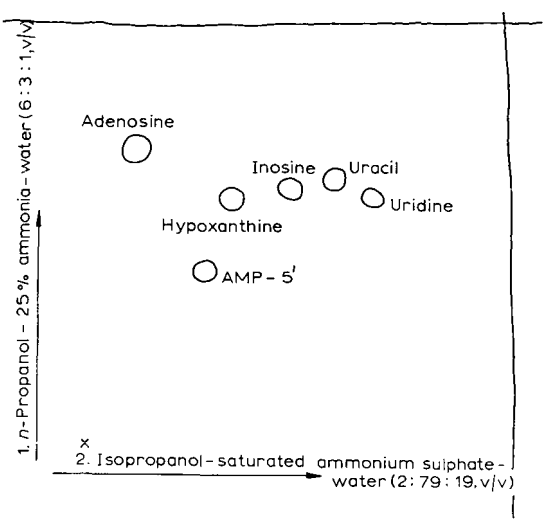

Fig. 1. Separation of some nucleo-derivatives on purified cellulose MN 300 layers[4]. First dimension: *n*-propanol–25 % ammonia–water (6:3:1, v/v), second dimension: isopropanol–saturated ammonium sulphate–water (2:79:19, v/v). The chromatography was carried out *without* chamber saturation.

* Preliminary communication.

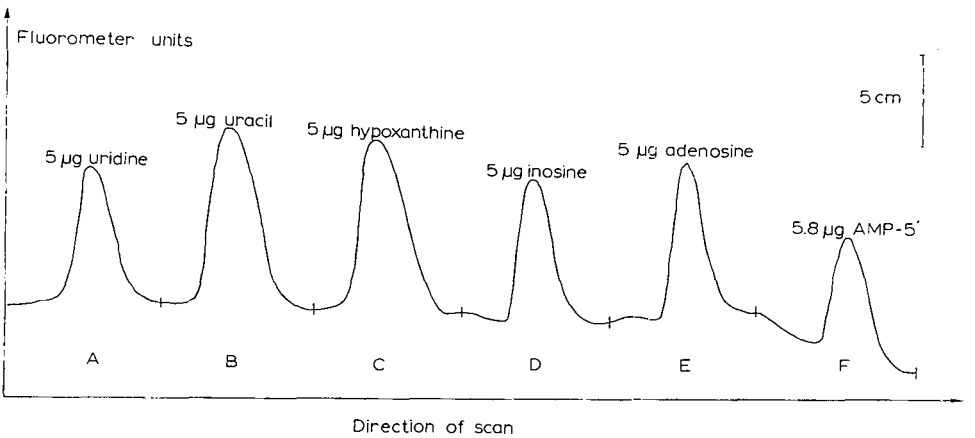

Fig. 2. Typical peaks produced by scanning the spots of adenosine, hypoxanthine, inosine, uracil, uridine (5 μg) and adenosine-5'-monophosphate (5.8 μg) using the CAMAG apparatus (lamp: 110–851; primary filter: 110–810; secondary filter: 110–816; aperture at door: 2 mm; sensitivity: 3 ×).

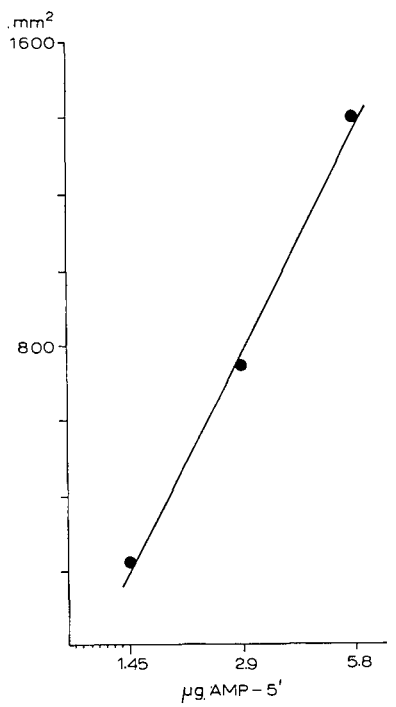

Fig. 3. Peak area plotted against log concentration of adenosine-5'-monophosphate.

Fluorometry was carried out using a Turner fluorometer model 111 fitted with a door for thin-layer plates (supplied by CAMAG*, Muttenz/BL, Switzerland). The experimental conditions are given in Fig. 2. The fluorometer units were recorded by a Varicord model 43 (Photovolt Co., New York).

Results

Fig. 1 shows the separation of some compounds in our chromatographic system. This TLC method has been recently developed in our laboratory and makes the characterisation of about sixty purine and pyrimidine derivatives possible. The procedure will be described in a further publication[4].

Typical curves produced from scanning spots are shown in Fig. 2. Linearity between the area under the peaks and the amount of material applied to the plate exists between 1–5 μg for adenosine-5'-monophosphate (Fig. 3).

The application of this method to the analysis of complex mixtures and full description of the procedure will be given in a subsequent paper**.

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1 E. STAHL (Editor), *Thin-layer Chromatography*, Springer Verlag, Berlin, Göttingen, Heidelberg, 1965.
2 K. RANDERATH, *Dünnschichtchromatographie*, Verlag Chemie, Weinheim, 1965.
3 G. PATAKI, *Dünnschichtchromatographie in der Aminosäure und Peptidchemie*, Walter de Gruyter, Berlin, 1966.
4 G. PATAKI, to be published.

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** *Note added in proof*

Since completion of this paper we have found that DNS-, DNP-, and, with some limitations, PTH-derivatives of amino acids can also be quantitatively estimated by direct fluorometry.

Notes

Displacement effect of water in gas chromatography

It has been found that when a mixture of water and organic components passes through a gas chromatographic column, the retention volumes of substances more strongly retained than water are reduced by comparison with their values in the absence of water. This effect is shown in the three superimposed chromatograms illustrated in Fig. 1 obtained with a hydrogen flame ionisation detector. The chromatogram of water alone was recorded at a much higher instrument sensitivity than the other two. Operating conditions, together with retention data and peak dimensions,

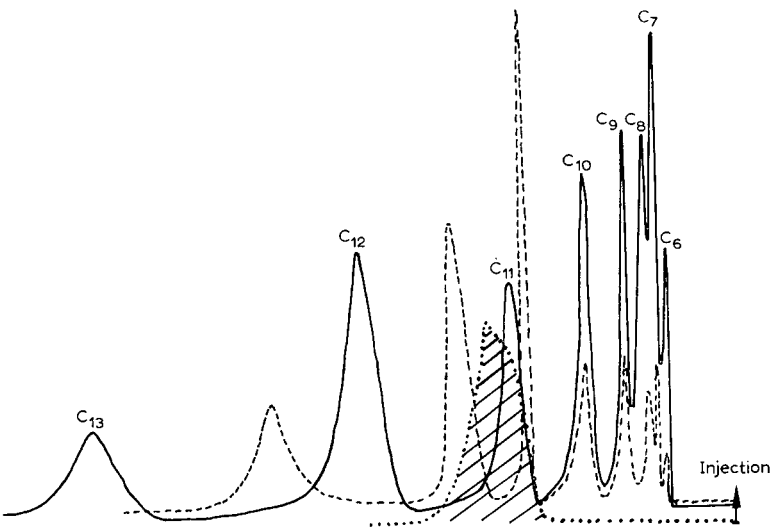

Fig. 1. Chromatograms of *n*-alkanes on polyethylene glycol in the presence, and the absence, of water. (——) *n*-alkanes alone; (– – – –) *n*-alkanes plus water; (· · · ·) water alone (water peak hatched).

are given in Table I. A similar effect was observed when an apolar silicone oil column was used instead of one containing the polar glycol. Results for the silicone column are given in Table II.

The principal features shown by these chromatograms are:

(i) Substances which normally elute before the water "front" have identical retention times in the presence and absence of water.

(ii) A substance having an almost identical retention to water emerges, in its presence, just ahead of the water "front" and, under these conditions, the peak is very much sharper.

(iii) Substances normally eluting after the water "front" are eluted more rapidly in the presence of water, although they do not overtake the water "front".

TABLE I

RETENTION DISTANCES OF *n*-ALKANES - POLAR COLUMN

Column: 6′, 10% polyethylene glycol 4000 on 60–100 mesh celite; temperature: 101°; carrier gas flow rate: 75 c.c.s. min^{-1} nitrogen at column exit; chart speed: 64″ h^{-1}; samples and size: (a) 0.2 μl n-C_6–n-C_{13} alkane blend, (b) as (a) with 7 μl water added.

n-Alkane	*Retention distances (mm)*		*Peak width at half height (mm)*	
	(*a*)	(*b*)	(*a*)	(*b*)
Hexane	21	21	—	—
Heptane	24	24	—	—
Octane	27	27	—	—
Nonane	33	33	2.0	2.0
Decane	43	43	3.8	3.0
Undecane	65	58	5.6	1.9
Dodecane	107	77	10.0	6.3
Tridecane	184	110	17.0	11.0

Water front appears at 57 mm.

(iv) The presence of a water peak is revealed by a hydrogen flame ionisation detector despite the generally accepted belief that this detector does not respond to water[1].

Discussion

As the retention times of substances which travel through a column more rapidly than water are unaffected, it follows that the influence of water is effected in the column, not at the point of injection. The unexpected sharpness of the *n*-undecane peak (Fig. 1 and Table I) which emerges with the water "front" can be attributed to a displacement effect of the water. This displacement may arise from a change in stationary phase character. In the vicinity of a water peak, as it passes through a column, the stationary phase is considered to be a mixture of the particular phase used and water. Consequently, partition coefficients, particularly of hydrophobic

TABLE II

RETENTION DISTANCES OF *n*-ALKANES - APOLAR COLUMN

Column: 6′ 10% silicone oil on 60–100 mesh celite; temperature: 124°; carrier gas flow rate: 70 c.c.s. min^{-1} nitrogen at column exit; chart speed: 64″ h^{-1}; samples and sizes: (a) 0.2 μl n-C_6–n-C_9 alkane blend, (b) as (a) with 7 μl water added.

n-Alkane	*Retention distances (mm)*	
	a	*b*
Hexane	74	65
Heptane	108	100
Octane	169	163
Nonane	286	283

Water front appears at 42 mm.

materials, will be decreased in this region of the column. In the example cited, *n*-undecane is probably virtually insoluble in the polyethylene glycol–water mixture postulated and therefore passes through the column as a very sharp "slug" just ahead of the water "front".

The unexpectedly rapid elution of substances retained more strongly than *n*-undecane is interpreted as reflecting that the water and the substance proceed together through the column for a period of time. The time during which water and the solute are proceeding together through the column is roughly inversely proportional to the difference in their retention times. During this period the partition coefficient of the solute is lower than normal and the component therefore moves more rapidly than normal, though not as fast as the water. The solute eventually falls behind the water peak region and for the remainder of its passage through the column partitions normally into the stationary phase. These observations show that identifications based on retention data obtained in the absence of water are suspect when applied to the analysis of aqueous solutions.

Conclusions

It is concluded that the gas chromatographic analysis of dilute aqueous solutions of hydrocarbons requires either the selection of a column which retains water after all of the component of interest have passed (a very polar column) or the calibration of the column with aqueous solutions of pure components prior to the running of unknown samples.

If the explanation of the origin of the displacement effect of water is correct, it is expected that the retentions of small amounts of substances eluting close to a major component will also be changed; the magnitude of the change would depend on both the quantity and the chemical nature of the major component. Thus, in trace analysis, difficulties in identification based on retention parameters obtained with pure substances can be anticipated. Very recently an effect of this type has been reported in the separation of esters of long chain fatty acids[2].

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1 A. B. LITTLEWOOD, *Gas Chromatography*, Academic Press, London, 1962, p. 294.
2 R. G. ACKMAN, *J. Gas Chromatog.*, 3 (1965) 15.

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Eine halbautomatische Mischbatterie zur Herstellung von Dichtegradienten

Kontinuierliche Dichtegradienten innerhalb bestimmter Volumenbereiche finden in Verbindung mit der Fraktionierung von Zellpartikeln[1] und Substanzgemischen unterschiedlicher Molekülgrösse[2] einen immer ausgedehnteren Anwendungsbereich. Die Forderungen, die bei der Herstellung der Dichtegradienten erhoben werden, sind: (1) eine schnelle und verlässliche Gradientenmischung; und (2) eine absolute Reproduzierbarkeit der linearen Dichteänderungen.

Es sind eine Reihe von Konstruktionen publiziert worden, die darauf beruhen, dass entweder unter Ausnutzung des hydrostatischen Eigendruckes des Lösungsmittels oder mit mechanischem Druck geeignete Lösungen unterschiedlicher Dichte gemischt und der Flüssigkeitsspiegel des Gradientengefässes manuell nachkorrigiert wird[3,4].

Die im Handel befindlichen Gradientenmischer arbeiten nach dem gleichen Prinzip und lassen trotz grossen technischen Aufwandes entweder jede Variationsmöglichkeit zur Programmierung der Gradienten oder die Nivellierung des Flüssigkeitsspiegels im Bezug zum Auslauf vermissen.

Die vorgeschlagene Bauweise der halbautomatischen Mischbatterie ist aus diesem Grunde so gestaltet, dass mechanisch angetriebene und mit Programmierscheiben gesteuerte Stempel die Lösungen unterschiedlicher Dichte in einem Mischteil mit meanderförmig eingeschnittenen Kanälchen treiben und deren Auslauföffnungen

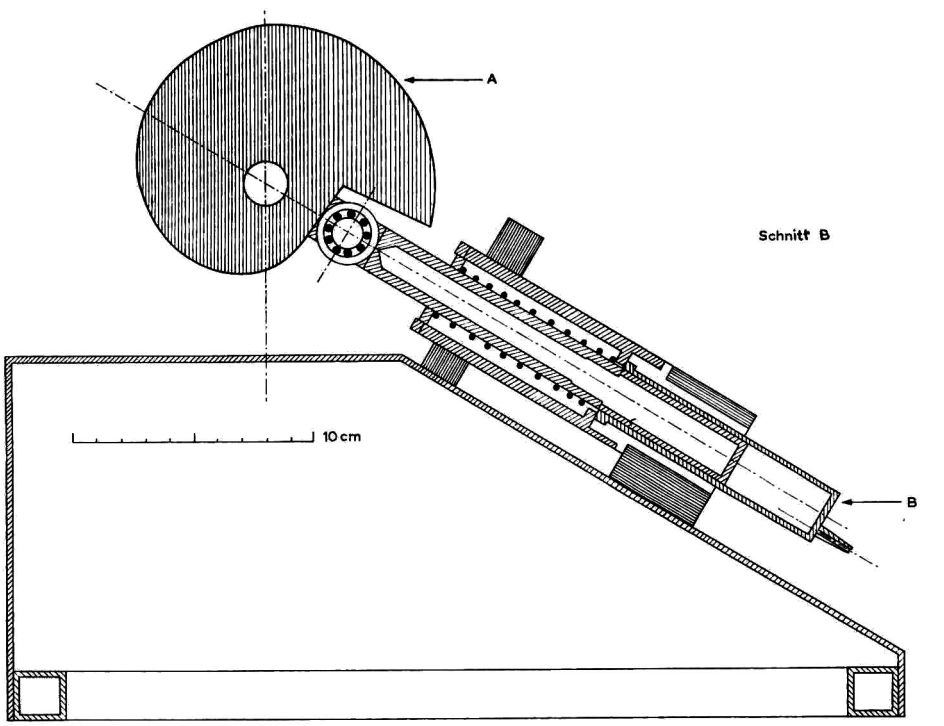
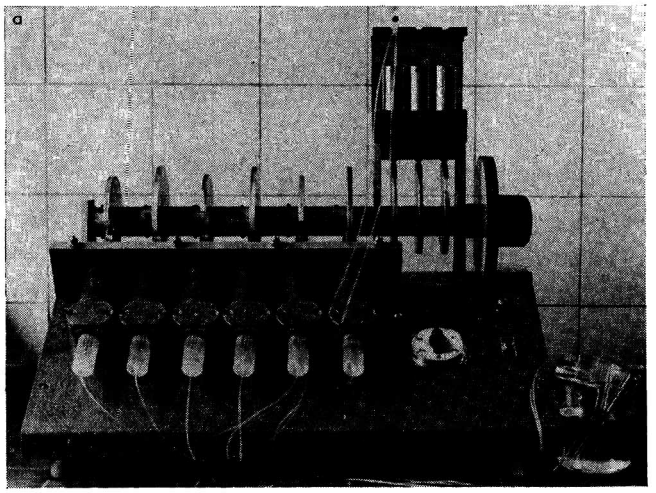


Fig. 1. Querschnittszeichnung zur Demonstration der Anwendung von Programmierscheibe und Spritze. A = Programmierscheibe; B = Nylon-Spritze.



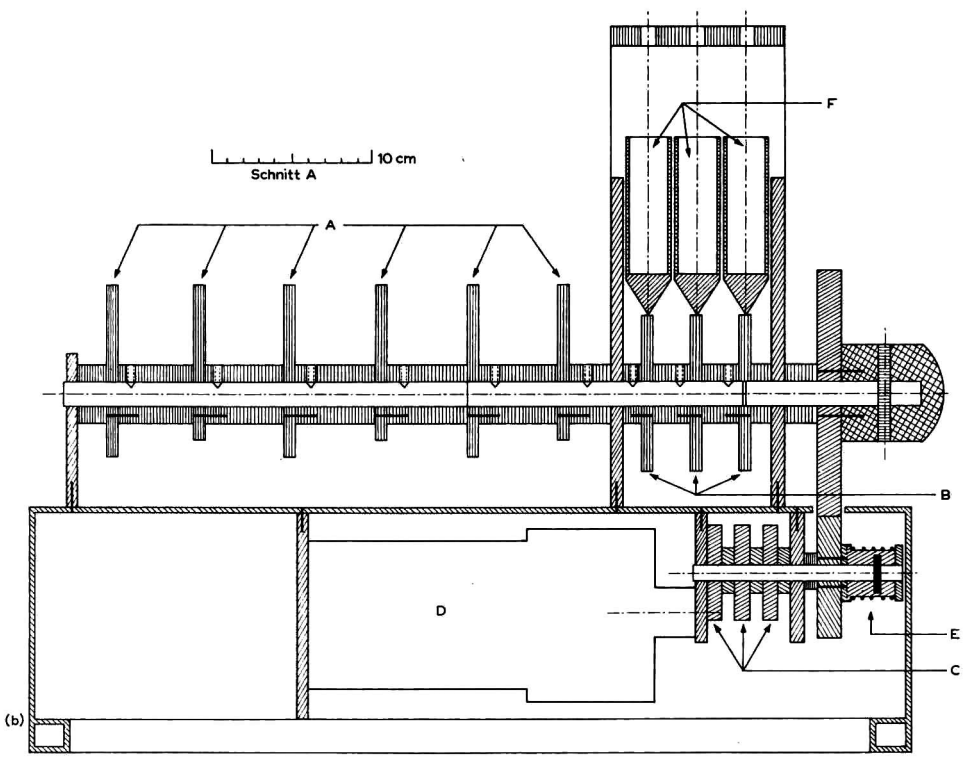


Fig. 2. (a) Vorderansicht der Mischbatterie; (b) Schnitt durch die Längsachse des Gerätes. A = Programmierscheiben in Form positiver und negativer logarithmischer Spiralen; B = Programmierscheiben mit linearem Abfall; C = Zwischengetriebe; D = Motor; E = Zahnkupplung zur Rückstellung; F = Gefässhalterungen.

durch eine gleichfalls gesteuerte Gefässhalterung so nivelliert werden, dass sie sich immer direkt über dem Flüssigkeitsspiegel befinden. Der gesamte Aufbau enthält die Mischvorrichtungen für gleichzeitig drei Gradientengefässe und gestattet deren reproduzierbare Herstellung innerhalb von 30 Min. Dadurch ist gewährleistet, dass sich vergleichbare kontinuierliche Gradienten herstellen lassen, die durch Änderung der Ausgangsdichten der zu mischenden Lösungen variiert werden können und jederzeit zu reproduzieren sind. Die gleichzeitige Anpassung der Auslauföffnungen im Bezug zum Flüssigkeitsspiegel mit derselben Antriebseinheit schaltet jeden Fehler aus, der durch eine manuelle Korrektur auftreten muss.

Beschreibung und Funktion der Bauteile

Zur Aufnahme der zu mischenden Lösungen dienen 6 Nylonspritzen der Fa. Delvenne (Mannheim) mit einem Fassungsvermögen von 20 ml, die mit einer Neigung von ungefähr 30° auf einem Pult montiert sind. Die Stempel werden in Kunststoffhalterungen geführt, die gleichzeitig die Rückstellfedern aufnehmen. Der Vorschub der Stempel wird durch Programmierscheiben gewährleistet, die auf einer gemeinsamen Achse befestigt sind und paarweise je einen Stempel mit abnehmender (konzentrierte Lösung) und zunehmender (verdünnte Lösung) Vorschubgeschwindigkeit antreiben. Die Scheiben wurden aus 6 mm starken Aluminium hergestellt und zur Steuerung des Vorschubes von insgesamt 40 mm bei einem Umlauf in Form positiver und negativer logarithmischer Spiralen geschnitten. Die Stempelhalterungen sind mit kugelgelagerten Rollen versehen, so dass die Übertragung der sich stetig ändernden Vorschubsgeschwindigkeit einwandfrei gewährleistet ist (Fig. 1). Die Programmscheiben sind auf einer gemeinsamen zentralen und ebenfalls kugelgelagerten Achse montiert, die durch einen Motor, Typ SK, der Fa. Siemens, Berlin (220 V, 3 U/Min.)

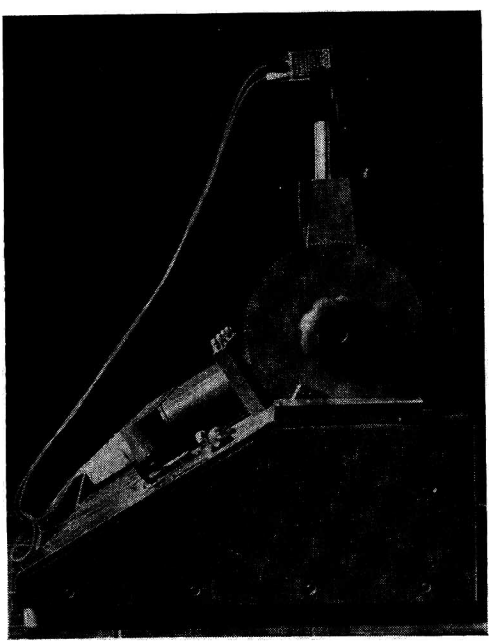


Fig. 3. Seitenansicht der Mischbatterie mit angeschlossenem Auslauf- und Mischteil.

und ein Zwischengetriebe von 4 U/Min. auf 1 U/Std. angetrieben wird. Die Rückstellung zur Gewinnung der Ausgangsposition erfolgt über eine Zahnkuppelung (Figs. 2a und 2b).

Zur Nivellierung des Flüssigkeitsspiegels im Zentrifugengefäss im Bezug zur Auslauföffnung wird die Kunststoffhalterung der Gefässe bei gleitender Führung durch ihr Eigengewicht gegen die Programmierscheibe mit linearem Abfall des Durchmessers gedrückt und entsprechend des Flüssigkeitszuflusses so gesenkt, dass sich die Auslauföffnung des Mischteiles immer über dem Flüssigkeitsspiegel befindet. Die Programmierscheiben zum Nivellieren der Gefässhalterungen sind auf die gleiche zentrale Achse montiert. Die Mischteile sind beweglich auf Stifte gelagert und enthalten meanderförmig eingefräste Kanäle, die die Mischung der spezifisch schwereren und leichteren Lösung gewährleisten. Ihr Eigengewicht drückt deren Auslauföffnung gegen die Wandung des Zentrifugenröhrchens, wodurch die Bildung von Flüssigkeitswirbeln vermieden werden soll (Fig. 3). Durch Anschluss einer Kontaktuhr kann nach vorgewählter Zeit der Antriebsmotor abgeschaltet werden.

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1 D. A. YPHANTIS UND D. F. WANGH, *J. Phys. Chem.*, 60 (1956) 630.
2 R. G. MARTIN UND B. N. AMES, *J. Biol. Chem.*, 236 (1961) 1372.
3 D. F. H. WALLACH UND V. B. KAMAT, *Proc. Natl. Acad. Sci. U.S.*, 52 (1964) 721.
4 S. SAMUELS, *Anal. Biochem.*, 7 (1964) 164.

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* Unter technischer Mitarbeit von H. FEINEIS.

Preparation of deproteinised tissue extracts for chromatography and assay of compounds related to glutathione

When extracting tissue for assay of glutathione and other thiols, the chief problem is to prevent losses either by oxidation of the thiol to disulphide or by binding to protein in thiol–disulphide exchanges. The extractants recommended for high recovery are salt-saturated 4 % (w/v) metaphosphoric acid or 3 % (w/v) sulphosalicylic acid[1]. Such extracts are not immediately suitable for chromatography because of the content of salts and involatile acids. Also direct colorimetric assays of thiol in extracts from brain or slightly fatty livers are ruined by opacity introduced by dispersed lipid material.

During studies on the metabolism of some foreign compounds in the rat, I wished to measure the effect of doses of these compounds on liver GSH and also to separate derivatives of GSH which had been formed *in vivo*. Extraction of components from tissues with ethanol is quite common and this paper describes the modifications of the technique needed to obtain good recoveries of GSH and its derivatives

in a clear solution sufficiently free of salt for high-resolution chromatography without further processing.

The extracting solution is prepared fresh by mixing 3 vol. ice-cold ethanol with 1 vol. ice-cold KH_2PO_4 (24 m*M*; pH 5.5) containing EDTA (0.1 m*M*). 2 g liver is homogenised in 25 ml of mixture with a high-speed smooth pestle homogeniser[2] and the homogenate centrifuged at 5,000 *g* for 10 min at 0°. The phosphate content of the clear pale yellow extract is less than the nominal 6 m*M* since crystallisation occurs from the initially supersaturated solution and the crystals are removed during the centrifugation.

At least 30 ml of extract can be applied without further processing to a Dowex column (15 × 1.3 cm) in the carbonate form for separation of basic, neutral and acidic amino acids as described by GAITONDE[3]. Alternatively the extract can be quickly concentrated to one tenth volume in a rotary evaporator at 35°: the opalescent concentrate is easily applied to chromatography paper and the resolution of the amino-acid constituents is not affected by other material in the extract.

The GSH content of liver extracted as described, and determined according to BEUTLER[4] *et al.*, is 85 % of that determined after deproteinisation with 3 % (w/v) sulphosalicylic acid. Recovery is decreased by the following:

(a) Alteration of the extraction pH outside the range 5–7. Since atmospheric oxidation in subsequent processing would be favoured by higher pHs, pH 5.5 was chosen as optimum.

(b) Increase of alcohol:buffer ratio above 3:1. Decreasing the ratio also proved unsatisfactory because it caused incomplete deproteinisation. Use of acetone–buffer in the proportion 2:1 gave 100 % recovery but the suitability of the extract for chromatography has not been assessed.

(c) Decrease of the extractant vol:tissue wt. ratio below 10:1. A similar effect was observed when 3 % sulphosalicylic acid was used as extractant.

There is no specific assay for S-derivatives of GSH. Standards were added to liver extract before centrifugation. The deproteinised extract was concentrated and subjected to paper chromatography. Ninhydrin-positive spots were cut out and eluted with ethanol (60 % v/v in water) and compared spectrophotometrically (565 mμ) with standards subjected only to chromatography. Recoveries ranged from 83–95 %.

Although of particular value for glutathione the method seems generally useful, particularly in view of the large volume of extract which can be applied to ion-exchange columns.

I am indebted to Miss H. MACKENZIE for skilled technical assistance.

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1 J. W. PATTERSON AND A. LAZAROW, in D. GLICK (Editor), *Methods of Biochemical Analysis*, Vol. 2, Interscience, New York, 1955, p. 259.
2 W. N. ALDRIDGE, R. C. EMERY AND B. W. STREET, *Biochem. J.*, 77 (1960) 326.
3 M. K. GAITONDE, *J. Neurochem.*, 8 (1961) 234.
4 E. BEUTLER, O. DURON AND B. M. KELLY, *J. Lab. Clin. Med.*, 61 (1963) 882.

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Thin-layer chromatography of Captan and Captax

Captan (N-trichloromethyl-thio-4-cyclohexene-1,2-dicarboximide) and Captax (2-mercaptobenzothiazole) have both extensive application. The former being utilized primarily as a general fungicide for the treatment of a wide variety of foliar soil and seed-borne diseases[1–4], and the latter as a fungicide[5–7] and accelerator for rubber vulcanization[8,9].

The analytical determinations of both have been mainly carried out by colorimetric techniques, *viz.* for Captan: procedures utilizing resorcinol[10–12], pyridine-alkali[13,14], and pyridine-tetraethylammonium hydroxide[15]; for Captax: bismuth nitrate[16] and selenious-sulfuric acid[17]. Chromatographic analyses, however, have been limited to both paper[18,19] and silicic acid column[20] procedures for Captan and paper techniques for Captax[21,22].

Our studies with Captan and Captax involve a variety of metabolic and feeding studies. This initial investigation was to determine the feasibility of thin-layer chromatography with its inherent advantages of speed, enhanced sensitivity and high resolving power for both residue analysis and elaboration of purity of standard and commercial samples of these compounds. Concomitantly it was of interest to compare two quantitation techniques, *viz.* densitometry and that of PURDY AND TRUTER[23] as to their efficacy with thin-layer chromatography.

Experimental

Detecting reagents. For Captan: 25 % resorcinol in glacial acetic acid[24]. For Captax: (1) Cupric chloride reagent[25]: solution I—aqueous solution of 3% cupric chloride, 6 % ammonium chloride and 6 % ammonium hydroxide; solution II—20 % aqueous hydroxylamine. Solutions I and II are mixed (1:2) prior to use. (2) Sulfuric acid–butanol reagent: (1:1) solution of conc. sulfuric acid and *n*-butanol, prepared daily.

Solvent systems. For Captan: chloroform; for Captax: isopropanol–ammonium hydroxide–carbon tetrachloride (50:10:40)[26].

Preparation of chromatoplates. The chromatoplates* utilized were 8 × 8 in. plates coated to a thickness of 250 μ with Camag DF-5 silica gel (for Captax) and TLC-7G Mallinckrodt silica gel for Captan determinations respectively. The plates were washed by ascending chromatography with chloroform–methanol (1:1), then activated in an oven at 75° for 30 min. Acetone solutions of Captax and chloroform solutions of Captan were spotted, chromatographed by the standard ascending technique, the developed plates sprayed with the indicated detector, and dried at room temperature in a hood for color development for Captax, and dried in an oven at 140° for five minutes for Captan.

Preparation of channel chromatoplates. The method consisted of dividing the plate into strips or channels a few mm wide, extending from several mm below the point of origin to the line marking the solvent front. One μl of a chloroform solution of Captan** (0.5, 1, 2, 4, 6, 8, 10, 16 and 20 μg/μl) was applied at the origin within each channel, developed by the ascending method and made visible by the indicated

* Pre-coated plates were obtained from Analtech, Wilmington, Del., U.S.A.

** Analytical standard obtained from California Chemical Co., Ortho Division, Richmond, Calif., U.S.A.

detecting reagent. Captax* standard solution in acetone (2, 4, 6, 8, 10, 16 and 20 $\mu g/\mu l$) was applied and treated in analogous manner as that described above.

Extraction of Captan from mouse tissue. An adult mouse (*ca.* 20 g) after sacrifice was homogenized in a Waring blender with 50 ml hexane and 1 ml of 5 *N* hydrochloric acid and spiked with Captan. The homogenate was filtered through 1 × 15 cm column of 1:1 (v/v) celite–sodium sulfate and then refiltered with an additional 20 ml hexane. The filtrate was then partitioned with 2 × 50 ml hexane–acetonitrile (2:1). The hexane–acetonitrile extract was backwashed with 10 ml of hexane and then concentrated to dryness under vacuum utilizing a Rinco evaporator. The final dilutions were made in chloroform.

Results and discussion

The resorcinol reagent detected Captan at R_F of 0.35 as a yellow spot (bright yellow fluorescence under U.V. of 3660 Å) with the lower limit of detection being 1 and 0.5 μg, respectively.

The cupric chloride detecting reagent revealed Captax at R_F 0.25 as a pale yellow spot (bright orange fluorescent spot under U.V. of 3660 Å) on a white background, the lower limit of detection being 2 μg. The sulfuric acid–butanol reagent also detected Captax as a pale yellow spot on a white background but the lowest detectable limit was 4 μg.

Densitometry and the technique of PURDY AND TRUTER[23] (plot of the log sample weight *vs.* the square root of the spot area) were evaluated for the quantitation of both Captax and Captan on channel chromatoplates.

For Captax. Fig. 1 is a plot of the log sample weight *vs.* maximum optical density obtained by utilizing both the cupric chloride** (Curve I) and sulfuric acid–butanol (Curve II) reagents. Measurements were accomplished at 420 λ utilizing a Photovolt Densitometer.***

Linearity from 2–16 μg is obtained utilizing the cupric chloride reagent (Curve I).

In contrast, the sulfuric acid–butanol reagent produced erratic results over the range from 4–20 μg.

Fig. 2 depicts the results of triplicate runs utilizing the measurement technique of PURDY AND TRUTER and is a plot of the log sample weight *vs.* the square root of the spot areas (width of channel × height of streak) determined both as a yellow streak after spraying with the cupric chloride reagent and as a quench at 2537 Å on a fluorescent background. The linearity obtained was, as in the densimetric procedure above, 2–16 μg.

For Captan. Fig. 3 is a plot of the log sample weight *vs.* maximum optical density obtained in an analogous manner to that described above for Captax. Linearity from 1–10 μg was obtained; however, a wide deviation was found to occur at concentrations from 14–20 μg. Fig. 4 depicts the results utilizing the technique of PURDY AND TRUTER. It can be seen that linearity occurs with greater precision and is extended to 1–20 μg

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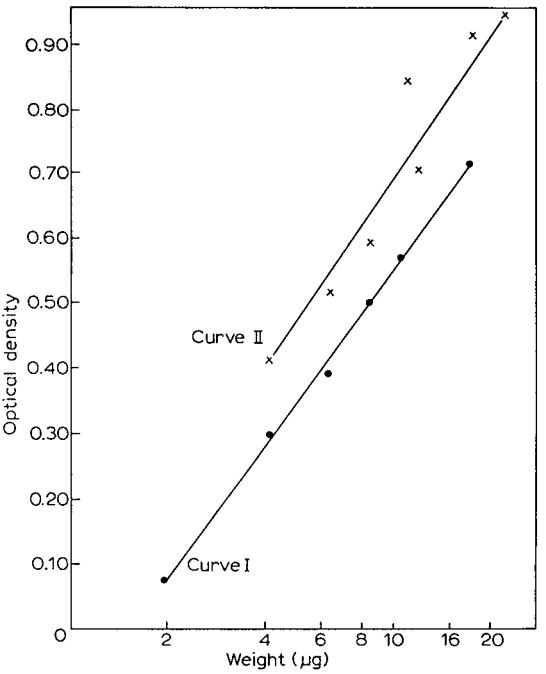
* Obtained from R. T. Vanderbilt Co., New York, N.Y., U.S.A.

** The utility of the cupric chloride reagent was limited to approx. 20–30 min after initial spraying due to the onset of yellowing of the chromatograms, and dictates the speed in which the densitometric measurements must be obtained.

*** Model 52-C with a motorized TLC stage, Model 520-A photomultiplier and a linear/log varicord recorder, Model 43, all obtained from Photovolt Corp., New York, N.Y., U.S.A.



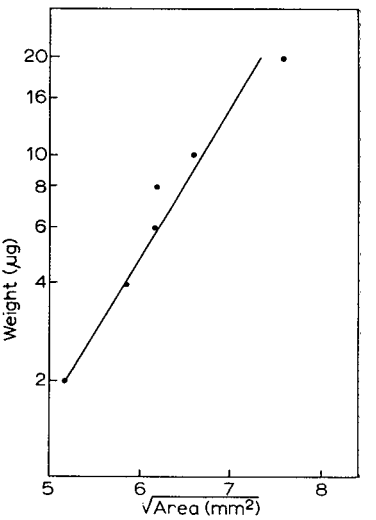
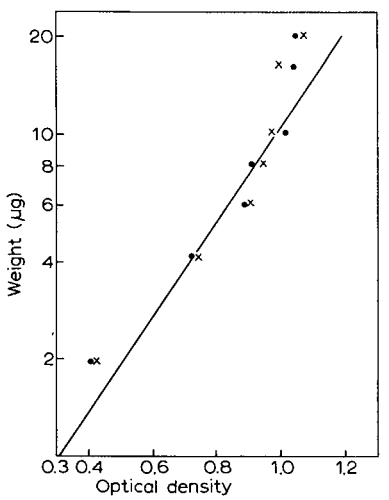


Fig. 1. Quantitation of Captax by densitometry.

Fig. 2. Quantitation of Captax by channel technique.



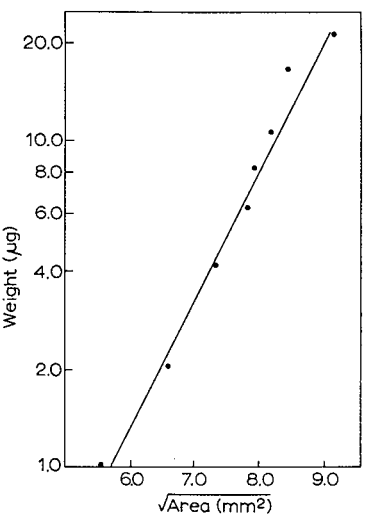


Fig. 3. Quantitation of Captan by densitometry.

Fig. 4. Quantitation of Captan by channel technique.

TABLE I

RECOVERY OF CAPTAN FROM MOUSE TISSUE

Replicate no.	Extract No. 1*			Extract No. 2*		
	Captan added (μg/ml)	Captan recovered (μg/ml)	Recovery (%)	Captan added (μg/ml)	Captan recovered (μg/ml)	Recovery (%)
1	40	38	95	40	41	102
2	40	48	120	40	41	102
3	40	40	100	40	45	110

* Extracts 1 and 2 were spiked with 200 and 800 μg respectively. The final dilutions in chloroform were 5 and 20 ml respectively, resulting in a final Captan conc. of 40 μg/ml for each solution. Fifty μl of sample was applied per spot and compared with Captan standards.

utilizing the latter method of quantitation which is dependent only upon spot area and not color density.

Table I depicts the recovery of Captan from mouse tissue extracts following addition of 200 and 800 μg amounts and demonstrates the utility both of the extraction procedure and the channel technique for the quantitation of Captan.

Conclusions

The quantitative determination of Captax and Captan on channel chromatoplates, both by densitometry and measurement of spot area (technique of PURDY AND TRUTER), was found to be feasible. The primary advantage of the latter procedure is both the speed and the inexpensive nature of the analysis.

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1 A. R. KITTLESON, *Science*, 115 (1952) 84.
2 P. E. HOCHSTEIN AND C. E. COX, *Am. J. Botany*, 43 (1956) 437.
3 D. S. DE ZEEUW, G. E. GUYER AND J. A. ANDERSON, *Plant Disease Reptr.*, 40 (1956) 727; *C.A.*, 50 (1956) 17291.
4 W. D. MCCLELLAN, *Plant Disease Reptr., Suppl.*, 192 (1950) 120; *C.A.*, 44 (1950) 9103.
5 D. WEIGHT AND D. A. L. SEILER, *Brit. Pat.*, 873, 602, appl. Dec. 19, (1957); *C.A.*, 56 (1962) 2454.
6 S. PAVELKA, *Textil*, 14 (1959) 225; *C.A.*, 58 (1963) 2536.
7 A. A. SOMMERVILLE AND C. E. BRADLEY, JR., *German Pat.*, 1,037,754 Aug. 28 (1958); *C.A.*, 55 (1961) 8048.
8 V. N. KARTSEV, *U.S.S.R. Pat.*, 130,667, Aug. 5 (1960); *C.A.*, 55 (1960) 5010.
9 G. FROMANDI AND S. REISSINGER, *Kautschuk Gummi*, 13 (1960) 255; *C.A.*, 55 (1961) 1045.
10 A. R. KITTLESON, *Anal. Chem.*, 24 (1952) 1173.
11 A. R. KITTLESON, *Anal. Chem.*, 27 (1955) 2015.
12 J. WAGNER, V. WALLACE AND J. M. LAWRENCE, *J. Agr. Food Chem.*, 4 (1956) 1035.
13 H. P. BURCHFIELD AND P. H. SCHULDT, *J. Agr. Food Chem.*, 6 (1958) 106.
14 H. MELTZER, *Nachrbl. Deut. Pflanzenschutzdienst (Berlin)*, 4 (1960) 193; *C.A.*, 55 (1961) 3577.

15 H. P. BURCHFIELD AND J. SCHECHTMAN, *Contrib. Boyce Thompson Inst.*, 19 (1958) 411; *C.A.*, 58 (1959) 333.
16 A. POPOV AND V. GYDEVA, *Compt. Rend. Acad. Bulgare Sci.*, 12 (1959) 419; *C.A.*, 55 (1961) 5003.
17 V. E. LEVINE AND M. NACHMAN, *J. Forensic Med.*, 10 (1963) 65.
18 L. C. MITCHELL, *J. Assoc. Offic. Agr. Chemists*, 39 (1956) 484.
19 L. C. MITCHELL, *J. Assoc. Ofic. Agr. Chemists*, 41 (1958) 781.
20 J. P. BARRETTE AND R. PAYFER, *J. Assoc. Offic. Agr. Chemists*, 44 (1961) 606.
21 M. STEPIEN AND R. GACZYNSKI, *Chem. Anal. (Warsaw)*, 6 (1961) 1045; *C.A.*, 57 (1962) 998.
22 S. MURAKAMI AND J. TSURUGI, *Nippon Gomu Kyokaishi*, 34 (1961) 253; *C.A.*, 56 (1962) 2542.
23 S. J. PURDY AND E. V. TRUTER, *Chem. Ind. (London)*, (1962) 506.
24 J. N. OSPENSON, D. E. PACK AND G. K. KOHN, *Analytical Methods, for Pesticides, Plant Growth Regulators and Food Additives*, Vol. III, Academic Press, New York, 1964, p. 7.
25 F. FEIGL, *Spot Tests in Organic Analysis*, Elsevier, Amsterdam, 1960, p. 246.
26 R. G. ACZYNSKI AND M. STEPIEN, *Przemysl Chem.*, 38 (1959) 571; *C.A.*, 54 (1960) 25934.

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A new spray reagent replacing sulphuric acid in thin-layer chromatography

Concentrated sulphuric acid is one of the most widely used general spray reagents in thin-layer chromatography. In addition to many advantages this reagent presents well known difficulties. The irritating action of its vapours on mucous membranes and their corrosive effects makes it impossible to use it outside well ventilated hoods. The use of special equipment such as chambers for spraying and visualisation of chromatograms is also indispensable. The spraying itself requires special precautions as oversprayed chromatograms give erroneous results.

The use of an aqueous solution of ammonium sulphate as a new spray reagent for TLC has been developed; it has all the advantages of sulphuric acid without its shortcomings. The thermal dissociation of this salt liberates adequate amounts of sulphuric acid, the excess of which is neutralised with ammonia from the dissociated salt after its decomposition.

The use of this reagent does not result in the formation of irritant and corrosive vapours, and does not require special equipment, or the use of separate rooms.

A 20 % aqueous solution of ammonium sulphate can be used, but better results are obtained with a 1:1 mixture of ammonium sulphate and ammonium hydrogen sulphate. The latter reagent is prepared by dissolving 20 g of ammonium sulphate in 100 ml water and adding 4 ml sulphuric acid ($d = 1.84$). This solution is then ready for use and can be stored for any period of time.

The chromatograms have to be sprayed quite heavily until they become transparent. No special precautions concerning the spraying technique are undertaken and overspraying is not critical.

The sprayed chromatograms are dried with hot air and visualised at elevated temperature. The minimal temperature required is different for various compounds, usually some 25° higher than when a sulphuric acid spray is used.

A practical method of heating the chromatograms is the use of ordinary hot plates (with metal cover) on which the dried chromatograms can be laid directly. This

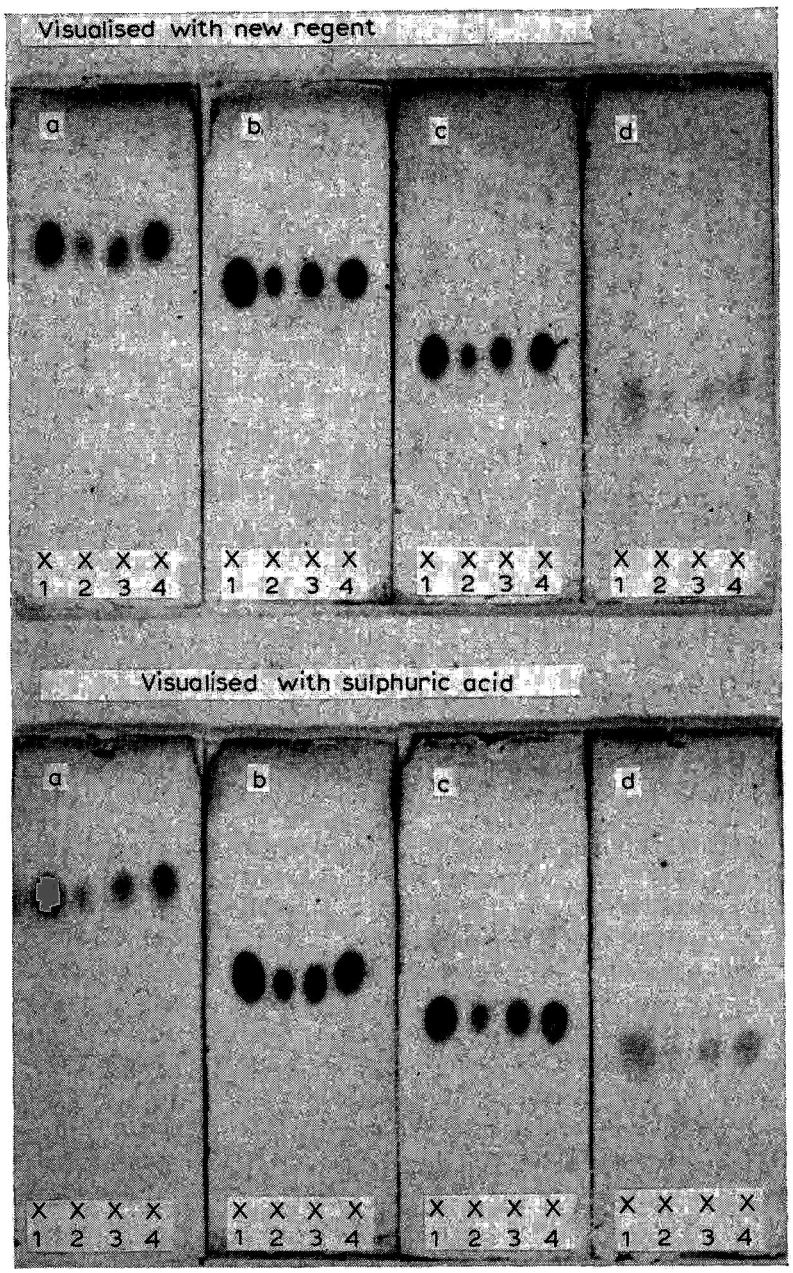

Fig. 1. TLC microchromatograms (8.5 × 3 cm) on Kieselgel G (0.3 mm layer). Solvent system: petroleum ether 50–70°–ethyl ether–acetic acid (6:4:0.1, v/v). a = Stearic acid; b = 12-ketostearic acid; c = 12-hydroxystearic acid; d = sebacic acid. 1 = 20 μg; 2 = 2 μg; 3 = 5 μg; 4 = 10 μg. Visualised with sulphuric acid: a, b, and c at 200° for 10 min; d at 225° for 20 min. Visualised with new reagent: a, b, and c at 225° for 15 min; d at 240° for 15 min.

is especially suitable for small slides. The temperature of the surface of hot plate is usually 400–500°, which is more than enough for the visualisation of all types of chromatograms. The use of hot plates reduces the time of development to 2–4 min.

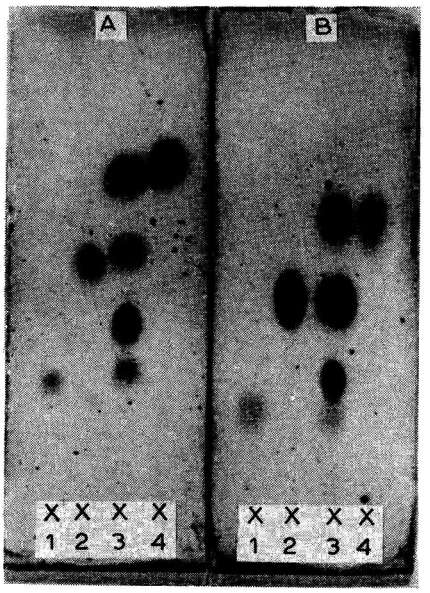

Fig. 2. TLC microchromatograms (run as in Fig. 1) visualised on hot plate. A = sulphuric acid; B = new reagent. 10 μg of each: 1 = sebacic acid; 2 = 12-ketostearic acid; 4 = stearic acid; 3 = mixture of (1), (2), (4) and 12-hydroxystearic acid.

The time required for the appearance of the spots is to a certain extent dependent on the character of organic compound, and the kind of functional groups it contains. This could be of some importance for the rapid preliminary evaluation of the type of substance appearing on the chromatograms. It is of practical value in the analysis of degradation products in the structural studies of natural compounds. This has been widely used in our laboratory in chemical studies on polyene antibiotics.

The comparative results obtained with the classical type sulphuric acid spray and the new reagent are given for a few examples in Figs. 1 and 2.

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Dünnschichtchromatographie der phenolischen Komponente der Steinkohlenteerfraktionen

Bei der Untersuchung phenolischer und kresolischer Mischungen von Substanzen stossen wir auf bestimmte Schwierigkeiten in der Erklärung des chromatographischen Verhaltens dieser Substanzen. Deshalb sind bereits interessante Arbeitsbedingungen zur chromatographischen Entwicklung der verschiedenen phenolischen Derivaten untersucht worden[1,2].

In der karbolischen Fraktion der Kohlendistillation fanden wir ungefähr 30 % karbolische Derivate vor. Diese Trennung, bei der die allgemeinen technischen Normen der Dünnschichtchromatographie angewandt wurden, ist komplizierter, da eine Ähnlichkeit im Verhalten einiger Derivate besteht.

Auf Grund der Notwendigkeit einer qualitativ vollkommenen Kenntnis der basischen Komponente dieser phenolischen Fraktion, haben wir eine indirekte Methode hinsichtlich des chromatographischen Studiums der Kieselgel G Schicht entwickelt, deren Ergebnisse wir in dieser Arbeit diskutieren.

Experimenteller Teil

Vorbereitung der Kieselgel G Schichten. Diese Schichten wurden mit den gebräuchlichen Methoden vorbereitet und wiesen nach dem Trocknen eine Schichtdicke von 400 μ auf. Das Trocknen wurde bei normaler Temperatur — 24 Stn. lang — ohne Aktivierung unternommen.

Bildung farbiger phenolischer Derivate. Mit dem Farbstoff, den wir durch Kupplung des phenolischen Derivates mit Echtviolettsalz[3] im alkalischen Medium erhielten, gelang es uns verschiedene der phenolischen Derivate, die sich in dieser Fraktion der Distillation des Steinkohlenteers befinden, zu identifizieren und zu trennen.

Ein Teil der phenolischen Fraktion, der die verschiedenen Typen phenolischer Derivate (Phenole, Kresole, usw.) in der Konzentration von 1:10 enthält, wird mit einer Mischung von Äthanol und Aceton (1:1) — woran 0.5 ml 10 % Na_2CO_3 pro 1 ml zugefügt und damit gut durchgeschüttelt — aufgelöst. Bei einer Temperatur von 10–15° kühlen und stehen lassen.

Separat wird eine Lösung (10 %) von Echtviolettsalz B (C.I. 37165) in einer Mischung von Äthanol–Wasser (1:1) bereitet und bis $\pm$10° abgekühlt; pro ml der Lösung des phenolischen Restes wird 1 ml der Lösung des Echtviolettsalzes B hinzugefügt und geschüttelt. Nach ungefähr 20 Min. periodischen Schüttelns filtriert man die Lösung, die dann für die Chromatographie verwendet wird.

Entwicklung. Trotz der Farbe des phenolischen Restes bleibt es notwendig die Flecken zu entwickeln um die Sensibilität ihrer Sichtbarkeit festzustellen, und sie in dieser Weise leichter charakterisieren und isolieren zu können.

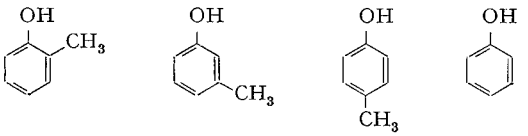
Nach der Entwicklung des Chromatogrammes, dessen Flecken zwischen gelb und braun variieren, wird dann das Chromatogramm mit H_2SO_4 besprüht, wodurch seine gelbliche Tonart sich in ein starkes Rot verändert. Mittels der Nuancen der roten Farbe ist es viel einfacher, die verschiedenen Phenole und Kresole zu unterscheiden.

Nachdem die Schicht mit H_2SO_4 besprüht worden ist, lässt man sie ungefähr 15 Min. stehen und stellt sie dann in einen Trockenschrank bei einer Temperatur von 60–70°, für ungefähr 10 Min.

Diskussion und Resultate

PETROWITZ, STAHL und andere[1,2] studierten das dünnschichtchromatographische Verhalten phenolischer Substanzen. Dabei wurden verschiedene Möglichkeiten angewandt, besonders um die gut sichtbare Unterscheidung der Phenole, *meta*- und *para*-Kresole zu bewerkstelligen.

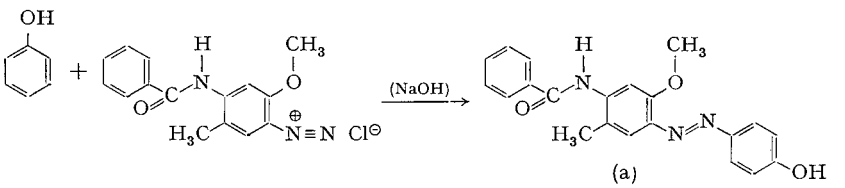
Wir stellen die Strukturen der phenolischen Komponente hiermit vor:



(1)

Man bemerkte, dass die Effekte + I unter hyperkonjugativ, die die Gruppe R—CH_3 auf R—OH ausübt keinen Intensitätsunterschied (der sich im chromatographischen Betragen widerspiegeln könnte) hervorruft, so dass eine deutliche Scheidung der Phenole nicht ermöglicht wird.

Wenn man jedoch den Monoazofarbstoff, der mittels jener Reaktion (2) zustande gekommen ist, chromatographiert, ist es möglich, besagte Fraktion der Dis-



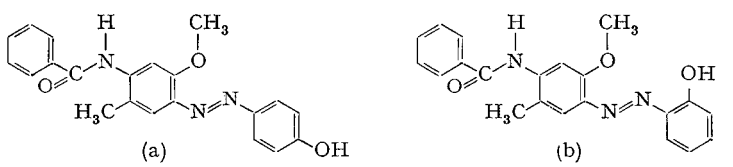
(2)

tillation des Steinkohlenteeres auch von anderen Mischungen phenolischer Derivattypen zu unterscheiden und mit Leichtigkeit zu identifizieren.

Die chromatographischen Untersuchungen verschiedener Distillationsteile der karbolischen Fraktion (Fig. 1) mit dem Objektiv die phenolischen Komponente zu trennen, zeigen uns nicht nur die Leichtigkeit mit der diese verschiedenen Komponente getrennt werden können, sondern haben uns ausserdem erlaubt das chromatographische Verhalten des so erlangten Farbstoffes zu studieren.

Die Lage des R—OH — die Hauptgruppe in der Bildung des Wasserstoffverbindung zwischen dem Farbstoff und den Gruppen Si—OH, welche sich an der Oberfläche des Kieselgels befinden — erklärt den Unterschied im Betragen dieser zwei Farbstofftypen (3a und 3b).

So rechtfertigt sich für Farbstoffe (3):



(3)

dass die Situation *p*-Hydroxyazo einen höheren hR_F Wert als die *o*-Hydroxyazo hat, was auch wirklich eintritt. Das zeigt uns auch, dass eine intermolekulare Wasserstoffverbindung der Typen (4) weit stärker ist als die, in der das Hydroxyl des Kieselgels

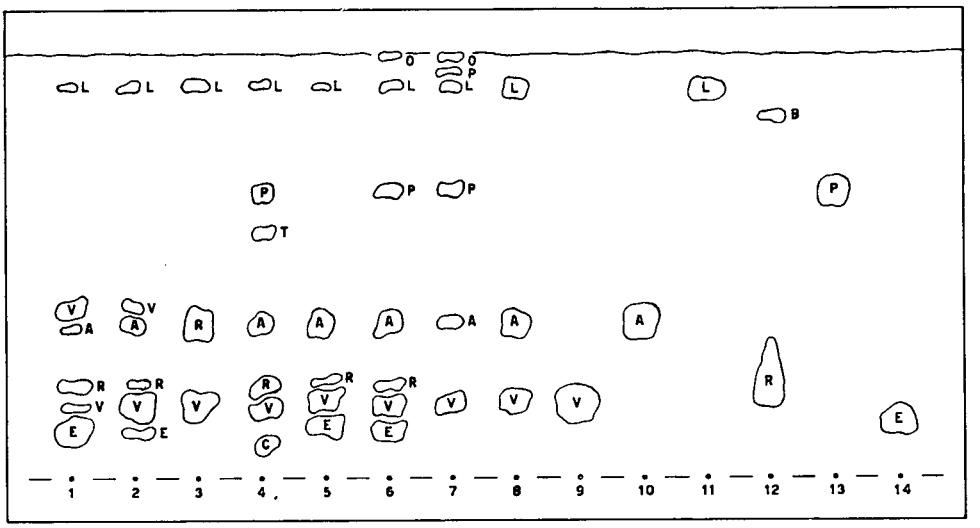
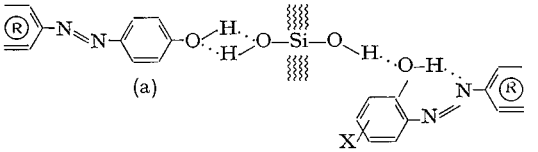


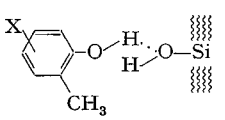
Fig. 1. Chromatogram auf einer 400 μ Kieselgel G Schicht (Merck) mit Benzin–Äthanol (10:1) als Lösungsmittel. Die Ordnung ist folgende: (1) Distillierte brutto Phenolfraktion bei 180°; (2) distillierte brutto Phenolfraktion bei 190–195°; (3) distillierte brutto Phenolfraktion bei 200°; (4) Distillationsrest dieser phenolischen Fraktionen; (5) brutto Phenol des Steinkohlenteers; (6) karbolisches Öl; (7) kreosotisches Öl; (8) Kresolmischung; (9) *m*-Kresol; (10) *o*-Kresol; (11) *p*-Kresol; (12) α-Naphthol; (13) β-Naphthol; (14) Phenol. Nach der Entwicklung mit konz. H_2SO_4 erscheinen in den Flecken folgende Farben: E (scharlach), P (blau), B (rötlich), L (rot), R (violett), A (korinth), V (bordo), C (braun), O (dunkelorange) und T (olive).

wie eine Wasserstoffspendergruppe zur Bildung der intermolekularen Verbindung eintritt. Eine (4a)-ähnliche Situation kann auch im *m*- und *o*-Kresol vorkommen, aber nur das *m*-Kresol ist fähig wirklich eine (4a)ähnliche Situation herbeizuführen.



(a) (4)

Deshalb befindet sich sein hR_F Wert = 17.2, bei dem Phenol (hR_F = 13.0). Hier übt die CH_3-Gruppe in *ortho*-Stellung zur Azogruppe einen sterischen Einfluss aus, der eine Erhöhung seines hR_F Wertes hervorruft. In dieser Weise wird die Wechselwirkung abgesetzt, was sich beim *o*-Kresol–AMB-Farbstoff bemerkbar macht (5).



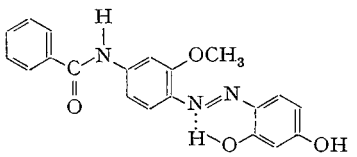
(5)

Die Lage der CH_3-Gruppe erschwert die Bildung einer zweiten Wasserstoffverbindung wie in (4).

Indem wir die hR_F Werte der Phenole und der verschiedenen Kresole (Isomere *o,m,p*-Kresol) vergleichen, bemerken wir, dass auch ein Unterschied zwischen dem

hR_F Wert im Benzol[3] des *o*-Kresols und den übrigen Kresolen und Phenolen besteht, was auch durch das gleiche Prinzip erklärt werden kann.

Andrerseits hat der Farbstoff (6):



(6)

eine doppelte Form, denn wir finden in ihm nicht nur die *o*-Hydroxyazo, sondern auch die *p*-Hydroxyazo Form.

Das führt zu einer (4a)-ähnliche Lage, die durch die molekularen charakteristischen Effekte der ersätzten Dihydroxyazoderivate verstärkt ist.

Dieses Vorkommen finden wir nicht nur in den phenolischen Derivaten, sondern auch in den von Naphtholen abgeleiteten Farbstoffen. So zeigt der AMB–β-Naphthol Farbstoff, der auch eine *o*-Hydroxyazo Struktur aufweist, einen hR_F Wert = 68.0, während sein Isomer — der AMB–α-Naphthol Farbstoff — einen erwarteten hR_F Wert = 23.5 hat.

Die Funktion der Amidgruppe (R—NH—CO—R'), die in Kupplungskomponenten gegenwärtig ist (Echtviolettsalz B), ist anscheinend in der Wechselwirkung des *Kieselgel-Farbstoffes* nicht sehr wichtig. Man bemerkt dies leichter, wenn man die respektiven molekularen Modelle studiert, bei denen, obgleich es sich um planare Moleküle handelt, das H-Atom und O-Atom der Amidgruppe sich sichtbar nach oben und unten in Richtung des Planes bewegen.

Diese Lage der senkrechten Richtung hinsichtlich des Planes macht die Bildung der Wasserstoffverbindung der Typen nicht einfach, und diese Gruppe wird durch die sterische Hinderung der Nachbargruppe R—CH_3 stark eingeschränkt.

Wissenschaftliche Abteilung von Naegeli S/A, Industrias Quimicas, Rio de Janeiro (Brasilien) ARNALDO N. ROSEIRA
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1 E. STAHL (Herausgeber), *Thin-layer Chromatography — A Laboratory Handbook*, Academic Press, New York, 1965.
2 W. WILDENHAIN UND G. HENSEKE, *J. Chromatog.*, 19 (1965) 438.
3 *The Colour Index*, Vol. 3, The Society of Dyers and Colourists and AATCC, Great Britain-U.S.A., 1956, p. 3323.

Eingegangen den 17. Dezember 1965

Paper chromatographic detection of plant phenols with antimony trichloride

Antimony trichloride has been used for detecting a variety of compounds in solution, including vitamin A[1], five-membered monoheterocyclic ring compounds[2], indoleacetic acid[3], and flavones[4]. It has also been used for detecting steroids, steroid glycosides[5–7] and biologically active quinones[8] on paper and thin-layer chromatograms. Only three references were found on the use of antimony trichloride for detection of flavonoids on paper and thin-layer chromatograms[9–11]. In these references no mention was made on the sensitivity of this spray reaction and only a few flavonoids were tested. For detection of flavonoids in solution[12,13] and phenols and terpenoids on thin-layer chromatograms[14] antimony pentachloride also has been used. This reagent is not suitable for paper chromatograms, however, because it blackens the paper upon warming to 100°.

During paper chromatographic investigation of the alcohol extractives of defatted soybean meal, fractions containing both saponins and isoflavones gave positive, though different, color reactions with a saturated chloroform solution of antimony trichloride. This report investigates the use of antimony trichloride as a spray reagent to detect naturally occurring phenolic compounds on paper chromatograms.

Experimental

Paper chromatography was performed on Schleicher & Schüll 589 Blue Ribbon paper with *n*-butanol–acetic acid–water (6:1:2) as the developing solvent. A series of concentrations of phenolic compounds was chromatographed to determine the sensitivity of the reaction. After air drying for 2 h, the chromatograms were examined under both visible and ultraviolet light (2537 Å and 3660 Å) and then sprayed with antimony trichloride in chloroform (34 g $SbCl_3$ in 100 ml chloroform), heated in an oven for 5 min at 100°, and reexamined under both visible and ultraviolet (3660 Å) light.

Results and discussion

The reactions of several phenolic compounds on paper chromatograms sprayed with $SbCl_3$ reagent are given in Table I. Each compound gives a spot on the chromatogram which either is colored when examined under visible light or fluoresces or absorbs under ultraviolet light. For those compounds giving visible colors or intensifying them, the least amount detectable is recorded; for those compounds giving no visible coloration, the least amount giving a color change under ultraviolet light is recorded. The flavone, flavanol, flavanones, a coumarin, phenol, and several phenolic acids tested gave distinct, visible colors and, for the flavonols, intensified colors after spraying with $SbCl_3$. Isoflavones did not develop a visible color after spraying with $SbCl_3$; however, definite changes in fluorescence were observed under ultraviolet light. The phenol, flavonol, flavone, and isoflavone compounds in concentrations of 1–5 μg are readily detected. Because flavanol, flavanones, and several phenolic acids are less sensitive to the reagent than the other phenolic compounds tested, from 10 to 20 μg are needed to develop color on paper chromatograms.

When sprayed with $SbCl_3$ the following compounds gave no visible coloration, a slight color change, or no color change: coumarin and scopoletin; phenolic esters:

TABLE I

COLOR REACTIONS OF PHENOLIC AND FLAVONOID COMPOUNDS WITH ANTIMONY TRICHLORIDE REAGENT

Class	*Sample*	*Amount detectable** (μg)	*Untreated U.V.*	$SbCl_3$–$CHCl_3$	
				Visible	*U.V.*
Isoflavone**	Daidzin	2	Trace light (sλ)***	—****	Yellow
	Daidzein	1	Trace blue (sλ)	—	Pale yellow
	Genistin	5	Dark (sλ)	—	Yellow
	Genistein	1	Dark (sλ)	—	Yellow
Flavone	Apigenin-7-glucoside	2	Dark	Trace yellow	Yellow
Flavonol	Kaempferol	1	Yellow	Yellow	Yellow
	Quercetin	1	Yellow	Yellow	Yellow
Flavanol	*d*-Catechin	10	Colorless	Brown → gray	Purple
Flavanone	Naringin	20	Colorless	Orange-brown	—
	Hesperidin	20	Colorless	Trace pink-brown	—
	7,4′-Dihydroxy-3′-methoxy-flavanone	10	Dark (sλ)	Orange	Orange
Coumarin	Coumarin	20	Dark (sλ)	—	Light
	Scopoletin	20	Bright blue	—	Blue-violet
	Esculin	20	Bright blue	Yellow	Blue

Phenol	Phloroglucinol	5	Colorless	Brown → gray	Purple
Phenolic ester	Chlorogenic acid	20	Blue	—	Trace gray-green
	Sinapine·SCN	20	Blue	—	Dark blue
Cinnamic acid	*p*-Coumaric acid	10	Purple (sλ)	Blue-purple	Purple
	Caffeic acid	10	Blue	Purple	—
	Ferulic acid	15	Blue	Purple	Blue-gray
	Isoferulic acid	15	Violet	Brown-purple	Gray
	o-Coumaric acid	20	White (pink tinge)	—	White
	Sinapic acid	20	Blue	—	Blue-green
Benzoic acid	β-Resorcylic acid	10	Blue-violet (sλ)	Pink	Pink-orange
	α-Resorcylic acid	15	Purple (sλ)	Yellow-brown	Yellow
	Gallic acid	20	Deep violet (sλ)	Trace red (fades)	Dark purple
	p-Hydroxybenzoic acid	20	Dark (sλ)	—	—
	Protocatechuic acid	20	Deep violet (sλ)	—	Trace purple
	Syringic acid	20	Violet (sλ)	—	Dark purple
	Vanillic acid	20	Deep violet (sλ)	—	—

* Quantities of 1, 2, 5, 10, 15 and 20 μg were spotted and developed; for those compounds giving visible color after spraying, the least amount visibly detectable is recorded; for those compounds giving no visible coloration, the least amount giving a color change when observed under U.V. light is recorded.

** Daidzin and genistin were obtained from dehulled, defatted soybean meal and were hydrolyzed to their respective aglycones.

*** (sλ) represents compounds visible only at 2537 Å.

**** Dash represents no color.

chlorogenic acid and sinapine·SCN; cinnamic acids: *o*-coumaric and sinapic; and benzoic acids: *p*-hydroxybenzoic, protocatechuic, syringic, and vanillic.

Many compounds besides the phenolics react with $SbCl_3$ to produce distinct colors in either visible or ultraviolet light. However, the visible and fluorescent colors obtained before and after treatment with $SbCl_3$ can aid in identifying the compounds under study. A mixture of soybean saponins sprayed with $SbCl_3$ gave several visible purple spots and a brown spot, and these spots had orange fluorescence. Although purple and brown spots similar to those for soybean saponins were observed with some phenolics, the soybean saponins differed in that they did not fluoresce or absorb before spraying with $SbCl_3$.

Acknowledgements

We thank Dr. A. BONDI, Hebrew University, Rehovoth, Israel, for the soybean saponin mixture; Dr. A. O. TAYLOR, University of Wellington, New Zealand, for 7,4′-dihydroxy-3′-methoxyflavanone; and Dr. F. L. AUSTIN of this laboratory, for sinapine·SCN.

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1 F. H. CARR AND E. H. PRICE, *Biochem. J.*, 20 (1926) 497.
2 V. E. LEVINE AND E. RICHMAN, *J. Biol. Chem.*, 101 (1933) 373.
3 I. IHASZ, *Magy. Kem. Folyoirat*, 69 (1963) 521.
4 R. NEU AND P. HAGEDORN, *Naturwiss.*, 40 (1953) 411.
5 R. NEHER AND A. WETTSTEIN, *Helv. Chim. Acta*, 34 (1951) 2278.
6 D. LAWDAY, *Nature*, 170 (1952) 415.
7 M. J. D. VAN DAM, G. J. DE KLEUVER AND T. G. DE HEUS, *J. Chromatog.*, 4 (1960) 26.
8 K. RANDERATH, *Thin-Layer Chromatography*, translated by D. D. LIBMAN, Academic Press, New York, London, 1963, p. 160.
9 V. A. BANDYUKOVA, *I'zuch. i Ispol'z. Lekarstv. Rastit. Resursov SSSR, Sb.*, (1964) 209.
10 L. HÖRHAMMER, H. WAGNER AND K. HEIN, *J. Chromatog.*, 13 (1964) 235.
11 M. K. MIKHAILOV, *Acta Chim. Acad. Sci. Hung.*, 10 (1957) 421.
12 G. B. MARINI-BETTÒLO AND A. BALLIO, *Gazz. Chim. Ital.*, 76 (1946) 410.
13 A. BALLIO AND F. POCCHIARI, *Gazz. Chim. Ital.*, 79 (1949) 913.
14 E. STAHL, *Chemiker-Ztg.*, 82 (1958) 323.

Received January 21st, 1966

Book Reviews

Vvedenie v obshchuyu teoriyu dinamiki sorbtsii i khromatografii (*Introduction to the General Theory of the Dynamics of Sorption and Chromatography*), by V. V. RACHINSKII, Izd. Akad. Nauk SSSR, Moscow, 1964, 136 pp., price 52 kopeks.

My teacher in chromatography used to say: "There are two kinds of chromatographers: those who develop the theory and those who use the method". The development of new types of chromatography on the basis of theoretical considerations and successful rationalization of the choice of experimental conditions show that these two tribes are no longer distinctly separate and any attempt to bridge the remaining gap is to be greeted. This is what Prof. RACHINSKII declares as the main goal of his little book: to contribute to the theoretical training of practising specialists in chromatography. To answer the question whether this goal has been achieved would first require a definition of a practical chromatographer. If this is a person familiar with thermodynamics (without necessarily being able to follow all the minutiae of specialized mathematical procedures), then this question can be answered in the affirmative.

The author's treatment starts from the general principles and proceeds, by logical deduction, to formulas which may in some cases be too difficult to solve without computers. By gradual simplification and introduction of special conditions one may then arrive at expressions which coincide with those published by earlier authors. The chapters follow in logical order: I. Dynamics of sorption and its practical importance. II. General formulation of the problem of the dynamics of sorption and methods of its solution. III. Theory of frontal dynamics of sorption of a single substance. IV. Theory of frontal chromatography. V. Theory of elution chromatography. VI. Theory of displacement chromatography. The book presents a review of pertinent earlier literature (especially valuable as a guide to Russian literature; references from Western literature do not extend beyond 1958, thus papers from *J. Chromatog.* naturally have not been quoted), a brief summary of the author's work and programme and perspective of work to be done. The author deliberately excludes questions which are of specialized interest in particular kinds of chromatography; likewise questions such as the relations between chemical structure and sorption behaviour, the influence of capacity gradients along the column or of the fluctuations of the rate of flow fall outside the scope of the book. The style, though concise, is admirably lucid, thus even readers with only a basic knowledge of Russian will be able to read the book; it is rather the exacting language of mathematics which may cause difficulties. An English translation of this small book would be useful as both a guide to earlier theoretical studies and a stimulus for further work.

I. M. HAIS (Hradec Králové)

Handbuch der Lebensmittelchemie (Analytik der Lebensmittel, Physikalische und Physikalisch-Chemische Untersuchungsmethoden), Zweiter Band/Teil 1, Schriftleitung J. SCHORMÜLLER, Springer-Verlag, Berlin, 1965, 944 pp., price DM 236.

In this review we shall limit ourselves to a discussion of the chapters dealing with separation methods which cover 224 pages and are thus equivalent to a monograph on the topic.

The chapter on paper chromatography was written by Dr. A. GRÜNE and covers all general aspects fairly well. It is regrettable that the author was too biased in favour of one brand of paper and thus omitted mentioning the commonly employed ion exchange papers. Also some sections are not at all clear, for example the discussion of comet formation (p. 545). Some problems important in the analysis of foods such as the separation of traces from large amounts of main product are not dealt with and actual applications are listed on only 2 pages and in a selected bibliography.

Column chromatography was dealt with by WOHLLEBEN, who wrote a good account of classical procedures with 20 odd examples but does not mention the use of modern automatic analysers such as the MOORE AND STEIN amino acid method.

SEHER writes a very detailed chapter on thin layer chromatography with good illustrations and an extensive bibliography.

DRAWERT presents a short introduction to gas chromatography containing a three-language glossary of gas chromatography terms and a 29-page table listing examples of the application of gas chromatography in food analysis.

Electrophoresis is treated by BELITZ in about 17 pages without any emphasis on the many really useful methods employed in food analysis.

HECKER condensed the essentials of his monograph on counter-current distribution to 32 pages.

These chapters are a short and adequate introduction to separation techniques but often confine themselves to principles (which every chemist knows today) rather than discussing their application in food analysis.

The Analytical Chemistry of the Noble Metals, by F. E. BEAMISH, Pergamon Press, Oxford, 1966, xiv + 608 pp., price £ 6.

The author is one of the foremost authorities on the analysis of the noble metals and has compiled a comprehensive treatise of their analytical methods.

868 references are discussed forming a source book not only for analysts but also for chemists working on the reactions of the noble metals. Two minor drawbacks (perhaps unavoidable in such a book) are that only work till 1961 is reported and that much is discussed in an uncritical manner leaving the task of evaluation to the reader. On the other hand many complete methods are given (in small print) making reference to the original papers superfluous. The reviewer feels that this book is essential to chemists working on any aspect of the noble metals be it radiochemistry, complex chemistry or analysis.

Analytical Applications of Ion Exchangers, by J. INCZEDY, translated by A. PÁLL, Pergamon Press, Oxford, 1966, 443 pp., price £ 5.5.0 net.

The German translation which appeared some time ago has already been reviewed in this journal. The English translation based on the original Hungarian edition (written in 1962) was written with the intention of presenting a brief summary of up-to-date methods applicable in the laboratory, as well as to give sufficient instructions for learning the techniques necessary to carry out separations described in the literature.

The author has certainly succeeded in the two tasks he set himself and has prepared one of the best hand- and reference books available on ion exchangers.

The translation is at times ugly but never unintelligible (perhaps a reading knowledge of German is at times necessary).

On looking through the reference lists at the end of each chapter one finds remarkably few printing errors but no papers later than 1962. Surely the author could have added at least a list of additional important references covering the last three years.

Dünnschichtchromatographie in der Aminosäure und Peptid-Chemie, by G. PATAKI, Walter de Gruyter & Co., Berlin, 1966, xx + 250 pages and 128 illustrations, price (bound) DM 38.00.

The rather universal application of chromatographic methods has resulted in the appearance of specialised monographs dealing with the use of a technique in a specialised field. There are now books on the gas chromatography of metal chelates or on agar electrophoresis in neurology.

The volume under discussion summarises the use of thin-layer chromatography and thin-layer electrophoresis in the field of amino acids and peptides.

All these specialised books contain a number of general chapters describing theory and techniques and these are usually most valuable because they are based more on personal opinions than is possible in a monograph dealing with all the applications of the technique. PATAKI has certainly written excellent chapters both on the technique and the theory of thin layer chromatography discussing the essentials in about 70 pages. The rest of the book is divided into the thin layer chromatography of amino acids and peptides (49 pages), application of TLC to the analysis of the amino acid sequence of proteins and peptides (62 pages), amino acids and related compounds in biological materials (27 pages), a reference list containing 457 references, and an author and a subject index.

All the sections are well written and well prepared and the reviewer has found it already invaluable as a reference book. It can be recommended equally as an introduction to thin layer methods and as a handbook for amino acid and peptide work.

MICHAEL LEDERER

News

Sixth International Symposium on Gas Chromatography and Associated Techniques

THEME AND SCOPE OF SYMPOSIUM

As with other physical techniques, gas chromatography has overlapped more and more with other subjects as it has matured. Coverage at this Symposium includes not only gas chromatography itself, but also other disciplines associated with gas chromatography. This is reflected in the extended title of the meeting.

The official language of the Symposium will be English.

LOCATION OF THE SYMPOSIUM

The Symposium will be held at the Cavalieri Hilton Hotel, which is situated on a hill (Monte Mario) on the outskirts of Rome. Please note that the venue has been changed from the Catholic University of the Sacred Heart to this hotel.

The exhibition will also be held in the hotel.

TECHNICAL PROGRAMME

The technical programme will consist of invited papers given by recognized authorities, submitted papers, and discussions. Invited papers and submitted papers will be presented and discussed in the four morning sessions of the meeting and the proceedings will be recorded for publication. On three of the afternoons there will be less formal discussions, the proceedings of which will be published in summary form.

Morning sessions

Prof. J. H. Purnell, Prof. A. I. M. Keulemans, Prof. A. Liberti, and Prof. S. R. Lipsky have kindly accepted invitations to give plenary papers opening the proceedings on each of the four morning sessions of the Symposium. The following submitted papers have been accepted for presentation:

Tuesday, 20th September: Theory and principles of chromatography

D. E. Martire, The thermodynamics of dilute solutions: aliphatic solutes in aromatic solvents and various solutes in paraffinic solvents.

A. V. Iogansen, G. A. Kurkchi and O. V. Levina, Evaluation of hydrogen-bond energies by means of gas–liquid chromatography.

O. L. Hollis and W. V. Hayes, Gas separations on microporous polymers.

J. C. Giddings and M. N. Myers, Developments in high-pressure chromatography.

H. G. Struppe, P. Jiru and O. Grubner, Interaction of water and oxygen with vanadium pentoxide catalyst by direct gas-chromatographic measurement.

R. Kaiser, Reversion gas chromatography: A method for determining traces of volatile substances in gases in the parts per thousand million range.

Wednesday, 21st September: Apparatus and techniques

D. GLASSER, A new design for a continuous gas chromatograph.
P. E. BARKER AND D. H. HUNTINGDON, A circular chromatographic machine for the preparative separation of liquid or gaseous mixtures.
D. DINELLI AND M. TARAMASSO, Design considerations and applications of a rotating unit for continuous production by gas chromatography.
G. GARZO AND D. FRITZ, Qualitative and quantitative detection of organometallic compounds by a modified flame ionization detector.
J. JANÁK AND V. SVOJANOVSKY, Working properties of a coupled flame ionization and sodium thermionic detector.
G. BURTON, A. B. LITTLEWOOD AND W. A. WISEMAN, A sensitive quantitative detector for gas chromatography using electrochemical measurement of oxygen.

Thursday, 22nd September: Novel applications of chromatography

E. GIL-AV, B. FEIBUSH AND R. C. SIGLER, Resolution of enantiomers by gas–liquid chromatography on an optically active stationary phase.
H. C. ROSE, W. KEANE, R. L. STERN AND B. L. KARGER, Studies of the mechanism of gas–liquid chromatographic separation of diastereoisomeric esters.
G. M. PETOV AND K. D. SHCHERBAKOVA, Gas-chromatographic investigation of adsorption and separation of terpenes on graphitized carbon black.
V. CANTUTI, G. ALBERINI AND G. P. CARTONI, Gas chromatography of heterocyclic nitrogen compounds.
W. D. ROSS AND R. E. SIEVERS, Ultra-trace analysis of beryllium by gas chromatography.
R. S. JUVET, JR. AND F. M. ZADO, Elution characteristics of metal chlorides from inorganic fused-salt liquid phases.

Friday, 23rd September: Techniques associated with chromatography

R. P. W. SCOTT, I. A. FOWLISS, D. WELTI AND T. WILKINS, Interrupted elution gas chromatography.
I. FLAMENT, Systematic analysis of food aromas by combination of gas chromatography, adsorption chromatography, thin-layer chromatography, and mass spectrometry.
F. CACACE AND G. CIRANNI, A combination of preparative gas chromatography and isotope dilution for precise quantitative analysis of isomeric reaction products.
P. A. T. SWOBODA, Freeze concentration of dilute aqueous solutions of volatile flavour components.
H. F. DYMOND AND K. D. KILBURN, The characterization of tobacco smoke by gas chromatography and a digital computer.
K. JONES, A. MCDOUGALL AND R. C. MARSHALL, Application of a digital computer to gas-chromatographic data from a laboratory-scale reaction.

Afternoon sessions

There will be two periods on each of three afternoons for informal discussions. Usually, there will be two discussions on unrelated subjects running concurrently, but in two instances, there will only be one discussion. One afternoon (Wednesday) is left free. The programme is as follows:

Tuesday, 20th September

1st Period: (*a*) Pollution studies; trace analysis
(*b*) Speed of chromatographic analysis
(*c*) Biomedical applications
2nd Period: (*a*) Liquid chromatography
(*b*) Documentation and publication of chromatographic papers and data

Thursday, 22nd September

1st Period: (*a*) Gas–solid chromatography
(*b*) Peak identification in gas chromatography
2nd Period: Commercial-apparatus specification

Friday, 23rd September

1st Period: (*a*) Auxiliary techniques associated with gas chromatography
(*b*) Flavour and essential oils
2nd Period: Brains Trust

The discussion sessions will last approximately one hour. In all but the last of these sessions, general discussion from the floor will follow an introduction from a speaker recognized for his work in the particular field.

Preprints

Preprints of submitted papers will be sent to registered delegates not later than 25th August, 1966. All delegates outside the United Kingdom will receive their copies via airmail.

Final proceedings

The bound volume of the final proceedings giving a complete record of all papers presented during the morning sessions and the ensuing discussions, together with critical summaries of the afternoon discussion sessions, will be published by the Institute of Petroleum within five months of the Symposium. It is expected that the price of the book will be about £5; delegates will have the opportunity of ordering one copy each at the Symposium, at a special price of £4.

EXHIBITION OF EQUIPMENT

An exhibition of the most up-to-date commercial gas chromatographic equipment, incorporating latest developments in the technique, will be held in the Cavalieri Hilton Hotel. The exhibition will be open throughout the meeting.

SOCIAL PROGRAMME

An attractive programme of social events has been arranged, and it is hoped that all delegates and their guests will participate.

Evening functions

There will be an opening reception for delegates at the Cavalieri Hilton Hotel at 8 p.m. on the Monday evening, and it is expected that a cocktail party will be held in a building of historic interest on another evening. In addition, Carlo Erba S.p.A. have kindly agreed to hold a cocktail party for delegates and their guests.

Ladies programme

It is hoped that many delegates will bring wives, for whom a special whole-day visit (including lunch) and also a half-day visit have been arranged to places of interest in or about Rome. In addition, everyone will have the opportunity of making reservations for extra excursions at the Van Ommeren stand in the exhibition foyer.

Rome was founded so long ago that her early history is part legend and part fact. As the former centre of the tremendous Roman Empire of the Caesars, the city and surrounding country have many wonderful buildings still standing, depicting the glory of those days. The religious upsurge of the Middle Ages attracted the great architects, sculptors, and painters of their generations, and the works of art and beauty they have left behind have made Rome perhaps the most fascinating city in the world to visit, particularly in September, when the weather is at its finest.

ACCOMMODATION

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The Organizing Committee have approved arrangements made by the official travel agents Phs. Van Ommeren (London) Ltd. to provide a combined return air flight and hotel stay in Rome at prices considerably below those that would normally be incurred. These special flight arrangements will operate from several European centres, and the Van Ommeren Company can, of course, fit in any private holiday requirements that members may wish to make or suggest special trips to other interesting places in Italy. A brochure/booking form giving all details will be sent to you on application.

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Location	*Hotel*	*Single room*	*Double room*
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Parioli area	Parco dei Principi	£6. 5.0 ($17.00)	£5.10.0 ($15.00)
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	Rivoli	£3.15.0 ($10.00)	£3. 0.0 ($ 9.00)
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	Massimo d'Azeglio	£5. 5.0 ($14.00)	£4.15.0 ($13.00)
	Continentale	£6.10.0 ($18.00)	£6. 5.0 ($17.00)
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It is emphasized that the prices quoted are approximate, and are included solely as a guide.

The Van Ommeren Company can make reservations at these or other hotels in Rome to suit delegates' wishes.

It would be greatly appreciated if those delegates who previously indicated an interest in accommodation at the Cavalieri Hilton Hotel would now complete the booking form enclosed in the travel brochure, and forward it as soon as possible to Messrs Phs. Van Ommeren (London) Ltd. The Van Ommeren Company have now taken over all the hotel bookings previously made at this hotel by the Symposium organization. It is regretted that accommodation is no longer available at the Student Hall of Residence at the Catholic University.

Coaches will be available to transport delegates to the Symposium venue from the railway terminal square, for those staying in the centre of Rome, and from a convenient spot in the Parioli area, for those staying in hotels in this neighbourhood.

REGISTRATION

Registration for the Symposium must be confirmed by completing the Registration Form (which will be sent to you on application) and sending it together with the registration fee to the Executive Secretary. Attendance at the Symposium is limited to 500, and registration will be dealt with in strict order of receipt. All applications will be acknowledged. Applications received after the first 500 will be placed on a waiting list. Intending delegates whose applications have been placed on the waiting list will be informed of that fact.

The registration fee is £10.0.0 (U.S. $33.00, to include cost of airmailing preprints) per delegate and £6.0.0 (U.S. $17.00) for delegates' guests taking part in the social programme only. These fees cover, for delegates, one set of preprints, all the social events, and morning coffee and afternoon tea, and for delegates' guests, all the social events and the whole-day and half-day excursions organized by the Symposium.

Priority of registration will be given until May 20th to delegates who have returned preliminary registration forms to the Executive Secretary.

REFRESHMENTS

Morning coffee and afternoon tea will be available without charge at the Cavalieri Hilton Hotel for delegates only throughout the duration of the Symposium.

Lunch

The Cavalieri Hilton Hotel is situated some distance from the centre of Rome, and lunch in the Hotel Restaurant is expensive. Unfortunately, there is no nearby cheaper alternative, except a coffee house within the hotel, which, however, is rather small. Special arrangements have, therefore, been made with the hotel that a buffet lunch will be provided for delegates at a total all-in cost of £4.0.0 ($11.00) for a set of tickets covering all four days of the Symposium. It is not possible to buy ticket for individual days only, and it is regretted that this facility is not open to delegates' guests.

Lunches will be served in the vestibule and gardens of the hotel, and will comprise a choice of two hot dishes, cold meats, dessert, and coffee.

Delegates wishing to take advantage of this offer must make reservations for lunch when they register for the Symposium; remittance of £4.0.0 ($11.00) for lunches must be sent with registration fee(s).

Tickets for lunch will be sent to delegates with their preprints.

Under the arrangement with the hotel, it is possible neither to add to, nor to subtract from, the number of delegates requiring lunch, and consequently luncheon cancellations (including the return of the £4.0.0 ($11.00) cost) cannot be accepted in Rome.

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Bibliography Section

Paper Chromatography

1. REVIEWS AND BOOKS

GIDDINGS, J. C. and KELLER, R. A. (Editors): *Advances in Chromatography*, Dekker, New York, 1965, 392 pp.

MARINI-BETTÒLO, G. B.: Trends in chromatographic techniques. *Riv. Ital. Sostanze Grasse*, 41, No. 3 (1964) 122–130; *Anal. Abstr.*, 12 (1965) 3611 — PC and TLC.

SAVIDAN, L.: *La chromatographie*, 2nd Ed., Dunod, Paris, 1963, 115 pp. — PC and TLC.

SCHORN, P.-J.: Chromatography. Survey of papers, adsorbents and devices. *Glas.-Instr.-Tech.*, 8 (1964) 385–386; *Anal. Abstr.*, 12 (1965) 5559 — PC and TLC.

WHEELER, T. E.: Paper chromatography of protein hydrolyzates. I. General considerations. *Serum Proteins Dysproteinemias*, (1964) 159–162; *C.A.*, 63 (1965) 4639b.

2. FUNDAMENTALS, THEORY AND GENERAL

SOCZEWINSKI, E.: Comparison of chromatographic behavior of organic electrolytes in structural studies. *Bull. Acad. Polon. Sci., Ser. Sci. Chim.*, 13 (1965) 209–12; *C.A.*, 63 (1965) 6801c.

STEWART, G. H.: The stationary phase in paper chromatography. In J. C. GIDDINGS and R. A. KELLER, (Editors): *Advances in Chromatography*, Vol. I, Dekker, New York, 1965, pp. 93–111.

3. TECHNIQUES I

BAILEY, R. E., PIETERS, H. P. AND BECK, J. H.: Semi-automatic transfer of volatile liquids to paper chromatograms. *Nature*, 204 (1964) 485.

BOULTON, A. A., CHARD, N. E. AND GRANT, L.: Quantitative analysis of fluorescent zones on paper strips. *Biochem. J.*, 96 (1965) 69P–70P — devised mainly for dansyl derivatives of amino acids.

BOULTON, A. A., CHARD, N. E. AND GRANT, L.: Heating attachment for a fluorimetric scanning device. *Biochem. J.*, 96 (1965) 82P — increase in sensitivity is possible due to removal of water etc.

BOULTON, A. A., CHARD, N. E. AND GRANT, L.: A fluorimetric scanning device. *Biochem. J.*, 96 (1965) 83P.

BOULTON, A. A., HOLDER, R. L. AND ROSS, H. F.: A computer automated method of estimating the areas under the peaks obtained by scanning paper partition chromatograms. *Biochem. J.*, 96 (1965) 70P.

BOULTON, A. A., HOLDER, R. L. AND ROSS, H. F.: A system for transcribing electrical signals produced by an automatic paper strip scanning device on the punched paper tape. *Biochem. J.*, 96 (1965) 83P.

BRIGGS, D. E., MILLIN, D. J. AND SALTMARSH-ANDREW, M. J.: Detection of "activated" compounds on paper. *Nature*, 204 (1964) 286–287.

CLARKE, E. G. C. AND SOWTER, S. A.: Continuous flow spotting for paper chromatograms. *Nature*, 202 (1964) 795.

DAVIS, J. E.: Spot and stripe applicator for chromatography. *U.S. Pat.*, 3,189,413 (Cl.23–253), June 15, 1965; Appl. Sept. 25, 1961, 16 pp.; *C.A.*, 63 (1965) 6317c.

KELLER, R. A. and ROMMEL, M. A.: Circumstances of multi-spot paper chromatograms of sodium orthophosphates: *in situ* identification of the species. *Nature*, 204 (1964) 873–875.

MENN, J. J., MCBAIN, J. B. AND DENNIS, M. J.: Detection of naturally occurring cholinesterase inhibitors in several crops by paper chromatography. *Nature*, 202 (1964) 697–698 — spraying with human blood plasma as the cholinesterase source and then with acetylcholine.

PONNAMPERUMA, C., KIRK, P., MARINER, R. AND TYSON, B.: A coincidence technique for paper chromatography. *Nature*, 202 (1964) 393–394.

SMITH, I.: Chromatography and electrophoresis on paper and thin layers: A teachers' guide. In J. C. GIDDINGS AND R. A. KELLER (Editors): *Advances in Chromatography*, Vol. I, Dekker, New York, 1965, pp. 61–92.

STICKLAND, R. G.: Readily adjustable chromatography spray. *Lab. Pract.*, 13 (1964) 523–524; *Anal. Abstr.*, 12 (1965) 5564 — PC and TLC.

WEISZ, H. UND SCHEPKY, G.: Über eine Kombination von Papierchromatographie und Ringofenmethode zur Untersuchung einiger toxikologisch interessanter organischer Stoffe. *Mikrochim. Acta*, (1965) 717–724.

4. TECHNIQUES II

MACEK, K., VEČERKOVÁ, J., AND STANISLAVOVÁ, J.: Neues Verfahren der systematischen Analyse von Arzneimitteln durch Papier- und Dünnschichtchromatographie. *Pharmazie*, 20 (1965) 605–616 — PC and TLC of 161 compounds.

6. ALCOHOLS

FIJOLKA, P. AND RADOWITZ, W.: (Qualitative paper-chromatographic analysis of polyols in polyesters.) *Plaste Kautschuk*, 12, No. 4 (1965) 207–209; *C.A.*, 63 (1965) 6295a.

7. PHENOLS

CHKANIKOV, D. I., PAVLOVA, N. N. AND GERTSUSKII, D. F.: (Production of halophenols from halophenoxyacetic acids in plants.). *Khim. v Sel'skom. Khoz.*, 3, No. 4 (1965) 56–60; *C.A.*, 63 (1965) 8250c.

8. SUBSTANCES CONTAINING HETEROCYCLIC OXYGEN

DUFF, R. B., BACON, J. S. D., MUNDIE, C. M., FARMER, V. C., RUSSELL, J. D. AND FORRESTER, A. R.: Catalpol and methylcatalpol: naturally occurring glycosides in *Plantago* and *Buddleia* species. *Biochem. J.*, 96 (1965) 1–5.

GALMARINI, O. L. AND STODOLA, F. H.: Fonsecin, a pigment from an *Aspergillus fonsecaeus* mutant. *J. Org. Chem.*, 30 (1965) 112–115.

JOHNSTON, S. E. AND ROUX, D. G.: Natural and synthetic diastereoisomeric (—)-3′,4′,7-trihydroxyflavan-3,4-diols. *Biochem. J.*, 96 (1965) 681–687.

JURD, L. AND WAISS JR., A. C.: Anthocyanins and related compounds. VI. Flavylium salt-phloroglucinol condensation products. *Tetrahedron*, 21 (1965) 1471–1483; *C.A.*, 63 (1965) 8304d.

KOEPPEN, B. H. AND ROUX, D. G.: C-Glycosylflavonoids. The chemistry of orientin and iso-orientin. *Biochem. J.*, 97 (1965) 444–448.

MARKHAM, K. R.: Gentian pigments. II. Xanthones from *Gentiana bellidifolia*. *Tetrahedron*, 21 (1965) 1449–1452; *C.A.*, 63 (1965) 8307h.

9. OXO COMPOUNDS

KERN, H., AND NAEF-ROTH, S.: The formation of phytotoxic pigments by fusaria of the Martiella group). *Phytopathol. Z.*, 53, No. 1 (1965) 45–64; *C.A.*, 63 (1965) 4704b.

10. CARBOHYDRATES

ALI, B. S., HEITEFUSS, R. AND FUCHS, W.: (The production of chitinase by *Entomophthora coronata*.) *Z. Pflanzenkrankh. Pflanzenschutz*, 72 (1965) 201–207; *C.A.*, 63 (1965) 4705a.

ANGYAL, S. J. AND GERO, S. D.: Cyclitols. Part XVII. The selectivity of protecting groups: tetrahydropyranyl esters. *J. Chem. Soc.*, (1965) 5255–5258.

BATES, C. J. AND PASTERNAK, C. A.: The incorporation of labelled amino sugars by *Bacillus subtilis*. *Biochem. J.*, 96 (1965) 155–158.

BINKLEY, W. W.: Isolation of planteose and nystose from cane final molasses. *Intern. Sugar J.*, 67 (1965) 204–206; *C.A.*, 63 (1965) 8594f.

BINNS, W. W. AND BLUNDEN, G.: Chemical identification of soluble starch and other starch products. *Pharm. J.*, 194 (1965) 577–578; *C.A.*, 63 (1965) 8595d — PC and TLC.

DOKUMOV, S. I.: The lactosuria of pregnancy as detected by means of ascending PC. *Gynaecologia*, 157 (1964) 95–102; *C.A.*, 63 (1965) 13789a.

DRABIKOWSKA, A.: (Determination of raffinose in molasses.) *Prace Inst. Lab. Badawczych Przemyslu Spozywczego*, 15 (1965) 1–11; *C.A.*, 63 (1965) 7193e.

JOHNSTON, I. R.: The composition of the cell wall of *Aspergillus niger*. *Biochem. J.*, 96 (1965) 651–658.

JOHNSTON, I. R.: The partial acid hydrolysis of a highly dextrorotatory fragment of the cell wall of *Aspergillus niger*. Isolation of the α-(1→3)-linked dextrin series. *Biochem. J.*, 96 (1965) 659–664.

LAKHERA, J. P.: Chromatographic examination of sugars in the tuber of *Pueraria tuberosa*. *Current Sci. (India)*, 34 (1965) 249; *C.A.*, 63 (1965) 2120g.

OHKUMA, S. AND SHINOHARA, T.: Identification of hexosamines by two-dimensional paper electrophoresis and chromatography. *Nature*, 202 (1964) 593.

SUAMI, T. AND YABE, K.: Amino cyclitols. VII. New synthetic route to myo-4-inosamine. *Bull. Chem. Soc. Japan*, 38 (1965) 855–856; *C.A.*, 63 (1965) 8464f.

TYLER, J. M.: The seed mucilage of *Lepidium sativum* (Cress). Part I. Identification of the components and some neutral oligosaccharides derived from the mucilage, together with the examination of a pentosan fraction. *J. Chem. Soc.*, (1965) 5288–5300.

TYLER, J. M.: The seed mucilage of *Lepidium sativum* (Cress). Part II. Products of hydrolysis of the methylated mucilage and the methylated degraded mucilage. *J. Chem. Soc.*, (1965) 5300–5310.

11. ORGANIC ACIDS AND LIPIDS

11a. Organic acids

LINDNER, K. UND JURICS, E. W.: Direkte densitometrische Methode zur Auswertung der Papierchromatogramme von Apfelsäure, Weinsäure und Citronensäure in Obst. *Z. Lebensm. Untersuch. Forsch.*, 128 (1965) 65–70.

MARCZAL, G.: (Qualitative studies of the tannin content of *Rubus idaeus* leaf.) *Herba Hung.*, 2 (1963) 343–360; *C.A.*, 63 (1965) 6787f.

PECHAN, Z., AND DUCHOŇ, J.: (Melanogen determination in urinary hydroxyindole conjugates and homovanilic acid.) *Med. Lab.*, 18, No. 4 (1965) 73–80; *C.A.*, 63 (1965) 7467f.

SHAH, N. C. AND GULATI, B. C.: Water-soluble constituents of palmarosa oil *Cymbopogon martini* var. *motia*. *Perfumery Essent. Oil Record*, 56 (1965) 435–437; *C.A.*, 63 (1965) 8115h.

VALLE, C. UND GARCÍA-OLMEDO, R.: (Papierchromatographie von Zuckerarten und ihre Anwendung in der Lebensmittelanalyse.) *An. Bromatologia (Madrid)*, 15 (1963) 127–177; from *Z. Lebensm. Untersuch. Forsch.*, 127 (1965) 297.

WILLIAMSON, I. P. AND KEKWICK, R. G. O.: The formation of 5-phosphomevalonate by mevalonate kinase in *Hevea brasiliensis* latex. *Biochem. J.*, 96 (1965) 862–871.

YOUNG, J. A., SCHWARTZMANN, G. AND MELTON, A. L.: Separation and identification of sodium salts of acetic, propionic, butyric and valeric acids by PC. *J. Assoc. Offic. Agr. Chemists*, 48 (1965) 622–624; *C.A.*, 63 (1965) 7331h.

11b. Lipids and their constituents

DAWSON, R. M. C. AND EICHBERG, J.: Diphosphoinositide and triphosphoinositide in animal tissues. Extraction, estimation and changes *post mortem*. *Biochem. J.*, 96 (1965) 634–643.

DE FREITAS, A. S. W. AND DEPOCAS, F.: Quantitative isolation and assay of neutral glyceride glycerol and phosphoglyceride glycerol. *Can. J. Biochem.*, 42 (1964) 195–202; *Anal. Abstr.*, 12 (1965) 2939.

13. STEROIDS

ADAM, Y., JOLY, P. AND VARENNE, J. Y.: (Estimation of the principal 17-ketosteroids of urine by PC. Estimation of dehydroepiandrosterone.) *Rev. Franc. Etudes Clin. Biol.*, 10 (1965) 94–98; *C.A.*, 63 (1965) 2071g

AMORMINO, V., CECCARINI, R. AND CINGOLANI, E.: (Evaluation of adrenocortical extracts for pharmaceutical use.) *Farmaco (Pavia), Ed. Prat.*, 19 (1964) 628–639; *C.A.*, 63 (1965) 8120e.

BENGTSSON, L. P., DEUTSCH, A., AND NILSSON, R.: Effect of estrogen on the acid-soluble nucleotide content of the rabbit vagina. *Acta Obstet. Gynecol. Scand.*, 43 (1965) 369–380; *C.A.*, 63 (1965) 10273e.

GUPTA, D. AND TANNER, J. M.: A new steroid found in the urine of children but not adults. *Nature*, 203 (1964) 187–188 — characterization according to chromatographic behaviour.

LAMBERT, M. AND PENNINGTON, G. W.: The estimation of polar steroids in liquor amnii. *J. Endocrinol.*, 32 (1965) 287–293; *C.A.*, 63 (1965) 7285d.

PETERSON, R. E.: Determination of peripheral plasma aldosterone. *Aldosterone Symp., Prague, 1963*, (pub. 1964) 145–161; *C.A.*, 63 (1965) 18569f.

SCHNEIDER, J. J.: Hydroxylation of deoxycorticosterone at the 6β, 11α, 15α, 15β, and 16α positions by surviving liver slices of the bullfrog. *Biochemistry*, 4 (1965) 689–693 — ΔR_{Mg} values for 9 hydroxyl groups.

15. TERPENE DERIVATIVES

TEGISBAEV, E. T., CHUMBALOV, T. K. AND ABUBAKIROV, N. K.: (Triterpenoid glycoside — silenoside obtained from the roots of *Silene latifolia*.) *Rast. Resursy*, 1, No. 1 (1965) 102–106; *C.A.*, 63 (1965) 4573c.

17. AMINES, AMIDES AND RELATED NITROGEN COMPOUNDS

HRDLIČKA, J. AND JANÍČEK, G.: Volatile amines as components of toasted oat flakes. *Nature*, 204 (1964) 1201 — R_F values for 15 amines.

JOH, Y.: Synthesis of 5-hydroxykynuramine hydrochloride (Mausamine). *J. Biochem.* (*Tokyo*), 58 (1965) 248–250.
MOKRANJAC, M. ST. AND VUJOVIC, B.: (Investigation of the transformation of ptomaines by PC.) *Acta Pharm. Jugoslav.*, 14 (1964) 89–94; *C.A.*, 63 (1965) 10228g.

18. AMINO ACIDS

BARBIROLI, G.: Papierchromatographische Trennung und Bestimmung von 22 Aminosäuren. *Mikrochim. Acta*, (1965) 652–656.
FUJIWARA, Y.: (Determination of amino acids with a photoelectric densitometer.) *Oita Daigaku Gakugei Gakubu Kenkyu Kiyo, Shizen Kagaku*, 2, No. 4 (1964) 49–58; *C.A.*, 63 (1965) 7651f.
HOCH, H., SINNETT, S. L., MILLER, P. O. AND MAHADY, I. B.: Dialyzable thyroid hormone-binding material in human serum. *Biochemistry*, 4 (1965) 931–939 — interaction with thyroxine on paper chromatogram.
KISS, A., EPERJESSY, A., KOVACS, V., AND FESZT, T.: (Chromatographic study of cerebral amino acids in experimental allergic encephalomyelitis.) *Rev. Med.* (*Targu-Mures*), 11, No. 1 (1965) 36–39; *C.A.*, 63 (1965) 10466e.
KUDRYASHOVA, N. A. AND KOLOBKOVA, E. V.: (Determination of tryptophan by PC.) *Byul. Gl. Botan. Sada*, No. 54 (1964) 75–80; *C.A.*, 63 (1965) 2103e.
MARTYNOV, V. F., BESPALOVA, Z. D. AND TITOV, M. I.: (Synthesis of the protected hexapeptide, carbobenzoxy-L-phenylalanyl-L-leucyl-L-leucyl-L-phenylalanyl-L-leucyl-L-leucine methyl ester.) *Vestn. Leningr. Univ., Ser. Fiz. Khim.*, 20, No. 2 (1965) 159–161; *C.A.*, 63 (1965) 8480e.
SCHOENTAL, R. AND RIVE, D. J.: Interaction of N-alkyl-N-nitroso-urethanes with thiols. *Biochem. J.*, 97 (1965) 466–474 — R_F values and detection of 20 compounds.
SHINDLE, V. A., JAIN, R. K. AND AGARWAL, I. P.: Paper chromatographic analysis of the enzymatic activity *in vitro*. II. Liver histidase in pregnancy. *J. Anal. Soc. India*, 14, No. 1 (1965) 1–6; *C.A.*, 63 (1965) 13629a.
WHEELER, T. E.: Paper chromatography of protein hydrolyzates. II. Method for PC of protein hydrolysates. *Serum Proteins Dysproteinemias*, (1964) 162–165; *C.A.*, 63 (1965) 4639c.

19. PEPTIDES; CHEMICAL STRUCTURE OF PROTEINS AND PEPTIDES

GORDON, D. F., JR. AND SPECK, M. L.: Bitter peptide isolated from milk cultures of *Streptococcus cremoris*. *Appl. Microbiol.*, 13 (1965) 537–542; *C.A.*, 63 (1965) 4680c.

21. PURINES, PYRIMIDINES, NUCLEOSIDES, NUCLEOTIDES, NUCLEIC ACIDS, BARBITURATES

ADLER, M. AND GUTMAN, A. B.: 3-Methylxanthosine. *Nature*, 204 (1964) 681.
CRESSWELL, R. M., MAURER, H. K., STRAUSS, T. AND BROWN, G. B.: (R_F values of pyrimidine and purine N-oxides.) *J. Org. Chem.*, 30 (1965) 408; from *J. Chromatog., Chromatog. Data*, 20 (1965) D10.
HALL, R. H.: A general procedure for the isolation of "minor" nucleosides from ribonucleic acid hydrolysates. *Biochemistry*, 4 (1965) 661–670 — R_F values for 22 nucleosides.
IKEHARA, M., TADA, H. AND MUNEYAMA, K.: Nucleosides and nucleotides. XXV. Purine cyclonucleosides. 2. Synthesis of 5′-deoxyguanosine via a 5′,8-cyclonucleoside. *Chem. Pharm. Bull.* (*Tokyo*), 13 (1965) 639–642; *C.A.*, 63 (1965) 8472d.
IMURA, N., IRIE, M. AND UKITA, T.: Enzymatic depolymerization of polyadenylic acid by bovine pancreatic ribonuclease-A. *J. Biochem.* (*Tokyo*), 58 (1965) 264–272.
NARBUTT-MERING, A. B. AND WEGLOWSKA, W.: (Detection of decomposition in thiopental-type preparations.) *Acta Polon. Pharm.*, 22 (1965) 13–20; *C.A.*, 63 (1965) 8126b.
PICARD, J., GARDAIS, A. AND DUBERNARD, L.: Presence of sulphated nucleotides in the epiphysial growth cartilage of the rat. *Nature*, 202 (1964) 1213–1214.

22. ALKALOIDS

ABDOH, Y. AND PIRELAHI, H.: Alkaloid content of tobacco seeds. *Nature*, 204 (1964) 791–792.
GAWRYCH, Z. AND WILCZYNSKA, I.: (Determination of non-hydrogenated and hydrogenated ergot alkaloids.) *Acta Polon. Pharm.*, 22 (1965) 1–12; *C.A.*, 63 (1965) 8126g.
ISRAILOV, L., ABDUAZIMOV, KH. A. AND YUNOSOV, S. YU.: (Alkaloids of *Ungernia ferganica* and *Convolvulus lineatus*.) *Dokl. Akad. Nauk. Uz.SSR*, 22, No. 3 (1965) 18–19; *C.A.*, 63 (1965) 7346e.
MINIMA, S. A. AND ADUEVSKAYA, G. I.: (Alkaloid content of *Physochlaina orientalis*.) *Aptechn. Delo*, 14, No. 2 (1965) 66–68; *C.A.*, 63 (1965) 4656a.

NIEZGODSKI, L. AND MANIKOWSKI, W.: Rapid method for the assay of alkaloids in toxicological investigations. I. Atropine, brucine, quinine and pilocarpine. *Poznan. Towarz. Przyjaciol Nauk, Wydzial Lekar., Prace Komisji Farm.*, 3 (1965) 107–113; *C.A.*, 63 (1965) 7547 e.

TSO, T. C., BURK, L. G., DIETERMAN, L. J. AND WENDER, S. H.: Scopoletin, scopolin and chlorogenic acid in tumours of interspecific *Nicotiana* hybrids. *Nature*, 204 (1964) 779–780.

VALENZUELA, M., BENAVIDES, V. AND NEGRETE, R.: (Separation of atropine and papaverine in mixtures.) *Anales Real Acad. Farm. (Madrid)*, 31 (1965) 44–47; *C.A.*, 63 (1965) 6785 b.

23. OTHER SUBSTANCES CONTAINING HETEROCYCLIC NITROGEN

ASATOOR, A. M.: Pyridoxine deficiency in the rat produced by D-penicillamine. *Nature*, 203 (1964) 1382–1383.

BARTHOLINI, G., PLETSCHER, A. AND BRUDERER, H.: Formation of 5-hydroxytryptophol from endogenous 5-hydroxytryptamine by isolated blood platelets. *Nature*, 203 (1964) 1281–1283 — PC and TLC.

DALE, J. E. AND MILFORD, G. F.: Role of endogenous growth substances in the fruiting of upland cotton. *New Phytologist*, 64, No. 1 (1965) 28–37; *C.A.*, 63 (1965) 10588 h.

GORDIN-SHARIR, A. AND WAREING, P. F.: Auxin and gibberellin-like substances in peanut (*Arachis hypogaea*). *Nature*, 204 (1964) 1308–1309.

NAGATSU, T. AND YAGI, K.: The disappearance of serotonin by monoamine oxidase inhibitor insensitive agent in rat liver homogenate. *J. Biochem. (Tokyo)*, 58 (1965) 302–304.

NICHOLS, R.: Fruit development of cacao (*Theobroma cacao*) in relation to cherelle wilt. II. Auxins and development of the seeds. *Ann. Botany* (London), 29 (1965) 181–196; *C.A.*, 63 (1965) 4669 e.

ROBINSON, J. D. AND GREEN, J. P.: Presence of imidazoleacetic acid riboside and ribotide in rat tissues. *Nature*, 203 (1964) 1178–1179 — R_F values for 20 compounds.

SCHWARTZ, S. L. AND MCKENNIS, JR., H.: Mammalian degradation of (—)-demethylcotinine. *Nature*, 202 (1964) 594–595.

24. ORGANIC SULPHUR COMPOUNDS

BRIDGES, J. W., KIBBY, M. R. AND WILLIAMS, R. T.: The structure of the glucuronide of sulphadimethoxine formed in man. *Biochem. J.*, 96 (1965) 829–836 — R_F values and colour reactions of 15 compounds.

NEGORO, K.: (Reaction of arenesulfonic acids with formaldehyde.) *Koru Taru*, 16 (1964) 510–514; *C.A.*, 63 (1965) 8277 h.

SEMENTOVS'KA, G. P.: (Use of a phenol–hypochlorite reaction for chromatographic analysis of sulfonamides.) *Zb. Aspirants'k. Prats. Odes'k. Tekhnol. Inst. Kharch. ta Kholodi'n. Prom.*, 1 (1963) 56–64; *C.A.*, 63 (1965) 8122 h.

STEPHAN, R. AND ERDMAN, J. G.: Detection of divalent sulphur compounds on paper and thin-layer chromatograms. *Nature*, 203 (1964) 749 — PC and TLC.

25. ORGANIC PHOSPHORUS COMPOUNDS

CLARK, V. M. AND WARREN, S. G.: Studies on phosphorylation. XXVIII. The formation of pyrophosphates from phosphoramidate monoesters. *J. Chem. Soc.*, (1965) 5509–5513.

27. VITAMINS

BRANDENBURG, C., PEPPLER, E. AND CREMER, H. D.: Beitrag zur papierchromatographischen Bestimmung von Thiamin. *Z. Lebensm. Untersuch. Forsch.*, 127 (1965) 10–16.

CARR, N. G. AND EXELL, G.: Ubiquinone concentrations in *Athiorhodaceae* grown under various environmental conditions. *Biochem. J.*, 96 (1965) 688–692.

DESMUKH, D. S., MURTHY, S. K., MAHADEVAN, S. AND GANGULY, J.: Studies on metabolism of vitamin A. Absorption of retinal (vitamin A aldehyde) in rats. *Biochem. J.*, 96 (1965) 377–382.

ROSE, A. H., ILAHI, M. AND KELEMEN, M. V.: Studies on the biosynthesis of biotin. Production of biotin and biotin-like compounds by a pseudomonad. *Biochem. J.*, 96 (1965) 319–327.

SOTOME, S. AND KITAHARA, K.: Polysaccharide-fermenting lactic acid bacteria. III. Nutritional requirements and existance of fermentation-promoting factors for sucrose and inulin. *Nippon Nogei Kagaku Kaishi*, 37 (1963) 351–356; *C.A.*, 63 (1965) 2148 c.

28. ANTIBIOTICS

RÖHR, M.: Papierchromatographische Trennung und Nachweis von Penicillin und dessen enzymatischen Spaltprodukten. *Mikrochim. Acta*, (1965) 705–707.

ROGERS, H. J., NEWTON, G. G. F. AND ABRAHAM, E. P.: Production and purification of bacilysin. *Biochem. J.*, 97 (1965) 573–578.

SANNIKOV, V. A.: (Chromatographic determination of the proportions of components in commercial preparations of neomycin.) *Materialy 2-oi (Vtoroi) Konf. Molodykh Uchenykh Leningr. Inst. Antibiotikov, Leningrad, Sb.*, (1964) 74–76; *C.A.*, 63 (1965) 8127b.

UMEZAWA, H., OKAMI, Y., HASHIMOTO, T., SUHARA, Y., HAMADA, M. AND TAKEUCHI, T.: A new antibiotic, kasugamycin. *J. Antibiotics (Tokyo), Ser. A*, 18 (1965) 101–103; *C.A.*, 63 (1965) 6786d.

30. SYNTHETIC AND NATURAL DYES

PEARSON, D. AND WALKER, A.: R_F values of permitted synthetic water-soluble coloring matters. *J. Assoc. Public Analysts*, 3 (1965) 45–48; *C.A.*, 63 (1965) 13930h — R_F values for 29 dyes.

31. PLASTICS AND THEIR INTERMEDIATES

KORN, O. AND WOGGON, H.: (Paper-chromatographic determinations of urea-based poly(vinyl chloride) stabilisers in plastic materials.) *Plaste Kautsch.*, 11 (1964) 278–280; *Anal. Abstr.*, 12 (1965) 5270.

32. PHARMACEUTICAL AND FORENSIC APPLICATIONS; METABOLISM OF DRUGS

ADAMANIS, F., PAWELCZYK, E., PLOTKOWIAKOWA, Z. AND DOMERACKI, S.: (Chromatographic analysis of molecular compounds. II. The effect of varying pH of the medium on the R_F values of some molecular compounds.) *Poznan. Towarz. Przyjaciol Nauk, Wydzial Lekar. Prace Komisji Farm.*, 3 (1965) 41–51; *C.A.*, 63 (1965) 8121e.

ISRAILI, A. H. AND SIDDIQUI, H. H.: *Withania coagulans*. II. Chemical investigation of the alcoholic extract of the whole fruit. *Indian J. Pharm.*, 27 (1965) 178–179; *C.A.*, 63 (1965) 7347d.

NIYOGI, S. K.: Detection of secobarbital metabolite in the urine of the rat. *Nature*, 202 (1964) 1225–1226.

OKUDA, S., KATAOKA, H. AND TSUDA, K.: Lupine alkaloids. II. Absolute configuration of lupine alkaloids. 1. *Chem. Pharm. Bull. (Tokyo)*, 13 (1965) 487–491; *C.A.*, 63 (1965) 7059h.

OKUDA, S., KATAOKA H. AND TSUDA, K.: Lupine alkaloids. III. Absolute configuration of lupine alkaloids. 2. *Chem. Pharm. Bull. (Tokyo)*, 13 (1965) 491–500; *C.A.*, 63 (1965) 7060f.

OKUDA, S., MURAKOSHI, I., KAMATA, H., KASHIDA, Y., HAGINIWA, J. AND TSUDA, K.: Lupine alkaloids. I. Minor alkaloids of Japanese *Sophora flavescens*. *Chem. Pharm. Bull. (Tokyo)*, 13 (1965) 482–487; *C.A.*, 63 (1965) 7059e.

PAWELCZYK, E. AND PLOTKOWIAKOWA, Z.: (Analysis of decomposition products of drugs. XII. Determination of naphazoline in the presence of its decomposition products.) *Dissertationes Pharm.*, 17 (1965) 75–79; *C.A.*, 63 (1965) 6793b.

VLASENKO, L. M.: (Chromatography in forensic chemistry.) *Aptechn. Delo*, 14, No. 2 (1965) 75–82; *C.A.*, 63 (1965) 2103a — PC and TLC.

33. INORGANIC SUBSTANCES

CHANG, C.-C. AND YANG, H.-H.: (Separation of rhenium, vanadium, molybdenum, and tungsten by paper chromatography.) *Hua Hsueh Tung Pao*, 32 (1965) 45–46; *C.A.*, 63 (1965) 7634d.

ERMOLENKO, I. N. AND LONGIN, M. L.: (Ion-exchange chromatography of inorganic cations on carboxycellulose paper.) *Zhur. Anal. Khim.*, 19 (1964) 425–430; *Anal. Abstr.*, 12 (1965) 3735.

HARDY, C. J. AND LYON, C. E.: Rapid methods for determining the relative amounts of zirconium-95 and niobium-95 based on anion-exchange paper chromatography and gamma spectrometry. *U.K. At. Energy Authority, Rept.*, AERE-R 4814, 1964; *Anal. Abstr.*, 12 (1965) 2749.

KULAEV, F. N.: (Precipitation chromatography on paper for qualitative analysis of inorganic ions.) *Izv. Vysshikh Uchebn. Zavedenii, Khim. i Khim. Tekhnol.*, 7, No. 6 (1964) 904–909; *C.A.*, 63 (1965) 6297b.

MAH, D. C., MAH, S. AND TUPPER, W. M.: Circular paper chromatographic method for determining trace amounts of cobalt, copper, nickel, and zinc in rocks and soils. *Can. J. Earth Sci.*, 2 No. 2 (1965) 33–43; *C.A.*, 63 (1965) 6304b.

SCHNEER-ERDEY, A. AND TÓTH, T.: Paper chromatography in organic qualitative analysis. *Talanta*, 11 (1964) 907–911; *Anal. Abstr.*, 12 (1965) 5023.

SOÓ, A., GIURGIU, M. AND CADARIU, I.: (Chromatographic studies of basic chromium complexes). *Stud. Univ. Babes-Bolyai, Cluj, Chim.*, 8, No. 2 (1963) 61–74; *Anal. Abstr.*, 12 (1965) 3854.

VARSHAL, G. M., BOGDANOVA, V. I., SENYAVIN, M. M. AND SAUNKIN, O. F.: (Distribution paper chromatography for the concentration of elements.) *Tr. Komis. po Analit. Khim., Akad. Nauk SSSR, Inst. Geokhim. i Analit. Khim.*, 15 (1965) 358–367; *C.A.*, 63 (1965) 7629d.

WERNER, G.: Trennung der seltenen Erden durch Papierchromatographie mit umgekehrten Phasen. *Angew. Chem.* 77 (1965) 920 — impregnation with dinonylnaphthalenesulphonic acid.

34. RADIOACTIVE AND OTHER ISOTOPIC COMPOUNDS

HALES, I., LANE, J., RICHARDS, M. AND STANLEY, P. G.: Abnormal iodinated proteins in two cases of anaplastic carcinoma of the thyroid. *Acta Endocrinol.*, 48 (1965) 513–521; *C.A.*, 63 (1965) 2203c.

KHARLAMOV, V. T. AND SHUBNYAKOVA, L. P.: (The use of paper chromatography for the determination of the decomposition of methionine-S^{35} during storage.) *Metody Analiza Radioaktivn. Preparatov Sb. Statei*, (1965) 58–67; *C.A.*, 63 (1965) 10201f.

SHIMAOKA, K. AND JASANI, B. M.: The application of two-dimensional PC and low-level counting to the study of triiodothyronine in plasma. *J. Endocrinol.*, 32 (1965) 59–64; *C.A.*, 63 (1965) 2074h.

SZANTAI, I., URAY, Z. AND KOVACS, V.: (Isotopic exchange between iodinated amino-acids and ^{131}I on chromatographic paper.) *Studii Cercetari Biochim.*, 8, No. 1 (1965) 81–83; *C.A.*, 63 (1965) 10302e.

35. MISCELLANEOUS COMPOUNDS AND COMPLEX MIXTURES

DERGE, K.: (Gas chromatographic analysis of aromatic substances.) *Riechstoffe Aromen*, 13 (1963) 89–90, 92; *C.A.*, 63 (1965) 6296f.

GYÖRGY, P., MURATA, K. AND IKEHATA, H.; Antioxidants isolated from fermented soybeans (tempeh). *Nature*, 203 (1964) 870–872.

Thin-layer Chromatography

1. REVIEWS AND BOOKS

GRUENE, A.: (Thin-layer chromatography on cellulose plates.) *Riechstoffe, Aromen, Koerperpflegemittel*, 15 (1965) 173, 175, 178; *C.A.*, 63 (1965) 6295h.

JURRIENS, G.: (Column and thin-layer chromatography of organic compounds, with $AgNO_3$ impregnated adsorbents.) *Riv. Ital. Sostanze Grasse*, 42, No. 3 (1965) 116–121; *C.A.*, 63 (1965) 7627c.

OPIENSKA-BLAUTH, J., KRACZKOWSKI, H. AND BRZUSZKIEWICZ, H.: (Thin-layer chromatography.) *Postepy Biochem.*, 11 (1965) 212–224; *C.A.*, 63 (1965) 6295g.

PARIS, M.: (TLC and application to the clinical biology laboratory.) *Pharmacien Biologiste*, 4, No. 37 (1965) 31–36; *C.A.*, 63 (1965) 13683b.

TRUTER, E. V.: The techniques of laminar chromatography. In J. C. GIDDINGS AND R. A. KELLER (Editors): *Advances in Chromatography*, Vol. I, Dekker, New York, 1965, pp. 113–150.

3. TECHNIQUES I

BACON, M. F.: A sample applicator for preparative layer chromatography. *Chem. Ind. (London)*, (1965) 1692–1693.

LIEU, V. T., FREI, R. W., FRODYMA, M. M. AND FUKUI, I. T.: A rapid method for the determination of substances resolved on thin-layer plates. *Anal. Chim. Acta*, 33 (1965) 639–647.

7. PHENOLS

BALOGH, B. AND ANDERSON, A. B.: Chemistry of the genus *Sequoia*. II. Isolation of sequirins, new phenolic compounds from the coast redwood (*Sequoia sempervirens*). *Phytochemistry*, 4 (1965) 569–575; *C.A.*, 63 (1965) 13620a — TLC and PC.

CREAVEN, P. J., PARKE, D. V. AND WILLIAMS, R. T.: A fluorimetric study of the hydroxylation of biphenyl *in vitro* by liver preparations of various species. *Biochem. J.*, 96 (1965) 879–885.

DYATLOVITSKAYA, F. G. AND MAKTAZ, E. D.: (Separate determination of volatile phenols in water using thin-layer chromatography.) *Gigiena i Sanit.*, 30, No. 6 (1965) 60–64; *C.A.*, 63 (1965) 6718a.

GROVER, P. L. AND SIMS, P.: The metabolism of γ-2,3,4,5,6-pentachlorocyclohex-1-ene and γ-hexachlorocyclohexane in rats. *Biochem. J.*, 96 (1965) 521–525.

PIFFERI, P. G.: (TLC on Silica Gel G of some phenolcarboxylic acids.) *Vitis*, 5, No. 1 (1965) 24–26; *C.A.*, 63 (1965) 7333b.

8. SUBSTANCES CONTAINING HETEROCYCLIC OXYGEN

ABERHART, D. J., CHEN, Y. S., DE MAYO, P. AND STOTHERS, J. B.: N.M.R. studies. VII. Mold metabolites. 4. Isolation and constitution of some ergot pigments. *Tetrahedron*, 21 (1965) 1417–1432; *C.A.*, 63 (1965) 8300d.

DEIBNER, R., BOURZEIX, M. AND CABIBEL-HUGUES, M.: (Separation of diglucoside anthocyanins by TLC and their spectrophotometric estimation.) *Ann. Technol. Agr.*, 13, (1964) 359–375; *C.A.*, 63 (1965) 2355h.

KUZNETSOVA G. A. AND KUZ'MINA, L. V.: (Identification of natural coumarins and furocoumarins by TLC.) *Rast. Resursy*, 1, No. 1 (1965) 149–151; *C.A.*, 63 (1965) 4662a.

SAAYMAN, H. M. AND ROUX, D. G.: Configuration of guibourtacacidin, and synthesis of isomeric racemates. *Biochem. J.*, 96 (1965) 36–42.

WHITTLE, K. J., DUNPHY, P. J. AND PENNOCK, J. F.: Plastochromanol in the leaves of *Hevea brasiliensis*. *Biochem. J.*, 96 (1965) 17C–19C — TLC and PC.

9. OXO COMPOUNDS

EL-DAKHAKHNY, M.:(Dimerization of thymoquinone and a side reaction in the preparation of its 2,4-dinitrophenylhydrazone.) *Ann. Chem.*, 685 (1965) 134–139; *C.A.*, 63 (1965) 8273b.

NATORI, S., SATO, F. AND UDAGAWA, S.: Anthraquinone metabolites of *Talaromyces avellaneus* and *Preussia multispora*. *Chem. Pharm. Bull. (Tokyo)*, 13 (1965) 385–386; *C.A.*, 63 (1965) 2046b — TLC and PC.

PACKTER, N. M.: Studies on the biosynthesis of quinones in fungi. Incorporation of 6-methylsalicylic acid into fumigatin and related compounds in *Aspergillus fumigatus* I.M.I.89353. *Biochem. J.*, 97 (1965) 321–332.

REIMANN, E. M.: Syn-anti isomerism in unsymmetrically substituted 2,4-dinitrophenylhydrazone benzophenones. II. *Proc. S. Dakota Acad. Sci.*, 43, No. 22 (1964) 170–175; *C.A.*, 63 (1965) 8165d.

SCHWARTZ, D. P., KEENEY, M. AND PARKS, O. W.: Thin-layer chromatography of carbonyl compounds. Separation of (aliphatic) dicarbonyl bis-(2,4-dinitrophenylhydrazones) into classes. *Microchem. J.*, 8 (1964) 176–179; *Anal. Abstr.*, 12 (1965) 5218.

SHEVCHENKO, Z. A. AND FAVORSKAYA, I. A.: (Thin-layer chromatography of 2,4-dinitrophenylhydrazones of isomeric ketones.) *Vestn. Leningr. Univ., Ser. Fiz. Khim.*, No. 10 (1964) 107–112; *Anal. Abstr.*, 12 (1965) 3956.

TSCHETTER, L.: Syn-anti isomerism in unsymmetrically substituted 2,4-dinitrophenylhydrazone benzophenones. I. *Proc. S. Dakota Acad. Sci.*, 43, No. 22 (1964) 165–169; *C.A.*, 63 (1965) 8165b.

10. CARBOHYDRATES

BECKER, M. J. AND SCHEFNER, A. M.; Thin-layer and paper chromatographic analyses of the carbohydrates in the cell wall of *Chlorella pyrenoidosa* 7-11-05. *Nature*, 202 (1964) 803–804 — TLC and PC.

11. ORGANIC ACIDS AND LIPIDS

11a. Organic acids

ADAMS, J. B.: Linkage of carbohydrates to hydroxyamino acids in mucopolysaccharides and mucoproteins. *Biochem. J.*, 97 (1965) 345–352 — TLC and PC.

BERGELSON, L. D., DYATLOVSKAYA, E. V. AND VORONKOVA, V. V.: (Eine Methode zur dünnschichtchromatographischen Bestimmung von Methylestern einfach ungesättigter Fettsäuren von C_{16}—C_{26}). *Izv. Akad. Nauk SSSR, Otd. Khim. Nauk*, (1965) 46–51; from *Z. Anal. Chem.*, 215 (1965) 132.

CLIFFORD, D. R. AND WOODCOCK, D.: Metabolism of phenoxyacetic acid by *Aspergillus niger* van Tiegh. *Nature*, 203 (1964) 763.

COVELLO, M. AND SCHETTINO, O.: (Application of thin-layer chromatography to the identification of antifermentatives in foods. II.) *Riv. Ital. Sostanze Grasse*, 41, No. 2 (1964) 75–78; *Anal. Abstr.*, 12 (1965) 3028.

JONES, D. F.: Examination of the gibberellins of *Zea mays* and *Phaseolus multiflorus* using thin-layer chromatography. *Nature*, 202 (1964) 1309–1310.

MIZUGAKI, M., UCHIYAMA, M. AND OKUI, S.: Metabolism of hydroxy fatty acids. V. Metabolic conversion of homoricinoleic and homoricinelaidic acids by *Escherichia coli* K 12. *J. Biochem. (Tokyo)*, 58 (1965) 273–278.

ŚLIWIOK, J. AND KWAPNIEWSKI, Z.: Dünnschicht-Chromatographie höherer Fettsäuren. *Mikrochim. Acta*, (1965) 657–659 — TLC and PC..

YUFERA, E. P., PARAREDA, J. S. AND ALBEROLA, J.: (Detection of citrus juice adulteration. I. Process for détermination of acids in orange juice through TLC.) *Rev. Agroquim. Tecnol. Alimentos*, 3 (1963) 349–356; *C.A.*, 63 (1965) 7578h.

11b. Lipids and their constituents

CLAUSEN, J., LOU, H. O. C., AND ANDERSEN, H.: Phospholipid and glycolipid patterns of infant and fetal brain. TLC studies. *J. Neurochem.*, 12 (1965) 599–606; *C.A.*, 63 (1965) 10399f.

EL-NOCKRASHY, A. S. AND MAHFOUZ, M. M.: (TLC of sardine phospholipids.) *Grasas Aceites (Seville, Spain)*, 16, No. 1 (1965) 5–7; *C.A.*, 63 (1965) 7331f.

HUGHES, B. P. AND FRAIS, F. F.: Muscle phospholipids: Thin-layer chromatographic studies on normal and diseased tissue. *Biochem. J.*, 96 (1965) 6P.

KASAI, N. AND YAMANO, A.: Lipids and endotoxins. TLC of lipid fractions. *Japan. J. Exptl. Med.*, 34 (1964) 329–344; *C.A.*, 63 (1965) 2042h.

LEIKOLA, E. AND JUKKA, H.: The structure of the red blood cells. *Farm. Aikakauslehti*, 74, No. 4 (1965) 135–153; *C.A.*, 63 (1965) 2041a.

PALLOTTA, U. AND MATARESE, L.: (Determination of elaidic acid in esterified oils by thin-layer chromatography.) *Riv. Ital. Sostanze Grasse*, 41, No. 4 (1964) 210–212; *Anal. Abstr.*, 12 (1965) 4244.

YAMAMOTO, K.: Study of plasma lipid of retinitis pigmentosa by TLC. *Nippon Ganka Gakkei Zasshi*, 68 (1964) 1619–1620; *C.A.*, 63 (1965) 4801b.

13. STEROIDS

COLLINS, W. P. AND SOMMERVILLE, I. F.: Quantitative determination of progesterone in human plasma by thin-layer and gas–liquid radiochromatography. *Nature*, 203 (1964) 836–839.

DANNENBERG, H. AND GROSS, H. J.: (Dehydrogenation of steroids. X. Bamford-Stevens reaction of 1,4-dien-3-one steroid tolylsulfonylhydrazones.) *Tetrahedron*, 21 (1965) 1611–1617; *C.A.*, 63 (1965) 8445g.

IBAYASHI, H., NAKAMURA, M., MURAKAWA, S., UCHIKAWA, T., TANIOKA, T. AND NAKAO, K.: Determination of urinary testosterone using thin-layer chromatography and gas chromatography. *Steroids*, 3 (1964) 559–568; *Anal. Abstr.*, 12 (1965) 5370.

KAZUNO, T., BETSUKI, S., TANAKA, Y. AND HOSHITA, T.: Stero-bile acids and bile alcohols. LXXV. Studies on bile of *Rana nigromaculata*. *J. Biochem. (Tokyo)*, 58 (1965) 243–247.

RITTER, F. J.: Light-induced oxidation and halogenation of Δ^4-3-ketosteroids in halogenated solvents. *Nature*, 202 (1964) 694–695.

SUZUKI, H.: TLC of steroids. Application of TLC to the separation and quantitative analysis of individual free corticoids and the tetrahydro compounds in urinary extracts and adrenal vein blood of dogs. *Nippon Naibumpi Gakkai Zasshi*, 40 (1965) 1358–1363; *C.A.*, 63 (1965) 2071c.

VLASSAK, W. AND WILLEMS, G.: (Rapid separation of α-ketosteroids by TLC.) *J. Pharm. Belg.*, 19, No. 3–4 (1964) 195–199; *C.A.*, 63 (1965) 7287d.

15. TERPENE DERIVATIVES

VAGUJFALVI, D. AND TYIHAK, E.: (Reagents for thin-layer chromatography. I. Reagents for thin-layer chromatography of essential oils.) *Herba Hung.*, 2 (1963) 361–382; *C.A.*, 63 (1965) 6781b.

17. AMINES, AMIDES AND RELATED NITROGEN COMPOUNDS

CHOULIS, N. H.: Quantitative TLC of noradrenaline. *Chim. Chronika (Athens, Greece)*, 30, No. 4 (1965) 52–54; *C.A.*, 63 (1965) 13641d.

KUEHL, JR., F. A., HICHENS, M., ORMOND, R. E., MEISINGER, M. A. P., GALE, P. H., CIRILLO, V. J. AND BRINK, N. G.: *para*-O-Methylation of dopamine in schizophrenic and normal individuals. *Nature*, 203 (1964) 154–155.

SULLIVAN, G. AND BRADY, L. R.: Thin-layer chromatographic separation of betaine, choline, and muscarine. *Lloydia*, 28 (1965) 68–70; *C.A.*, 63 (1965) 8180g.

18. AMINO ACIDS

CHIARI, D., RÖHR, M. UND WIDTMANN, G.: Die Anwendung von Zellulosepulvern zur dünnschicht-chromatographischen Trennung von Aminosäuren. *Mikrochim. Acta*, (1965) 669–673.

HEATHCOTE, J. G. AND JONES, K.: The rapid resolution of complex mixtures of the naturally occurring amino acids by two-dimensional thin-layer chromatography on cellulose powder. *Biochem. J.*, 97 (1965) 15P — TLC and PC.

PEDERSEN, B. N.: Mapping of the free amino acids in the cells of bacteria from genus *Bacillus* by TLC. *Kgl. Vet. Land-Bohöjskole, Åreskr.*, (1965) 144–152; *C.A.*, 63 (1965) 4683a.

PONDER, E. AND PONDER, R. V.: Chromatographic identification of lysins in normal tissues and tumours of mice. *Nature*, 204 (1964) 995.

ROSSETTI, V.: (A multiple spraying method for TLC of amino acids.) *Biochim. Appl.*, 11 (1964) 225–230; *C.A.*, 63 (1965) 4633h.

WALEY, S. G.: Structural studies of α-crystallin. *Biochem. J.*, 96 (1965) 722–728 — TLC and PC.

WEISER, M., HASITSCHKA, P. AND STOECKL, W.: (TLC demonstration of free amino acids in the blood of the horse, ox, pig and hen.) *Zentr. Vetarinaermed., Reihe A*, 12 (1965) 395–399; *C.A.*, 63 (1965) 7229g.

21. PURINES, PYRIMIDINES, NUCLEOSIDES, NUCLEOTIDES, NUCLEIC ACIDS, BARBITURATES

BOGAN, J., RENTOUL, E. AND SMITH, H.: Detection of barbiturates and related drugs by thin-layer chromatography. *Forensic Sci. Soc. J.*, 4 (1964) 147–154; *Anal. Abstr.*, 12 (1965) 3418 — R_F values of 29 barbiturates.

HAWRYLYSHYN, M., SENKOWSKI, B. Z. AND WOLLISH, E. G.: Thin-layer chromatography of fluoropyrimidines. *Microchem. J.*, 8 (1964) 15–22; *Anal. Abstr.*, 12 (1965) 2882.

22. ALKALOIDS

JOHNE, S. AND GROEGER, D.: (Synthesis of radioactively labeled compounds. XIII. Synthesis of 9-pegene-1,2,3,10-^{14}C (deoxyvasicine-1,2,3,10-^{14}C).) *Z. Chem.*, 5 (1965) 228–229; *C.A.*, 63 (1965) 8431d.

KAESS, A. AND MATHIS, C.: Essai des préparations pharmaceutiques par la chromatographie fonctionelle sur couche mince. I. Préparations contentant des alcaloïdes du groupe de l'atropine. *Ann. Pharm. Franc.*, 23 (1965) 267–274 — R_F values for 11 alkaloids.

RELYVELD, P.: Dimeric indole alkaloids from *Aspidosperma excelsum*. *Pharm. Weekblad*, 100 (1965) 614–615; *C.A.*, 63 (1965) 7254c.

23. OTHER SUBSTANCES CONTAINING HETEROCYCLIC NITROGEN

HOLTZ, P., STOCK, K. AND WESTERMANN, E.: Formation of tetrahydropapaveroline from dopa mine *in vitro*. *Nature*, 203 (1964) 656–658.

24. ORGANIC SULPHUR COMPOUNDS

KIGER, J. L. AND KIGER, J. G.: Procédés de diagnose differentielle rapide et en série des dérivés usuels de la phénothiazine. *Ann. Pharm. Franc.*, 23 (1965) 489–500 — R_F values for 32 phenothiazine derivatives.

MARGASIŃSKI, Z., DANIELAK, R., POMAZAŃSKA, T. AND RAFAŁOWSKA, H.: (Separation of phenothiazine derivatives by means of thin-layer chromatography.) *Acta Polon. Pharm.*, 21 (1964) 253–256; *Anal. Abstr.*, 12 (1965) 4821.

NEIDLEIN, R., KLUEGEL, G. AND LEBERT, U.: (Thin-layer chromatographic separation of some oral antidiabetic compounds and sulfonamides.) *Pharm. Ztg.*, No. 20 (1965) 651–652; *C.A.*, 63 (1965) 6788b.

SUZUOKI-ZIRO, NISHIKAWA, K. AND NUMATA, M.: The fate and urinary metabolites of thiamine propyl disulfide in rats. *J. Biochem. (Tokyo)*, 58 (1965) 279–284.

27. VITAMINS

FRASER, D. R. AND KODICEK, E.: Vitamin D esters: their isolation and identification in rat tissues. *Biochem. J.*, 96 (1965) 59P–60P — 1st dimension on $AgNO_3$ impregnated silica gel, 2nd dimension reversed phase partition.

HAYASHI, M. AND KAMIKUBO, T.: TLC of vitamin B_{12}. *Bitamin*, 31 (1965) 362–368; *C.A.*, 63 (1965) 2103d.

MCKENNA, M., HENNINGER, M. D. AND CRANE, F. L.: A second naphthoquinone in spinach chloroplasts. *Nature*, 203 (1964) 524–525.

OSNITSKAYA, L. K., THRELFALL, D. R. AND GOODWIN, T. W.: Ubiquinone-40 and vitamin K_2 (40) in *Chromatium vinosum*. *Nature*, 204 (1964) 80–81 — silica gel impregnated with rhodamine.

RAO, M. K. G., RAO, S. V. AND ACHAYA, K. T.: Separation and estimation of tocopherols in vegetable oils by TLC. *J. Sci. Food Agr.*, 16, No. 3 (1965) 121–124; *C.A.*, 63 (1965) 4868a.

STONE, K. J. AND HEMMING, F. W.: The incorporation of (2-^{14}C, 4*R*-$^{3}H_1$) mevalonate and (2-^{14}C, 4*S*-$^{3}H_1$) mevalonate into squalene, ubiquinone and ergosterol of *Aspergillus fumigatus* Fresenius. *Biochem. J.*, 96 (1965) 14C–17C — TLC and PC.

28. ANTIBIOTICS

CIFERRI, O., ALBERTINI, A. AND CASSANI, G.: Origin of the sarcosine molecules of actinomycins. *Biochem. J.*, 96 (1965) 853–861.

29. INSECTIDES AND OTHER PESTICIDES

EL-REFAL, A. AND HOPKINS, T. L.: TLC and cholinesterase detection of several phosphorothiono insecticides and their oxygen analogs. *J. Agr. Food Chem.*, 13 (1965) 477–481; *C.A.*, 63 (1965) 13968d.

LIEBMANN, R. AND SCHUHMANN, H.: (Identification of chloropropham and *m*-chloroaniline by thin-layer chromatography.) *Chem. Tech. (Berlin)*, 16 (1964) 267–268; *Anal. Abstr.*, 12 (1965) 4851.

30. SYNTHETIC AND NATURAL DYES

BUNT, J. S.: Analysis of algal pigments by thin-layer chromatography. *Nature*, 203 (1964) 1261–1263.

ITO, Y., FUJII, T., NOZAWA, Y. AND TAKEYAMA, A.: TLC of the pigments isolated from some *Trichophyton* species. *J. Invest. Dermatol.*, 44 (1965) 345–347; *C.A.*, 63 (1965) 2047c.

SMITH, L. W., BREIDENBACH, R. W. AND RUBENSTEIN, D.: TLC of plant pigments on mannitol or sucrose. *Science*, 148 (1965) 508–509; *C.A.*, 63 (1965) 2047h.

STELZER, H.: (Identification of synthetic coloring in paprika.) *Nutr. Bromatol. Toxicol.*, 2, No. 4 (1963) 177–179; *C.A.*, 63 (1965) 10583f.

32. PHARMACEUTICAL AND FORENSIC APPLICATIONS; METABOLISM OF DRUGS

FRESEN, J. A.: (Thin-layer chromatography of drug mixtures fractionated according to MUEHLEMANN and BUERGIN.) *Pharm. Weekblad*, 100 (1965) 532–536; *C.A.*, 63 (1965) 6783f.

MAROZZI, E. AND FALZI, G.: (TLC in forensic toxicology. Identification of non-volatile toxic organic substances.) *Farmaco (Pavia)., Ed. Prat.*, 20 (1965) 302–314; *C.A.*, 63 (1965) 7331d.

PARIS, R., ROUSSELET, R., PARIS, M. AND FRIES, J.: La chromatographie en couche mince sur film: application aux alcaloïdes et aux drogues à alcaloïdes, aux flavonoïdes et aux anthocyanes. *Ann. Pharm. Franc.*, 23 (1965) 473–479 — R_F values for 27 alkaloids, for 26 flavonoids, and for 6 anthocyans.

SANDRI-CAVICCHI, G. AND QUAGLIO, M. P.: (Microchemical differentiation of analgesic drugs.) *Boll. Chim. Farm.*, 104 (1965) 300–304; *C.A.*, 63 (1965) 6790d.

TURNER, L. K.: Some application of accelerated TLC in toxicology. *Forensic Sci. Soc. J.*, 5 (1965) 94–96; *C.A.*, 63 (1965) 13681e.

33. INORGANIC SUBSTANCES

GAGLIARDI, E. AND LIKUSSAR, W.: Trennung der Erdalkalien mittels Dünnschichtchromatographie. *Mikrochim. Acta*, (1965) 765–769.

GAGLIARDI, E. AND POKORNY, G.: Trennung der Halogenide und Pseudohalogenide mittels Dünnschichtchromatographie. *Mikrochim. Acta*, (1965) 699–704.

HAMMERSCHMIDT, H. AND MÜLLER, M.: Identification of fillers and coating materials: thin-layer chromatographic method. *Paper Mkr. London*, 147, No. 5 (1964) 46–65; *Anal. Abstr.*, 12 (1965) 4659.

POLLARD, F. H., BURTON, K. W. C. AND LYONS, D.: Thin-layer chromatography in inorganic chemistry. *Lab. Pract.*, 13 (1964) 505–511; *Anal. Abstr.*, 12 (1965) 5008 — A review with 31 references.

WYSOCKA, B.: Detection of Hg in biological material by means of TLC. *Dissertationes Pharm.*, 17, No. 1 (1965) 99–102; *C.A.*, 63 (1965) 4635e.

34. RADIOACTIVE AND OTHER ISOTOPIC COMPOUNDS

BOUCKE, G.: (Activity measurements on thin-layer chromatograms.) *Atompraxis*, 11 (1965) 263–267; *C.A.*, 63 (1965) 7629h.

35. MISCELLANEOUS COMPOUNDS AND COMPLEX MIXTURES

DANIELS, D. G. H. AND MARTIN, H. F.: Antioxidants in oats: light-induced isomerization. *Nature*, 203 (1964) 299.

LUECK, E. AND COURTIAL, W.: (TLC separation of the preservatives benzoic acid and sorbic acid). *Deut. Lebensm.-Rundschau*, 61, No. 3 (1965) 78–79; *C.A.*, 63 (1965) 2308c.

Gas Chromatography

1. REVIEWS AND BOOKS

ETTRE, L. S.: Fifth symposium on gas chromatography held in Eastern Germany, May, 1955. *J. Gas Chromatog.*, 4 (1966) 16–19 — a brief review of 55 contributions.

HORNING, E. C. AND VANDENHEUVEL, W. J. A.: La chromatographie en phase gazeuse. Application aux problèmes biochimiques et biologiques. *Chim. Anal.* (Paris), 48 (1966) 12–20 — a review with experimental data of many authors.

LIPSETT, M. B. (Editor): *Gas chromatography of steroids in biological fluids*, Plenum Press, New York, 1965, xix + 315pp. — lectures read at the proceedings of the workshop on gas-liquid chromatography of steroids in biological fluids, February 25–27, 1964, Warrenton, Virginia.

LITTLEWOOD, A. B.: Informal symposium of the gas chromatography discussion group. *J. Gas Chromatog.*, 4 (1966) 32–36 — detailed review of 5 papers read on September 24, 1965, at Leeds University.

MATTICK, L. R. AND SZYMANSKI, H. A. (Editors): *Lectures on gas chromatography–1964. Agricultural and biological applications*, Plenum Press, New York, 1964, 256 pp. — a series of lectures given at the 1964 Cornell University Pesticide Workshop and the 1964 Canisius College Gas Chromatography Institute.

PERRY, J. A.: Chicago gas chromatography discussion group. *J. Gas Chromatog.*, 4, No. 1 (1966) 16A-20A — brief report on 14 papers read at the fifth annual spring meeting, June 16–17, 1965.

ROCHICCIOLI, C.: Progrès récents dans la micro-analyse des gaz par absorption infrarouge. *Chim. Anal.* (*Paris*), 48 (1966) 3–11 — coupling of the method with GC is mentioned.

2. FUNDAMENTALS, THEORY AND GENERAL

2b. Gas–solid systems

GRUBNER, O., RÁLEK, M. AND ZIKÁNOVÁ, A.: Calculation of the mass-transfer coefficients by means of a more exact theory of gas–solid chromatography. I. Comparison of columns charged with glass spheres and materials of high internal porosity. *Collection Czech. Chem. Commun.*, 31 (1966) 852–861 — application of statistical moments as the characteristic properties of a peak; experiments support the theory (see E. KUČERA, *J. Chromatog.*, 19 (1965) 237).

KING, JR., J. AND BENSON, S.W.: Electrostatic interactions in gas–solid chromatography. *Anal. Chem.*, 38 (1966) 261–265 — the retention times of rare gases and methane in γ-Al_2O_3 can be calculated because of the linear relationship between retention times and polarizabilities.

SCOTT, C. G.: Contemporary gas–solid chromatography. *J. Gas Chromatog.*, 4 (1966) 4–7 — a comparison of retentions in gas–solid and gas–liquid chromatography systems.

2c. Thermodynamics and theoretical relationships

GOLAY, M. J. E.: Study of some of the parameters involved in split-stream chromatographic injection. *Nature*, 209 (1966) 502 — equations constitute two essential constraints on a mixing tube, giving as they do an upper bound on its cross-section and a lower bound on its length.

KNOX, J. H.: Evidence for turbulence and coupling in chromatographic columns. *Anal. Chem.*, 38 (1966) 253–261 — the column–particle diameter ratio ρ influences the peak broadening in the mobile phase: at reduced velocities in excess of 20, trans-column processes are the main contributors to this; turbulence occurs in GC columns at high velocities and Reynolds numbers in excess of about 3.

MARTIRE, D. E.: Solute adsorption at the gas–liquid interface in gas–liquid chromatography. *Anal. Chem.*, 38 (1966) 244–250 — solute adsorption at the gas–liquid interface affects the retention data significantly; $V_{Rg}^{\circ} = k_s V_L + k_a A_L$; standard substances for Kováts indexing system differ in units for n-paraffins and in tens of units for derivatives at a coating of 1–12% by weight.

PRETORIUS, V. AND SMUTS, T. W.: Turbulent flow chromatograph: A new approach to faster analysis. *Anal. Chem.*, 38 (1966) 274–281 — in gas chromatography turbulent flow can lead to analysis times about one tenth of those obtained by using laminar flow, in liquid chromatography it can shorten analysis times by as much as a factor of 10^4.

2d. General

CHOVIN, P. AND LEBBE, J.: Characterization of the polarity of stationary phases used in gas chromatography. *J. Gas Chromatog.*, 4 (1966) 37–41 — a criticism of statements by LAZARE and ROUMAZEILLES (at the meeting of the Société Chimique de France, Oct. 23rd, 1964) that the product of the relative retention α of two homologous n-paraffins and of the column temperature T is a constant.

EVANS, M. B.: Retention indices of solutes on squalane, dinonyl phthalate, and polyethylene glycol 400. *J. Gas Chromatog.*, 4 (1966) 1–3 — 38 C_5–C_8 hydrocarbons on squalane at 22°, 30°, 40°, 55°, 60°, 70°, 80° and 100°, 15 C_5—C_8 hydrocarbons on DNP at 60°, 80° and 100°, 48 compounds on PEG 400 at 60°, 80°, and 100°.

HALÁSZ, I.: A simple approach to the rate theory of chromatography. *J. Gas Chromatog.*, 4 (1966) 8–11 — some nomenclature problems are discussed.

SEHER, A. AND KÜHNAST, R.: Die quantitative Auswertung von Gas-Chromatogrammen, IV. Beziehung zwischen Retentionszeit und Peakfläche. *Fette-Seifen-Anstrichmittel*, 67 (1965) 754–762.

3. TECHNIQUES I

3a. Detectors

BATT, L. AND CRUICKSHANK, F. R.: Irreproducibility in the quantitative calibration of a flame ionisation detector. *J. Chromatog.*, 21 (1966) 296–300.

BRODY, S. S. AND CHANEY, J. E.: Flame photometric detector. The application of a specific detector for phosphorus and for sulfur compounds – selective to subnanogram quantities. *J. Gas Chromatog.*, 4 (1966) 42–46 — P-emission at 526 mμ and S-emission at 394 mμ; minimum sensitivity of malathion and parathion was 0.25 ng; response linearity 6 p.p.b. to 60 p.p.m.

CHAIGNEAU, M.: État actuel de la spectrométrie de masse. *Chim. Anal. (Paris)*, 47 (1965) 619–626 — three schemes for coupling mass spectrometric and GC analyzers.

HENNEBERG, D. AND SCHOMBURG, G.: Ein kleines Massenspektrometer als spezifischer gaschromatographischer Detektor. *Z. Anal. Chem.*, 215 (1966) 424–430 — coupling with capillary GC; applied for lead alkyls at *m/e* 207 and for trimethylsilyl ethers at *m/e* 75.

HUGHES, D. E. P.: Flame ionization detector for gas chromatography. *J. Chem. Educ.*, 42 (1965) 450.

LUNDIN, R. E., ELSKEN, R. H., FLATH, R. A., HENDERSON, N., MON. T. R. AND TERANISHI, R.: Time-averaged proton magnetic resonance analysis of micro samples from open-tube gas chromatographs. *Anal. Chem.*, 38 (1966) 291–293 — single eluent containing 0.3 μmole of hydrogen from capillary column is trapped with a microcell and analysed with time averaging the output of an analytical 60-Mc. per sec spectrometer over a period of approximately one day.

3b. Column performance and filling studies

ALTENAU, A. G.: New adsorbents for gas chromatography. *Dissertation Abstr.*, 25 (1964) 787–788.

DEWAR, M. J. S. AND SCHROEDER, J. P.: *p*-Alkoxy- and *p*-carbalkoxybenzoates of diphenols. A new series of liquid crystalline compounds. *J. Org. Chem.*, 30 (1965) 2296–2300 — 10 of 17 liquid crystalline *p*-phenylene and *p,p'*-biphenylene esters of *p*-alkoxy- and *p*-carbalkoxybenzoic acids exhibit very broad mesomorphic ranges and high transition temperatures.

HALÁSZ, I. AND GERLACH, H.-O.: Aerogel columns in gas chromatography. *Anal. Chem.*, 38 (1966) 281–286 — aerogel particles have a size of about 1 μ; columns give values of 0.01 cm for h and 0.035 cm for H at high carrier gas velocities ($h = Lw^2/16\,t_R^2$; $H = Lw^2/16(t_R')^2$).

HOLLIS, O. L.: Separation of gaseous mixtures using porous polyaromatic polymer beads. *Anal. Chem.*, 38 (1966) 309–316 — beads are synthetised from styrene, *tert.*-butylstyrene and ethylvinylbenzene with divinylbenzene as cross-linker by suspension with a diluent to give a highly porous structure; separation of N_2, O_2 and A at –78°; N_2, NO, CO_2, and N_2O at 27°; alcohols, ethers, water, etc. at different temperatures up to 200°.

HUPE, K. P., BÖHNISCH, W. AND QUITT, H.: Temperaturverteilung in gaschromatographischen Säulen bei Anwendung der Temperaturprogrammierung. *Chem. Ing. Tech.*, 37 (1965) 146–151.

MYERS, M. N. AND GIDDINGS, J. C.: High inlet pressure micro column system for use in gas chromatography. *Anal. Chem.*, 38 (1966) 294–297 — plate heights as low as 0.082 mm obtained in columns with 13-μ particles of alumina at 100–150 atm. pressure.

NOVÁK, J. AND JANÁK, J.: Formamide as a stationary phase in gas chromatography. *Anal. Chem.*, 38 (1966) 265–270 — one mole of formamide gives a flame ionisation signal of 0.3 gram atom of paraffinic carbon; the volatility at 40° is lower than that of dimethylformamide at 0°; the selectivity is 3 times higher than on PEG; the C term is 2–5 times lower than that of common stationary phases; no tailing of water or unpolar stationary liquids on untreated support materials.

SCHWARTZ, R. D., BRASSEAUX, D. J. AND MATHEWS, R. G.: High-resolution capillary adsorption columns for gas chromatography. *Anal. Chem.*, 38 (1966) 303–306 — stainless steel tubes are coated with colloidal sols containing hydrophobic silica; application to C_5–C_8 hydrocarbons.

URONE, P. AND PARCHER, J. F.: Support effects on retention volumes in gas chromatography. *Anal. Chem.*, 38 (1966) 270–274 — retention minima at 0.6% and maxima at 2% of liquid phase were observed for the solutes on squalane-coated surface-active solids.

WAKSMUNDZKI, A., SUPRYNOWICZ, Z. AND MEIDZIAK, I.: The effect of amount of binary fixed phase on the adsorption properties of support in gas chromatography. *J. Gas Chromatog.*, 4 (1966) 74–76.

WALKER, J. Q. AND JAGIELSKI, B. P.: Dual chromatography offers many uses. *Hydrocarbon Process. Petrol. Refiner*, 44 (1965) 171–172.

3c. Apparatus, accessories and materials for GC

ALPERSTEIN, M. AND BRADOW, R. L.: A gas sampling and injection valve for vacuum service. *Anal. Chem.*, 38 (1966) 366–367 — capability of leak free operation with collection volume pressures of 10^{-4} torr.

FERRIN, C. R.: Connection between chromatographic column and valve system. *U.S. Pat.*, 3,198,001 (August 3, 1965).

FRISONE, G. J.: A simple and precise soap-bubble flow meter. *Chemist-Analyst*, 54 (1965) 56.

GROCHOVSKA, H.: (Laboratory elutriation columns for fractionation of grains of solid substances). *Przemysl Chem.*, 45 (1966) 38–40 — useful for fractionation of crushed GC support materials.

HAAHTI, E., NIKKARI, T. AND KÄRKKÄINEN, J.: Design and operation of circulating chain detector for liquid chromatography. *J. Gas Chromatog.*, 4 (1966) 12–15 — construction and application details are given.

JENTZSCH, D. AND WEBER, H.: Gas chromatography valving apparatus. *U.S. Pat.*, 3,203,249 (August 31, 1965) — four-way valve combines the functions of a by-pass and a backflush valve.

NOREM, S. D. AND HILL, H. I.: Chromatography apparatus. *U.S.Pat.*, 3,199,274 (Aug. 10, 1965).

PRESCOTT, B. O. AND WISE, H. L.: Clean gases for gas chromatography. *J. Gas Chromatog.*, 4 (1966) 80–84 — accessories and method for cleaning of gas for flame ionization detector.

ROGER, L.: L'appareillage de Laboratoire au 7e Salon International de la Chimie, 21 avril – 2 mai 1965. *Chim. Anal. (Paris)*, 48 (1966) 32–38 — exhibition data of GC apparatus are given.

STERNBERG, J. C. AND LITLE, R. L.: Electrical discharge pyrolyzer for gas chromatography. *Anal. Chem.*, 38 (1966) 321–330 — solid sample is placed on a porous graphite felt electrode which serves as the downstream electrode in a flow-through tubular discharge chamber; construction schemes and data are given; the literature is not complete.

TĂTARU, E. AND PIRINGER, O.: (Automatic apparatus for the analysis of deuterium and other gas mixtures). *Rev. Chim. (Bucuresti)* 16 (1965) 290–292.

VILLALOBOS, R.: Gas chromatograph. *U.S.Pat.*, 3,201,971 (Aug. 24, 1965) — splitter columns.

VILLALOBOS, R.: Chromatographic method. *U.S.Pat.* 3,201, 922 (Aug. 24, 1965) — for the separation of components with long and short retention times.

WILHITE, W. F.: Developments in micro gas chromatography. *J. Gas Chromatog.*, 4 (1966) 47–50 — constructive details of a space GC apparatus for the gas analysis of the atmosphere of Mars; 1 μl of gas sample; thermistor cell volume 0.1 μl, sensitivity better than 5 p.p.m.; two-column system for CO_2, H_2, A, O_2, N_2.

4. TECHNIQUES II

4a. Preparative-scale GC

BARKER, P. E. AND HUNTINGDON, D. H.: The preparative scale separation of multicomponent mixtures by continuous gas liquid chromatography. *J. Gas Chromatog.*, 4 (1966) 59–66 — a new circular chromatography column is described; complete separation of $(C_2H_5)_2O$ and CH_2Cl_2 in a purity of 97% at a feed rate of 117.2 ml/h; unfortunately, no European literature on the moving bed technique is given (PICHLER, SIMMON, BENEDECK, etc.).

CAREL, A. B. AND PERKINS, JR., G: Gas chromatographic fractionation as a supplement and replacement for laboratory distillation. *Anal. Chim. Acta*, 34 (1966) 83–100 — working properties of a preparative chromatograph for 1–200 ml samples is described; quantitative trapping.

4d. Special techniques

CHER, M. AND HOLLINGSWORTH, C. S.: Repeated gas chromatographic sampling technique in gas phase chemical kinetics. Photolysis of acetone. *Anal. Chem.*, 38 (1966) 353–356 — schema of apparatus is given.

DAVIS, T. C., PETERSEN, J. C. AND HAINES, W. E.: Inverse gas–liquid chromatography. A new approach for studying petroleum asphalts. *Anal. Chem.*, 38 (1966) 241–243 — retention behaviour of test substances clarifies the chemical composition of the asphalt; the principle was used earlier by J. ROMOVÁČEK AND Z. NOVOTNÝ (*Brennstoff-Chem.*, 43 (1962) 118) for the characterisation of the properties of wash oils.

YAMAMOTO, D. AND FUKUDOME, A.: "Sublimation chromatography" of some aromatic amines on montmorillonite clay–cellulose column. *Anal. Chim. Acta*, 34 (1966) 240–243 — a type of frontal to displacement chromatography without carrier gas.

4f. Measurement of physico-chemical and related values

KUGE, Y. AND YOSHIKAWA, Y.: (A new method for the determination of the relative surface area by gas chromatography). *Bull. Chem. Soc. Japan*, 38 (1965) 948–951.

SMOLKOVÁ, E., KRIŠTOFÍKOVÁ, FELTL, L. AND GRUBNER, O.: Determination of the surface of powder substances by the method of thermal desorption using organic vapours as the sorbates. *Collection Czech. Chem. Commun.*, 31 (1966) 450–456 — measuring of ZnO, TiO_2, MgO, Al_2O_3 etc. of 0.1–100 m^2/g with benzene, *n*-hexane and *n*-heptane.

5. HYDROCARBONS AND HALOGEN DERIVATIVES

5b. Other hydrocarbons

CHOVIN, P. AND LEBBE, J.: Dosage des hydrocarbures aromatiques dans l'air. *Cahiers Notes Docum. Inst. Nat. Sécur.*, No. 39 (1965) 63–72 — C_6–C_8 hydrocarbons on bis(β-cyanoethoxy)formamide at 85°.

HŘIVNÁČ, M. AND JANÁK, J.: (A contribution to the determination of naphthalene in coal tar wash oils). *Paliva*, 46 (1966) 51–55 — on PEG 400 at 210° with benzonitrile as internal standard.

PURCELL, J. E. AND ETTRE, L. S.: Support-coated open tubular columns. II. Application in trace analysis. *J. Gas Chromatog.*, 4 (1966) 23–27.

RASNYANSKAYA, A. Ya., TARASOV, A. I., TIKHOVA, A. P. AND MUZYCHENKO, V. P.: (Chromatographic determination of the composition of the 40°–140° hydrocarbon fraction of olefins obtained on thermic cracking of paraffin). *Khim. Tekhnol. Topliv i Masel*, No. 1 (1966) 58–61 — retention data of 27 C_4–C_9 hydrocarbons on triester of monochlorhydrin with pentaerythritol and *n*-valeric acid.

YEW, F. F. H. AND MAIR, B. J.: Isolation and identification of C_{13} to C_{17} alkylnaphthalenes, alkylbiphenyls, and alkyldibenzofurans from the 275° to 305° dinuclear aromatic fraction of petroleum. *Anal. Chem.*, 38 (1966) 231–237 — GLC, NMR, U.V. and other methods.

6. ALCOHOLS

DANIELS, D. G. H.: Gas chromatography of long-chain α,ω-diols and related compounds. *J. Chromatog.*, 21 (1966) 305–306 – C_{10}–C_{26} on E-301 at 245°.

ENTENMAN, C., JAEGER, J. AND SAKHEN, R. A.: An improved method for the determination of ethanol in blood by gas chromatography. *Clin. Chem.*, 11 (1965) 806.

HESSEL, D. W. AND MODGLIN, F. R.: The quantitative determination of ethanol and other volatile substances in blood by gas–liquid partition chromatography. *J. Forensic Sci.*, 9 (1964) 255–264.

ŠKRABÁNEK, P. AND NOVÁK, J.: Bestimmung des Blutaethylalkohols mittels der Gaschromatographie. *Blutalkohol*, 3 (1966) 271–277 — after extraction with butyl acetate on polypropylene sebacate at 80°–100° with cyclopentane as internal standard.

8. SUBSTANCES CONTAINING HETEROCYCLIC OXYGEN

VOGEL, J. AND DESHUSSES, J.: Dosage du maltol (méthyl-2-hydroxy-3-pyrane) par chromatographie en phase gazeuse. *Mitt. Gebiete Lebensm. Hyg.*, 56 (1965) 41–45 — on DEG.

9. OXO COMPOUNDS

BOGUSLAVSKAYA, L. S., KARTASHOVA, N. A. AND BARAMZINA, A. N.: (Identification and quantitative estimation of impurities in chloral). *Zh. Prikl. Khim.*, 39 (1966) 232–234 — on silicone elastomer at 135°.

10. CARBOHYDRATES

APPLEGARTH, D. A.: The gas liquid chromatography of carbohydrate derivatives. *Can. Pulp Paper Ind.*, 16, No. 7 (1963) 34–37.

BROWER, H. E., JEFFERY, J. E. AND FOLSOM, M. W.: Gas chromatographic sugar analysis in hydrolyzates of wood constituents. Quantitative determination of mixtures of arabinose, xylose, mannose, galactose, and glucose as their trimethylsilyl ether derivatives. *Anal. Chem.*, 38 (1966) 362–364 — on SE-52 and EGS by PTGC 110°–190°, 2°/min.

CHEMINAT, A. AND BRINI, M.: Séparation par chromatographie en phase gazeuse de quelques monosaccharides. *Bull. Soc. Chim. France*, (1966) 80–83 — retention data of 23 trimethylsilyl derivatives on SE-30 at 180°, Apiezon L at 210° and SE-52 at 170°.

HOROWITZ, M. I. AND DELMAN, M. R.: Gas chromatography of hexosaminitols. *J. Chromatog.*, 21 (1966) 300–302 — retention data of 9 derivatives on butanediol succinate polyester by PTGC 125°–215°, 4°/min.

11. ORGANIC ACIDS AND LIPIDS

11a. Organic acids

CANVIN, D. T.: Analysis of some organic acids by gas–liquid chromatography. *Can. J. Biochem.*, 43 (1965) 1281–1287.

CATTANEO, C., LUCCHESI, M., DERITIS, G. C., PIETROPAOLO, C., ROSSI, P. AND FRISANI, G.: (Gas chromatographic separation of the C_{12}–C_{20} fatty acids in the lipids of mycobacteria). *Ann. Inst. "Carlo Forlanini"*, 24 (1964) 353–361.

HETMAN, N. E., ARLT, JR., H. G., PAYLOR, R. AND FEINLAND, R.: Gas chromatographic analysis of tall oil rosin acids. *J. Am. Oil Chemists' Soc.*, 42 (1965) 255–256.

HORII, Z. I., MAKITA, M., TAKEDA, I., TAMURA, Y. AND OHNISHI, Y.: Gas chromatography of aromatic acids. *Chem. Pharm. Bull. (Tokyo)*, 13 (1965) 636–638.

MÖLLER, K. E.: Neues auf dem Gebiet der Kochschen Carbonsäuresynthese. *Brennstoff-Chem.*, 47 (1966) 10–16 — methyl esters of decene acids on PEG 400 at 80°.

OSTERHAUS, E.: Reinigung von Essigsäure-isoamylester zur Verwendung als Extraktionsmittel für gaschromatographische Untersuchungen. *Z. Anal. Chem.*, 215 (1966) 241–243.

PARKER, P. L. AND LEO, R. F.: Fatty acids in blue-green algal mat communities. *Science*, 148 (1965) 373–374 — C_{12}–C_{18} methyl esters on DEGS.

RUSEVA-ATANASOVA, N. AND JANÁK, J.: Multi-dimensional chromatography using different developing methods. IV. Rational identification of fatty acid esters by means of program med distribution of fractions in two-dimensional (GC–TLC) chromatograms. *J. Chromatog.*, 21 (1966) 207–212 — the gas chromatogram (Apiezon L at 220°, Reoplex 400 at 200°) is eluted on to the logarithmically travelling start line of a thin layer (silica gel coated with $AgNO_3$); spots of esters formed a geometrically additive pattern.

UCCIANI, E., PRETELLI, E. AND NAUDET, M.: Sur la photobromuration des chaines grasses bromées. I. Cas du bromo-9(10)-octadécanoate de méthyle. *Bull. Soc. Chim. France*, (1966) 136–141 — on SE–30 and PEGSucc. at 213°.

VOGEL, J. AND DESHUSSES, J.: Identification des acides déhydroacétique, sorbique, benzoïque, *ortho*-chlorobenzoïque, *para*-chlorobenzoïque, salicylique; des esters méthylique, éthylique, propylique et butylique de l'acide *para*-hydroxybenzoïque par chromatographie en phase gazeuse. *Mitt. Gebiete Lebensm. Hyg.*, 56 (1965) 35–37 — on DEGSucc. at 195°.

WHITE, JR., H. B.: A complementary thin-layer and gas–liquid chromatographic procedure for fatty acid analysis. *J. Chromatog.*, 21 (1966) 213–222 — after TLC group separation GC on LAC-728 by PTGC 100°–210°, 3°/min.

11b. Lipids and their constituents

BOWERS, C, Y., HAMILTON, J. G., MULDREY, J. E., MIYAMASU, W. T., REYNOLDS, G. A. AND SCHALLY, A. V.: The simultaneous determination of both the quantity and the fatty acid composition of the triglycerides in three to ten microliters of plasma. *J. Am. Oil Chemists' Soc.*, 43 (1966) 2–6.

EMKEN, E. A., FRANKEL, E. N. AND BUTTERFIELD, R. O.: Homogeneous catalytic hydrogenation of unsaturated fats: metal acetylacetonates. *J. Am. Oil Chemists' Soc.*, 43 (1966) 14–18.

GRIECO, D. AND PIEPOLI, G.: Composition of the fatty acids contained in the lipids extracted from oil seeds and fruits. *Riv. Ital. Sostanze Grasse*, 41 (1964) 283–287.

MALLARD, T. M. AND CRAIG, B. M.: Quantitative analysis for oleic and petroselinic acids in glycerine oils. *J. Am. Oil Chemists' Soc.*, 43 (1966) 1–2 — on *o*-phthalic ethylene glycol polyester (1,2) by PTGC 160°–225°, 2.25°/min.

13. STEROIDS

CLAUDE, J. R. AND BEAUMONT, J. L.: Les transformations de la molécule de cholestérol *in vitro* – leur importance au cours des analyses chromatographiques. *J. Chromatog.*, 21 (1966) 189–201 — TLC and GC; retention data of 6 cholesterol compounds on SE-52 at 260° and cyanosilicone at 210°.

GOTTFRIED, H.: Gas chromatography of adrenocortical steroids. *J. Endocrinol.*, 31, No. 2 (1965) 22–23.

HEITZMAN, R. J. AND THOMAS, G. H.: The identification by gas chromatography of the eight androstane-3,17-diols and the occurrence of two of these in pregnant cow's urine. *Biochem. J.*, 96, No. 2 (1965) 22P.

HEYNS, W., HENDRIKX, A. AND DE MOOR, P.: (Gas chromatographic determination of 11-deoxy-17-ketosteroids in urine). *Acta Clin. Belg.*, 19 (1964) 346–359.

15. TERPENE DERIVATIVES

HAYASHI, S., YANO, K., YOKOYAMA, N. AND MATSUURA, T.: (Catalytic effect of the support in GLC of terpene compounds). *J. Chem. Soc. Japan*, 85 (1964) 553–557.

HUDGINS, W. R. AND SCHEUER, P. J.: Chemotaxonomic value of some fingerprint gas chromatograms of oils from genus *Pelea*. *Naturwiss.*, 51 (1964) 511–512.

RENWICK, J. A. A., PITMAN, G. B. AND VITÉ, J. P.: Detection of a volatile compound in hind guts of male *Ips confuses* (LeConte) (*Coleoptera*: *Scolytidae*). *Naturwiss.*, 53 (1966) 83–84 — on Apiezon L and DEGSucc. at 110° and 95°.

TERANISHI, R., FLAT, R. H. AND MON, T. R.: Gas chromatography of terpenoid compounds with open tube and spring-packed columns. *J. Gas Chromatog.*, 4 (1966) 77–79 — large-bore open tubular columns, 0.03 in. I.D., 1000 ft., and spring-packed columns, 0.04 in. I.D., 200 ft.; pressure programming yields better resolution with 0.01 in. I.D. and permits lower temperature conditions with 0.03 in. I.D. open tubular columns.

17. AMINES, AMIDES AND RELATED NITROGEN COMPOUNDS

CAPELLA, P. AND HORNING, E. C.: Separation and identification of derivatives of biologic amines by gas–liquid chromatography. *Anal. Chem.*, 38 (1966) 316–321 — on F-60 by PTGC 100°–200°, 1.5°/min.; hydroxyl groups are converted into trimethylsilyl ether groups, and primary amines into eneamines or Schiff bases.

HEYNS, K., STUTE, R. AND WINKLER, J.: Zur gaschromatographischen Trennung von Aminen an Kapillarsäulen. *J. Chromatog.*, 21 (1966) 302–304 — GC separation of amines and pyridines on N,N-bis-hydroxyethyl-trimethylenediamine by PTGC 20°–100°.

JONES, R. M.: Gas chromatographic separation of hydrazine, monomethylhydrazine, and water. *Anal. Chem.*, 38 (1966) 338–340 — on Dowfax 9N9 ($C_9H_{19}\cdot C_6H_4\cdot(O\cdot CH_2\cdot CH_2)_9\cdot OH$) on Teflon-6 at 110°.

18. AMINO ACIDS

BLAU, K. AND DARBRE, A.: Analyse quantitative des acides aminés par chromatographie en phase gazeuse. *Chim. Anal.* (*Paris*), 47 (1965) 631–633 — as trifluoroacetyl derivatives.

21. PURINES, PYRIMIDINES, NUCLEOSIDES, NUCLEOTIDES, NUCLEIC ACIDS, BARBITURATES

MARTIN, H. F. AND DRISCOLL, J. L.: Gas chromatographic identification and determination of barbiturates. *Anal. Chem.*, 38 (1966) 345–346 — in the form of methylated compounds on SE-30 at 175°.

22. ALKALOIDS

ARTUSI, G. C.: (Microdetermination of papaverine by gas chromatography). *Ateneo Parmense*, 34 (1963) 550–554.

23. OTHER SUBSTANCES CONTAINING HETEROCYCLIC NITROGEN

JANÁK, J., HOLÍK, M. AND FERLES, M.: Untersuchungen in der Pyridinreihe. X. Gaschromatographische Trennung einiger Stellungsisomeren der Pyrideinreihe. *Collection Czech. Chem. Commun.*, 31 (1966) 1273–1280 — retention data and Kováts indices of isomeric 1,3-dimethylpiperideines on tris(β-cyanoethoxymethyl)-γ-picoline at 50°–90°.

24. ORGANIC SULPHUR COMPOUNDS

CARRUTHERS, W.: Photolysis of organic sulfides. *Nature*, 209 (1966) 908.

26. METALLO-ORGANIC COMPOUNDS

EISENTRAUT, K. J. AND SIEVERS, R. E.: Volatile rare earth chelates. *J. Am. Chem. Soc.*, 87 (1965) 5254–5256 — trifluoroacetyl acetonates of 15 rare earth elements on Apiezon H on Teflon support at 157° or 185°.

EVANS, C. S. AND JOHNSON, C. M.: The separation of some alkyl selenium compounds by gas chromatography. *J. Chromatog.*, 21 (1966) 202–206 — retention data of C_1–C_3 derivatives on Polymetaphenylether, Carbowax 20M and Silicone Oil DC 550 at many temperatures in the range 35°–175°.

FREIDLINA, R. KH., KUZ'MINA, N. A. AND CHUKOVSKAYA, E. TS.: (Hydride transfer in the reactions of silicon hydrides with vinyl alkyl and other ethers in the presence of pentacarbonyl iron). *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1966) 176–178 — on silicone elastomer NF at 134°.

29. INSECTICIDES AND OTHER PESTICIDES

GIUFFRIDA, L.: Isolation of microquantities of pesticides by gas chromatography for infrared analysis. *J. Assoc. Offic. Agr. Chemists*, 48 (1965) 354–357.

HENKEL, H. G.: Über eine Möglichkeit zur direkten gas-chromatographischen Bestimmung herbizidwirksamer N-Phenylharnstoff-Derivate. *J. Chromatog.*, 21 (1966) 307–311 — on Versamid 900 by PTGC 180°–250°.

KAWAI, S.: (Determination of organic phosphorus insecticides in preparations by gas chromatography). *Japan Analyst*, 14 (1965) 360–363.

31. PLASTICS AND THEIR INTERMEDIATES

GUYOT, A. AND GUILLOT, J.: Application de la chromatographie gazeuse à l'étude cinétique des réactions de copolymérisation. *J. Chim. Phys.*, 61 (1964) 1434–1438.

SCHOLZ, R. G., BEDNARCZYK, J. AND YAMAUCHI, T.: Gas chromatographic analysis of polymers after oxidative degradation. *Anal. Chem.*, 38 (1966) 331–334.

33. INORGANIC SUBSTANCES

33a. Permanent and rare gases

BOYS, F. L.: Dual carrier gas chromatography: Analysis of samples containing hydrogen. *J. Gas Chromatog.*, 4 (1966) 20–22 — the simultaneous injection of portions of a sample into two identical columns, using carrier gases of different thermal conductivities, gives chromatograms with good sensitivity and linearity for all components.

HANIN, M. AND VILLENEUVE, D.: Dosage de l'oxygène et de l'azote dans les aciers par chromatographie en phase gazeuse après fusion réductrice sous gaz neutre. *Chim. Anal. (Paris)*, 47 (1965) 634–642 — CO, H_2, N_2; schemes and designs are given.

33b. Volatile inorganic compounds

SIEVERS, R. E., WHEELER, JR., G. AND ROSS, W. D.: Microanalysis of titanium by gas chromatography. *Anal. Chem.*, 38 (1966) 306–309 — TiO_2 is converted by CCl_4 into $TiCl_4$ in a microreactor at 450° directly in the carrier gas stream; deviation 1.1% rel.

34. RADIOACTIVE AND OTHER ISOTOPIC COMPOUNDS

BRUNER, F., CARTONI, G. P. AND LIBERTI, A.: Gas chromatography of isotopic molecules on open tubular columns. *Anal. Chem.*, 38 (1966) 298–303 — separation of methane, ethane, ethylene, propane and their deuteroanalogues and isotopic oxygen (masses 16 and 18) at very low temperatures; in most cases the heavier isotope is eluted before the lighter.

WHITE, E. R. AND DAVIS, H. G.: Detection of volatile carbon-14-labeled compounds by a modified gas chromatography–ionization chamber technique. *Anal. Chim. Acta*, 34 (1966) 105–107 — oxidation products of propylene-3-^{14}C.

35. MISCELLANEOUS COMPOUNDS AND COMPLEX MIXTURES

AHRENDT-LARSEN, B. AND HASEN, H. L.: Gas chromatographic investigations of the flavor stability of beer. *Wallerstein Lab. Commun.*, 27, No. 92 (1964) 41–48.

BAYER, E.: Quality and flavor by gas chromatography. *J. Gas Chromatog.*, 4 (1966) 67–73 — wine, rum and brandy flavours have been correlated with the quality of the products.

BRODERICK, J.: Techniques to supplement gas liquid chromatography. *J. Am. Perfumer Cosmet.*, 80, No. 2 (1965) 39–48.

CAMPBELL, M. H.: Gas chromatographic analysis of solvent used in reactor fuel reprocessing and fission product recovery. *Anal. Chem.*, 38 (1966) 237–240 — retention data of 13 compounds of different chemical structure (alkyl phosphates, phosphonates, amines, hydrocarbons) on Apiezon N at 300°.

GIL-AV, E., CHARLES-SINGLER, R., FISCHER, G. AND NUROK, D.: Resolution of optical isomers by gas liquid partition chromatography. *J. Gas Chromatog.*, 4 (1966) 51–58 — retention data of 19 C_4–C_{19} alkanes as α-alkanoyl oxypropionates on squalane, PPG and FS-1265, and of 12 amino acids as N-TFA esters of 2-*n*-butanol and/or 2-*n*-octanol on PPG, butanediol succinate and trifluoropropyl methyl polysiloxane by capillary GC at 120°–160°.

HRUTFIEORD, B. F.: Lignin degradation product analysis by gas chromatography. *Tappi*, 48 (1965) 48–54.

KOMAROV, P. N.: (Oxidation of *p*-xylene initiated by γ-radiation). *Zh. Fiz. Khim.*, 40 (1966) 243–247 — at 125°.

REYMOND, D., MUEGGLER-CHAVAN, F., VIANI, R., VUATAZ, L. AND EGLI, R. H.: Gas chromatographic analysis of stream volatile aroma constituents: Application to coffee, tea and cocoa aromas. *J. Gas Chromatog.*, 4 (1966) 28–31 — analysis of air surrounding solution shows enrichment of some classes of aroma constituents due to the low solubility coefficients; many Kovats indices are given.

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CHROMATOGRAPHIC DATA

SUPPLEMENT TO THE
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TABLE 1

TLC R_F VALUES OF SOME AMINES

(R. GNEHM, H. U. REICH AND P. GUYER, *Chimia (Aarau)*, 19 (1965) 587)

Thin layer: Silica gel G.
Solvent: Chloroform–methanol–17% ammonia (2:2:1).
Detection: 0.5% potassium permanganate and 0.5% potassium persulphate.

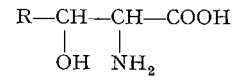
Compound	R_F
Triethylenetetramine	0.05
Diethylenetriamine	0.10
Ethylenediamine	0.17
Piperazine	0.21
N-Methyl-ethylenediamine	0.21
N-Aminoethyl-piperazine	0.23
N-(β-Hydroxyethyl)-ethylenediamine	0.28
N-Methyl-monoethanolamine	0.29
Monoethanolamine	0.34
2-Methyl-piperazine	0.43
Diethanolamine	0.47
N-Methyl-(β-hydroxyethyl)-ethylenediamine	0.50
N-Hydroxyethyl-piperazine	0.52
2-(2-Aminoethoxy)-ethanol	0.54
cis-2,5-Dimethyl-piperazine	0.54
N,N′-Bisaminoethyl-piperazine	0.58
Triethanolamine	0.59
N-Methyl-piperazine	0.62
Ethylene glycol	0.63
N-Methyl-diethanolamine	0.65
Triethylenediamine	0.68
N,N′-Bishydroxyethyl-piperazine	0.70
Diethylene glycol	0.70
trans-2,5-Dimethyl-piperazine	0.71
N-Ethyl-piperazine	0.75
Hexamethylene tetramine	0.78
Morpholine	0.81
N,N′-Dimethyl-piperazine	0.92
N,N′-Diethyl-piperazine	0.96

TABLE 2

PC R_F VALUES OF β-HYDROXY-α-AMINO ACIDS

(H. GEIPEL, J. GLOEDE, K.-P. HILGETAG AND H. GROSS, *Chem. Ber.*, 98 (1965) 1680)

Solvents: S_1 = Butanol–acetic acid–water (4:1:1).
S_2 = Butanol–methyl ethyl ketone–water–ammonia (5:3:1:1).
Detection: Ninhydrin.



Compound	*R*	R_F		
		S_1	S_2 *allo*	S_2 *threo*
Threonine	CH_3	0.28	0.11	0.18
β-Hydroxynorvaline	C_2H_5	0.39	0.29	0.36
β-Hydroxynorleucine	C_3H_7	0.50	0.40	0.39
α-Amino-β-hydroxyoenanthic acid	C_4H_9	0.63	0.52	0.60
α-Amino-β-hydroxycaprylic acid	C_5H_{11}	0.74	0.65	0.72
α-Amino-β-hydroxypelargonic acid	C_6H_{13}	0.77	0.74	0.80
α-Amino-β-hydroxycapric acid	C_7H_{15}	0.81	0.81	0.86
α-Amino-β-hydroxyundecanoic acid	C_8H_{17}	0.86	0.87	0.91

TABLE 3

PC R_F VALUES OF 22 AMINO ACIDS

(G. BARBIROLI, *Mikrochim. Ichnoanal. Acta*, (1965) 655)

Paper: Whatman No. 2.
Development: Circular.
Solvent: Phenol–*n*-butanol–methyl ethyl ketone–propionic acid–acetic acid–water (20:20:50:10:10:20).
Detection: Ninhydrin (0.5 g ninhydrin in 5 ml glacial acetic acid and 95 ml acetone); after dipping heating to 105° for 20 min.

Amino acid	R_F	*Detection limit* (μg)
Cystine + cysteine	0.13	1.0
Histidine	0.16	1.0
Lysine	0.19	1.0
Aspartic acid + asparagine	0.22	1.0
Arginine	0.24	4.0
Serine	0.27	0.5
Glycine	0.30	0.5
Glutamic acid + glutamine	0.34	0.5
Threonine	0.39	1.0
Hydroxyproline	0.43	2.0
Alanine	0.47	0.5
Tyrosine	0.51	1.0
Proline	0.59	2.0
Tryptophan	0.62	4.0
Valine	0.62	1.0
Methionine	0.68	1.0
Phenylalanine	0.72	4.0
Leucine + isoleucine	0.80	1.0

TABLE 4

TLC R_F VALUES OF AMINO ACIDS

(D. CHIARI, M. RÖHR AND G. WIDTMANN, *Mikrochim. Ichnoanal. Acta*, (1965) 672)

Thin layer: Cellulose powder Schleicher & Schüll No. 143.

Solvents: S_1 = Phenol–methanol–water (35:50:15).
S_2 = Phenol–triethanolamine–*n*-propanol–methanol–water (35:8:25:25:15).
S_3 = Phenol–triethanolamine–ethanol–water (35:8:50:15).
S_4 = Phenol–triethanolamine–methanol–water (20:25:50:15).
S_5 = Collidine–*n*-butanol–acetone–water (8:40:40:20).
S_6 = Collidine–*sec.*-butanol–acetone–water (8:40:40:20).
S_7 = Collidine–*sec.*-butanol–acetone–water (20:40:20:10).
S_8 = Collidine–isopropanol–acetone–water (8:40:40:20).
S_9 = Collidine–methanol–acetone–water (8:40:40:20).
S_{10} = *n*-Butanol–acetone–diethylamine–water (40:40:20:8).
S_{11} = 95% ethanol–5% ammonia (70:30).
S_{12} = *n*-Butanol–acetone–conc. ammonia–water (40:40:8:20).
S_{13} = Methanol–acetone–conc. ammonia–water (40:40:8:20).
S_{14} = *n*-Propanol–water (80:20).
S_{15} = Isopropanol–methanol–formic acid–water (60:20:8:20).
S_{16} = *sec.*-Butanol–acetone–formic acid–water (40:40:8:20).
S_{17} = *n*-Butanol–formic acid–water (60:20:20).
S_{18} = *n*-Butanol–acetic acid–water (40:10:50).
S_{19} = Ethanol–acetic acid–water (40:10:50).
S_{20} = Ethanol–acetic acid–water (40:20:40).

Temperature: 22°.

Detection: Ninhydrin (0.2 g ninhydrin + 70 ml methanol + 25 ml water + 3 ml glacial acetic acid); after spraying, the plate is heated to 70° for 30 min; the spots are stabilised by spraying with copper nitrate solution.

Amino acid	$R_F \times 100$																			
	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}	S_{13}	S_{14}	S_{15}	S_{16}	S_{17}	S_{18}	S_{19}	S_{20}
Leucine	79	80	73	88	40	42	42	38	55	62	72	60	68	68	83	84	84	70	83	85
Valine	74	67	68	83	23	28	25	24	43	50	67	44	63	57	76	72	81	54	75	80
Methionine	66	67	65	80	28	31	32	26	42	53	65	49	63	58	70	68	78	54	72	78
Alanine	50	42	35	62	12	13	13	12	25	36	58	28	50	45	61	49	76	35	60	73
Glutamic acid	30	25	22	50	5	6	11	5	40	15	42	8	22	20	53	40	72	32	52	70
Threonine	45	44	34	62	13	16	18	15	40	58	57	53	75	69	50	36	74	33	52	70
Serine	28	25	22	48	8	8	13	8	20	43	50	34	56	52	43	28	70	26	43	65
Lysine	16	23	14	40	0	0	0	0	2	37	42	24	49	42	40	16	75	27	43	72
Histidine	24	35	28	45	6	6	10	5	14	40	49	32	51	47	29	13	68	24	41	66
Glycine	30	25	20	43	6	7	10	6	15	28	46	20	41	35	44	30	68	27	46	65
Phosphatidylserine	28	13	10	34	0	0	0	0	9	45	50	0	0	0	22	5	66	26	24	60
Tyrosine	50				26	28	32	22	37	44	60	36	57	55	62	61	71	48	65	72
Tryptophan	50	51	48	65	32	30	38	22	32	60	60	58	58	58	62	68	64	58	72	68
Aspartic acid	15	16	15	37	5	5	12	4	17	8	33	6	15	17	44	30	70	25	45	65
Phenylalanine	72	77	58	88	37	36	65	27	48	65	68	57	65	65	76	74	78	65	78	78
Proline	68				15	18	42	15	30	42	58	30	51	48	60	46	78	40	63	75
Hydroxyproline	44				10	12	30	10	20	30	52	19	39	35	44	28	74	30	48	78

TABLE 5

PC R_F VALUES OF SUGAR HYDRAZONES

(H.-H. STROH, A. ARNOLD AND H.-G. SCHARNOW, *Chem. Ber.*, 98 (1965) 1409)

Paper: Schleicher & Schüll 2043 b.
Solvent: Acetone–water (4:1).

Hydrazone of	R_F
D-Ribose	0.33
L-Arabinose	0.21
D-Xylose	0.22
D-Galactose	0.10
D-Mannose	0.12
D-Glucose	0.09
L-Rhamnose	0.18

TABLE 6

PC R_F VALUES OF SUGAR DIALKYL AND ARALKYL HYDRAZONES

(H.-H. STROH AND H.-G. SCHARNOW, *Chem. Ber.*, 98 (1965) 1595, 1597)

Paper: Schleicher & Schüll 2043bM.
Solvent: 96% ethanol–benzene (3:1).

Compound	R_F
D-Mannose N,N-dimethylhydrazone	0.31
D-Galactose N,N-dimethylhydrazone	0.35
L-Arabinose N,N-dimethylhydrazone	0.51
L-Rhamnose N,N-dimethylhydrazone	0.58
D-Mannose N,N-diethylhydrazone	0.56
D-Galactose N,N-diethylhydrazone	0.58
L-Arabinose N,N-diethylhydrazone	0.66
L-Rhamnose N,N-diethylhydrazone	0.66
D-Mannose N,N-di-*n*-propylhydrazone	0.73
D-Galactose N,N-di-*n*-propylhydrazone	0.82
L-Arabinose N,N-di-*n*-propylhydrazone	0.78
D-Lyxose N,N-di-*n*-propylhydrazone	0.76
D-Mannose N,N-di-*n*-butylhydrazone	0.64
D-Galactose N,N-di-*n*-butylhydrazone	0.79
L-Arabinose N,N-di-*n*-butylhydrazone	0.86
D-Galactose N-methyl-N-ethylhydrazone	0.41
L-Rhamnose N-methyl-N-ethylhydrazone	0.72
D-Mannose N-methyl-N-*n*-propylhydrazone	0.52
D-Galactose N-methyl-N-*n*-propylhydrazone	0.57
L-Arabinose N-methyl-N-*n*-propylhydrazone	0.57
L-Rhamnose N-methyl-N-*n*-propylhydrazone	0.72
D-Mannose N-methyl-N-*n*-butylhydrazone	0.62
D-Galactose N-methyl-N-*n*-butylhydrazone	0.62
L-Arabinose N-methyl-N-*n*-butylhydrazone	0.71
L-Rhamnose N-methyl-N-*n*-butylhydrazone	0.87
D-Mannose benzylhydrazone	0.45
D-Galactose benzylhydrazone	0.56
D-Mannose β-phenylethylhydrazone	0.53
D-Mannose γ-phenyl-*n*-propylhydrazone	0.50
D-Galactose γ-phenyl-*n*-propylhydrazone	0.59

TABLE 7

PC R_F VALUES OF SUGAR MONOALKYLHYDRAZONES

(H.-H. STROH AND H.-G. SCHARNOW, *Chem. Ber.*, 98 (1965) 1594)

Paper: FN 12 (Spezialpapierfabrik Niederschlag).
Solvent: *n*-Butanol–acetone–water (2:7:1).

Compound	R_F
D-Mannose methylhydrazone	0.37
D-Galactose methylhydrazone	0.23
L-Arabinose methylhydrazone	0.47
L-Rhamnose methylhydrazone	0.37
D-Mannose ethylhydrazone	0.40
D-Galactose ethylhydrazone	0.45
L-Arabinose ethylhydrazone	0.40
L-Rhamnose ethylhydrazone	0.38
D-Mannose *n*-propylhydrazone	0.41
D-Galactose *n*-propylhydrazone	0.46
L-Arabinose *n*-propylhydrazone	0.44
L-Rhamnose *n*-propylhydrazone	0.40
D-Mannose *n*-butylhydrazone	0.51
D-Galactose *n*-butylhydrazone	0.47
L-Arabinose *n*-butylhydrazone	0.48
L-Rhamnose *n*-butylhydrazone	0.48
D-Mannose *n*-pentylhydrazone	0.62
D-Galactose *n*-pentylhydrazone	0.51
L-Arabinose *n*-pentylhydrazone	0.73
L-Rhamnose *n*-pentylhydrazone	0.62
D-Mannose *n*-hexylhydrazone	0.80
D-Galactose *n*-hexylhydrazone	0.64
L-Arabinose *n*-hexylhydrazone	0.80

TABLE 8

PC R_F VALUES OF SUGAR AZINES

(H.-H. STROH, A. ARNOLD AND H.-G. SCHARNOW, *Chem. Ber.*, 98 (1965) 1409)

Paper: Schleicher & Schüll 2043b.
Solvent: Acetone–water (4:1).

Azine of	R_F
D-Ribose	0.19
L-Arabinose	0.23
D-Xylose	0.18
D-Galactose	0.11
D-Mannose	0.08
D-Glucose	0.06
L-Rhamnose	0.25
D-Fructose	0.27
L-Sorbose	0.16
Maltose	0.14
Lactose	0.12

TABLE 9

TLC R_F VALUES OF SOME CAROTENOIDS

(K. EGGER AND H. VOIGT, *Z. Pflanzenphysiol.*, 53 (1965) 70)

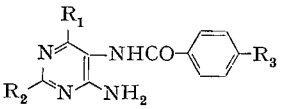
Thin layer: Polyamide (Merck) + 15% cellulose MN 300.
Solvents: S_1 = Light petroleum (b.p. 100–120°)–methanol–methyl ethyl ketone (8:1:1).
S_2 = Light petroleum–methanol–methyl ethyl ketone (4:1:1).
S_3 = Light petroleum–methanol–methyl ethyl ketone (2:1:1).
S_4 = Methanol–methyl ethyl ketone (1:1).
S_5 = Water–methanol–methyl ethyl ketone (1:5:5).
S_6 = Water–methanol–methyl ethyl ketone (1:3:3).
S_7 = Water–methanol–methyl ethyl ketone (1:2:2).

Compound	R_F						
	S_1	S_2	S_3	S_4	S_5	S_6	S_7
Physaliene	1.00	1.00	0.95	0.30	0.00	0.00	0.00
β-Carotene	1.00	1.00	1.00	0.80	0.25	0.10	0.05
Lycopene	0.95	0.90	0.75	0.60	0.05	0.00	0.00
Cryptoxanthin	0.62	0.70	0.76	0.74	0.39	0.21	0.09
Rubixanthin	0.45	0.60	0.64	0.45	0.15	0.04	0.00
Lycoxanthin	0.29	0.37	0.40	0.32	0.08	0.00	0.00
3,4-Dihydroxy-β-carotene	0.50	0.58	0.72	0.81	0.56	0.37	0.20
Lutein	0.35	0.57	0.93	0.95	0.68	0.45	0.24
Zeaxanthin	0.30	0.54	0.78	0.82	0.55	0.35	0.18
Isozeaxanthin	0.34	0.56	0.92	0.91	0.57	0.36	0.19
Escholtzxanthin	0.12	0.22	0.25	0.22	0.08	0.01	0.00
Lycophyll	0.08	0.20	0.22	0.20	0.07	0.00	0.00
Dihydrocanthaxanthin, red.	0.22	0.30	0.37	0.34	0.18	0.08	0.04
Tareoxanthin	0.28	0.42	0.84	0.92	0.78	0.62	0.43
Violaxanthin	0.30	0.52	0.76	0.93	0.88	0.73	0.55
Neoxanthin	0.22	0.42	0.82	0.96	0.90	0.78	0.60
3,4,4′-Trihydroxy-β-carotene	0.31	0.50	0.88	0.92	0.76	0.53	0.38
3,4,3′,4′-Tetrahydroxy-β-carotene	0.18	0.33	0.86	0.95	0.81	0.62	0.44
Equinenone	0.91	0.92	0.90	0.72	0.35	0.18	0.06
Euglenanone	0.62	0.68	0.81	0.80	0.54	0.34	0.17
Rhodoxanthin	0.28	0.42	0.43	0.40	0.14	0.07	0.02
Dihydrorhodoxanthin	0.81	0.85	0.87	0.87	0.69	0.47	0.27
Canthaxanthin	0.58	0.65	0.79	0.80	0.55	0.37	0.20
Dihydrocanthaxanthin	0.55	0.61	0.70	0.65	0.35	0.17	0.08
Dehydroadonirubin	0.47	0.59	0.74	0.76	0.48	0.30	0.15
Astacin	0.34	0.50	0.69	0.72	0.42	0.25	0.12
Capsanthin	0.24	0.42	0.79	0.81	0.62	0.42	0.25
Capsorubin	0.19	0.37	0.74	0.76	0.60	0.42	0.25
Fucoxanthin	0.27	0.44	0.84	0.98	0.95	0.85	0.75
β-Apo-8′-carotenic acid	0.28	0.38	0.30	0.15	0.05	0.00	0.00
Torularhodin	0.06	0.10	0.09	0.02	0.01	0.00	0.00

TABLE 10

PC R_F VALUES OF 4-AMINO-5-BENZAMIDOPYRIMIDINE DERIVATIVES
(S.-C. J. FU, E. CHINOPOROS AND H. TERZIAN, *J. Org. Chem.* 30 (1965) 1917)

Paper: Whatman No. 1.
Solvents: S_1 = Methanol–conc. hydrochloric acid–water (70:20:10).
S_2 = *n*-Butanol–2 *N* ammonium hydroxide–ethanol (20:5:2).

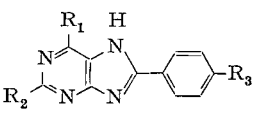


Substituents			R_F	
R_1	R_2	R_3	S_1	S_2
CH_3	H	H	0.65	0.86
H	H	H	0.51	0.86
NH_2	H	H	0.50	0.68
OH	CH_3	H	0.68	0.58
OH	NH_2	CH_3	0.49	0.45
SH	SH	H	0.62	0.48
SH	H	H	0.41	0.22

TABLE 11

PC R_F VALUES OF 8-PHENYLPURINE DERIVATIVES
(S.-C. J. FU, E. CHINOPOROS AND H. TERZIAN, *J. Org. Chem.*, 30 (1965) 1917)

Paper: Whatman No. 1.
Solvents: S_1 = Methanol–conc. hydrochloric acid–water (70:20:10).
S_2 = *n*-Butanol–2 *N* ammonium hydroxide–ethanol (20:5:2).



Substituents			R_F	
R_1	R_2	R_3	S_1	S_2
CH_3	H	H	0.61	0.81
H	H	H	0.13	0.72
NH_2	H	H	0.46	0.70
OH	CH_3	H	0.65	0.53
OH	NH_2	CH_3	0.27	0.38
SH	SH	H	0.31	0.76

TABLE 12

TLC R_F VALUES (RELATIVE) OF PURINES, PYRIMIDINES, NUCLEOSIDES AND NUCLEOTIDES

(R. G. STRICKLAND, *Anal. Biochem.*, 10 (1965) 116)

Thin layer: DEAE-cellulose.
Technique: Gradient elution; two-dimensional chromatography.
Solvents: S_1 = 75 ml of 1.0 *M* NH_4HCO_3 in one chamber and 85 ml of 0.15 *M* NH_4HCO_3 in the second chamber.
S_2 = 75 ml of 2.0 *M* ammonium formate, pH 4.2, in one chamber and 85 ml of 0.2 *M* ammonium formate, pH 2.8, in the second chamber.

Compound	R_{CMP}*	
	S_1	S_2
Adenine	0.55	0.98
Cytosine	1.36	1.38
Guanine	0.52	0.98
Uracil	1.13	1.02
Adenosine	0.92	0.99
Cytidine	1.39	1.38
Guanosine	0.84	0.98
Uridine	1.30	1.02
AMP	0.76	0.94
ADP	0.65	0.26
ATP	0.63	0.06
CMP	1.00	1.00
CDP	0.89	0.64
CTP	0.86	0.18
GMP	0.52	0.38
GDP	0.50	0.14
GTP	0.50	0.02
UMP	0.92	0.73
UDP	0.86	0.18
UTP	0.81	0.06
NAD	0.98	0.29
NADP	0.87	0.36
UDP-glucose	1.00	0.45

* R_F value relative to that of CMP.

TABLE 13

ELECTROPHORETIC MOBILITIES OF NUCLEOTIDES

(G. R. BANKS AND D. COHEN, *J. Chem. Soc.*, (1966) 6209)

Paper: Whatman No. 4.
Applied voltage: 500 V for 3 h.
Buffer: 0.05 *M* ammonium acetate pH 5.5.

Compound	*Mobility**
Adenosine-5′-phosphate	+3.0
Adenosine-5′-sulphatophosphate	+5.5
Adenosine	—0.7
Adenosine-5′-sulphatophosphate (2′,3′)-sulphate	+8.1
Unknown compound	—8.2

* + indicates migration towards the anode, — migration towards the cathode.

TABLE 14

PC R_F VALUES OF NUCLEOTIDES

(G. R. BANKS AND D. COHEN, *J. Chem. Soc.*, (1965) 6209)

Paper: Whatman No. 4.
Solvents: S_1 = *n*-Propyl alcohol–ammonia (d = 0.88)–water (6:3:1).
S_2 = Isobutyric acid–0.5 *N* ammonia (5:3, v/v).

Compound	R_F	
	S_1	S_2
Adenosine-5′-phosphate	0.16	0.35
Adenosine-5′-sulphatophosphate	0.25	0.38
Adenosine	0.63	0.73
Adenosine-5′-sulphatophosphate (2′,3′)-sulphate	—	0.23
Unknown compound	0.80	0.84

TABLE 15

PC R_F VALUES OF POLYNUCLEOTIDES

(E. OHTSUKA, M. W. MOON AND H. G. KHORANA, *J. Am. Chem. Soc.*, 87 (1965) 2956)

Paper: Whatman No. 40 or No. 1.
Solvents: S_1 = Propan-2-ol–conc. ammonia–water (7:1:2).
S_2 = Ethyl alcohol–1 *M* ammonium acetate, pH 3.8 (7:3).
S_3 = Propan-1-ol–conc. ammonia–water (55:10:35).
S_4 = Isobutyric acid–conc. ammonia–water (66:3:13).

Compound	R_F			
	S_1	S_2	S_3	S_4
β-Cyanoethyl thymidine 5′-phosphate		0.65		
5′-O-Phosphorylthymidylyl-(3′ → 5′)-N^6-anisoyldeoxycytidine	0.11	0.43		
5′-O-Phosphorylthymidylyl-(3′ → 5′)-deoxycytidine	0.03	0.17		
5′-O-Phosphorylthymidylyl-(3′ → 5′)-N^6-anisoyl-3′-O-acetyldeoxycytidine		0.52		
β-Cyanoethyl N-benzoyldeoxyadenosine 5′-phosphate		0.80		
5′-O-Phosphoryl-N-benzoyldeoxyadenylyl-(3′ → 5′)-N-acetyldeoxyguanosine		0.28		
5′-O-Phosphoryldeoxyadenylyl-(3′ → 5′)-deoxyguanosine		0.12	0.26	
5′-O-Phosphoryl-N^6-anisoyldeoxycytidylyl-(3′ → 5′)-N^6-benzoyldeoxyadenosine		0.40		0.78
5′-O-Phosphoryldeoxycytidylyl-(3′ → 5′)-deoxyadenosine		0.09		0.61
5′-O-Phosphoryl-N^6-anisoyldeoxycytidylyl-(3′ → 5′)-N^6-benzoyl-3′-O-acetyldeoxyadenosine		0.49		
5′-O-Phosphorylthymidylyl-(3′ → 5′)-N-acetyldeoxyguanosine		0.25		
5′-O-Phosphorylthymidylyl-(3′ → 5′)-N-3′-O-diacetyldeoxyguanosine		0.35		

TABLE 16

PC R_F VALUES OF POLYNUCLEOTIDES

(T. M. JACOB AND H. G. KHORANA, *J. Am. Chem. Soc.*, 87 (1965) 2971)

Paper: Whatman No. 40 or No. 1.
Solvents: S_1 = Ethanol–1 *M* ammonium acetate, pH 7.5 (7:3).
S_2 = Propan-2-ol–water (70:30) (with ammonia in the vapour phase).

Compound	R_F*	
	S_1	S_2
d-TrTpTpC^{An}	1.97	
d-TrTpTpC^{An}pT	1.59	2.07
d-TtTpTpC^{An}pTpTp	1.24	1.86
d-TrTpTpC^{An}pTpTpC^{An}	0.86	1.67
d-TrTpTpC^{An}pTpTpC^{An}pT	0.57	1.17
d-TrTpTpC^{An}pTpTpC^{An}pTpT	0.33	0.86
d-TrTpTpC^{An}pTpTpC^{An}pTpTpC^{An}	0.23	0.73
d-TrTpTpC^{An}pTpTpC^{An}pTpTpC^{An}pT	0.12	
d-TrTpTpC^{An}pTpTpC^{An}pTpTpC^{An}pTpT	0.066	
d-TrTpTpC^{An}pTpTpC^{An}pTpTpC^{An}pTpTpC^{An}	0.03	

* R_F relative to that of pT.

TABLE 17

PC R_F VALUES OF POLYNUCLEOTIDES

(T. M. JACOB AND H. G. KHORANA, *J. Am. Chem. Soc.*, 87 (1965) 2971)

Paper: Whatman No. 40 or No. 1.
Solvent: Propan-1-ol–conc. ammonia–water (55:10:35).

Compound	R_F*
d-TrTpTpC	1.9
d-TrTpTpCpT	1.79
d-TrTpTpCpTpT	1.59
d-TrTpTpCpTpTpC	1.35
d-TrTpTpCpTpTpCpT	1.2
d-TrTpTpCpTpTpCpTpT	1.03
d-TrTpTpCpTpTpCpTpTpC	0.89
d-TrTpTpCpTpTpCpTpTpCpT	0.61
d-TrTpTpCpTpTpCpTpTpCpTpT	0.49
d-TrTpTpCpTpTpCpTpTpCpTpTpC	0.36
d-TpTpC	
d-TpTpCpT	1.03
d-TpTpCpTpT	0.76
d-TpTpCpTpTpC	0.58
d-TpTpCpTpTpCpT	0.47
d-TpTpCpTpTpCpTpT	0.32
d-TpTpCpTpTpCpTpTpC	0.24
d-TpTpCpTpTpCpTpTpCpT	0.17
d-TpTpCpTpTpCpTpTpCpTpT	0.13
d-TpTpCpTpTpCpTpTpCpTpTpC	0.11

* R_F relative to that of pT.

TABLE 18

PC R_F VALUES OF POLYNUCLEOTIDES

(S. A. NARANG, T. M. JACOB AND H. G. KHORANA, *J. Am. Chem. Soc.*, 87 (1965) 2988)

Paper: Whatman No. 40 or No. 1.
Solvents: S_1 = Propan-2-ol–conc. ammonia–water (7:1:2).
S_2 = Ethanol–1 *M* ammonium acetate, pH 7.5 (7:3, v/v).
S_3 = Propan-1-ol–conc. ammonia–water (55:10:35).

Compound	R_F*		
	S_1	S_2	S_3
d-MMTr-$G^{Ac}pA^{Bz}$		3.8	
d-MMTr-$G^{Ac}pA^{Bz}pA^{Bz}$		3.5	
d-MMTr-$G^{Ac}pA^{Bz}pA^{Bz}pG^{Ac}$		3.17	
d-MMTr-$G^{Ac}pA^{Bz}pA^{Bz}pG^{Ac}pA^{Bz}$		2.7	
d-MMTr-$G^{Ac}pA^{Bz}pA^{Bz}pG^{Ac}pA^{Bz}pA^{Bz}$		1.98	
d-MMTr-GpA	7.29	3	1.97
d-MMTr-GpApA	3.56	2.05	1.8
d-MMTr-GpApApG	0.74	0.97	1.49
d-MMTr-GpApApGpA	0.27	0.4	1.39
d-MMTr-GpApApGpApA	0.09	0.15	1.19
d-GpA		2.17	1.09
d-GpApA		0.7	0.86
d-GpApApG		0.22	0.48
d-GpApApGpA		0.07	0.36
d-GpApApGpApA			0.27

* R_F relative to that of d-pA.

TABLE 19

PC R_F VALUES OF POLYNUCLEOTIDES

(S. A. NARANG, T. M. JACOB AND H. G. KHORANA, *J. Am. Chem. Soc.*, 87 (1965) 2988)

Paper: Whatman No. 40 or No. 1.
Solvents: S_1 = Propan-2-ol–conc. ammonia–water (7:1:2).
S_2 = Propan-1-ol–conc. ammonia–water (55:10:35).

Compound	R_F*	
	S_1	S_2
d-CpA	3.6	1.30
d-CpApA	0.85	0.96
d-CpApApC	0.23	0.80
d-CpApApCpA	—	0.76
d-CpApApCpApA	—	0.55

* R_F relative to that of d-pA.

TABLE 20

PC R_F VALUES OF POLYNUCLEOTIDES

(S. A. NARANG AND H. G. KHORANA, *J. Am. Chem. Soc.*, 87 (1965) 2981)

Paper: Whatman No. 40 or No. 1.
Solvents: S_1 = Propan-2-ol–conc. ammonia–water (7:1:2).
S_2 = Propan-1-ol–conc. ammonia–water (55:10:35).
S_3 = Propan-2-ol–water (70:30) with water in the vapour phase.

Compound	R_F		
	S_1	S_2	S_3
Pyridinium deoxyinosine 5′-phosphate			0.40
Pyridinium 3′-O-acetyldeoxyinosine 5′-phosphate			0.64
DMTr-TpT	0.78		
DMTr-TpTpI	0.51		
DMTr-TpTpIpT	0.30	1.70*	
DMTr-TpTpIpTpT	0.14	1.54*	
DMTr-TpTpIpTpTpI	0.07	1.40*	
DMTr-TpTpIpTpTpIpT		1.13*	
DMTr-TpTpIpTpTpIpTpT		0.93*	
DMTr-TpTpIpTpTpIpTpTpI		0.82*	
DMTr-TpTpIpTpTpIpTpTpIpT		0.54*	
DMTr-TpTpIpTpTpIpTpTpIpTpT		0.39*	
DMTr-TpTpIpTpTpIpTpTpIpTpTpI		0.23*	
TpTpI	0.256	1.17*	
TpTpIpT	0.21	0.87*	
TpTpIpTpT		0.76*	
TpTpIpTpTpI		0.59*	
TpTpIpTpTpIpT		0.43*	
TpTpIpTpTpIpTpT		0.37*	
TpTpIpTpTpIpTpTpI		0.28*	
TpTpIpTpTpIpTpTpIpT		0.14*	
TpTpIpTpTpIpTpTpIpTpT		0.11*	
TpTpIpTpTpIpTpTpIpTpTpI		0.092*	

* R_F relative to that of pT.

TABLE 21

PC R_F VALUES OF D-*threo*-2,3-DIHYDROXY-4-PENTENAL AND RELATED COMPOUNDS
(R. M. SAUNDERS AND C. E. BALLOU, *J. Org. Chem.*, 30 (1965) 3219)

Paper: Whatman No. 1.
Solvent: S_1 = Butan-1-ol–95% ethanol–water (4:1:1).

Compound	R_F
D-*threo*-2,3-Dihydroxy-4-pentenal	0.76
5,6-Didehydro-5,6-dideoxy-3,4-O-isopropylidene-D-*arabo*-hexitol	0.85
5,6-Didehydro-5,6-dideoxy-D-*arabo*-hexitol	0.43
5,6-Didehydro-5,6-dideoxy-L-*xylo*-hexitol	0.49
2,3-O-Isopropylidene-D-*threo*-2,3-dihydroxy-4-pentenal	0.90

TABLE 22

TLC R_F VALUES AND COLOUR REACTIONS OF SOME ORGANIC ACIDS
(I. P. TING AND W. M. DUGGER, Jr., *Anal. Biochem.*, 12 (1965) 574)

Thin layer: Silica gel H.
Solvent: Water-saturated diethyl ether–formic acid (7:1).
Detection: Acid ammonium molybdate solution.

Acid	R_F	*Colour**	*Comments*
α-Ketoglutaric	0.56	B	
Ascorbic	0.30	B	Sodium salt applied
Aspartic	0.04	FB	
β-Hydroxybutyric	0.81	FB	
cis-Aconitic	0.61	FB	
Citramalic	0.64	B	
Citric	0.37	B	
Citraconic	0.91	FB	
Fumaric	0.96	LB	Sodium salt applied
Galacturonic	0.06	B	
Glucuronic	0.09	B	Sodium salt applied
Glutamic	0.05	FB	
Glutaric	0.91	NR	
Isocitric	0.42	B	Sodium salt applied
Isocitric lactone	0.63	B	
Lactic	0.65	B	
Malic	0.53	B	
Malonic	0.69	FB	
Oxalic	0.07	B	Usually streaks
Phosphoric	—	Y	R_F increases with incr. concn.
Succinic	0.86	FB	Sodium salt applied
Tartaric	0.23	B	
Tartronic	0.06	B	Streaks
Sudan Red G	0.88	—	Standard dye

* B = blue; FB = faint blue; LB = light blue; NR = no apparent reaction; Y = yellow.

TABLE 23

PC R_F VALUES OF MYOINOSITOL TETRAHYDROPYRANYL ETHERS

(S. J. ANGYAL AND S. D. GERO, *J. Chem. Soc.*, (1965) 5255)

Paper: Whatman No. 1.
Solvents: S_1 = Butan-1-ol–ethanol–water (40:11:19).
S_2 = Butan-1-ol–ethanol–water (6:1:1).
S_3 = Ethyl methyl ketone saturated with water.

Compound	R_F		
	S_1*	S_2*	S_3
Myoinositol	1.00	1.00	—
1-O-(2-Tetrahydropyranyl)-myoinositol	3.18	6.65	0.09
5-O-(2-Tetrahydropyranyl)-myoinositol	3.60	8.90	0.17
2-O-(2-Tetrahydropyranyl)-myoinositol	3.76	9.10	0.16
1,2-Di-O-(2-tetrahydropyranyl)-myoinositol	4.87	—	—
4-O-(2-Tetrahydropyranyl)-myoinositol	—	9.25	0.19

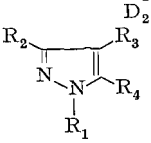
* Values relative to that of myoinositol.

TABLE 24

PC R_F VALUES OF SOME PYRAZOLES

(S. TABAK, I. I. GRANDBERG AND A. N. KOST, *Zh. Analit. Khim.*, 20 (1965) 871)

Paper: Acetylated paper (Soviet paper equal to Whatman No. 1).
Impregnation: I_1 = Vapours of chloroform (15–20 min).
I_2 = Vapours of chlorobenzene (15–20 min).
Solvent: 80% ethanol.
Detection: D_1 = Ultra violet light.
D_2 = Iodine vapours.



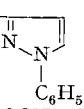
Compound				R_F	
R_1	R_2	R_3	R_4	I_1	I_2
H	CH_3	H	CH_3	0.60	0.60
H	C_6H_5	H	C_6H_5	—	0.17
CH_3	CH_3	H	CH_3	0.70	0.90
C_6H_5	CH_3	H	CH_3	0.18	0.16
C_6H_5	H	H	Cl	—	0.24
C_6H_5	CH_3	H	Cl	0.18	0.07
C_6H_5	Cl	H	H	0.20	0.15
H	C_6H_5	H	NH_2	0.53	0.35
iso-C_3H_7	CH_3	H	NH_2	—	0.80
iso-C_3H_7	*p*-$C_6H_4NH_2$	H	NH_2	0.30	0.10
CH_3	*p*-$C_6H_4NH_2$	H	NH_2	0.30	0.20
C_6H_5	H	H	NH_2	0.26	0.16
C_6H_5	CH_3	H	NH_2	0.26	0.20
C_6H_5	H	NH_2	H	0.45	0.15
C_6H_5	NH_2	H	H	0.21	0.18
$CH_2C_6H_5$	CH_3	H	NH_2	0.70	0.76
CH_3	CH_3	NO_2	CH_3	0.40	0.30

(*continued on p. D15*)

TABLE 24 (*continued*)

Compound				R_F	
R_1	R_2	R_3	R_4	I_1	I_2
CH_3	CH_3	SCl	CH_3	0.50	0.33
iso-C_3H_7	CH_3	NO	CH_3	0.64	0.28
CH_2CH_2CN	C_6H_5	H	C_6H_5	—	0.23
C_6H_5	H	OCH_3	H	0.21	—
C_6H_5	H	HgCl	H	0.24	—
C_6H_5	H	HgBr	H	0.20	—
p-$NO_2C_6H_4$	CH_3	NO_2	Cl	0.10	0.10
p-$HO_3SC_6H_4$	H	H	C_6H_5	0.20	0.17
NO*	CH_3	H	C_6H_5	0.30	0.22
$COCH=CHCO_2H$	CH_3	H	α-furyl	0.68	0.60
$COCH=CHCO_2H$	C_6H_5	H	α-furyl	0.20	0.20
iso-C_3H_7	CH_3	X**	OH	0.49	0.44
iso-C_3H_7	C_6H_5	X**	OH	0.30	0.25
CH_2CH_2CN	CH_3	X**	OH	0.00	0.15
$CH_2CH_2C_6H_5$	CH_3	X**	OH	0.23	0.20
C_6H_5	CH_3	CH_2OH	Cl	0.19	—
C_6H_5	CH_3	H	Y***	0.63	0.79
C_6H_5	CH_3	H	Z***	0.56	0.40
C_6H_5	H	H	T***	—	0.30
C_6H_5	H	H	Z***	—	0.20
C_6H_5	H	H	Y***	—	0.60
C_6H_5	H	Y***	H	—	0.59
C_6H_5	Z***	H	H	—	0.10
C_6H_5	Y***	H	H	—	0.60
C_6H_5	CH_3	H	T***	—	0.40

* Pyrazoline derivative.

** X = 

*** Y = —HN—COCH=C(OH)CH_3; Z = —HN—C(CH_3)=CHCOOC_2H_5; T = —N=C(CH_3)—CH=C(OH)—CH_3.

TABLE 25

PC R_F VALUES OF CYTOSINE DERIVATIVES

(T. J. DELIA, M. J. OLSON AND C. BOSWORTH BROWN, *J. Org. Chem.*, 30 (1965) 2766)

Paper: Whatman No. 1.

Solvents: S_1 = 1% aqueous ammonium sulphate–propan-2-ol (1:2).
S_2 = 5% disodium hydrogen phosphate–isoamyl alcohol (3:2).
S_3 = *tert.*-Butanol–methyl ethyl ketone–88% formic acid–water (40:30:15:15).
S_4 = *n*-Butanol–acetic acid–water (4:1:1).
S_5 = Propan-2-ol–water–28% ammonium hydroxide (7:2:1).
S_6 = *n*-Propanol–water (3:1).

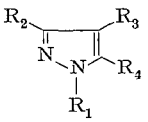
Compound	R_F					
	S_1	S_2	S_3	S_4	S_5	S_6
Cytosine 3-N-oxide	0.33	0.84	0.42	0.31	0.44	0.21
Cytidine 3-N-oxide	0.37	0.87	0.39	0.26	0.38	—
N^4-Acetoxycytosine	0.91	0.71	0.78	—	—	0.85
N^4-Hydroxycytosine	0.88	0.68	0.58	—	0.79	0.76
Cytosine	0.63	0.77	0.45	—	—	—
Cytidine	—	0.83	0.34	—	—	—

TABLE 26

PC R_F VALUES OF SOME PYRAZOLES

(S. TABAK, I. I. GRANDBERG AND A. N. KOST, *Zh. Analit. Khim.*, 20 (1965) 872)

Paper: Acetylated paper (Soviet paper equal to Whatman No. 1).
Impregnation: I_1 = in vapours of chloroform.
I_2 = in vapours of chlorobenzene .
I_3 = in vapours of isoamyl chloride.
I_4 = in vapours of benzyl chloride.
(In all cases equilibration for 15–20 min).
Solvent: 80% ethanol.
Detection: D_1 = Ultra violet light.
D_2 = Iodine vapours.



Compound				R_F			
R_1	R_2	R_3	R_4	I_1	I_2	I_3	I_4
C_6H_5	H	H	OH	0.57	0.40	0.60	—
C_6H_5	CH_3	H	OH	0.45	0.20	—	—
C_6H_5	H	OH	H	0.40	0.17	0.11	—
C_6H_5	OH	H	H	0.40	0.30	0.25	—
C_6H_5	OH	H	C_6H_5	0.40	—	—	—
C_6H_5	CH_3	H	SH	0.70	—	—	—
CH_3	CH_3	$COCH_3$	CH_3	—	0.68	—	—
CH_3	CH_3	COC_3H_7	CH_3	0.69	—	—	—
$CH_2C_6H_5$	CH_3	COC_6H_5	CH_3	—	0.70	—	—
C_6H_5	CH_3	$COCH_3$	CH_3	0.50	0.30	—	0.34
C_6H_5	CH_3	COC_6H_5	CH_3	0.80	0.80	—	—
C_6H_5	H	$COCH_3$	Cl	—	0.24	—	—
C_6H_5	CH_3	$COCH_3$	Cl	0.20	0.20	0.20	—
C_6H_5	Cl	$COCH_3$	H	0.34	0.80	0.18	—
H	CH_3	$COCH_3$	CH_3	0.20	0.18	—	—
$COCH_3$	C_6H_5	H	CH_5	0.87	0.30	—	0.40
COC_6H_5	CH_3	H	C_6H_3	0.50	—	—	—
H	CH_3	H	COOH	—	0.30	0.50	0.40
H	C_6H_5	H	COOH	0.30	—	—	—
C_5H_{11}	C_4H_9	H	COOH	0.70	0.32	0.70	0.20
C_6H_5	H	H	COOH	0.40	0.20	0.36	0.30
C_6H_5	CH_3	H	COOH	0.54	0.36	0.50	0.45
C_6H_5	COOH	H	Cl	0.50	0.20	0.30	0.28
C_6H_5	CH_3	COOH	Cl	0.27	—	—	—
$CH_2C_6H_5$	CH_3	H	COOH	0.70	—	—	—

TABLE 27

PC R_F VALUES OF SOME VOLATILE ALIPHATIC AMINES

(J. HRDLIČKA AND G. JANÍČEK, *Nature*, 204 (1964) 1201)

Paper: Not specified.
Solvent: *n*-Butanol–acetic acid–water (4:1:5).
Detection: Ninhydrin.

Compound	R_F	*Colour*
N,N'-Di-1,4-diaminobutane	0.03	red-violet
1,3-Diaminopropane	0.10	red-violet
1,4-Diaminobutane	0.12	red-violet
1,5-Diaminopentane	0.15	violet
Ethanolamine	0.15	violet
Methylamine	0.30	violet
Dimethylamine	0.33	brown-violet
Ethylamine	0.39	violet
Isopropylamine	0.45	grey-violet
n-Propylamine	0.50	violet
Isobutylamine	0.58	violet
n-Butylamine	0.61	violet
n-Amylamine	0.71	violet

TABLE 28

PC AND TLC R_F VALUES OF 6-HYDROXYCATECHOLAMINES AND SOME METABOLITES

(J. W. DALY, J. BENIGNI, R. MINNIS, Y. KANAOKA AND B. WITKOP, *Biochemistry*, 4 (1965) 2518)

Paper: Whatman No. 1.
Solvents: S_1 = Nitroethane–acetic acid–water (90:28:12).
S_2 = Methyl ethyl ketone–propionic acid–water (15:5:6).
S_3 = Methanol–butanol–benzene–water (2:1:1:1).
S_4 = Butanol–acetic acid–water (4:1:1).
S_5 = Butanol–pyridine–acetic acid–water (4:2:1:1).
Thin layer: Silica gel.
Solvents: S_6 = Butanol–acetic acid–water (4:1:1).
S_7 = Butanol–ammonia–ethyl acetate (3:1:1).
Detection: D_1 = Gibbs reagent.
D_2 = Ninhydrin.
D_3 = Diazotized *p*-nitroaniline.

Compound	$R_F \times 100$									
	PC					*TLC*		*Color**		
	S_1	S_2	S_3	S_4	S_5	S_6	S_7	D_1	D_2	D_3
6-Hydroxydopamine	17	48	55	23	50	50	Dec.	Br	BG	R
3-O-Methyl-6-hydroxydopamine	39	60	70	47	55	56	35	PB	B	YO
N-Methyl-3-O-methyl-6-hydroxydopamine	57	71	75	59	63	44	33	PB	G	YO
2,4-Dihydroxy-5-methoxyphenylacetic acid	56	88	70	80	80	88	12	PG	—	YO
2,4-Dihydroxy-5-methoxyphenethanol	67	93	87	88	90	90	53	PG	—	YO
6-Hydroxynorepinephrine	09	42	Dec.	26	43	36	Dec.	Br	P	R
3-O-Methyl-6-hydroxynorepinephrine	28	61	71	49	60	56	28	P	RP	YO
6-Hydroxyepinephrine	14	45	Dec.	32	31	44	Dec.	Br	GP	R
3-O-Methyl-6-hydroxyepinephrine	32	58	70	50	59	50	25	P	GP	YO
2,4-Dihydroxy-5-methoxymandelic acid	28	76	58	72	57	90	06	P	—	YO
2,4-Dihydroxy-5-methylphenyl glycol	44	69	78	85	83	85	31	P	—	YO

* B = blue; Br = brown; G = gray; O = orange; P = purple; R = reddish; Y = yellow.

TABLE 29

TLC R_F VALUES OF SOME PRIMARY AROMATIC AMINES

(I. GEMZOVÁ AND J. GASPARIČ, *Collection Czech. Chem. Commun.*, 31 (1966) 2527)

Thin layer: Aluminium oxide neutral, activity III, without binder (Reanal, Budapest).
Solvent: Benzene.
Detection: 1 % *p*-Dimethylaminobenzaldehyde in 95 parts ethanol and 5 parts conc. hydrochloric acid.

Compound	R_F	*Colour*
Aniline	0.41	yellow
2-Methylaniline	0.49	yellow
3-Methylaniline	0.42	yellow
4-Methylaniline	0.35	yellow
2-Chloroaniline	0.71	yellow
3-Chloroaniline	0.54	yellow
4-Chloroaniline	0.49	yellow
3-Bromoaniline	0.57	yellow
4-Bromoaniline	0.54	yellow
3-Iodoaniline	0.60	yellow
2-Methoxyaniline	0.42	yellow
3-Methoxyaniline	0.28	yellow
4-Methoxyaniline	0.19	yellow
2-Ethoxyaniline	0.47	yellow
4-Ethoxyaniline	0.21	yellow
2-Nitroaniline	0.45	yellow
3-Nitroaniline	0.30	yellow
4-Nitroaniline	0.20	orange
2,3-Dimethylaniline	0.44	yellow
2,4-Dimethylaniline	0.41	yellow
2,5-Dimethylaniline	0.49	yellow
2,6-Dimethylaniline	0.60	yellow
3,4-Dimethylaniline	0.37	yellow
2,5-Dichloroaniline	0.78	yellow
2,4-Dichloroaniline	0.78	yellow
2,5-Dimethoxyaniline	0.25	orange
2,4-Dinitroaniline	0.17	yellow
2-Methyl-3-chloroaniline	0.62	yellow
2-Methyl-4-chloroaniline	0.56	yellow
2-Methyl-5-chloroaniline	0.65	yellow
2-Methyl-6-chloroaniline	0.75	yellow
3-Chloro-4-methylaniline	0.53	yellow
2-Methoxy-5-methylaniline	0.38	yellow
2-Methoxy-4-chloroaniline	0.64	orange
2-Methoxy-5-chloroaniline	0.61	yellow
2-Methyl-3-nitroaniline	0.55	yellow
2-Methyl-4-nitroaniline	0.27	yellow
2-Methyl-5-nitroaniline	0.34	yellow
2-Nitro-4-methylaniline	0.41	orange
3-Nitro-4-methylaniline	0.24	yellow
2-Chloro-4-nitroaniline	0.37	yellow
2-Nitro-4-chloroaniline	0.49	yellow
2-Methoxy-4-nitroaniline	0.35	yellow
2-Methoxy-5-nitroaniline	0.31	yellow
2-Nitro-4-cyanoaniline	0.14	yellow
4-Aminobenzoic acid methyl ester	0.15	yellow
2,4-Dimethoxy-5-chloroaniline	0.33	orange
2,5-Dimethoxy-4-chloroaniline	0.36	orange
2-Methoxy-4-chloro-5-methylaniline	0.48	yellowish orange
2,6-Dinitro-4-methylaniline	0.68	yellow

(*continued on p. D19*)

TABLE 29 (*continued*)

Compound	R_F	*Colour*
2,4-Dinitro-6-chloroaniline	0.51	yellow
2,6-Dichloro-4-nitroaniline	0.69	faint yellow
1,2-Phenylenediamine	0	yellow
1,3-Phenylenediamine	0.07	yellowish brown
1,4-Phenylenediamine	0.08	red
4-Methyl-1,3-phenylenediamine	0.06	yellowish brown
3-Nitro-1,2-phenylenediamine	0.19	red-violet
4-Nitro-1,2-phenylenediamine	0.02	yellow
4-Nitro-1,3-phenylenediamine	0.05	yellow
2-Nitro-1,4-phenylenediamine	0.12	orange
2-Chloro-1,4-phenylenediamine	0.12	yellowish brown
N-Acetyl-1,3-phenylenediamine	0.36	yellowish brown
N-Acetyl-1,4-phenylenediamine	0	yellow
2,5-Dichloro-4-acetaminoaniline	0.05	yellow
1,2-Aminophenol	0.05	red
1,3-Aminophenol	0.15	yellow
1,4-Aminophenol	0.01	yellow
4-Aminoacetophenone	0.17	yellow
6-Amino-2-phenylbenzothiazole	0.26	orange
2-Amino-6-methoxybenzothiazole	0.02	yellow
1-Naphthylamine	0.47	yellow
2-Naphthylamine	0.43	yellow
4-Aminodiphenyl	0.42	yellow
4,4′-Diaminodiphenyl	0.49	yellow
2-Nitro-4,4′-diaminodiphenyl	0.06	orange
2,2′-Diaminodiphenyl	0.23	yellow
3,3′-Dimethyl-4,4′-diaminodiphenyl	0.13	orange
3,3′-Dimethoxy-4,4′-diaminodiphenyl	0.17	orange
1-Aminoanthracene	0.47	orange
2-Aminoanthracene	0.43	orange

TABLE 30

TLC R_F VALUES OF SOME PRIMARY AROMATIC AMINES

(I. GEMZOVÁ AND J. GASPARIČ, *Collection Czech. Chem. Commun.*, 31 (1966) 2529)

Thin layer: Aluminium oxide neutral, activity III, without binder (Reanal, Budapest).
Solvent: Chloroform.
Detection: 1 % *p*-Dimethylaminobenzaldehyde in 95 parts ethanol and 5 parts conc. hydrochloric acid.

Compound	R_F
4-Nitro-1,2-phenylenediamine	0.20
2-Amino-5-nitrophenol	0.13
1,2-Phenylenediamine	0.25
N-Acetyl-1,4-phenylenediamine	0.11
1,4-Aminophenol	0.07
1-Amino-3-methoxy-4-acetaminobenzene	0.19
2-Amino-6-methylbenzothiazole	0.25
2-Amino-6-methoxybenzothiazole	0.23
2-Aminophenol-4-diethylaminosulphonamide	0.27
2,5-Dimethyl-1,4-phenylenediamine	0.22
N-Acetyl-2,5-dichloro-1,4-phenylenediamine	0.44

TABLE 31

PC R_F VALUES OF *p*-SUBSTITUTED 1-ARYL-3,3-DIMETHYLTRIAZENES

(M. MATRKA, J. MARHOLD, Z. SÁGNER AND V. ŠTĚRBA, *Collection Czech. Chem. Commun.*, 30 (1965) 3957)

Paper: Whatman No. 1.
Impregnation: I_1 = 10% kerosene (fraction 190–220°) in hexane.
I_2 = 10% paraffin oil in hexane.
I_3 = 10% 1-bromonaphthalene in hexane.
Solvents: S_1 = Ethanol–water (1:1).
S_2 = *n*-Propanol–water (1:1).
S_3 = Acetone–water (1:1).
S_4 = 50% acetic acid.
S_5 = Butanol–pyridine–water (5:3:3).
Detection: 3% resorcinol in 2 *M* hydrochloric acid in 50% ethanol; after spraying the chromatograms are exposed to ammonia vapours.

Compound	$R_F \times 100$								
	I_1S_1	I_1S_2	I_1S_3	I_2S_1	I_2S_2	I_2S_3	I_3S_4	I_3S_5	I_3S_1
1-Phenyl-3,3-dimethyltriazene	32	68	32	18	78	35	90	51	0
1-*p*-Tolyl-3,3-dimethyltriazene	12 33*	69	13	21	68	10	96	51	0
1-*p*-Nitrophenyl-3,3-dimethyltriazene	46	86	25	32	78	25	11	51	0
1-*p*-Chlorophenyl-3,3-dimethyltriazene	25	71	18	16	58	18	80	51	0
1-*p*-Bromophenyl-3,3-dimethyltriazene	13	40	16	20	63	13	72	49	0
1-*p*-Carboxyphenyl-3,3-dimethyltriazene	76	83	83	79	87	86	72	40	64
1-*p*-Methoxyphenyl-3,3-dimethyltriazene	55	58	43	54	87	50	96	57	0
1-*p*-Ethoxyphenyl-3,3-dimethyltriazene	24	50	16	28	79	32	96	49	0
1-*p*-Hydroxyphenyl-3,3-dimethyltriazene	85	91	95	81	94	89	67	50	0
1-*p*-Aminophenyl-3,3-dimethyltriazene	59	92	70	60	88	57	81	47	0 22*
1-Diphenyl-3,3-dimethyltriazene	20	60	05	06	45	06	84	—	—

* Two spots were found in these systems, the first is the main compound, the second an impurity.

TABLE 32

TLC R_F VALUES OF 12 NAPHTHYLAMINEMONOSULPHONIC ACIDS

(E. ASMUS AND G. SCHULZE, *Z. Anal. Chem.*, 217 (1966) 180)

Thin layer: MN-Cellulosepulver 300G (Macherey, Nagel & Co.).
Solvent: *n*-Butanol–*n*-propanol–water–conc. ammonia (10:5:4:1).
Detection: U.V. light.

Compound	R_F	*Colour*
1-Naphthylamine-2-sulphonic acid	0.54	violet
1-Naphthylamine-3-sulphonic acid	0.43	violet
1-Naphthylamine-4-sulphonic acid	0.36	blue-violet
1-Naphthylamine-5-sulphonic acid	0.36	yellow-green
1-Naphthylamine-6-sulphonic acid	0.42	blue
1-Naphthylamine-7-sulphonic acid	0.47	bright blue
1-Naphthylamine-8-sulphonic acid	0.56	yellow-green
2-Naphthylamine-1-sulphonic acid	0.50	violet
2-Naphthylamine-5-sulphonic acid	0.38	blue
2-Naphthylamine-6-sulphonic acid	0.39	violet
2-Naphthylamine-7-sulphonic acid	0.40	blue-violet
2-Naphthylamine-8-sulphonic acid	0.43	blue-violet

TABLE 33

PC R_F VALUES OF SOME XANTHINES

(G. HAUCK, *Deut. Apotheker-Ztg.*, 105 (1965) 214)

Paper: Schleicher & Schüll 2043b.
Solvents: S_1 = *n*-Butanol–formic acid–water (12:1:7).
S_2 = *n*-Butanol satd. with 10% ammonia.
Detection: D_1 = U.V.-250 (photoprint).
D_2 = Dragendorff reagent and 1% $AgNO_3$ in 5% sulphuric acid.

Xanthine	R_F	
	S_1	S_2
1,3-Dimethyl-7-(N-methyl-N-hydroxyethyl-3-amino-2-hydroxypropyl)-xanthine	0.24	0.60
Mercuri-1-allyl-3,7-dimethylxanthine	0.31	0.07
3,7-Dimethylxanthine (theobromine)	0.43	0.24
1,3-Dimethyl-7-(2,3-dihydroxypropyl)-xanthine	0.49	0.53
1,3,7-Trimethylxanthine (caffeine)	0.60	0.26
1,3-Dimethylxanthine (theophylline)	0.62	0.28
1,3-Dimethyl-7-(2-hydroxyethyl)-xanthine	0.62	0.59
1,3-Dimethyl-8-chloroxanthine	0.70	0.93
1-Allyl-3,7-dimethylxanthine	0.80	0.78
1-(2-Hydroxypropyl)-3,7-dimethylxanthine	0.82	0.83
1-Hexyl-3,7-dimethylxanthine	0.89	0.86

TABLE 34

TLC R_F VALUES OF SOME MERCURY-FREE DIURETICS

(R. NEIDLEIN, H. KRÜLL AND M. MEYL, *Deut. Apotheker-Ztg.*, 105 (1965) 482)

Thin layer: Silica gel GF_{254} (Merck).
Solvent: Toluene–xylene–1,4-dioxan–isopropanol–25% ammonia (1:1:3:3:2).
Detection: U.V. 254 mμ and 350 mμ; Fearon reagent.

Compound	*Trade name*	R_F	*Colour with Fearon reagent*
2-Acetylamino-1,3,4-thiadiazole-5-sulphonamide	Diamox	0.10	Orange-yellow
7-Chloro-2-ethyl-1,2,3,4-tetrahydro-4-oxo-6-sulphamyl-quinazoline	Aquamox	0.53	Slight rust red
6-Chloro-7-sulphamyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1-dioxide	Esidrix	0.41	Rust red
6-Trifluoromethyl-7-sulphamyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1-dioxide	Olmagran	0.43	Rust red
1-Oxo-3-(3′-sulphamyl-4′-chlorophenyl)-3-hydroxy-isoindoline	Hygroton	0.59	Orange-yellow
4-Chloro-N-(2-furylmethyl)-5-sulphamoyl-anthranilic acid	Lasix	0.25	Blue-grey
N-*p*-Chlorophenyl-2,4-diamino-*sym*-triazine hydrochloride	Orpidan	0.70	—

TABLE 35

TLC R_F VALUES OF SOME PHARMACEUTICALS

(S. EL GENDI, W. KISSER AND G. MACHATA, *Mikrochim. Acta*, (1965) 125)

Thin layer: Kieselgel G with fluorescence indicator.
Solvents: S_1 = Chloroform with 10% acetone.
S_2 = Methanol.
S_3 = Chloroform with 10% ethanol.
S_4 = Chloroform with 20% ethanol.
Detection: D_1 = U.V. light.
D_2 = Potassium iodoplatinate.

Compound	R_F			
	S_1	S_2	S_3	S_4
Ephedrine	0.04	0.15	0.12	0.16
Nicotine	0.18	0.55	0.41	0.58
Brucine	0.03	0.10	0.25	0.32
Strychnine	0.05	0.13	0.24	0.30
Atropine	0.02	0.12	0.06	0.09
Quinine	0.02	0.41	0.25	0.31
Theobromine	0.11	0.50	0.38	0.56
Theophylline	0.19	0.53	0.50	0.64
Coffeine	0.35	0.64	0.61	0.74
Morphine	0.06	0.15	0.06	0.10
Codeine	0.04	0.18	0.20	0.23
Hydromorphone (Dilaudid)	0.01	0.21	0.15	0.19
Dionine (Ethylmorphine)	0.03	0.31	0.34	0.38
Papaverine	0.40	0.78	0.82	0.93
Narcotine	0.42	0.65	0.83	0.83
Cocaine	0.35	0.60	0.70	0.76
Prothipendyl (Dominal)	0.04	0.18	0.20	0.25
Sordinol	0.08	0.36	0.39	0.47
Captodiamine (Covatix)	0.18	0.51	0.59	0.79
Methaqualone (Revonal)	0.70	0.89	0.79	0.79
Octamylamine (Octinum D)	0.05	0.23	0.32	0.37
Pheniramine (Avil)	0.03	0.11	0.12	0.15
Tolazoline (Priscol)	0.04	0.16	0.10	0.12
Synopen (Halopyramine)	0.04	0.19	0.21	0.25
Diphenhydramine (Benadryl)	0.08	0.22	0.31	0.33
Antazoline (Antistine)	0.04	0.17	0.20	0.23
Neo-antergan (Pyrilamine)	0.10	0.32	0.47	0.57
Diphenylpyraline (Mepiben)	0.09	0.35	0.42	0.49
Thenalidine (Sandosten)	0.11	0.40	0.51	0.65
Tripelennamine (Pyribenzamine)	0.10	0.43	0.50	0.64
Clemizole (Allercur)	0.45	0.80	0.81	0.91
Tetracaine (Pantocaine)	0.15	0.47	0.40	0.48
Nupercaine (Dibucaine)	0.15	0.59	0.46	0.57
Phenacaine (Holocaine)	0.17	0.49	0.46	0.55
Lidocaine (Xylocaine)	0.37	0.71	0.68	0.86
Novesine (Dorsacaine)	0.17	0.64	0.58	0.73
Procaine (Novocaine)	0.10	0.53	0.37	0.39
Tutocaine (Butamine)	0.12	0.56	0.48	0.58
Propylhexedrine (Eventine)	0.03	0.13	0.11	0.14
Methamphetamine (Pervitin)	0.04	0.17	0.14	0.18
Reactivan	0.06	0.25	0.19	0.23
Methadone (Heptadone)	0.16	0.29	0.35	0.55
Tepanil (Regenon)	0.20	0.38	0.53	0.66
Perphenazine (Decentan)	0.06	0.48	0.29	0.33
Pethidine (Dolantin)	0.20	0.50	0.60	0.69

(*continued on p. D23*)

TABLE 35 (*continued*)

Compound	R_F			
	S_1	S_2	S_3	S_4
Phenmetrazine (Preludin)	0.12	0.57	0.43	0.51
Hydroxyzine (Atarax)	0.15	0.52	0.56	0.71
Ritalin (Rilatin)	0.12	0.54	0.55	0.68
Prethcamide (Micoren)	0.84	0.32	0.61/66	0.70/79
Tryptizol	0.36	0.27	0.55	0.36
Valium	0.89	0.54	0.83	0.82
Librium (Methaminodiazepoxide)	0.09	0.76	0.79	0.89
Taractan (Truxal)	0.20	0.45	0.57	0.72
Levomepromazine (Nozinan)	0.09	0.44	0.45	0.54
Promethazine (Phenergan)	0.09	0.38	0.49	0.58
Imipramine (Tofranil)	0.15	0.37	0.44	0.52
Thioridazine (Melleril)	0.09	0.24	0.38	0.44
Chlorpromazine (Largactil)	0.10	0.24	0.37	0.39

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