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Microchemical Journal, Volume 20, Number 3, September 1975

Briefs

An Auxiliary Table for Concentration Calculations in Atomic-Absorption Flame Photometry. J. RAMIREZ-MUÑOZ, *Beckman Instruments, Inc., Irvine, California 92664*

A table has been prepared for atomic-absorption work which gives concentrations in ppm and percent of substance in the original sample for a given series of standards as a function of the dilution ratio used during the steps of sample preparation. The table covers the most frequent concentration ranges of minor and micro components and dilution ratios from 1 : 10 to 1 : 10,000.

Microchem. J. **20,** 259–268 (1975).

Thin-Layer Chromatography of Steroids. X. R_M Correlations of Cholesterol Esters, Ethers, Carbonic Acid Esters and Halogen Derivatives. GABRIELLA D. SZÖNYI AND B. MATKOVICS, *Biochemical, Genetic Group, "A. J." University, Szeged, Hungary.*

A study was made of the TLC properties of the 3-beta-substituted esters, ethers, carbonic acid esters, and halogen derivatives of cholesterol. A regularity can be observed with regard to the polarity change of the esterifying component from C_6 to C_{16} in the cyclohexane–chloroform system.

Microchem. J. **20,** 269–277 (1975).

Thin-Layer Chromatography of Steroids. XI. Conclusions from R_M-Values of Estrone and Its Derivatives. D. G. SZÖNYI AND B. MATKOVICS, *Biochemical, Genetical Groups, "A. J." University, Szeged, Hungary.*

Derivatives with only a slight difference in molecular weight, in the case of 3-substituted ether side-chains, are difficult to separate by means of TLC. Ethers containing an iso, unsaturated, cycloalkyl or aromatic side-chain can readily be separated. The same holds for esters.

Microchem. J. **20,** 278–286 (1975).

Spectrophotometric Determination of Palladium Using Arsenazo I. H. KHALIFA AND Y. M. ISSA, *Chemistry Department, Faculty of Science, Cairo University, Giza, Egypt.*

Arsenazo I is used for the determination of palladium. The optimum conditions favoring the formation of the complex are extensively investigated. The complex is on a 1 : 1 ratio. As little as 0.64 ppm of Pd has been determined.

Microchem. J. **20,** 287–291 (1975).

Simultaneous Spectrophotometric Determination of Molybdenum and Selenium, and of Molybdenum and Tellurium. D. A. WARNER AND J. PAUL, *Chemistry Department, University of Bridgeport, Bridgeport, Connecticut 06602.*

A procedure is described for the solvent extraction of molybdenum–phenylfluorone complex and its application to the simultaneous determination of molybdenum and selenium and of molybdenum and tellurium.

Microchem. J. **20,** 292–298 (1975).

A Rapid Potentiometric Method for Analysis of Some Minerals and Basic Refractories. H. KHALIFA AND A. I. ATALLA, *Chemistry Department, Ministry of Industry, Cairo, Egypt.*

Analyses of phosphate, quartzite, and fluorspar-minerals, chromite, chrome magnesite, and magnesite chrome brick-basic refractories are described. These are based on the titration of excess EDTA with Hg(II) nitrate using a silver amalgam indicating electrode.

Microchem. J. **20,** 299–304 (1975)

The Application of Elemental Analysis to the Determination of the Composition of a Mixture of Pyridine Compounds. E. A. REICH, M. A. CARROLL, A. POST, AND J. E. ZAREMBO, *Smith Kline & French Laboratories, 1500 Spring Garden Street, Philadelphia, Pennsylvania 19101.*

The composition of a gross mixture of pyridine compounds was determined by use of standard microanalytical determinations followed by simple mathematical calculations. The results were corroborated by spectral data.

Microchem. J. **20,** 305–308 (1975).

Detection of Steroids with Molybdovanadophosphoric Acids on Thin-Layer Chromatograms. RONALD M. SCOTT AND RICHARD T. SAWYER, *Department of Chemistry, Eastern Michigan University, Ypsilanti, Michigan 48197.*

Two vanadium analogues of phosphoric acid have been studied for use as detection reagents for steroids on thin-layer plates. Characteristic colors were obtained.

Microchem. J. **20,** 309–312 (1975).

Reactor for Photo-Electrochemical Studies. G. F. ATKINSON, *Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1.*

Apparatus is described which is suitable for small-scale preparations.

Microchem. J. **20,** 313–314 (1975).

Marine Adhesives. V. Amino Acids Content of *Mytilus Edulis* Byssal Threads. ALLAN F. KRIVIS AND C. O. CHIU, *Department of Chemistry, The University of Akron, Akron, Ohio 44325.*

Byssal threads secreted by the mussel, *Mytilus edulis,* have been analyzed to determine their amino acid content. Based on chromatographic data, the threads contain at least 19 amino acids. The overall composition of the threads appears to be similar to glycoproteins.

Microchem. J. **20,** 315–318 (1975).

A Micromethod for Lead in Canned Baby Juices. YUEN S. LEE, NELSON T. LAO, AND JOSEPH P. CRISLER, *Division of Laboratories, D. C. Department of Human Resources, Washington, D. C. 20001.*

The method has advantages over conventional wet digestion methods which require time, attention, acid handling, hood space, and possible loss of lead due to multiple extraction. The data presented indicate some infants may be ingesting double the estimate permissible maximum daily intake of 300 μg of lead, above which the entire amount of lead cannot be excreted and accumulation in the body begins.

Microchem. J. **20,** 319–323 (1975).

The Pyrolytic Identification of Organic Molecules. IV. The Pyrolytic Behavior of the Isomeric Butanols. G. INGRAM AND S. M. H. RIZVI, *Department of Chemistry, Portsmouth Polytechnic, Burnaby Road, Portsmouth, Hants., England.*

A study of the pyrolytic behavior of the butanol isomers has been made in order to establish a basis for their identification from the thermolysis pattern of each butanol. The identity of the volatile products was established by gas chromatographic analysis. Each isomer was found to decompose via degradation reactions characteristic of the structural arrangement of each alcohol molecule for which mechanisms are proposed.

Microchem. J. **20,** 324–352 (1975).

The Determination of Organic Substances by the Oxidation with Permanganate. XVIII. The Oxidation of Malonic Acid. A. BERKA, M. KOŘÍNKOVÁ, AND J. BAREK, *Department of Analytical Chemistry, Charles University, Albertov 2030, Prague 2, Czechoslovakia.*

On the basis of successive oxidation, a titration determination, involving complete oxidation to carbon dioxide and water, was developed.

Microchem. J. **20,** 353–359 (1975).

Determination of Sulfate in Waters. B. W. BUDESINSKY, *Phelps Dodge Corporation, Morenci, Arizona 85540.*

Determination of sulfate in industrial waters is performed by shaking with Dowex 50-X8 resin and titration of aqueous acetone solution with barium perchlorate using Dimethylsulfonazo III as the indicator. Only phosphate and arsenate interfere.

Microchem. J. **20,** 360–362 (1975).

Volumetric Determination of Pt(IV) in the Presence of Ir, Pd, and Rh with Ferrous Ammonium Sulfate in Alkaline Mannitol Medium. NAZIR CHUGHTAI, JAN DOLEŽAL, AND JAROSLAV ZÝKA, *Department of Analytical Chemistry, Charles University, Albertov 2030, Prague 2, Czechoslovakia.*

A potentiometric reductimetric method for the determination of Pt(IV)-Pt(II) with a standard Fe(II) solution in an alkaline medium of mannitol is described. Ir, Pd, and Rh do not interfere.

Microchem. J. **20,** 363–366 (1975).

The Oxidation of Aminophenazone and Phenazone with Ceric Sulfate. HANA TOMÁNKOVÁ AND JAROSLAV ZYKA, *The State Institute for Drug Control and the Department of Analytical Chemistry, Charles University, Prague 2, Czechoslovakia.*

The present paper supplements the previous investigation of oxidation of organic compounds, the present work being done in strongly acidic and carbonate media, using ceric sulfate as the oxidant.

Microchem. J. **20,** 367–378 (1975).

Guanosine Tetra- and Penta phosphate Analysis: PEI-Cellulose Thin-Layer Purification and Luciferin–Luciferase Liquid Scintillation Quantitation. KNOX VAN DYKE, *Department of Pharmacology, West Virginia University Medical Center, Morgantown, West Virginia 26506.*

A system is described that can separate and quantitate in picomole amounts various guanosine tetra- and pentaphosphates. It uses a crude firefly luciferin–luciferase system in which the various derivatives probably transphosphorylate ADP to produce the ATP necessary to emit light with the luciferin–luciferase system.

Microchem. J. **20,** 379–387 (1975).

Comments on the Potentiometric Titration of Halides Including Fluoride with a Mixed Titrant. WALTER SELIG, *Chemistry Department, Lawrence Livermore Laboratory, Livermore, California 94550*

An attempt was made to verify work reported by others on the potentiometric titration of halides including fluoride with a mixed titrant of silver nitrate and thorium nitrate. The platinum indicator electrode can be used to monitor the titration of bromide and chloride. The reported results on fluoride could not be verified. There seems to be no theoretical basis on which to expect the platinum electrode to respond to changes in fluoride ion concentration.

Microchem. J. **20,** 388–391 (1975).

Polarographic Investigation of the Zimmermann Reaction Mechanism. K. G. BLASS AND R. J. THIBERT, *Department of Chemistry, University of Windsor, Windsor, Ontario, Canada N9B 3P4.*

Polarographic examination of the Zimmermann reaction adds further experimental evidence to the proposed theory of methylene group attack to the para position of *m*-dinitrobenzene. It also supports the theory that nitro anion formation can occur at high concentrations of 3β-hydroxyandrost-5-en-17-one compared to *m*-dinitrobenzene. Furthermore, polarographic analysis shows that the purple chromogen measured in the determination of steroids by the Zimmermann reaction does not contain a nitro anion.

Microchem. J. **20,** 392–397 (1975).

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An Auxiliary Table for Concentration Calculations in Atomic-Absorption Flame Photometry

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INTRODUCTION

In spite of the fact that many modern atomic-absorption spectrophotometers are designed to read concentrations of analyte directly, many flame spectroscopists still prefer to plot analytical working curves from readings obtained with sets of standards. Curves which relate readings (absorbance or relative absorbance values) with concentrations are usually prepared on the basis of the actual concentration of the standard solutions measured in the working session. However, this procedure then necessitates subsequent transformation of the observed test solution concentrations into the concentrations actually present in the original sample.

When computer techniques are used to handle experimental readings for calculating original concentrations (linear or nonlinear interpolation), a step should be introduced which uses the dilution ratio factor. This dilution ratio factor, d, used as a concentration factor, $1/d$, leads to concentration values in original samples.

Manual calculations and programs can be simpler if functional concentration scales are used. In other words, readings are related to original concentrations (i.e., test solution or standard solution concentrations multiplied by $1/d$). The same criterion can be used when adjusting the instrument's readout module to give concentrations directly.

The auxiliary table given in this note covers the most common dilution ratios and the most frequent concentration ranges in standards. This table facilitates rapid calculation of functional concentration ranges in manual plotting of curves, and calibration of concentration readouts. Use of the table is illustrated with several analytical situations typical of AAS programs. Also, guidelines are given relative to dilution procedures.

AUXILIARY TABLE

The table has been simplified by assuming that exact weights of sample (2.0000, 1.0000, 0.5000 g, etc.) are being diluted to the most frequent volumes used in volumetric flasks.

TABLE 1
AUXILIARY TABLE

Analyte concentration in standards (ppm)	Analyte concentration in original samples											
	Dilution ratio used											
	1:10		1:20		1:25		1:50		1:100		1:200	
	ppm	%	ppm	%	ppm	%	ppm	%	ppm	%	ppm	%
100	10^3	0.1	2×10^3	0.2	2.5×10^3	0.25	5×10^3	0.5	10^4	1.0	2×10^4	2.0
50	500	0.05	10^3	0.1	1.25×10^3	0.125	2.5×10^3	0.25	5×10^3	0.5	10^4	1.0
20	200	0.02	400	0.04	500	0.05	10^3	0.1	2×10^3	0.2	4×10^3	0.4
10	100	0.01	200	0.02	250	0.025	500	0.05	10^3	0.1	2×10^3	0.2
5	50	0.005	100	0.01	125	0.0125	250	0.025	500	0.05	10^3	0.1
2	20	0.002	40	0.004	50	0.005	100	0.01	200	0.02	400	0.04
1	10	0.001	20	0.002	25	0.0025	50	0.005	100	0.01	200	0.02
0.5		—	10	0.001	13	0.0013	25	0.0025	50	0.005	100	0.01
0.2		—		—		—	10	0.001	20	0.002	40	0.004
0.1		—		—		—		—	10	0.001	20	0.002

Analyte concentration in standards (ppm)	Analyte concentration in original samples											
	Dilution ratio used											
	1:250		1:500		1:1000		1:200		1:500		1:10000	
	ppm	%	ppm	%	ppm	%	ppm	%	ppm	%	ppm	%
100	2.5×10^4	2.5	5×10^4	5.0	10^5	10.0		—		—		—
50	1.25×10^4	1.25	2.5×10^4	2.5	5×10^4	5.0	10^5	10.0		—		—
20	5×10^3	0.5	10^4	1.0	2×10^4	2.0	4×10^4	4.0	10^5	10.0		—
10	2.5×10^3	0.25	5×10^3	0.5	10^4	1.0	2×10^4	2.0	5×10^4	5.0	10^5	10.0
5	1.25×10^3	0.125	2.5×10^3	0.25	5×10^3	0.5	10^4	1.0	2.5×10^4	2.5	5×10^4	5.0
2	500	0.05	10^3	0.1	2×10^3	0.2	4×10^3	0.4	10^4	1.0	2×10^4	2.0
1	250	0.025	500	0.05	10^3	0.1	2×10^3	0.2	5×10^3	0.25	5×10^3	0.5
0.5	125	0.0125	250	0.025	500	0.05	10^3	0.1	2.5×10^3	0.25	5×10^3	0.5
0.2	50	0.005	100	0.01	200	0.02	400	0.04	10^3	0.1	2×10^3	0.2
0.1	25	0.0025	50	0.005	100	0.01	200	0.02	500	0.05	10^3	0.1

The first column of Table 1 shows the actual concentration in ppm of calibration standards (1 ppm = 1×10^{-6} g/ml). The other columns give the functional concentration (real concentration of analyte in the sample) as a function of the dilution ratio used in the steps of sample preparation. Real concentrations of the analyte are expressed both in ppm and percentage.

As this table is primarily intended to be used in the determination of minor and microcomponents, special attention has been given to analytes present in samples from 1.0 to 0.001% (10^4 to 10 ppm). Exceptionally, some data have been included for concentrations of 10.0, 5.0, and 2.0%.

Dilution ratios shown in Table 1 are adequate to cover most of the cases which are commonly found in atomic-absorption analysis. Dilution operations are among the best known and frequently done techniques in the laboratory work. However, dilution, besides weighing, can be one of the major sources of operational errors in atomic-absorption work. Errors are introduced in the preparation process when small pipets and/or flasks are used, or too small original samples are weighed. In Table 2 some recommended weights (if enough sample is available), and volume of volumetric flasks for the first dilution step are summarized. In this table, also volumes of aliquots (pipets) and volumes of volumetric flasks for the second dilution step are given. Observe that sample weights of 0.5000 g or over are given, and volumes are never under 5.00 ml.

Last column of Table 2 gives an indication of the percentage of original sample in solution after all the dilution process. This value is important in atomic-absorption work when it is desirable to maintain the amount of solubilized sample under some limits, in order to avoid deposits, clogging effects, or memory. If so, dilution ratio lower than 1:100 should be chosen. Percentage values of last column of Table 2 have been given with a minimum of significant figures, to indicate only the concentration level. The real number of accurate figures will depend in each case on the accuracy of the weighing and/or diluting steps.

In the case of analytical schedules which have to cover wide concentration ranges for a given analyte, Table 1 can be used as follows: (a) The total analytical concentration range can be supposed to be divided into sections corresponding to a change of concentration equal to an order of magnitude (or about an order of magnitude); for instance, if the total concentration range to be examined covers from 5.0 to 0.005%, it can be divided into three subranges (5.0–0.5, 0.5–0.05, and 0.05–0.005%). (b) A single sample weighing and a preliminary dilution is performed (for instance, 1.0000 g of sample is dissolved and diluted to 100 ml, i.e., 1:100 dilution ratio). (c) Two other dilutions are prepared

TABLE 2
DILUTION RATIOS

Dilution ratios	First dilution step		Second dilution step		Concentration of original sample in final solution (%)
	Sample weight (g)	Volume (ml)	Aliquot (ml)	Final volume (ml)	
1:10	1.0000	10.00			10.0
	5.000	50.0			
	10.000	100.0			
1:20	0.5000	10.00			5.0
	2.5000	50.0			
	5.000	100.0			
1:25	1.0000	25.0			4.0
	2.0000	50.0			
	4.0000	100.0			
1:50	0.5000	25.0			2.0
	1.0000	50.0			
	2.0000	100.0			
1:100	0.5000	50.0			1.0
	1.0000	100.0			
	2.0000	200.0			
	2.5000	250.0			
	5.000	500.0			
	10.000	1000.			
1:200	0.5000	100.0			0.5
	1.0000	200.0			
1:250	1.0000	250.0			0.4
	2.0000	500.0			
1:500	0.5000	250.0			0.2
	1.0000	500.0			
			20.0 of 1:100	100.0	
1:1000	0.5000	500.0			0.1
	1.0000	1000.			0.1
			10.00 of 1:100	100.0	
			25.0 of 1:100	250.0	
1:2000	0.5000	1000.			0.05
			5.00 of 1:100	100.0	
			10.00 of 1:100	200.0	
1:5000			20.0 of 1:100	1000.0	0.02
			20.0 of 1:1000	100.0	
1:10000			10.00 of 1:100	1000.0	0.01
			10.00 of 1:1000	100.0	

from this preliminary solution to end in the dilution ratios 1:1000 and 1:10000. (d) A single series of standards prepared (in this case, standards with concentrations of 5, 2, 1, and 0.5 ppm of analyte). (e) The three solutions prepared are measured by using the single series of standards. See in Table 1 the analyte concentrations which correspond to

standards containing 5, 2, 1, 0.5 ppm under the dilution ratios 1:100, 1:1000, and 1:10000. The table is used to choose the standards for a given concentration (initial concentration) and a given dilution, or to choose the necessary dilution for a given concentration (initial concentration) to make to coincide the final solutions with a given series of standards.

If strong interference effects are observed, it might be recommendable to prepare three series of standards for this example. Each series should be compensated by adding the necessary concentration of matrix components according to the dilution ratio used (1/100, 1/1000, and 1/10000 of the initial concentration of the matrix components, respectively).

As previously indicated, the table has been prepared on the basis of exact weights of sample. However, the table still can be applied for other sample weights, W. If instead of 1.0000 g, the sample weights were 1.0340 or 0.8295 g, the concentration read in the direct concentration readout or functional scale can be multiplied by the corresponding factor, 1/W; then C (1/W) would be C (1.0000/1.0340) or C (1.0000/0.8295), respectively.

SOME EXAMPLES

In Table 3, a few examples have been summarized. Data of Table 3 come from some recent experimental work done at the author's laboratory. They correspond to metallurgical samples: determination of minor and microcomponents in aluminum and ferrous alloys. One percent and 0.5% preliminary solutions were prepared for aluminum and ferrous alloys, respectively (dilution ratios: 1:100 and 1:200). Then further diluted solutions were prepared. In some cases the full range of concentrations were covered with three dilution ratios (such are the cases of Pb, Zn, and Mg in aluminum alloys, and of Cr and Cu in ferrous alloys). The same range of concentrations was used for the standards at any dilution ratio, thus permitting the operator to work with identical instrumental settings; in other words, at the same instrumental sensitivity.

Dilutions and standards were chosen taking into account the sensitivity achievable with the instruments and burner used. Data shown in Table 3 correspond to operation with Beckman Autolam burner with Beckman Models 485 and 495 Atomic-Absorption Spectrophotometers[1], by using air-acetylene flame for all elements, except Sn (nitrous oxide-acetylene flame).

[1] The same ranges are applicable to Beckman Models 448, 440, and 444 Atomic-Absorption Spectrophotometers.

TABLE 3
EXAMPLES, METALLURGICAL ANALYSIS

Analyte	Sample	Analyte concentration ranges in original samples (%)	Recommended dilution ratio	Concentration of standards (ppm)
Pb	Aluminum alloys	4.0–0.5	1:1000	50, 20, 10, 5
		0.8–0.1	1:2000	
		0.2–0.01	1:100	
Zn	Aluminum alloys	5.0–0.5	1:10000	5, 2, 1, 0.5
		0.5–0.05	1:10000	
		0.5–0.01	1:100	
Ni	Aluminum alloys	1.0–0.1	1:10000	10, 5, 2, 1
		0.1–0.01	1:100	
Mn	Aluminum alloys	1.0–0.05	1:1000	10, 5, 2, 1, 0.5
Mg	Aluminum alloys	5.0–0.5	1:10000	5, 2, 1, 0.5
		0.5–0.05	1:1000	
		0.05–0.005	1:100	
Cr	Aluminum alloys	0.5–0.05	1:100	50, 20, 10, 5
Fe	Aluminum alloys	1.0–0.1	1:1000	10, 5, 2, 1
Cu	Aluminum alloys	10.0–0.5	1:10000	10, 5, 2, 1
Sn	Aluminum alloys	0.2–0.02	1:100	20, 10, 5, 2
Co	Ferrous alloys	0.1–0.01	1:200	10, 5, 2, 1
Cr	Ferrous alloys	20.0–10.0	1:20000	10, 5, 2, 1
		1.0–0.5	1:1000	
		0.1–0.05	1:200	
Cu	Ferrous alloys	5.0–1.0	1:5000	10, 5, 2, 1
		0.5–0.1	1:200 or 1:500	
		0.1–0.05	1:200	
Mn	Ferrous alloys	1.0–0.5	1:10000	10, 5, 2, 1
		0.5–0.1	1:200 or 1:10000	
Ni	Ferrous alloys	5.0–1.0	1:5000	10, 5, 2, 1
		0.1–0.05	1:200	

If working with burners which permit more sensitivity in the instrumental operation, other dilutions and/or sets of standards can be chosen. For instance, by working with the Beckman Laminar Flow Burner, in hot operation, instead of the dilutions and standards given in Table 3 for Zn, the ranges 2–0.2 ppm Zn and 0.2–0.02 ppm Zn can be investigated at dilution ratios 1:10000 and 1:1000, respectively, with standard containing (according to Table 1) 2, 1, 0.5, and 0.2 ppm Zn.

The choosing of appropriate dilutions as a function of the sensitivity achieved in the instrumental operation has been fully discussed in another paper (*1*).

Experimental values obtained in these research projects were calcu-

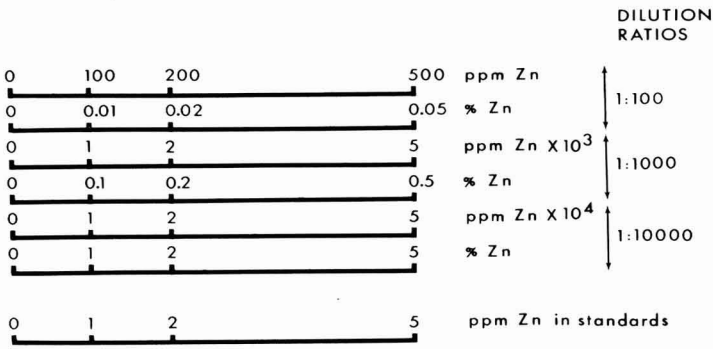

FIG. 1. Functional concentration scales used in zinc determination in aluminum alloys. Autolam burner.

KEY PUNCH FORM - GENERAL PURPOSE X255

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INTERPOLATION METHOD IN ATOMIC ABSORPTION

ZINC IN AL-ALLOYS READS. ABSORBANCE-NO SCALE EXPANSION AUTOLAM BURNER

ZN-5	1:100		.480	.483		.005	.100	.101		.01	.190	.190			
						.02	.345	.340		.03	.474	.470			
						.05	.618	.620						Ø	
ZN-7	1:1000		.275	.273		.05	.095	.089		.1	.180	.181			
						.2	.330	.331						Ø	
ZN-8	1:10000		.254	.251		.5	.087	.088		1.	.180	.180			
						2.	.333	.330						9	

FIG. 2. Input table for computer calculation of zinc concentration in three aluminum alloys. First column contains sample identification and identification of dilution ratio. Figures in the next two columns are absorbance readings (in duplicate) for the sample solutions. The other columns show functional concentration of standards and their absorbance readings (in duplicate). No conversion factors have been used.

ZINC IN AL-ALLOYS REDGS.ABSORBANCE-NO SCALE EXPANSION AUTOLAM BURN PAGE 1

SYSTEM		STANDARDS C	A	ERROR	C	A	ERROR	CS ADDED	ABSORBANCE	CS FOUND	DIFFERENCE
ZN-5	1 100	0.005 0.020 0.050	0.100 0.342 0.619	0.000 0.987 0.068	0.010 0.030	0.190 0.472	-0.066 -0.536	0.0	0.4815	0.0311	0.0311
ZN-7	1 1000	0.050 0.200	0.092 0.330	0.001 0.000	0.100	0.180	-0.003	0.0	0.2740	0.1594	0.1594
ZN-8	1 10000	0.500 2.000	0.087 0.331	0.000 0.000	1.000	0.180	-0.002	0.0	0.2525	1.4422	1.4422

Note. From a previous paper (2): C = conc. expressed as ppm; A = absorbance; and A = 0.0436 Cppm/PCLppm, where PCL = percentual concentration limits.

FIG. 3. Output Table. Results of % Zn in aluminum alloys are shown in column CS FOUND (Concentration of samples, found). Values 0.0 are shown under CS ADDED because the samples have been treated by the computer as unknowns.

lated by graphic interpolation on analytical working curves plotted with functional concentration scales similar to those shown in Fig. 1 for Zn determinations.

Functional concentration values were used for standards when preparing input tables for batch operation in an off-line computer. Concentrations for standards given for interpolation programs, as shown in Fig. 2, correspond to concentrations in % Zn calculated according to Table 1 for standard solutions containing only 5, 3, 2, 1, and 0.5 ppm Zn. Just for information purposes, dilution ratios for each sample are shown in input tables besides the sample identification number. No conversion factors are included in the input tables. In the example shown in Fig. 2, three different sets of functional concentrations for standards are used. In routine analysis of samples examined at the same dilution ratio, standards are given only once, and a flag -1 is used instead of the flag 0 to keep the same standards for the whole series of samples. Results appear in output tables (Fig. 3) directly in % Zn in original samples.

SUMMARY

A table has been prepared for atomic-absorption work which gives concentrations in ppm and percent of analyte in original sample for a given series of standards as a function of the dilution ratio used during the steps of sample preparation. The use of original concentrations simplifies the calculation of concentration in manual graphical interpolation and allows the operator the use of simple interpolation computer programs, if desired, without the need of introducing constant or variable conversion factors. The table covers the most frequent concentration ranges of minor and micro components and dilution ratios from 1:10 to 1:10000.

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MICROCHEMICAL JOURNAL 20, 269–277 (1975)

Thin-Layer Chromatography of Steroids. X. R_M-Correlations of Cholesterol Esters, Ethers, Carbonic Acid Esters and Halogen Derivatives

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In this paper an account is given of the layer-chromatographic behavior of the steroid groups indicated in the title, and of the conclusions that can be drawn from this.

The TLC[2] study of these compounds was justified by the need to supplement our chemical analyses, but in addition our aim was to find a practical method simplifying comparisons.

As regards earlier publications relating to other steroid groups, mention should be made of the investigations of Feher *et al.* (*1–3*) and of ourselves (*4,5*).

MATERIALS AND METHODS

The TLC plates were prepared with Kieselgel G nach Stahl (Reanal, Budapest), with Desaga type layering.

The plates measured 20 × 20 cm, with a layer thickness of 0.25 mm. Activation was carried out at 110°C for 1 hr.

In all cases a 1-dimensional ascending system was employed. The distance varied in the range 17–18 cm. Presaturation was not performed.

To the evaluation the plates were developed with a 1:1 mixture of orthophosphoric acid–water, and the color reaction brought about by heating under an infrared lamp. The positions of the spots were detected under a Camag analytical quartz lamp (366 nm).

The following groups of derivatives were examined.

1. *Cholesterol 3-beta-substituted esters:*
   Cholesterol formate, acetate, propionate, butyrate, valerate, laurate, tridecanoate, myristate, palmitate, stearate, undecylen-10-ate, oleate, benzoate and cinnamate.
2. *Cholesterol 3-beta-substituted ethers:*

[1] To whom all correspondence and reprint request should be addressed.
[2] Abbreviation used: TLC = thin-layer chromatography.

Cholesterol methyl, ethyl, *n*-propyl, *n*-butyl, *n*-amyl, *n*-octyl, *n*-lauryl, isopropyl, tert-butyl and benzyl ethers.

3. *Cholesterol 3-beta-substituted carbonic acid esters:*
   Cholesterol methyl, ethyl, *n*-amyl, *n*-octyl, isopropyl, isobutyl and benzyl carbonates.
4. *Cholesterol halogen derivatives*
   Cholesteryl chloride, iodide, bromide.

(The physico-chemical characteristics of these substances will be reported in a subsequent work.)

The *solvent systems* were as follows.

For groups 1–3:

I. Cyclohexane-methanol (99 : 1).
II. Cyclohexane-chloroform (6 : 4).
III. Cyclohexane-chloroform (8 : 2).
IV. Benzene-petroleum ether (1 : 1).

For group 4:

V. Cyclohexane.
VI. Cyclohexane-*n*-heptane (1 : 1).
VII. Isooctane.
VIII. Isooctane-carbon tetrachloride (95 : 5).

RESULTS AND DISCUSSION

For the analysis of the TLC properties, not only R_f values, but also the R_M values defined by Bate-Smith and Westall (*6*) were taken into consideration. The experimentally obtained R_f and R_M values are listed in Tables 1–4.

In connection with the separation of the cholesterol esters it must be mentioned that the finding of a system suitable for the separation of cholesterol and its derivatives posed many problems. The reason is that there is a very large difference in chromatographic mobility between cholesterol and its esters (this also applies to the ethers and carbonic acid esters).

It is clear from the data of Table 1 that the weakly polar systems are suitable for separative purposes in the TLC practice of this group of compounds.

As an illustration of the TLC correlations in the different systems, the R_M values have been plotted as a function of the MW. If the changes observed in system II are followed, it can be seen (Fig. 1) that in a certain section of the plot R_M is directly proportional to MW. For this linear section $\Delta R_{M(CH_2)} = -0.04$. The members containing an esterified side-chain shorter than C_6 or longer than C_{16} (and also $C_{12,13}$ do not obey this rule.

TABLE 1

No.	Substance	$R_M{}^f$ Systems applied[a] I	II	III	IV
1.	Cholesterol (Chol.)	0.03 1.51	0.032 1.48	start —	0.035 1.44
2.	Chol. formate	0.625 −0.22	0.447 0.09	0.215 0.56	0.675 −0.32
3.	Chol. acetate	0.57 −0.12	0.345 0.28	0.10 0.95	0.41 0.16
4.	Chol. propionate	0.68 −0.33	0.445 0.09	0.16 0.72	0.53 −0.05
5.	Chol. butyrate	0.64 −0.25	0.495 0.02	0.23 0.52	0.71 −0.38
6.	Chol. valerate	0.647 −0.26	0.662 −0.29	0.245 0.49	0.918 −1.05
7.	Chol. caproate	0.645 −0.26	0.54 −0.07	0.252 0.47	0.809 −0.63
8.	Chol. caprylate	0.80 −0.60	0.59 −0.16	0.262 0.45	0.775 −0.54
9.	Chol. caprinate	0.83 −0.66	0.637 −0.24	0.28 0.41	0.81 −0.63
10.	Chol. undecanoate	0.737 −0.45	0.657 −0.28	0.325 0.32	0.90 −0.95
11.	Chol. laurate	0.717 −0.40	0.647 −0.08	0.35 0.27	0.905 −0.98
12.	Chol. tridecanoate	0.767 −0.52	0.535 −0.06	0.335 0.30	0.75 −0.48
13.	Chol. myristate	0.747 −0.47	0.675 −0.32	0.33 0.31	0.92 −1.06
14.	Chol. palmitate	0.86 −0.79	0.717 −0.40	0.38 0.21	0.87 −0.82
15.	Chol. stearate	0.84 −0.72	0.712 −0.39	0.395 0.18	0.85 −0.75
16.	Chol. undecylen-10-ate	0.712 −0.39	0.587 −0.15	0.252 0.47	0.862 −0.79
17.	Chol. oleate	0.84 −0.72	0.677 −0.32	0.335 0.30	0.845 −0.74
18.	Chol. benzoate	0.657 −0.28	0.537 −0.06	0.27 0.43	0.83 −0.69
19.	Chol. cinnamate	0.50 0.00	0.435 0.11	0.155 0.74	0.707 −0.39

[a] In each series, the upper values are the R_f ones and the lower values are the R_M ones.

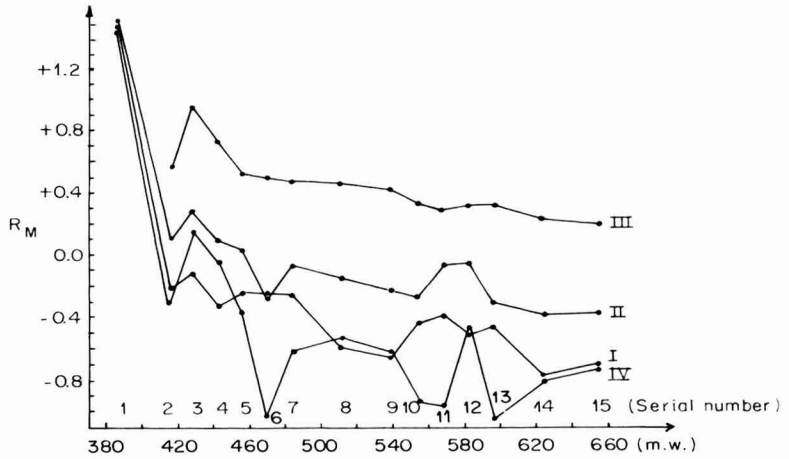

FIG. 1. Correlation of R_M and molecular weight for cholesterol esters Nos. 1–15.

Figure 2 shows the similar correlations for the esters containing an unsaturated carbon chain or an aromatic ring. Systems I and IV offer the best possibilities of separation, and it follows clearly from the R_M values that the polarity of the molecule is increased to a greater extent in the over mentioned systems than in any others in the examined series.

Table 2 shows that etherification results in compounds more polar than the corresponding esters, but the lengthening of the etherified side-chain is accompanied by a stronger polarity decrease. The decrease is greatest for the members containing 1–3 carbon atoms; with more than 5 carbon atoms in the side-chain the difference in polarity becomes increasingly less. These changes are well illustrated by the $\Delta R_{M(CH_2)}$ values for system IV (Fig. 3). Further, the similar chromatographic characteristics are confirmed by the fact that the systems which worked well in

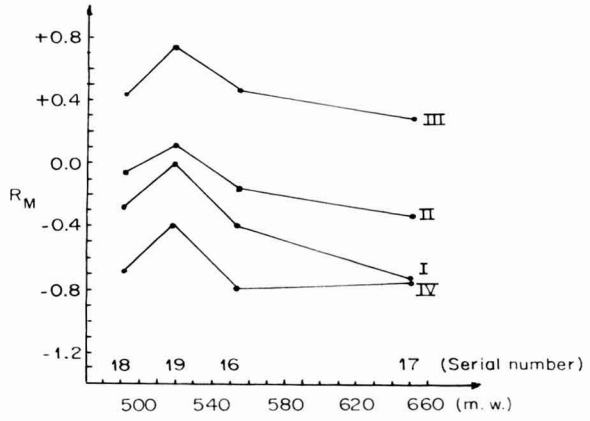

FIG. 2. Correlation of R_M and molecular weight for cholesterol esters Nos. 16–19.

TABLE 2

No.	Substance	R_M[f] Systems applied[a] I	II	III	IV
1.	Cholesterol	0.03 1.51	0.032 1.48	start —	0.035 1.44
2.	Chol. methyl ether	0.37 0.23	0.24 0.50	0.135 0.81	0.377 0.22
3.	Chol. ethyl ether	0.442 0.10	0.307 0.35	0.17 0.69	0.505 −0.01
4.	Chol. propyl ether	0.522 −0.04	0.405 0.17	0.22 0.54	0.645 −0.26
5.	Chol. butyl ether	0.53 −0.05	0.422 0.14	0.27 0.43	0.727 −0.42
6.	Chol. amyl ether	0.57 −0.12	0.487 0.02	0.285 0.40	0.795 −0.59
7.	Chol. octyl ether	0.627 −0.22	0.575 −0.13	0.36 0.25	0.897 −0.94
8.	Chol. lauryl ether	0.595 −0.17	0.62 −0.21	0.39 0.19	0.907 −0.99
9.	Chol. isopropyl ether	0.475 0.04	0.345 0.28	0.185 0.64	0.552 −0.09
10.	Chol. tert-butyl ether	0.407 0.17	0.31 0.35	0.18 0.66	0.572 −0.12
11.	Chol. benzyl ether	0.495 0.01	0.392 0.19	0.21 0.57	0.69 −0.35

[a] In each series, the upper values are the R_f ones and the lower values are the R_M ones.

the case of the esters were also excellently suited for the separation of the esters.

Figure 3 (dashed line) similarly shows the R_M and MW correlations for the isopropyl, tert-butyl and benzyl ethers. It is clearly seen that in spite of the molecular weights being the same, they can be chromatographed together with the members substituted with aliphatic chains.

The chromatography of the carbonic acid esters can be compared with that of the previous two groups. The polarity changes are similar to those described for the ethers. The derivatives containing iso-chain and aromatic esterifying components, however, exhibit a smaller R_M difference from the straight-chain carbonic acid esters, in contrast with what was found for the esters (Figs. 4, 5).

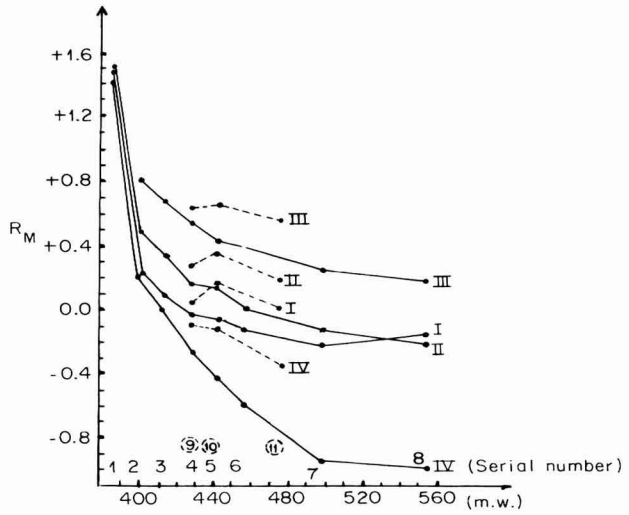

FIG. 3. Variation of R_M for cholesterol esters Nos. 1–11 as a function of molecular weight.

TABLE 3

		$R_M{}^f$			
		Systems applied[a]			
No.	Substance	I	II	III	IV
1.	Cholesterol	0.03	0.032	start	0.035
		1.51	1.48	—	1.44
2.	Chol. methyl carbonate	0.252	0.347	0.177	0.61
		0.47	0.27	0.67	−0.19
3.	Chol. ethyl carbonate	0.32	0.432	0.221	0.68
		0.33	0.12	0.55	−0.33
4.	Chol. amyl carbonate	0.377	0.582	0.36	0.80
		0.22	−0.14	0.25	−0.60
5.	Chol. octyl carbonate	0.482	0.655	0.43	0.87
		0.03	−0.28	0.12	−0.82
6.	Chol. isopropyl carbonate	0.345	0.50	0.24	0.75
		0.28	0.00	0.50	−0.48
7.	Chol. isobutyl carbonate	0.41	0.56	0.31	0.77
		0.16	−0.10	0.35	−0.52
8.	Chol. benzyl carbonate	0.335	0.48	0.26	0.72
		0.30	0.03	0.45	−0.41

[a] In each series, the upper values are the R_f ones and the lower values are the R_M ones.

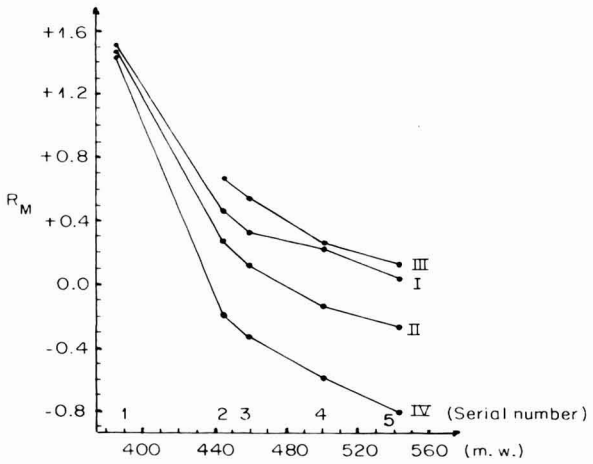

FIG. 4. Variation of R_M for cholesterol carbonic acid esters Nos. 1–5 as a function of molecular weight.

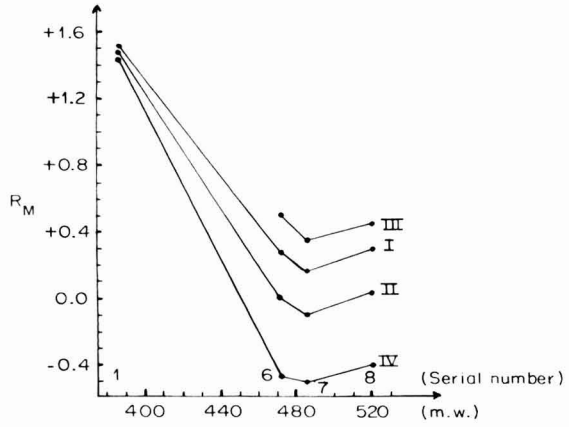

FIG. 5. Variation of R_m for cholesterol carbonic acid esters Nos. 6–8 as a function ob molecular weight.

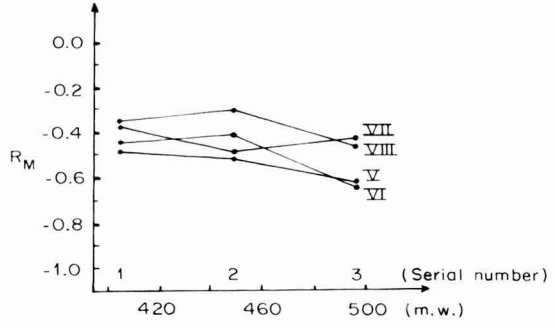

FIG. 6. Variation of R_M for halogen derivatives of cholesterol as a function of molecular weight.

TABLE 4

No.	Substance	$R_M{}^f$ Systems applied[a] V	VI	VII	VIII
1.	Chol. chloride	0.75	0.74	0.70	0.69
		−0.48	−0.45	−0.37	−0.35
2.	Chol. iodide	0.765	0.723	0.73	0.667
		−0.51	−0.41	−0.43	−0.30
3.	Chol. bromide	0.81	0.81	0.75	0.745
		−0.63	−0.64	−0.48	−0.46

[a] In each series the upper values are the R_f ones and the lower values are the R_M ones.

The TLC behavior of the halogen derivatives examined is a completely different type from that of the above three groups.

Their well-reproducible separation can no longer be solved with the systems used to separate the esters and the ethers. Significant chromatographic differences between these derivatives can be attained only by using apolar solvents (Fig. 6).

SUMMARY

A study was made of the TLC properties of the 3-beta-substituted esters, ethers, carbonic acid esters and halogen derivatives of cholesterol.

As regards the TLC properties it can be stated as a general correlation that the same solvent systems are suitable for the separation of these groups of compounds (with the exception of the halogen derivatives). As a result of our experiment, four such systems could be found among many others tried.

A regularity can be observed with regard to the polarity change of the esterifying component from C_6 to C_{16} in the cyclohexane-chloroform (6:4) system.

The cholesterol 3-beta-ethers are more polar than the esters, but with the lengthening of the etherifying side-chain the polarity decreases more rapidly than in the case of the esters; from the point of a TLC separation technique this is a very advantageous property.

As regards their properties the cholesterol carbonic acid derivatives can be correlated with the cholesterol ethers.

Compared with the above groups the 3-beta halogen cholesterol derivatives exhibit different properties. Their separation can be achieved with apolar solvents.

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Thin-Layer Chromatography of Steroids. XI. Conclusions from R_M-Values of Estrone and Its Derivatives

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As a continuation of earlier work (*1,2*), a study was made of the R_f and R_M correlations of estrone and its 3-ether derivatives, its oximes and the lactams obtainable from the oximes by Beckmann rearrangement (*3*). The present work deals with the conclusions to be drawn from these.

MATERIALS AND METHODS

The details of our TLC[2] method were described earlier (*4*).

In the present work the following groups of compounds were subjected to TLC studies:

1. Estrone and its 3-ether-substituted derivatives (Estrone methyl, ethyl, *n*-propyl, *n*-butyl, isopropyl, allyl, cyclopentyl and benzyl ethers) and two ester derivatives, the acetate and benzoate (serial nos. 1–11).
2. The oximes of estrone-17-one, corresponding to the derivatives listed in group 1/serial nos. 1-11/.
3. Estrone-D-homo-lactams /3-hydroxy-17-keto-17a-aza-D-homoestra-1,3,5/10/-triene and 3-substituted ether-derivatives/, similarly corresponding to the members of group 1/serial nos. 1–3 and 5–11/.

The systems applied for all three groups were as follows:

I. Benzene-methanol (95 : 5).
II. Benzene-methanol (9 : 1).
III. Benzene-ethanol (9 : 1).
IV. Benzene-ether (1 : 1).
V. *n*-heptane-acetone (2 : 1).
VI. *n*-heptane-acetone (3 : 1).
VII. Chloroform-acetone (9 : 1).

[1] To whom reprint requests should be addressed.

[2] Abbreviations used: TLC = thin-layer chromatography; estrone = 3-hydroxy-estra-1,3,5/10/-triene-17-one; MW = molecular weight.

VIII. Cyclohexane-chloroform-acetic acid (7:2:1).
IX. Acetic acid-cyclohexane-chloroform (1:2:2).

RESULTS AND DISCUSSION

As reported earlier for the TLC study of cholesterol and its derivatives, in the case of the estrone derivatives too an attempt was made to determine the chromatographic properties by calculation (Bate-Smith and Westall) of the R_f and R_M values (*5*). The experimental results corresponding to the ketone, 17-one-oxime and D-homo-lactam groups are listed in Tables 1–3.

For any system the estrone skeleton and its oxime and lactam exhibit the lowest R_f values, compared with their etherified derivatives. As regards a separation technique, therefore, etherification may be very ad-

TABLE 1

		R_M[a]								
		Systems applied[a]								
No.	Substance	I	II	III	IV	V	VI	VII	VIII	IX
1.	Estrone (E.)	0.39	0.35	0.45	0.76	0.39	0.22	0.66	0.18	0.495
		0.19	0.27	0.09	−0.50	0.19	0.55	−0.29	0.66	0.01
2.	E. methyl ether	0.69	0.71	0.72	0.89	0.54	0.46	0.945	0.44	0.775
		−0.35	−0.39	−0.41	−0.91	−0.07	0.07	−1.23	0.10	−0.53
3.	E. ethyl ether	0.69	0.715	0.73	0.88	0.565	0.48	0.955	0.43	0.785
		−0.35	−0.40	0.43	−0.86	−0.11	0.03	−1.33	0.12	−0.56
4.	E. *n*-propyl ether	0.71	0.725	0.76	0.915	0.54	0.53	front	0.43	0.81
		−0.39	−0.42	−0.50	−1.03	−0.07	−0.05	—	0.12	−0.63
5.	E. *n*-butyl ether	0.72	0.735	0.74	0.89	0.60	0.525	front	0.33	0.815
		−0.41	−0.44	−0.45	−0.91	−0.17	−0.04	—	0.31	−0.64
6.	E. isopropyl ether	0.72	0.73	0.71	0.905	0.575	0.51	front	0.36	0.805
		−0.41	−0.43	−0.39	−0.98	−0.13	−0.02	—	0.25	−0.61
7.	E. allyl ether	0.71	0.73	0.73	0.91	0.55	0.465	front	0.35	0.795
		−0.39	−0.43	−0.43	−1.00	−0.09	0.06	—	0.27	−0.59
8.	E. cyclopentyl ether	0.73	0.75	0.75	0.92	0.595	0.54	front	0.39	0.815
		−0.43	−0.48	−0.48	−1.06	−0.17	−0.07	—	0.19	−0.64
9.	E. benzyl ether	0.73	0.745	0.76	0.92	0.53	0.425	front	0.39	0.80
		−0.43	−0.46	−0.50	−1.06	−0.05	0.13	—	0.19	−0.60
10.	E. acetate	0.61	0.66	0.64	0.83	0.47	0.33	0.94	0.33	0.70
		−0.19	−0.29	−0.25	−0.69	0.05	0.30	−1.19	0.31	−0.37
11.	E. benzoate	0.68	0.70	0.75	0.88	0.47	0.35	front	0.385	0.76
		−0.33	−0.37	−0.48	−0.86	0.05	0.27	—	0.20	−0.50

[a] In each series, the upper values are the R_f-values and the lower ones are the R_M-values.

TABLE 2

No.	Substance	R_M[f] Systems applied[a] I	II	III	IV	V	VI	VII	VIII	IX
1.	Estrone-17-one oxime	0.05	0.35	0.33	0.40	0.18	0.13	0.10	0.27	0.48
		1.28	0.27	0.31	0.17	0.66	0.82	0.95	0.43	0.03
2.	E. methyl ether ox.	0.47	0.64	0.50	0.74	0.40	0.26	0.31	0.48	0.75
		0.05	−0.25	0.00	−0.45	0.17	0.45	0.35	0.03	−0.48
3.	E. ethyl ether ox.	0.49	0.62	0.52	0.79	0.43	0.29	0.33	0.47	0.775
		0.02	−0.21	−0.03	−0.57	0.12	0.39	0.31	0.05	−0.53
4.	E. *n*-propyl ether ox.	0.45	0.61	0.55	0.71	0.45	0.31	0.35	0.49	0.76
		0.09	−0.19	−0.09	−0.39	0.09	0.35	0.27	0.02	−0.50
5.	E. *n*-butyl ether ox.	0.53	0.62	0.544	0.84	0.41	0.305	0.37	0.47	0.785
		−0.05	−0.21	−0.07	−0.72	0.16	0.36	0.23	0.05	−0.56
6.	E. isopropyl ether ox.	0.51	0.57	0.54	0.81	0.44	0.295	0.34	0.46	0.77
		−0.02	−0.12	−0.07	−0.63	0.10	0.38	0.29	0.07	−0.52
7.	E. allyl ether ox.	0.51	0.61	0.53	0.70	0.41	0.275	0.33	0.45	0.77
		−0.02	−0.19	−0.05	−0.37	0.16	0.42	0.31	0.09	−0.52
8.	E. cyclopentyl ether ox.	0.50	0.585	0.538	0.82	0.45	0.315	0.37	0.48	0.79
		0.00	−0.15	−0.06	−0.66	0.09	0.34	0.23	0.03	−0.57
9.	E. benzyl ether ox.	0.49	0.60	0.534	0.79	0.40	0.23	0.36	0.50	0.78
		0.02	−0.17	−0.06	−0.57	0.17	0.52	0.25	0.00	−0.55
10.	E. acetate ox.	0.05	0.295	0.36	0.50	0.30	0.12	0.09	0.27	0.53
		1.28	0.38	0.25	0.00	0.37	0.86	1.00	0.43	−0.05
11.	E. benzoate ox.	0.05	0.25	0.33	0.49	0.25	0.14	0.10	0.31	0.515
		1.28	0.48	0.31	0.02	0.48	0.79	0.95	0.35	−0.02

[a] In each series, the upper values are R^f-values and the lower ones are the R_M-values.

vantageous. It is clear from the tabulated data that, for both the base compounds and the derivatives, the R_f values decrease in the order ketone > oxime > D-homo-lactam, in agreement with the polarities. If the various solvent systems are considered, for example in the case of system IV, it can be readily observed that while the ketones and oximes show the greatest mobility here, the lactams can not be separated in this system.

If it is desired to analyze the correlations of the TLC properties and structures of the ethers, it is to be expected that the members differing from one another by one CH_2 group will in principle exhibit linear variations.

However, it was found that the estrone methyl, ethyl, *n*-propyl and *n*-butyl ethers most often possessed very close R_f values. It can be ob-

TABLE 3

No.	Substance	R_M[f] Systems applied[a] I	II	III	IV	V	VI	VII	VIII	IX
1.	Estrone-D-homo-lactam	start	0.14	0.145	start	start	start	start	start	0.29
		—	0.79	0.77	—	—	—	—	—	0.39
2.	E. methyl ether l.	0.19	0.35	0.29	"	"	"	0.16	0.25	0.61
		0.63	0.27	0.39				0.72	0.48	−0.19
3.	E. ethyl ether l.	0.18	0.365	0.31	"	"	"	0.18	0.25	0.62
		0.66	0.24	0.35				0.66	0.48	−0.21
5.	E. *n*-butyl ether l.	0.20	0.40	0.34	"	"	"	0.19	0.24	0.68
		0.60	0.17	0.29				0.63	0.50	−0.33
6.	E. isopropyl ether l.	0.21	0.41	0.34	"	"	"	0.195	0.24	0.67
		0.57	0.16	0.29				0.61	0.50	−0.31
7.	E. allyl ether l.	0.19	0.39	0.34	"	"	"	0.17	0.22	0.63
		0.63	0.19	0.29				0.69	0.55	−0.23
8.	E. cyclopentyl ether l.	0.20	0.39	0.32	"	"	"	0.18	0.26	0.65
		0.60	0.19	0.33				0.66	0.45	−0.27
9.	E. benzyl ether l.	0.19	0.365	0.32	"	"	"	0.20	0.26	0.65
		0.63	0.24	0.33				0.60	0.45	−0.27
10.	E. acetate l.	0.10	0.24	0.24	"	"	"	0.13	0.11	0.34
		0.95	0.50	0.50				0.82	0.91	0.29
11.	E. benzoate l.	0.15	0.29	0.31	"	"	"	0.15	0.246	0.63
		0.75	0.39	0.35				0.75	0.48	−0.23

[a] In each series, the upper values are R_f-values and the lower ones are the R_M-values.

served in general in the various solvent systems that lengthening of the etherifying side-chain by one CH_2 unit leads to a negative R_M change, which proves the decrease in polarity of the molecules. This change best approaches linearity in solvent system II ($\Delta R_{M(CH_2)} = -0.01$ or -0.02). There are thus very small differences between the members of the group, which points to similar properties and very difficult co-chromatographability (Fig. 1).

Agreeing conclusions are reached if the iso, unsaturated or cyclic and aromatic ether derivatives are examined. Precisely as a consequence of the larger chemical differences, the efficiency of their separation is much higher. From this respect systems V and VI can be regarded as the most suitable (Fig. 2).

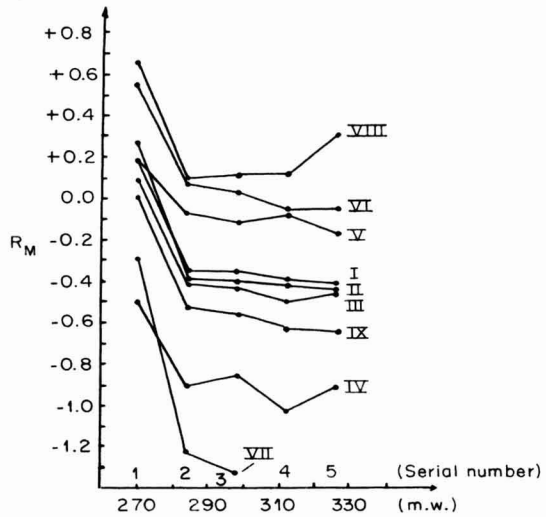

FIG. 1. Variation of R_M for estrone and its 3-ether-substituted derivatives Nos. 1–5 in 9 different solvent systems as a function of molecular weight.

The TLC studies indicate that besides estrone its 3-acetate and 3-benzoate can be well separated, with the exception of systems V, VI and VIII. More apolar system prepared, separation will be better (Fig. 3).

With estrone-17-one oxime and its C_1–C_4 ethers a linear correlation was obtained between the MW and R_M values in system VII. This change can be evaluated with $\Delta R_{M(CH_2)} = -0.04$. In a certain case,

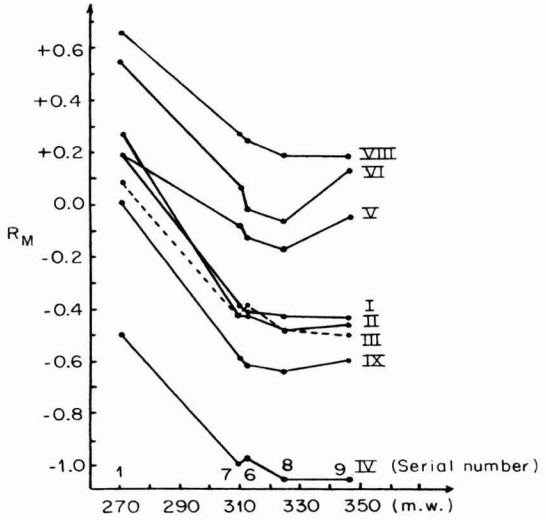

FIG. 2. Variation of R_M for estrone ethers Nos. 6–9 as a function of molecular weight.

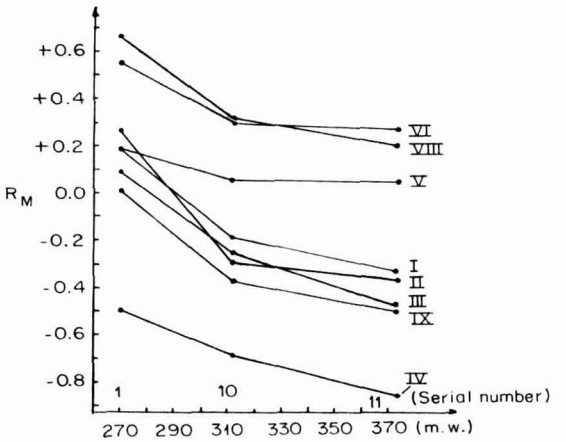

FIG. 3. Variation of R_M for two esters (acetate and benzoate) of estrone, similarly in 9 solvent systems.

therefore, under certain conditions there is a clear-cut polarity-decrease between the members, but under other conditions polarity-decreases and increases may alternate, or the polarity-increase may be enhanced (Fig. 4).

The better possibility of separation for the ethers of estrone with serial numbers 6–9 also holds good for the oximes of these derivatives. Here too the best separation is obtained in systems V and VI. It is interesting to observe that in these systems the shapes of the curves are very similar (Figs. 2 and 5), while at the same time it can be clearly seen that the R_M differences between the oxime members decrease (Fig. 5).

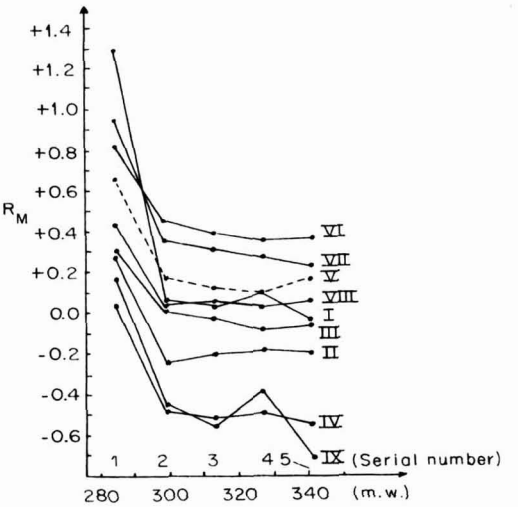

FIG. 4. Variation of R_M for estrone-17-one oxime ethers Nos. 1–5.

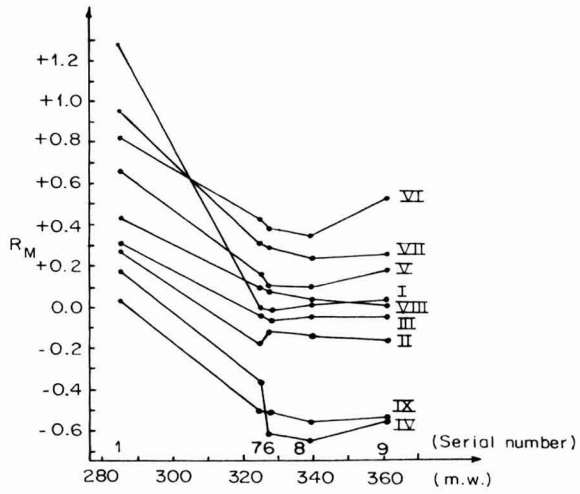

FIG. 5. Variation of R_M for estrone-17-one oxime ethers Nos. 6–9.

While the chromatography of the esters (acetate and benzoate) could earlier be brought into correlation with the separation of the ethers, for these two esters of the oestrone oximes very small R_M change is obtained compared to the 17-one oxime, as seen in the nonoximated base compound (estrone) (Fig. 6). Systems I and V are suitable for their separation.

In spite of the fact that the lactams have the same MW's as the oximes, they exhibit large chromatographic differences. It was pointed out earlier that in just those systems where the ethers and oximes can be well separated, the lactams are not moved from the start line.

The long-applied chloroform-containing systems play an important part in the separation of the lactams, as do those systems in which the

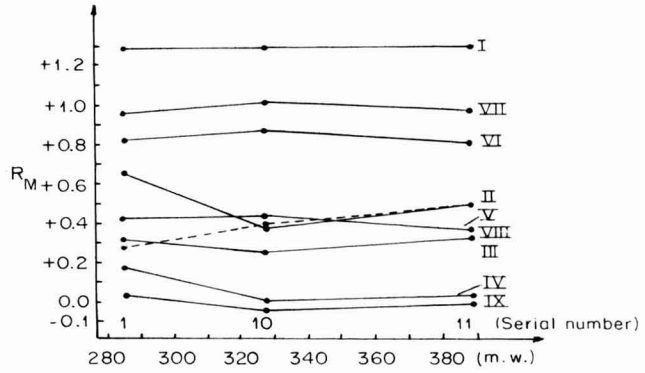

FIG. 6. Variation of R_M for two ester derivatives of estrone-17-one oximes.

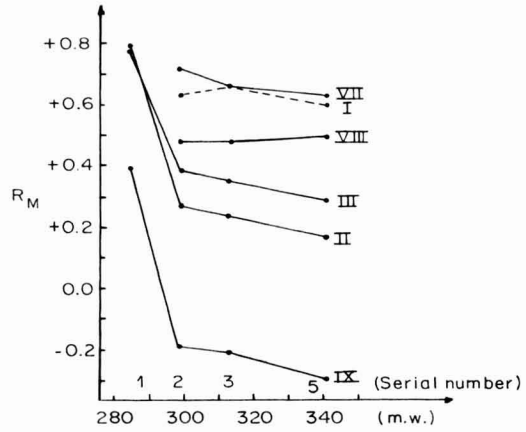

FIG. 7. Variation of R_M for ethers Nos. 1–3 and 5 of estrone-D-homo-lactams.

nonpolar component is in great excess over the polar one (systems II and III).

In system II the difference or polarity change between lactams 1–3 can be measured with $\Delta R_{M(CH_2)} = -0.03$ or -0.035 (Fig. 7).

For compounds 6–9 an ever poorer separation can be observed in the direction of the ethers, the oximes and the lactams. In the case of the lactams different R_f values can be detected only for system VII, and even then the values are very close (Fig. 8).

The situation is the reverse for the esterified lactams, which can be much better chromatographed than the corresponding oximes. Their separation can be solved in each of the systems tried, and best in the 3-component mixtures, naturally disregarding those systems in which all of the lactam stays on the start line (Fig. 9).

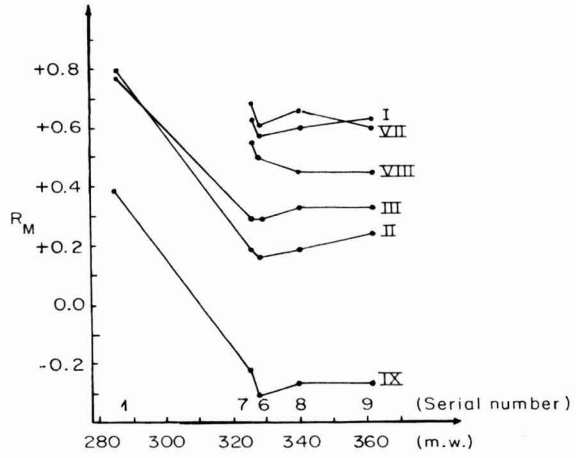

FIG. 8. Variation of R_M for ethers Nos. 6–9 of estrone-D-homo-lactams.

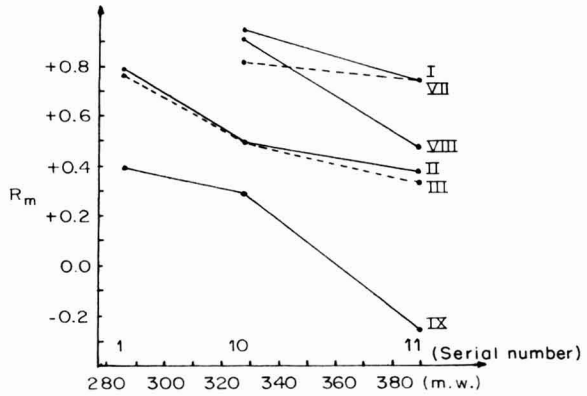

FIG. 9. Variation of R_M for two esters of estrone-D-homo-lactams.

SUMMARY

With the increase of the 3-substituted ether side-chain, the polarity of the molecules decreases. However, this decrease is not accompained by a great variation in the TLC properties, and so derivatives the molecular weights of which are not very different are difficult to separate.

On the other hand, ethers containing an iso, unsaturated, cycloalkyl or aromatic side-chain can readily be separated.

The separation of the esters similarly causes no problems.

For the ketones the linear polarity-decrease as a function of the molecular weight is the most pronounced for the oximes, but it also appears for the lactams in certain systems.

For the D-homo-lactams, which have the same molecular weights as the oximes, the separation can be solved only in those systems where the nonpolar component is in great excess over the polar one.

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MICROCHEMICAL JOURNAL 20, 287–291 (1975)

Spectrophotometric Determination of Palladium Using Arsenazo I

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INTRODUCTION

Arsenazo I [2-(*o*-arsonophenylazo)-1,8-dihydroxynaphthalene3,6-disulphonic acid, trisodium salt] was synthesised and used for the photometric determination of thorium (*4*), zirconium (*7*), aluminum (*8*) and uranium (*9*). Fritz *et al.* (*2*) used arsenazo I (AI) as a metallochromic indicator for rare-earths and yttrium in direct titration with EDTA at pH 5 to 6.5. The acid–base properties of AI have been investigated by us (*5*). The present investigation is a systematic spectrophotometric study of Pd–AI complexes, in solution.

EXPERIMENTAL METHODS

The water used was always twice distilled from all glass equipment. The chemicals were all of the highest purity available.

Solutions

The 0.001 *M* solution of AI (BDH, England, MW 614.28) was 0.6143 g/liter. The 0.01 *M* $PdCl_2$ solution (Prolabo, France) was prepared and standardized using EDTA (*6*). Lower concentrations whenever required were prepared by accurate dilution. Solutions containing 1 mg anion or cation for testing the interfering effect were prepared as recommended. The modified Britton and Robinson universal, acetate and hexamine buffer solutions were prepared as mentioned before (*1*).

RESULTS AND DISCUSSION

The optimum conditions for the determination resulted from a careful investigation of all factors involved in the procedure. Measurements have shown that the optimum pH values for developing the violet Pd–AI complexes are 2 and 12. Measuring the absorption spectra of AI at the recommended pH values, the Pd–AI at pH 2 and 12 against water and against each other, the difference curves showed that the wavelengths 565 and 585 nm are suitable for palladium, respectively, Fig. 1.

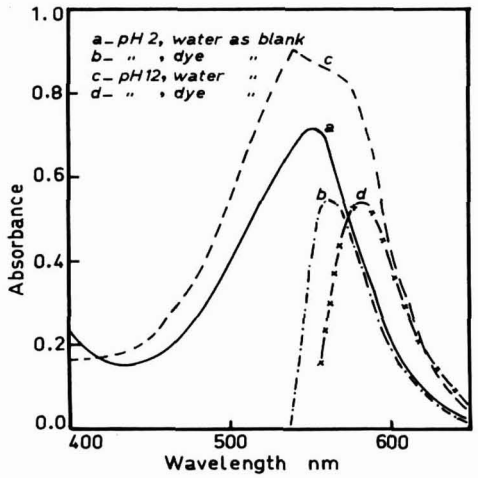

FIG. 1. Absorption spectra of 5×10^{-5} *M* Pd–AI complex.

The sequence of addition for Pd at pH 2 is not restricted to a certain one, although the sequence dye–metal–buffer is obtional at pH 12.

Experiments on the effect of time and temperature proved that 1 hr is required for full development of the color of the Pd complex which remains constant thereafter. Raising the temperature decreases the time required for complete color development.

As to the solvent affect absorbance measurements proved that ethanol, isopropanol, acetone or dioxane in different percentages have no influence on the color intensity of the complexes, while pyridine decreases the absorbance appreciably due to its tendency to complex with metal ions.

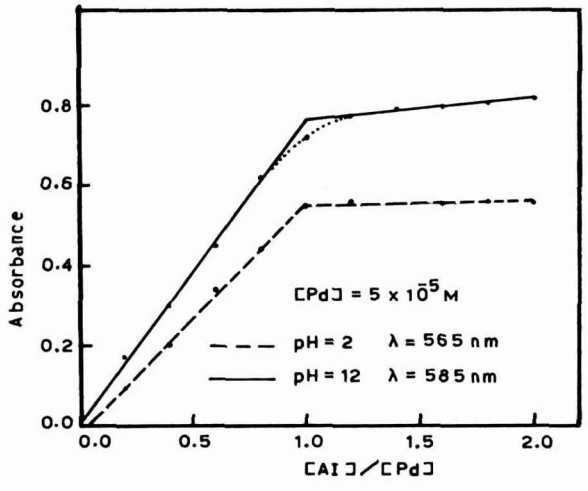

FIG. 2. Molar ratio method for Pd–AI complex.

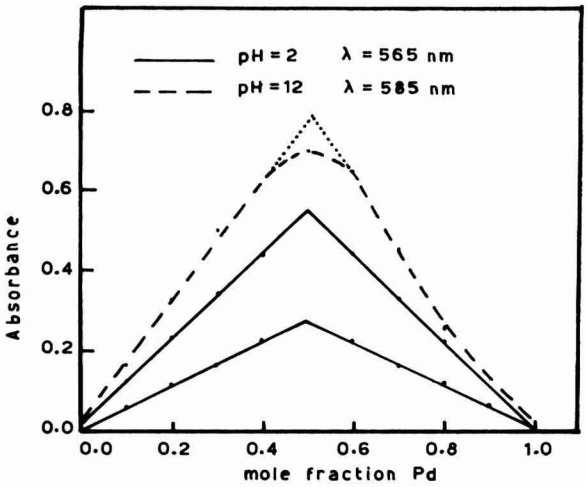

FIG. 3. Continuous variation method for Pd–AI complex.

Investigation of the molecular structure of the Pd–AI complexes, in the light of the results obtained by the molar ratio and continuous variation methods as practical ones and straight line, slope ratio and limiting logarithmic methods (*3*) as mathematical derivations from the former reveals the formation of 1:1 Pd:AI complex Figs. 2 and 3.

The log stability constants of the Pd–AI complex calculated from the molar ratio and limiting logarithmic methods amount to 3.49 and 3.63 at pH 2 and 12, respectively.

A systematic study of the influence of foreign ions led to the conclusion that the presence of the following ions in 20-fold the amount of Pd (at pH 2) has no influence: Li^+, Na^+, K^+, Rb^+, Be^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Al^{3+}, Th^{4+}, U^{4+}, Pt^{4+}, Rh^{3+}, Au^{3+}, F^-, Cl^-, Br^-, $B_4O_7^{2-}$, SO_4^{2-}, VO_3^-. On the other hand the following ions should not be present: Cu^{2+}, Cr^{3+}, Fe^{3+}, La^{3+}, Y^{3+}, Ru^{3+}, I^-, CN^-, $C_2O_4^{2-}$ EDTA, CDTA and citrate ions.

Regarding Beer's law, it was found to be obeyed satisfactorily in the range 1.0–10.6 ppm of Pd at pH 2, 0.64–6.39 ppm of Pd at pH 12 (Fig. 4) and the values of the molar absorption coefficients amount to 11,505 ± 245, 14,495, respectively. Such high values indicate the sensitivity of the method.

Procedure

To determine Pd: to a solution containing 10.0 to 106 μg Pd add 1.5 ml 0.001 *M* AI, 6 ml universal buffer pH 2, make with water up to 10 ml and measure the absorbance after 1 hr at 565 nm. Alternatively: to a solution containing 6.4 to 63.9 μg Pd add 1 ml 0.001 *M* AI, and allow to stand for 1 hr, add 6 ml universal buffer solution of pH 12, make with

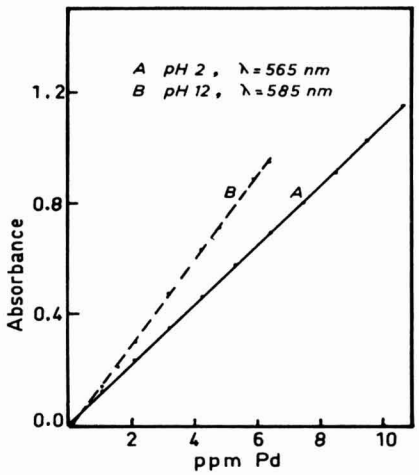

FIG. 4. Validity of Beer's law for Pd–AI complex.

water up to 10 ml and measure the absorbance at 585 nm against a blank solution. Compute the concentration by extrapolation from a standard calibration curve prepared in the same manner.

The present method affords a new means for the spectrophotometric determination of Pd in pure solutions or in presence of a variety of cations which do not interfere with such determination, with fair accuracy and high precision. The high selectivity of the method proposed for Pd stems from the fact that constituents of the buffer used act as masking agents for many cations.

SUMMARY

A new simple rapid method for the spectrophotometric determination of palladium using arsenazo I is given. The optimum conditions favoring the formation of the complex are extensively investigated; the molecular structure was found to be 1:1. Beer's law is obeyed for the ranges 1.0–10.6 or 0.64–6.39 ppm of Pd.

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MICROCHEMICAL JOURNAL 20, 292–298 (1975)

Simultaneous Spectrophotometric Determination of Molybdenum and Selenium, and of Molybdenum and Tellurium

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Among the several spectrophotometric methods that have been reported for the determination of molybdenum (*2, 3, 5, 6, 8, 9, 11*) the phenylfluorone procedure has the advantage that only few elements interfere (*5*). Halasz *et al.* (*5*), in that procedure, formed the molybdenum–phenylfluorone complex under acidic conditions and determined its absorbance in the aqueous phase in the presence of gum acacia as stabilizer.

Only few spectrophotometric procedures have been reported for selenium (*1, 4, 10*) while still fewer have appeared in the literature for the spectrophotometric determination of tellurium (*7, 12*). The use of the dithiocarbamates for the determination of selenium (*2*) and tellurium (*12*) individually suffers from the disadvantage that this group of reagents is nonselective, forming chelates with a relatively large number of interfering elements including molybdenum (*1*). However, regarding interference caused by the latter element, since selenium and tellurium do not form complexes with phenylfluorone, the possibility exists of firstly forming and removing molybdenum–phenylfluorone by selective solvent extraction in the presence of selenium or tellurium followed by the determination of selenium or tellurium in the remaining aqueous phase by diethyldithiocarbamate. In this way the molybdenum interference may be determined, thereby providing a method for the simultaneous determination of molybdenum and selenium, and molybdenum and tellurium and at the same time extending the usefulness of diethyldithiocarbamate as an analytical reagent in spite of its general nonselectivity. This paper describes a procedure for the solvent extraction of molybdenum–phenylfluorone complex and its application to the simultaneous determination of molybdenum and selenium and of molybdenum and tellurium.

[1] Taken from a thesis submitted in partial fulfillment of the MS degree.

[2] To whom all inquiries should be sent.

MATERIALS AND METHODS

Reagents. All reagents were of chemically pure grade. Distilled water was used throughout. All reactions were carried out at room temperature unless otherwise indicated.

Phenylfluorone, 0.03% solution. Transfer 0.060 g phenylfluorone to a 200 ml volumetric flask. Add 2.0 ml of hydrochloric acid (37%) and dilute to volume with 95% ethanol. Prepare fresh solutions daily.

Molybdenum solutions. Transfer 0.1840 g of ammonium molybdate (tetrahydrate) to a 100 ml volumetric flask. Dissolve with water and make up to volume. Prepare other solutions of ammonium molybdate from it by dilution.

Selenium solutions. Transfer 0.330 g of sodium selenite to a 100 ml volumetric flask. Dissolve with water and make up to volume. Prepare other solutions of sodium selenite from it by dilution.

Tellurium solutions. Transfer 0.1989 g of potassium tellurite to a 100 ml volumetric flask. Dissolve in water and make up to volume. Prepare other solutions of potassium tellurite from it by dilution. Prepare fresh solutions daily.

Sodium diethyldithiocarbamate solution, 4% (w/v)
Ammonium perchlorate solution, 10% (w/v)
Hydrochloric acid (5 *N*)
2-Ethyl-1-hexanol
Chloroform.

RECOMMENDED PROCEDURE FOR SIMULTANEOUS DETERMINATION OF MOLYBDENUM AND SELENIUM

Pipette 4.0 ml of the test solution containing molybdenum and selenium into a 50 ml separatory funnel and add 4.0 ml of phenylfluorone solution. Gently mix and let stand for 10 min. Add 2.0 ml ammonium perchlorate solution and mix gently. Extract the molybdenum–phenylfluorone complex by shaking with 25.0 ml chloroform for 20 sec. After the two phases have separated, withdraw the organic phase. Filter into cuvettes and read at 515 nm against a similarly treated blank. Molybdenum is thereby determined.

Carefully transfer the aqueous phase which remains after extraction of the molybdenum–phenylfluorone complex into a test tube. Heat the test tube in a water bath for 15 min. Cool to room temperature and retransfer the aqueous phase quantitatively into the separatory funnel. Add 1.0 ml of hydrochloric acid followed by 2.0 ml of sodium diethyldithiocarbamate solution. Mix and let stand for 5 min. Add 2.0 ml of ammonium perchlorate solution and immediately extract with 10.0 ml of 2-ethyl-1-hexanol by shaking for 20 sec. After the two phases have separated withdraw the organic phase and dry it with anhydrous sodium

sulfate. Filter and read at 380 nm against a similarly treated blank, 30 min after extraction. Selenium is thereby determined.

SIMULTANEOUS DETERMINATION OF MOLYBDENUM AND TELLURIUM

Pipette 4.0 ml of the test solution containing molybdenum and tellurium into a separatory funnel and add 4.0 ml of phenylfluorone solution. Gently mix and let stand for 10 min. Extract the molybdenum–phenylfluorone complex by shaking with 25.0 ml chloroform for 20 sec. Withdraw the organic phase and after filtering it, read at 515 nm against a similarly treated blank. Molybdenum is thereby determined.

Transfer the aqueous phase which remains into a test tube and proceed to determine tellurium as its diethyldithiocarbamate complex in exactly the same manner as that reported for selenium determination as outlined above, employing 2-ethyl-1-hexanol as the extracting solvent in the presence of perchlorate ions.

RESULTS AND DISCUSSION

Applying the procedures as outlined, straight-line calibration graphs were obtained for all three elements in the following concentration ranges: molybdenum, 0–10 μg/ml as Mo; selenium, 0–150 μg/ml as Se; tellurium, 0–250 μg/ml as Te. The calibration graphs for selenium and tellurium were constructed as if molybdenum were present so that these calibration graphs were made after the procedure for molybdenum had been applied.

Preliminary work on the simultaneous determination of molybdenum and tellurium had indicated that if molybdenum phenylfluorone was extracted by chloroform in the presence of perchlorate ions that a pink

TABLE 1
EFFECT OF PERCHLORATE IONS ON EXTRACTION OF MOLYBDENUM-PHENYLFLUORONE BY CHLOROFORM[a]

Molybdenum (μg)	Perchlorate present		Perchlorate absent	
	Absorbance		Absorbance	
	Aqueous phase	Solvent phase	Aqueous phase	Solvent phase
1.3	0	0.04	0	0.04
2.5	0	0.10	0	0.09
5.0	0	0.19	0	0.18
10.0	0	0.39	0	0.36
20.0	0	0.75	0	0.70

[a] Readings taken 15 min after extraction. The absorbance readings in the solvent phase remained unchanged 40 min after extraction.

TABLE 2
ACCURACY OF CALIBRATION GRAPHS FOR MOLYBDENUM

Molybdenum present (μg)	Perchlorate present	Perchlorate absent
	Mo found (μg)	Mo found (μg)
1.0	0.8	1.1
2.0	2.0	—
4.0	4.2	3.7
8.0	8.4	8.2
16.0	—	16.4

color was discharged in the remaining aqueous phase containing the tellurium. This resulted in nonreproducible recoveries of tellurium as the diethyldithiocarbamate complex. However, as is shown in Table 1, the molybdenum–phenylfluorone complex is completely extracted by chloroform whether perchlorate ions are present or not. This is essentially the only difference in procedure for the simultaneous determination of molybdenum and selenium, and molybdenum and tellurium. The results in Table 1 also show that extraction of the molybdenum–phenylfluorone in the presence of perchlorate ions is slightly more sensitive than without perchlorate and that this difference is not related to the efficiency of extraction of the molybdenum–phenylfluorone complex by chloroform since in both cases, the aqueous phase which remains after extraction of the complex gives 100% transmission reading. The results recorded in Table 2 show that the solvent extraction procedure for the determination of molybdenum as the phenylfluorone complex in the presence or absence of perchlorate ions is sensitive, accurate and reproducible.

The results recorded in Tables 3 and 4 demonstrate that the calibration graphs for selenium and tellurium gave accurate and reproducible results in recovery experiments. It is to be noted that for complete extraction of selenium and tellurium diethyldithiocarbamates, perchlorate

TABLE 3
ACCURACY OF SELENIUM CALIBRATION GRAPH[a]

Selenium present (μg)	Selenium found (μg)
63.0	62.5
125.0	125.0
200.0	200.0
250.0	250.0
300.0	300.0

[a] As if molybdenum were present.

TABLE 4
ACCURACY OF TELLURIUM CALIBRATION GRAPH[a]

Tellurium present (μg)	Tellurium found (μg)
31.0	30.5
125.0	125.0
250.0	250.0
300.0	300.0
400.0	400.0

[a] As if molybdenum were present.

ions must be present. To conclude these studies, the recoveries of molybdenum and selenium, and of molybdenum and tellurium from mixtures were determined by the recommended procedures. The results of those experiments recorded in Tables 5 and 6, respectively, show that the method of simultaneous determination of molybdenum and selenium and of molybdenum and tellurium is rapid, accurate and reproducible.

Of the various solvents tested for the extraction of selenium and tellurium diethyldithiocarbamates in the presence of perchlorate ions, 1-butanol, isoamyl alcohol, methyl isobutyl ketone and 2-ethyl-1-hexanol were the only solvents that gave 100% extraction of both diethyldithiocarbamates. The selenium diethyldithiocarbamate complex was stable even after 1 hr of extraction in all four extracting solvents but 2-ethyl-1-hexanol showed the greatest sensitivity and was selected as the extracting solvent. On the other hand, the tellurium diethyldithiocarbamate complex was found to be unstable in methyl isobutyl ketone 10 min after extraction, but stable in 1-butanol, isoamyl alcohol and 2-ethyl-1-hexanol even after 1 hr of extraction. Again, the greatest sensitivity was obtained in 2-ethyl-1-hexanol as the extracting solvent for tellurium diethyldithiocarbamate.

TABLE 5
SIMULTANEOUS DETERMINATION OF MOLYBDENUM AND SELENIUM

	Mo (μg)		Se (μg)	
Mixtures	Present	Found	Present	Found
A	1.0	0.8	50.0	49.5
B	2.0	2.1	75.0	75.0
C	8.0	7.6	100.0	100.0
D	10.0	10.0	150.0	150.0
E	16.0	15.5	300.0	300.0

TABLE 6
SIMULTANEOUS DETERMINATION OF MOLYBDENUM AND TELLURIUM

Mixtures	Mo (μg)		Te (μg)	
	Present	Found	Present	Found
A	2.5	2.5	31.0	3.1.0
B	4.5	4.5	62.0	62.0
C	9.0	8.8	125.0	125.0
D	18.0	17.8	250.0	250.0
E	20.0	20.0	400.0	400.0

SUMMARY

A method is reported for the extraction of molybdenum–phenylfluorone by chloroform. The extraction is complete whether perchlorate ions are present or not but the extractions in the presence of perchlorate ions gave a somewhat more sensitive procedure for the spectrophotometric determination of molybdenum in the solvent phase as the molybdenum–phenylfluorone complex.

A procedure is reported for the simultaneous determination of molybdenum and selenium, and molybdenum and tellurium. The method involves first the formation and solvent extraction of the molybdenum–phenylfluorone complex by chloroform in the presence of perchlorate ions, followed by determination of selenium in the remaining aqueous phase as selenium–diethyldithiocarbamate complex after solvent extraction with 2-ethyl-1-hexanol in the presence of perchlorate ions. A similar procedure is reported for the simultaneous determination of molybdenum and tellurium except that in the determination of molybdenum, the phenylfluorone complex is extracted by chloroform in the absence of perchlorate ions. Tellurium is determined in the remaining aqueous phase as tellurium–diethyldithiocarbamate complex after solvent extraction by 2-ethyl-1-hexanol solvent extraction in the presence of perchlorate ions.

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A Rapid Potentiometric Method for Analysis of Some Minerals and Basic Refractories

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Potentiometric titration of unconsumed EDTA with Hg(II) which has been recently applied for the analysis of alloys (*5–7*), pigments (*8,9*) and ores (*10*) is extended to the analysis of phosphate, quartzite and fluorspars, chromite, chrome–magnesite and magnesite–chrome bricks, with the advantage over classical methods, of avoiding tedious and time consuming separations. Phosphate mineral is used in the manufacture of phosphoric acid, fertilizers, in medicine and in steel industry. Quartzite is used in glass manufacture, refractories and abrasives. Fluorspar is used as a flux in many metallurgical processes and in ceramic manufacture. Chrome magnesite bricks remain the most aggravating of all steel plant refractories. Chromite bricks are used as neutral zone for separating magnesite and dolomite from silica and fire clay bricks. Complexometric methods have been reported for the determination of phosphorus in phosphates (*3,4,17*), for Ca in fluorspar (*13*), for Ca and Mg (*15,20*), for Al (*2,18*), for Fe (*16,19*), for the determination of Cr in presence of Fe (*1,14*) and for analysis of Cr, Fe, Al, Ca and Mg (*12*). However, most of these methods depend on visual end point determination which is a source of error in comparison with the present potentiometric method.

EXPERIMENTAL METHODS

The water used was always twice distilled from all glass equipment. The chemicals were of the highest purity available.

Solutions

The 0.05 *M* solutions of EDTA and Hg(II) nitrate, the hexamine or hexamine–NaOH buffer solutions were prepared and standardized or checked for pH, by recommended procedures.

Apparatus

As previously described, the titrant being delivered from a 1/100 or 1/50 graded microburette with its tip immersed in the solution, together with the silver amalgam and the calomel electrodes.

Procedures

a. Phosphate rocks. In a 150 ml beaker digest 1 g fine dry sample with 10 ml conc HNO_3, evaporate gently to near dryness, add water, boil, filter (Whatman 41) from SiO_2, wash it with hot water and make the filtrate with water up to 100 ml. With the sequence: aliquot portion, EDTA, 8 ml 10% hexamine and water up to 30 ml, in a 150 ml beaker, proceed to find ml EDTA equivalent to one or more of the components by titrating unconsumed EDTA with Hg at the desired pH, using silver amalgam as the indicator electrode coupled with SCE. For Fe + Al + Ca: Boil 5 ml aliquot with 10–12 ml EDTA, cool, add hexamine, water and drops of 0.5 *M* NaOH to pH 11 (initial potential = +0.11 V) and titrate unconsumed EDTA (EP-break = 50–65 mV/0.1 ml Hg).

For Fe + Al: Boil 5 ml with 4 ml EDTA, cool, add hexamine, water and titrate EDTA at pH 7 (EP-break = 90–100 mV/0.1 ml Hg).

For Al: Boil 5 ml with NaOH, filter from $Fe(OH)_3$ (Whatman 41), wash with hot water, add to the filtrate 3 ml EDTA, boil, cool, add hexamine, water and titrate EDTA at pH 7 (EP-break = 110–120 mV/0.1 ml Hg).

For P: Precipitate the phosphomolybdate as usual from 5 ml aliquot, filter by suction, wash with 1% HNO_3, dissolve the residue in conc NH_4OH, add 5–6 ml 0.05 *M* Mg-nitrate dropwise, filter (Whatman 42), wash with 5% NH_4OH, dissolve the precipitate in 1% HNO_3, add 5–7 ml EDTA, hexamine, water and titrate at pH 11 (EP-break = 50–60 mV/0.1 ml Hg). Find ml EDTA $\equiv$ Mg $\equiv$ P.

b. Quartzite. In a platinum dish treat 2 g fine dried sample with HF, 3 drops of conc HNO_3, evaporate and repeat this process twice more, extract the residue with 30 ml 2% HNO_3, make the solution with water up to 100 ml and continue as in (a) to find EDTA $\equiv$ Fe + Al + Ca, Fe + Al and Al, with the respective EP-breaks of 82, 155 and 120 mV/0.1 ml Hg. Alternatively, to find EDTA $\equiv$ Fe: dissolve the $Fe(OH)_3$ in conc HNO_3, add EDTA, hexamine, water and titrate at pH 6–7 (EP-breaks = 120 mV/0.1 ml Hg).

c. Fluorspars. In a platinum crucible fuse 0.5 g fine dried sample with Na_2CO_3 at 800°C, extract the melt with 30% HNO_3, boil, filter from SiO_2 and make the filtrate with water up to 100 ml.

For Ca: To 5 ml add 8–10 ml EDTA, hexamine, water, NaOH to pH 11 and titrate with Hg (EP-break = 60 mV/0.1 ml).

d. Chromite, chrome magnesite and magnesite chrome bricks. In a silica crucible fuse 0.5 g of a very fine sample with 4 g $KHSO_4$, dissolve the melt in 50 ml 2% HNO_3, boil, filter from SiO_2 (Whatman 41), wash it with hot water and make the filtrate with water up to 100 ml.

For Fe + Al + Cr(III): Boil 5 ml with 5 ml EDTA for 15 min, cool,

add hexamine, water and titrate with Hg at pH 7 (EP-break = 90–100 mV/0.1 ml).

For Fe + Al: Warm 5 ml + 5 ml EDTA to 50°C, cool, add hexamine, water and titrate with Hg at pH 6–7 (EP-break = 90 mV/0.1 ml).

For Fe: Boil 5 ml with 1 g Na_2O_2 to convert Cr(III) to Cr(VI) and Al to soluble aluminate, filter from $Fe(OH)_3$ (Whatman 41), wash with hot water, dissolve $Fe(OH)_3$ in 2% HNO_3, add 4 ml EDTA, hexamine, water and titrate with Hg at pH 6–7 (EP-break = 100 mV/0.1 ml).

For Mg: Boil 10 ml, add 1 g NH_4Cl and ammonia till just alkaline, filter from oxides of Fe, Al and Cr (R_2O_3) (Whatman 41), wash with hot water, add to the filtrate 10 ml NH_4Cl–NH_4OH buffer pH 10 and titrate Mg^{2+} ions with EDTA using EBT as indicator to the appearance of pure blue color.

RESULTS AND DISCUSSION

Table 1 lists representative results of analysis of the above mentioned minerals and refractories by the present method and by classicals ones which are in excellent agreement, indicating that the present method is quite reliable for such an analysis.

The classical methods of estimation of Fe, Al and Ca in phosphate rocks are very tedious and time consuming. If Al is determined as the oxide serious errors may arise from the coprecipitation of other elements. When the solution is made alkaline with ammonia, $FePO_4$ and $AlPO_4$ are precipitated. If the percentages of Fe and Al are not enough for precipitating all the PO_4^{3-} ions, a portion of Ca is coprecipitated leading to errors in the real content of either Al or Ca. For the classical determination of total Ca all the PO_4^{3-} ions should be separated as $FePO_4$ by the ferric chloride–acetate method. The fact that neither phosphate nor molybdenum ions are chelated with EDTA was the basis of the indirect potentiometric determination of phosphate. Thus the PO_4^{3-} ions are separated as the phosphomolybdate which, on dissolution in NH_4OH and addition of Mg ions, yields Mg NH_4PO_4. Magnesium equivalent to phosphate is then determined by the present method.

Consideration of the present procedures shows that the lack of selectivity of EDTA has been overcome by the selective control of pH which renders possible back titrating heavy plus alkaline earth cations at pH 11 and heavy cations only at pH 6–9 potentiometrically (*11*). In case iron is back titrated at pH 11 the iron versenate solution only acquires a slight reddish tint due to the formation of the hydroxo complex $Fe(OH)Y^{2-}$ and to a lesser extent $Fe(OH)_2Y^{3-}$ but there is no tendency for the reaction,

$$Fe(OH)_2Y^{3-} + OH^- \rightleftharpoons Fe(OH)_3 + Y^{4-},$$

TABLE 1
ANALYSIS OF SOME MINERALS AND REFRACTORIES[a]

%	Fe_2O_3	Al_2O_3	P_2O_5	CaO
Phosphate rocks				
a_1[b]	6.00	3.77	26.81	40.15
b_1	6.01	3.78	26.84	40.20
a_2	5.40	0.88	25.57	45.40
b_2	5.40	0.88	25.59	45.45
a_3	5.62	1.74	18.80	43.60
b_3	5.64	1.74	18.82	43.66
a_4	4.36	3.43	28.90	42.50
b_4	4.35	3.43	28.86	42.53

Fluorspars

	Ca	
a_1, b_1	47.60	47.63
a_2, b_2	46.66	46.68
a_3, b_3	46.50	46.52

Quartzite

	Fe_2O_3		Al_2O_3		CaO	
a_1, b_1	0.94	0.94	1.42	1.43	0.86	0.86
a_2, b_2	0.86	0.86	1.50	1.50	0.90	0.90

	Fe_2O_3	Al_2O_3	Cr_2O_3	MgO
Chrome bricks				
a_1	13.58	20.23	36.84	24.37
b_1	13.60	20.22	36.80	24.40
a_2	13.70	19.94	37.00	24.41
b_2	13.71	19.96	36.94	24.45
Chrome magnesite				
a_1	12.80	14.78	24.63	44.14
b_1	12.78	14.77	24.60	44.20
Magnesite chrome				
a_1	12.61	1.60	7.50	75.70
b_1	12.60	1.60	7.50	75.80

[a] All titrations were made twice for the sake of reproducibility.
[b] a = by the present method; b = by classical methods.

to occur unless the pH value exceeds 11. Cr(III) is prevented from the normal action of EDTA by oxidizing it to Cr(VI) with Na_2O_2 in alkaline medium. At the same time Fe separates as $Fe(OH)_3$ and Al goes into solution as AlO_2^-. The general convenience of EDTA in back titrating its excess potentiometrically with Hg(II) recommends the present method for simple routine analysis. Thus after dissolution of a phosphate sample, one aliquot is used for back titrating Fe + Al + Ca another for Fe + Al, a third for Al and a fourth for phosphate. With few exceptions, the potential breaks are large enough for determining the end points with fair accuracy and precision.

SUMMARY

Rapid and quite reliable procedures for analysis of phosphate, quartzite and fluorspar-minerals; chromite, chromemagnesite and magnesite chrome bricks-basic refractories are described. They are based on titrating unconsumed EDTA with Hg(II)-nitrate using silver amalgam as the indicator electrode, with breaks of reasonable order of magnitude lying within the immediate vicinity of the expected end points.

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The Application of Elemental Analysis to the Determination of the Composition of a Mixture of Pyridine Compounds

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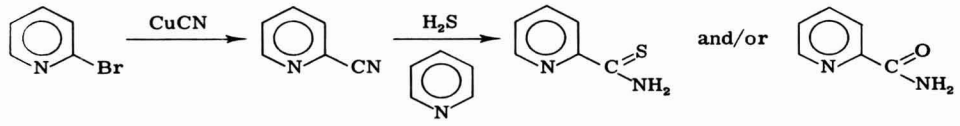
INTRODUCTION

Recently, we were presented with the problem of determining the composition of a reaction mixture of pyridine compounds. The sample was derived from the following series of reactions.



While this problem might be attacked many ways, we resolved it by utilizing microelemental analyses and feel that our solution to this problem would be of interest to analytical chemists.

Generally, mixtures of this type would be resolved by gas–liquid chromatography or high performance liquid chromatography. In these procedures, reference compounds would be required for their respective identification by retention time and, for quantitation, the determination of their respective response factors. In this particular problem, compounds D and E were not available and the need for rapid results was most important, hence the time element obviated the use of chromatography for separation and quantitation of the components.

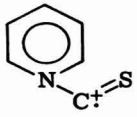
From the structures of the possible compounds in the mixture, microelemental analytical assays appeared capable of easily and readily determining the amount of each of the components. The mixture discussed here has uniquely three compounds which have one element not common to any of the others: compound A contains bromine; compound D contains sulfur; and compound E contains oxygen. The amount of each of these compounds, if present in the mixture, may be determined by a single microelemental quantitative analysis for the unique

elements. On the other hand, compounds B and C have none of these elements but do have carbon, hydrogen and nitrogen which are common to each as well as to compounds A, D and E. By determining their contribution of carbon and nitrogen to the mixture, one is thus left with the carbon and nitrogen due to only compounds B and C. The contribution of each of these latter two components is therefore determined by a series of simultaneous equations taking advantage of the carbon/nitrogen ratios of these two compounds, thereby establishing their percentage in the mixture. An example of how this was carried out is presented.

EXPERIMENTAL METHODS

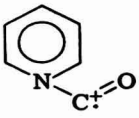
The elemental composition of the mixture was determined by standard microanalytical procedures. Carbon, hydrogen and nitrogen were determined on a Perkin–Elmer Model 240 CHN Analyzer (*3*). Samples for bromine and sulfur were combusted by the Schoniger technique (*4*). Bromine was determined by titration on the Aminco–Cotlove Titrator (*1*). Sulfur was determined by titration with barium chloride using Sulfanazo III indicator (*2*). Oxygen was determined by difference. The sample contained 64.99% carbon, 3.86% hydrogen, 23.93% nitrogen, 2.06% bromine, negligible sulfur and 5.16% oxygen.

Spectral data was obtained to corroborate the elemental information. Infrared spectroscopy showed the presence of an absorption peak at 6.1 μm that could be attributed to the amide carbonyl or amide thiocarbonyl. The mass spectrum of the sample confirmed the sulfur assay as no thioketopyridyl fragment,



(m/e 122)

was found; however, the ketopyridyl fragment,



(m/e 106)

was detected.

CALCULATIONS

The theoretical composition of each of the possible components is given in Table 1.

Since no sulfur was found, compound D was not present. To determine the amount of compound A which theoretically contains 50.58%

TABLE 1
THE ELEMENTAL COMPOSITION OF POSSIBLE COMPOUNDS

Element	A	B	C	D	E
C	38.01%	69.22%	75.92%	52.15%	59.01%
H	2.55	3.87	6.37	4.38	4.95
N	8.87	26.91	17.71	20.27	22.94
Br	50.58	0.00	0.00	0.00	0.00
S	0.00	0.00	0.00	23.20	0.00
O	0.00	0.00	0.00	0.00	13.10

bromine and is the only bromine containing compound, the following proportion was used:

$$2.06 : 50.58 = \chi : 100;$$
$$\chi = 4.07\%.$$

By similar calculation, the amount of compound E, based on the oxygen content, was determined:

$$5.16 : 13.10 = \chi : 100;$$
$$\chi = 39.39\%.$$

The carbon and nitrogen contributions from compounds A and E were calculated as follows:

For compound A,

$$.0407 \times 38.01\% = 1.55\% \text{ carbon};$$
$$.0407 \times 8.87\% = 0.36\% \text{ nitrogen}.$$

For compound E,

$$.3939 \times 59.01\% = 23.24\% \text{ carbon};$$
$$.3939 \times 22.94\% = 9.04\% \text{ nitrogen}.$$

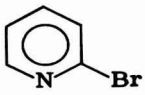
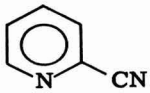
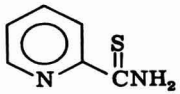
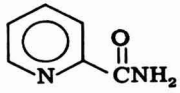
The total carbon found experimentally is 64.99%; the total nitrogen 23.93%. From these values, the net carbon and nitrogen due to compounds B plus C were obtained.

$$64.99 - (1.55 + 23.24) = 40.20\% \text{ carbon};$$
$$23.93 - (0.36 + 9.04) = 14.53\% \text{ nitrogen}.$$

By the use of the following simultaneous equations, the percentages of B and C were then obtained.

$$69.22\ (\%\ B) + 75.92\ (\%\ C) = 40.20;$$
$$26.91\ (\%\ B) + 17.71\ (\%\ C) = 14.53;$$
$$B = 48.24\%;$$
$$C = 9.09\%.$$

TABLE 2
THE COMPOSITION OF THE REACTION MIXTURE

Compound	Structure	Composition
A		4.07%
B		48.24
C		9.09
D		N.D.
E		39.39
	Total	100.8%

The composition of the reaction mixture is given in Table 2.

SUMMARY

The composition of a gross mixture of pyridine compounds was determined rapidly and inexpensively by the use of standard microanalytical techniques. Spectral data corroborated the elemental results.

ACKNOWLEDGMENTS

The authors thank Dr. E. White and Mr. R. Warren for the infrared and mass spectroscopic data and interpretations.

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MICROCHEMICAL JOURNAL 20, 309–312 (1975)

Detection of Steroids with Molybdovanadophosphoric Acids on Thin-Layer Chromatograms

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Received October 7, 1974

INTRODUCTION

The use of molybdophosphoric acid as a reagent for spray detection of steroid hormones has been described by Neher(*2*). The purpose of this study was to investigate the specificity and sensitivity of molybdovanadophosphoric acids as detection reagents for steroid hormones.

The free heteropoly molybdovanadophosphoric acids $H_4[PMo_{11}VO_{40}] \cdot 34H_2O$, $H_4[PMo_{10}V_2O_{40}] \cdot 32H_2O$ and their sodium and ammonium salts were first prepared by Tsigdinos and Hallada and were shown by photometric titration with $NaOCH_3$ to be tetraprotic. $H_4[PMo_{11}VO_{40}]$ has a low dielectric constant (*4*). Conductivity and pH measurements by Hallada, Tsigdinos, and Hudson showed that $H_4[PMo_{11}VO_{40}]$ and $H_4[PMo_{10}V_2O_{40}]$ are strong electrolytes, the latter being a more stable compound in solution (*1*).

MATERIALS AND METHODS

The materials and methods followed in this study were previously described by Scott and Pietrzak (*3*). Gelman instant thin-layer chromatograms (I.T.L.C.) type SA were employed to obtain the reported results. Steroids analyzed were a gift of Dr. Merlyn M. Minick, Eastern Michigan University, and included estradiol, estrone, dehydroisoandrosterone acetate, hydrocortisone acetate, pregnenolone, desoxycorticosterone acetate, methyl testosterone, methyl prednisolone acetate, prednisone, and progesterone. Steroids were dissolved in Mallinckrodt Nanograde chloroform. Development reagents used were $H_4[PMo_{11}VO_{40}] \cdot 15H_2O$ and $H_4[PMo_{10}V_2O_{40}] \cdot 15H_2O$ (hereafter V_1 and V_2, respectively) courtesy of the Climax Molybdenum Company of Michigan, Ann Arbor, Michigan. Reagents were tested at various concentrations in water, acetone, 0.01 *M* HCl and 50% aqueous H_2SO_4. In preliminary experiments, the I.T.L.C. sheets were spotted with 2.0 μl pipettes, and the reagents were applied directly using a Pasteur pipette. In later experiments spotted I.T.L.C. sheets were developed in lined

TABLE 1
SENSITIVITY AND COLOR REACTION

Steroid	Minimum amounts detected (mg)		Minimum amounts producing characteristic color (mg)			
	V_1	V_2	V_1		V_2	
Progestrone	1×10^{-4}	1×10^{-3}	1×10^{-3}	Yellow	1×10^{-2}	Yellow
Pregnenolone	1×10^{-4}	1×10^{-4}	1×10^{-2}	Brown	1×10^{-2}	Brown
Dehydroisoandrosterone	1×10^{-5}	1×10^{-4}	1×10^{-3}	Blue-black	1×10^{-3}	Red-brown
Desoxycorticosterone acetate	1×10^{-6}	1×10^{-6}	1×10^{-3}	Brown	1×10^{-4}	Red
Hydrocortisone	1×10^{-3}	1×10^{-3}	1×10^{-4}	Blue	1×10^{-5}	Red
Estradiol	1×10^{-7}	1×10^{-7}	1×10^{-2}	Yellow	1×10^{-2}	Yellow
Estrone	1×10^{-7}	1×10^{-7}	1×10^{-2}	Yellow	1×10^{-2}	Yellow
Methyl testosterone	8×10^{-5}	8×10^{-5}	8×10^{-3}	Pink-brown	8×10^{-3}	Blue
Methylprednisolone acetate	3×10^{-3}	3×10^{-3}	3×10^{-3}	Blue-gray	3×10^{-3}	Blue
Prednisone	1×10^{-3}	1×10^{-3}	1×10^{-3}	Tan	1×10^{-3}	Blue

chambers using a solvent system of chloroform–acetone (9:1, v/v) (Mallinckrodt Nanograde quality) and the reagent was applied by spraying. After applying reagent either by direct application or by spraying, plates were developed by heating them at 105°C for a minimum of 15 min in the oven. Fluorescence at 280 nanometers was checked with an ultraviolet light.

RESULTS

Gelman I.T.L.C. types SA, SG, and A, as well as Kodak Chromagrams and Whatman silic acid-impregnated papers were initially screened for use with these reagents and Gelman I.T.L.C. type SA was selected for the study. The reagents were prepared in concentrations of 0.1, 1, 5, 10, 15, and 25% w/v in water, acetone, 0.01 *M* HCl, and 50% aqueous H_2SO_4. In general sensitivity of detection improved with increased reagent concentration up to a concentration of 5%. For steroids present at very low concentration, the reagent produced a blue or blue-gray color, whereas at higher concentrations of steroid the reagent produced characteristic colors. These colors were not dependent on the choice of solvent used for the reagent. In all cases the dilute region at the edge of the steroid spot developed as a blue ring surrounding the central characteristic color. Optimum color development with either V_1 or V_2 was obtained using a concentration of 5% w/v in 50% aqueous H_2SO_4, then heating the plates for 15 min at 105°C. Shorter heating intervals produced only blue spots. The various color reactions obtained using the two reagents, and the lowest concentration at which characteristic color appears is reported in Table 1. The color reactions seen on TLC plates where reagent was applied directly were also observed on TLC plates developed in chloroform–acetone (9:1, v/v) and sprayed with reagent. Table 2 presents the fluorescence of steroids and their sen-

TABLE 2
FLUORESCENCE AND SENSITIVITY (mg)

Steroid	V_1		V_2	
Progestrone	Yellow	1×10^{-3}	Yellow	1×10^{-3}
Pregnenolone	Blue	1×10^{-2}	Blue-green	1×10^{-3}
Dehydroandrosterone	Blue	1×10^{-3}	—[a]	—
Desoxycorticosterone acetate	Pink	1×10^{-3}	—	—
Hydrocortisone	Yellow	1×10^{-7}	Yellow	1×10^{-7}
Estradiol	Yellow	1×10^{-2}	Yellow	1×10^{-3}
Estrone	Yellow	1×10^{-2}	Yellow	1×10^{-3}
Methyl testosterone	Yellow	—	Blue	—
Methylprednisolone acetate	Tan	—	Blue	—
Prednisone	Blue	—	Blue	—

[a] Not determined.

sitivities for detection when scanned with light of 280 nm. Fluorescence was, in general, less intense than was obtained with reagent-free 50% aqueous H_2SO_4 followed by heating at 105°C for 15 min.

DISCUSSION

The results indicate that both V_1 and V_2 produce characteristic colors when reacted with various steroids. The two reagents produce a particularly dramatic reaction with the estrogens. While Gelman I.T.L.C. type SA plates worked the best with these reagents, the same sensitivities and color reactions were observed with the other thin-layer products mentioned. In light of the characteristic colors produced by steroids detected with silicotungstic acid and phosphotungstic acid (*3*), the colors produced by $H_4[PMo_{11}VO_{40}]$ and $H_4[PMo_{10}V_2O_{40}]$ as detection reagents should be useful in establishing the identity of steroids.

SUMMARY

Two vanadium analogues of phosphomolybdic acid, $H_4[PMo_{11}VO_{40}]$ and $H_4[PMo_{10}V_2O_{40}]$, have been studied for use as detection reagents for steroids on thin-layer plates. They were found to produce characteristic colors with steroids after spraying and heating at 105°C.

ACKNOWLEDGMENTS

The authors are indebted to the Climax Molybdenum Company of Michigan, Ann Arbor, Michigan, and Dr. George Tsigdinos for the gracious loan of the reagents under study.

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MICROCHEMICAL JOURNAL 20, 313–314 (1975)

Reactor for Photo-Electrochemical Studies

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The equipment shown in Fig. 1 has been useful in studying the effect of ultraviolet radiation on the redox potential displayed by ferriin solutions (*tris*-1,10-phenanthroline iron(III) blue complex).

The sample is maintained at the desired temperature in a water-jacketed reaction kettle (Kontes Glass K296110) fitted with a dense polyethylene lid machined on the pattern of the Leeds and Northrup coulometric analyzer cell lid (067513) and bored to suit the parts to be fitted. This is conveniently held in place by the same clamp which secures the standard reaction kettle head. The quartz finger for the lamp leaves enough room for operating a magnetic stirring bar beneath it. The glass sleeve supporting the uv lamp bears a sidearm with a septum socket through which cooling air for the lamp is introduced using a length of Teflon tubing extending down into the quartz finger, and a second sidearm through which the coolant escapes. The length of this sleeve is determined by the dimensions of the lamp. The uv lamp itself (Ultraviolet Products Inc., Model PCQ) is silvered on all surfaces within the standard taper joint at the top. It is proposed to extend this silvering along the lamp to the top of the reaction kettle to minimize the irritating glare of the lamp (even though the glass sleeve is screening much of the harmful radiation) and possibly by internal reflections to direct some more of the light into contact with the solution being studied.

Appropriate holes bored in the kettle lid admit the stem of a platinum gauze electrode 25-mm diameter × 25-mm cylinder length, which is arranged to surround the quartz finger at a distance of about 3 mm. The reference electrode is prepared in a Hildebrand half-cell vessel modified by replacing the tip by a male Luer joint which engages the Teflon syringe needle shown. This needle thus serves as a Luggin capillary. It has been found that if the bore of the needle is distorted at the end by some such procedure as cutting off a short bit with not too sharp scissors, a plug of agar gel can be retained easily in the last few millimeters of the needle tubing.

The cell has been used to prepare ferriin by electrolytic oxidation of ferroin (the corresponding iron(II) red complex) either by replacing the

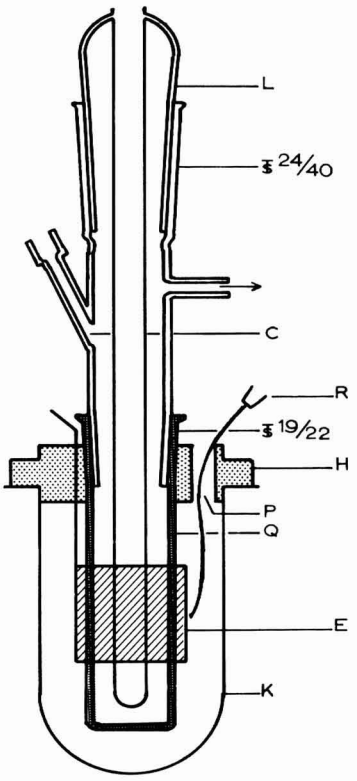

FIG. 1. Reactor Assembly. K, reaction kettle; Q, quartz finger; H, polyethylene lid; C, coolant inlet; L, ultraviolet lamp; E, platinum electrode (cylindrical gauze); P, port (typical) in lid; R, Teflon syringe needle for reference electrode.

quartz finger by a fritted glass sealing tube containing the counter-electrode, or by leaving the lamp in place and inserting a micro-scale gas dispersing tube (Ace Glass 9435C) on the end of a length of amber gum rubber tubing leading to a separate counter-electrode chamber.

The apparatus conveniently holds 50 to 75 cm^3 of sample, and is thus suited to small-scale preparations as well as studies of samples prepared in other equipment.

ACKNOWLEDGMENTS

The financial support of the National Research Council of Canada, and the technical assistance of Miss Dorothy McCulloch is gratefully acknowledged.

MICROCHEMICAL JOURNAL 20, 315–318 (1975)

Marine Adhesives

V. Amino Acid Content of *Mytilus Edulis* Byssal Threads

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Received October 17, 1974

Byssal threads secreted by the mussel, *Mytilus edulis,* have been analyzed to determine their amino acid content. Based on chromatographic data, the threads contain at least 19 amino acids. The overall composition of the threads appears to be similar to glycoproteins.

INTRODUCTION

The adhesives secreted by several marine organisms, such as the mussel and the barnacle, have been studied by a number of different groups of scientists in several countries. Since these adhesives have certain distinct advantages compared to other types of adhesives, there is a great desire to be able to produce these materials in the plant and/or the laboratory. Synthesis of these adhesives requires that their composition and structure be known. Therefore, analyses of the adhesives have been a major part of the studies reported to date.

Unfortunately, many of these reports have disagreed regarding the adhesive composition (*1,2,4,9*). These reported disparities range in importance from truly fundamental data differences to relatively trivial matters. A discussion of some of these disparities will be found in previous publications in the present series (*4–6*).

Some of the reasons for these differences have been found and explained by the present authors and their co-workers (*4–6*). It was found that the adhesives are extremely sensitive to the conditions used for sample preparation, and it is possible to degrade the adhesives, partially or totally, by small changes in the experimental procedures. For example, it was found that two hexosamines, or only one hexosamine, or even no hexosamines would be found in the adhesive simply by varying the hydrolytic temperatures on the order of only 5–10°C. Thus, relatively minor variations in experimental conditions could explain why reports have appeared which indicate that no hexosamines, one hexosamine, or two hexosamines are present in these marine adhesives.

Disparities also have appeared regarding the presence or absence of other components in the adhesive. For example, some workers report the presence of amino acids (*2,4,9*), while others report the absence of amino acids (*1*). In addition, there is some question regarding the particular amino acids which were found.

The work to be described in the present report was undertaken to obtain further data regarding the presence of amino acids in the adhesive secreted by the mussel, *Mytilus edulis*.

EXPERIMENTAL METHODS

Live specimens of *Mytilus edulis* were purchased from Marine Biological Laboratory, Woods Hole, Me., and were placed in a salt-water aquarium in our laboratories. After a period of time, samples of byssal threads which had been spun by the mussels were harvested. The threads were cleaned thoroughly by abrasion with a brush, dried, and cut into convenient sizes.

Milligram quantities of byssal thread were hydrolyzed in a sealed tube with 6 *M* hydrochloric acid at 108°C for approximately 24 hr. The hydrolysate was filtered and evaporated several times. After the remaining hydrochloric acid had been removed, the residue was taken up in 1 ml of water. Aliquots of the dissolved residue were chromatographed, using two thin layer chromatographic procedures for the separation of amino acids (*3,7*); both procedures are based on cellulose as the stationary support. The spotted plates were developed in one dimension with isopropanol–methylethylketone–1 *M* hydrochloric acid, and in the second dimension with either methanol–water–pyridine or *t*–butanol–methylethylketone–acetone–methanol–water–0.88 *M* ammonia. The solvent front was allowed to ascend 15 cm from the origin in the first dimension. The plate was air dried for about 15 min to remove the solvent and then warmed to 60°C. The yellow band at the solvent front was scraped off the plate, and the plate was developed in the second dimension until the front had moved about 16–17 cm. The fully developed plate was air dried for ½ hr and then heated to 100°C for 15 min. After cooling, the plates were sprayed with a Ninhydrin-copper reagent (*8*) and then heated at 110°C for 15 min to visualize the spots.

In separate experiments, 1 mg amounts of byssal threads were heated with 1 ml of water and barium hydroxide in a sealed tube at about 125°C for 24 hr. The cooled hydrolysate was adjusted to pH 6 with dilute sulfuric acid and the mixture boiled. The barium sulfate which formed was filtered off, washed, and the washings were combined. The solution was evaporated to dryness and redissolved in 1 ml of 0.1 *M* hydrochloric acid. Chromatograms of aliquots of this solution were run as described previously.

RESULTS AND DISCUSSION

A typical thin layer chromatogram of acid-hydrolyzed mussel byssal thread is shown in Fig. 1. Eighteen amino acids were detected on these chromatograms. In addition, several similar chromatograms confirmed the presence of hexosamines (*5,6*), along with the amino acids.

The amino acids whose spots are shown in Fig. 1 were identified from the chromatographic data on the basis of R_f values of pure materials run under similar conditions and on added standards. Table 1 lists the compounds corresponding to the hydrolysate spots shown in Fig. 1.

The list of compounds in Table 1 includes two amino acids, cystine and tryptophan, which had not been found by previous workers (*2*). The latter compound is destroyed under the acid conditions usually employed for hydrolysis of these adhesives, and would not be detected. Thus, the chromatograms of acid-hydrolyzed material (Fig. 1) did not show a spot corresponding to tryptophan; only after alkaline hydrolysis of the byssal threads did a spot corresponding to tryptophan appear on the chromatograms. The other amino acid, cystine, appeared to be present in very small quantities, and its absence in past analyses may be due to this factor. Alternatively, it may be that a specific marine environment is needed for this amino acid to be present in the adhesive.

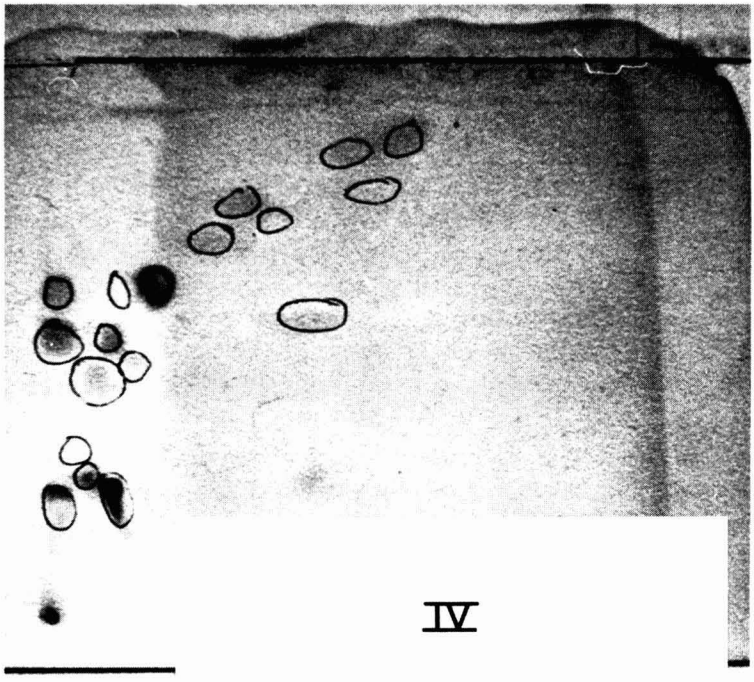

FIG. 1. Typical chromatogram of acid-hydrolyzed *Mytilus edulis* byssal threads.

TABLE 1
AMINO ACIDS DETECTED CHROMATOGRAPHICALLY FROM HYDROLYZED *Mytilus edulis* BYSSAL THREADS

Alanine	Lysine
Arginine	Methionine
Aspartic Acid	Phenylalanine
Cystine	Proline
Glutamic Acid	Serine
Glycine	Threonine
Histidine	Tryptophan
Hydroxyproline	Tyrosine
Isoleucine	Valine
Leucine	

Previous work (*5,6*) has shown that at least two hexosamines are present in mussel byssal threads in addition to the amino acids listed in Table 1. The hexosamine content, however, is in the range of a minor component. Therefore, the data presently reported, combined with our previous work, indicate that the byssal threads are composed of glycoproteins.

SUMMARY

Mytilus edulis byssal threads have been analyzed and found to contain at least 19 amino acids. Results to date indicate that the byssal threads are glycoprotein in nature.

ACKNOWLEDGMENT

The authors thank the National Institute of Dental Research which helped support this work through Contract No. PH-43-67-1172.

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MICROCHEMICAL JOURNAL 20, 319–323 (1975)

A Micromethod for Lead in Canned Baby Juices[1]

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INTRODUCTION

The effects and consequences of lead poisoning are well known. It can cause damage to the brain and nervous system resulting in permanent loss of muscle control, mental retardation, or blindness (*2*). Environmental contamination by lead is now receiving great attention, particularly of children living in poorly maintained housing who may be eating paint chips. Many cities have started blood lead screening programs for children ages 1–6. In the District of Columbia, the program has been underway for more than 3 yr. As an example, in April 1974, we tested 1168 children for lead levels at neighborhood health clinics and through a mobile unit. More than 25% of the children tested had elevated blood lead levels high enough to cause possible kidney damage, as well as other health problems. Twelve children were found to have acute lead poisoning.

Experts are puzzled about the causes of lead poisoning. Lead is coming from everywhere—from a multiple number of sources, such as automobile emmissions, paint chips, or lead water pipes. However, it has been estimated that from normal foods and beverages, approximately 3.3—5.0 μg of lead per kilogram of body weight has been ingested daily, as compared to 1.3 μg absorbed from the atmosphere in an urban environment (*6*). A mean of 1.04 ppm, 0.50 ppm, and 0.36 ppm of lead were reported by Dr. Lamm in evaporated skimmed, infant formula concentrate, and evaporated milks, respectively (*4*). The purpose of this survey was twofold; to investigate possible lead contamination from dietary sources such as baby juices which infants are often fed daily, and to determine whether a micro blood lead method (*1*) would be feasible to determine lead in baby juices.

[1] Presented at Environmental Protection Session of the 34th Institute of Food Technologists' Annual Meeting on May 13, 1974, New Orleans, La.

MATERIALS AND METHODS

Apparatus

A Perkin-Elmer 403 Atomic Absorption Spectrometer (AAS)[2] equipped with three-slot burner heads, intensitron lead hollow cathode lamps, and model 165 recorders, was used. The 403 was equipped with a deuterium background corrector and microsampling system. The lead resonance line at 2833 A was used. A Thermolyne hot plate and Oxford microliter pipets with disposable tips were also used.

Reagents

Hydrogen peroxide, 30%, analytical reagent
Acetic acid, 4%, analytical reagent.
Citric acid, 2%, lead free.
Nitric acid, lead free.
Perchloric acid, lead free.
Dithizone, lead free.

Methods

Investigations were conducted during late 1973 and early 1974. Canned baby juices and food containing vegetables and meats in glass jars were purchased from several major grocery chain stores in the District of Columbia. These samples were submitted by food inspectors of the Department of Environmental Services to the central laboratory for lead analysis.

A micromethod which had been developed and used successfully for the determination of blood lead for children in our laboratory (*1*) was used for the determination of lead in the juices.

Briefly, duplicate samples of 10 μg of juices were pipeted into nickel cups. Four standards of 0.4 ppm of lead as lead nitrate, prepared daily, were used in each tray.

A tray, containing 20 cups of sample and standards, was then placed on a thermolyne hot plate at 140°C and the liquids were allowed to evaporate to dryness. Each cup was treated with 20 μg of 30% of hydrogen peroxide and taken to dryness. After cooling, the samples were subsequently atomized in an air-acetylene flame on the 403 AAS. Peak heights were read from a strip-chart recorder and compared with those from standards.

Baby food consisting of vegetables and meats packed in glass jars were digested with nitric and perchloric acids and extracted with dithizone. The extracts were analyzed for lead by AAS.

[2] The use of vender's name is for identification and does not constitute an endorsement.

Some baby juice cans were leached with 4% acetic acid following an FDA method recommended for leaching lead from glazed pottery. Other cans were leached using 2% citric acid solution. Additional empty cans were treated with lead-free apple juice. Glass jars were treated in the same manner.

RESULTS

Results obtained from 80 cans of baby juices indicated that lead content varied from 0.10 to 1.18 ppm, with an average of 0.49, 0.43, and 0.19 ppm for apple, mixed fruits, and orange juices, respectively. A comparative study of 25 other baby foods containing vegetables and meats in glass jars gave a very low lead of 0.05 ppm. Bottled orange juice, prune juice, apple juice, and mixed juice revealed no lead present (Table 1).

Leaching the empty cans for 24 hr at room temperature (can was covered with watch glass) indicated an average of 0.54 ppm of lead by 4% acetic acid and 0.40 ppm by 2% citric acid (Tables 2 and 3). As shown in the tables, more lead was detected as the time of leaching progressed. Further, a study using lead-free apple juice (predetermined) to leach the empty cans indicated an average of 0.45 ppm being leached out as compared to 0.30 ppm in the original juices (Table 4). There was not any detectable lead after 96 hr of leaching in glass jars under the same experimental condition. Different batches of cans varied as to the degree of leaching.

DISCUSSION

The micromethod used for blood lead has been discussed elsewhere (*1*). We experienced no difficulty in determining lead in various juices in

TABLE 1
LEAD CONTENT IN CANNED BABY JUICES AND FOODS

Products	No. samples	Range (ppm)	Median (ppm)
Canned baby juices			
Apple juice	20	0.13–1.18	0.49
Orange juice	30	0.09–0.63	0.19
Mixed juice	30	0.21–0.66	0.43
Baby foods (glass-jars)			
Vegetables	13	0.01–0.14	0.04
Meats	12	0.01–0.20	0.06
Bottled Apple juice	1	—	0.04
Bottled Orange juice	1	—	0.02
Bottled Mixed juice	1	—	0.02
Bottled Lime juice	1	—	0.02
Bottled Prune juice	1	—	0.07

TABLE 2
CAN LEACHING STUDY BY 4% ACETIC ACID

Can No.	Original Content	Pb (ppm)		
		Juices[a]	24 Hrs.[b]	48 Hrs.[b]
222	Orange juice	0.08	0.50	0.53
260	Orange-banana	0.07	0.55	0.59
261	Mixed fruits	0.10	0.41	0.44
263	Orange-pineapple	0.33	0.57	0.57
276	Apple-grape	0.38	0.49	0.59
277	Apple-cherry	0.47	0.68	0.73
278	Apple-cherry	0.40	0.60	0.78

[a] Original juice.
[b] Leached by acetic acid.

TABLE 3
CAN LEACHING STUDY BY 2% CITRIC ACID

Can no.	Original content	Pb (ppm)		
		Juices[a]	24 Hrs.[b]	48 Hrs.[b]
196	Apple juice	0.13	0.45	0.87
197	Orange-apricot	0.53	0.20	0.56
229	Apple-pineapple	0.10	0.63	1.63
232	Apple juice	0.42	0.22	0.35
236	Apple juice	0.34	0.73	1.50
262	Apple juice	0.10	0.06	0.13
285	Orange-apple	0.34	0.53	0.66

[a] Original juice.
[b] Leached by acetic acid.

TABLE 4
CAN LEACHING STUDY BY LEAD FREE APPLE JUICE

Can No.	Original Content	Pb (ppm)	
		Juices[a]	48 Hrs.[b]
316	Prune-orange	0.12	0.67
318	Orange-apricot	0.20	0.45
319	Mixed fruits	0.35	0.34
321	Orange-apple-banana	0.41	0.56
330	Orange-apple	0.48	0.41
333	Prune-orange	0.21	0.25

[a] Original juice.
[b] Leached by lead free apple juice.

this study. The peroxide treatment is adequate to destroy much of the organic matrix, and the deuterium background corrector provided a clear separation of the smoke peak from the lead peak.

Our findings indicate that a consumption of 4.2 oz of some baby juices would give an intake of about 146 μg of lead. This is about half of the estimated maximum daily permissible intake of 300μg of lead as established by Dr. King's study (*3*).

Using Dr. Lamm's data on infant formula and our results on baby juices, calculations indicate that an infant consuming 32 oz (four 8-oz bottles) of milk formula and 8 oz (two 4-oz cans) of canned baby juice daily, is approaching an intake of 600 μg of lead per day.

The British Government has established a standard of 0.5 ppm as the maximum for lead in baby food, including evaporated formula (*5*). The FDA is considering establishing a limit of 0.25 ppm (*3*).

SUMMARY

For juices, the microprocedure has proved to be simple, convenient, accurate, and there is no sample preparation. It has the advantages over conventional wet digestion methods which require time, attention, acid handling, hood space, and possible lead loss due to multiple extraction.

These data indicate some infants may be ingesting double the estimate permissible maximum daily intake of 300 μg of lead, above which (*3*) the entire amount of lead cannot be excreted and accumulation in the body begins.

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The Pyrolytic Identification of Organic Molecules

IV. The Pyrolytic Behavior of the Isomeric Butanols

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INTRODUCTION

In a previous publication (*9*) we presented the results of an investigation of the pyrolytic behavior of the lower homologues of the aliphatic alcohols. In this communication we present the results of a similar study with the four butanol isomers. These model compounds were selected to test the validity of our pyrolysis gas chromatographic system for the analysis of liquid samples having the same element composition, but a different structural arrangement.

Pyrolytic studies of the butanols have been made by previous investigators, who have used a variety of pyrolysis conditions and analytical procedures. Hurd (*6*) has described classical studies of the large-scale pyrolysis of *n*-butanol and iso-butanol. Generally, the butanols were pyrolyzed in the presence of catalysts and the products were analyzed by chemical methods. Barnard (*1*) employed a static system and a temperature range of 573°–629°[1] for his study of the pyrolysis of *n*-butanol, and a temperature of between 487°–620° for the pyrolysis of tert-butanol (*2*). Chemical and gas chromatographic methods were used to identify and determine the products formed. Wolf and Rosie (*16*) followed the pyrolysis of the four isomers over a temperature range of about 400°–1000° and identified the products formed by means of gas chromatography.

In our investigation we have pyrolyzed each of the isomers over a temperature range of 300°–1150° and identified the products by means of gas chromatographic analysis. Each isomer gave its own characteristic thermal decomposition pattern (which we have designated as the "pyrometron" (*9*). The four isomers were distinguishable from each other by means of the different breakdown products formed according to specific reactions pertaining to the structural arrangement of the individ-

[1] All temperature measurements are in °C.

ual alcohol. Mechanisms are proposed for the reaction products obtained.

EXPERIMENTAL

Apparatus

The pyrolysis and gas chromatographic units were the same as those used in the previous investigation (*9*). One modification was the addition of heating tape around the metal connecting tube between the outlet of the pyrolysis tube and the column inlet to maintain the tube at about 200°.

The response from each detector used (thermal conductivity and flame ionization detector), connected in series, was recorded on a Philips PM8010 dual pen flat-bed recorder at a chart speed of 25 mm/min.

Gas Chromatographic Column Systems

The columns and conditions for the pyrolysate analyses have been described in Part III (*9*). Briefly, *Column 1* was Carbowax 1540 on 20/60 mesh Teflon 6 at 80°, used for polar molecules. *Column 2* was 80/100 mesh Porapak T at 160°, used as a general purpose column. *Column 3* was 80/100 mesh silica gel at 50°, used for the permanent gases. Hydrogen was determined with the same column but with nitrogen instead of helium carrier gas. *Column 4* was 30/60 mesh silica gel at 60°, used for the determination of acetylene. *Column 5* was 100/150 mesh Porasil B at 56° for the determination of the lower hydrocarbons and at 90° for C_4 and above hydrocarbons, used for verification of hydrocarbons.

The katharometer detector was maintained at the same temperature of each column. Helium at a flow rate of 50 ml/min was employed as the carrier gas with each column, but nitrogen at a flow rate of 50 ml/min was used for the determination of hydrogen.

Test Compounds

Samples of the pure alcohols were double distilled and stored over 5 Å molecular sieve material. Samples of 1 μl were measured out for injection into the pyrolysis tube by means of 1 μl Hamilton micro syringes. Calibrations were performed in the manner described in Part II (*4*) of the series.

Pyrolysis and Analytical Procedures

The four isomers were subjected to flash vaporization pyrolysis in the stream of the carrier gas in steps of approximately 50° over the range of 300°–1150°. Each isomer was individually examined with columns 1 to 4

over the specified temperature range to give the pyrometrons recorded as Tables 1 to 4. The values given are the peak areas in cm^2 for each volatile product obtained with each temperature step of the pyrolysis of 1 μl of sample.

RESULTS

The peak areas on the column 1 pyrograms corresponding to the concentration of *n*-, iso- and sec-butanol in the pyrolysates increased steadily at temperatures between 350° and 650°. This anomaly could be explained in the case of iso- and sec-butanol. The retention time of water (13.5 cm) on the column was close enough to the retention time of isobutanol (17.1 cm) and sec-butanol (11.1 cm) for each water peak on the pyrogram to be overshadowed by the large alcohol peak. This resulted in the apparent absence of water production and increase in the alcohol peak areas as shown in their respective pyrometrons (Tables 2A and 3A). At high temperatures, the alcohol and water peaks were resolved due to the decrease in concentration of the alcohols through their decomposition. In the case of *n*-butanol, its retention time on column 1 was 25.0 cm, and water was completely resolved and measurable from the start of its production at 450°. No explanation can be given to account for the increase in the *n*-butanol concentration at temperatures between 350° and 550°. The logical explanation would be the presence of an unresolved pyrolysis product, but this was not confirmed with column 2 analysis. No trouble was experienced when the thermal degradation of the alcohols was followed with column 2 analysis. The values recorded in the appropriate pyrometrons represent a more realistic pattern of their degradation. The degradation of the four butanols was followed with column 2 analysis to confirm the formation of the products identified with column 1. With column 2 analysis 1-butene and acetaldehyde had very similar retention times to water, 1.75, 2.1, and 2.2 cm, respectively. The 1-butene and acetaldehyde peaks coalesced, and measurement of peak areas was not possible to begin with. At lower temperatures 1-butene, acetaldehyde, and water were unresolved as an indented broadish peak due to the low concentration of the two former products. At higher temperatures when acetaldehyde and 1-butene concentrations had decreased, resolution of the 1,3-butadiene (a decomposition product of 1-butene which had a similar retention time as 1,3-butadiene) and water occurred. A similar effect was observed when 2-methylpropene was involved as a decomposition product. In this case 1,3-butadiene, the decomposition product of 2-methylpropene, was also resolved from the water peak when the concentration of the hydrocarbon was low due to decomposition at higher pyrolysis temperatures.

Ethanol and benzene had retention times of 6.55 and 6.6 cm, respec-

tively, when separated on column 1, but their retention times were well separated on column 2. With column 1 analysis the benzene peak obtained at pyrolysis temperatures below 850° would also include ethanol if formed as a product. Ethanol when present was determined with column 2 analysis. Benzene, however, is not a significant product in the case of the thermal degradation of the butanols, as it is formed from thermal rearrangement of C_4 and C_3 hydrocarbons and, to a lesser extent from lower hydrocarbons, and not directly from the butanols as a major thermal reaction product.

n-Butanol

The products found with column 1 analysis and with katharometer detection in order of elution were: (a) a peak representing 1-butene which at 600° merged with the second peak (b). This peak was the unresolved permanent gases, H_2, CO, CH_4, etc., which began as a separate peak at 400° in trace amounts. Peak (c) was acetaldehyde, (d) cyclohexane, (e) propionaldehyde in trace amounts, (f) *n*-butyraldehyde, (g) methanol, (h) benzene, (i) toluene in a trace quantity and detected by the flame ionization detector in measurable amounts only, (j) water, and (k) a peak of unpyrolyzed n-butanol up to a temperature of 750°.

The thermal degradation of *n*-butanol was followed with column 2 analysis over the range of 300°–1050°. The presence of *n*-butyraldehyde, the trace amount of propionaldehyde, benzene, and water was confirmed also on the series of pyrolyses. A trace quantity of ethanol was also detected with the FID sensor, being too small for katharometer detection. A trace quantity of allene was also detected between 900°–1050°. Propylene was detected and its production followed with column 2 analysis. Its presence was confirmed in separate pyrolyses with column 5 (Porasil B) analysis. Analysis with column 5 showed that propane, equivalent to about 10% of the propylene concentration was also a product of the decomposition of *n*-butanol.

The presence of 1-butene was confirmed with column 5 analysis. Examination of 1-butene at pyrolysis temperatures of 600° and 700° showed that the hydrocarbon was completely decomposed at 700° and that 1,3-butadiene was one of the products formed. 1,3-Butadiene appeared above 700° in small quantities as a separate peak with column 2 analysis.

The products found with column 3 (silica gel) analysis in order of elution were carbon monoxide, methane, ethane, and ethylene. Hydrogen production was followed with nitrogen as the carrier gas on the same column. Acetylene production was followed with column 4 analysis.

The relevant results of the degradation of *n*-butanol, expressed in peak area cm^2, are collectively shown in the pyrometron, Table 1A and 1B.

TABLE 1A

PYROMETRON OF n-BUTANOL: COLUMN 1 ANALYSIS[a]

T°	*n*-Butanol	Water	Methanol	*n*-Butyraldehyde	Propionaldehyde	Acetaldehyde	1-Butene	Benzene	Toluene	Cyclo-hexane
350	104.1									
400	115.0						T[b]			
450	118.0	0.75					T			
500	130.4	1.12	T	T			0.13			
550	132.3	1.05	0.28	0.3		0.17	0.64			
600	128.3	1.04	0.52	0.6		0.73	1.94			
650	103.2	2.4	1.2	1.15		2.19	T			
700	51.9	6.0	3.15	1.75	T	7.5	X[c]	0.81		
750	7.4	10.7	2.5	1.14	0.22	4.0		0.9	T	T
800	X	11.0	1.26	X	T	T		2.67	T	1.86
850		11.4	0.25					3.87	0.7	1.19
900		11.6	X					6.57	0.24	0.42
950		10.8						9.5	T	0.36
1000		11.4						10.7	T	0.15
1050								8.6	X	0.1

[a] Peak area cm^2.

[b] Denotes a trace amount.

[c] No product detected.

TABLE 1B

PYROMETRON OF n-BUTANOL: COLUMN 2[a], 3[b], AND 4[c] ANALYSIS[d]

T°	n-Butanol[a]	Propylene/Propane[a]	1,3-Butadiene[a]	Allene[a]	Carbon monoxide[b]	Methane[b]	Ethane[b]	Ethylene[b]	Hydrogen[b]	Acetylene[c]
350	121.3									
400	121.0								T[e]	
450	119.2								T	
500	117.0	T							3.24	
550	108.5	0.75				T			10.0	
600	101.9	1.89			T	0.28	0.16	T	11.28	
650	50.5	10.0			1.0	1.98	1.05	4.4	25.6	
700	7.0	28.0			9.9	10.9	3.22	17.1	65.4	
750	T	28.6			22.8	22.9	4.22	29.4	202.4	
800	X[f]	12.1	0.47		22.5	23.7	2.75	35.9	286.0	
850		3.8	0.53		28.8	31.0	1.84	36.2	328.4	1.04
900		1.2	0.25	T	28.8	32.2	0.7	30.7	388.0	3.6
950		0.61	0.21	T	28.9	32.6	0.3	20.5	444.0	7.04
1000		0.28	0.03	T	29.4	32.6	T	11.3	621.0	9.54
1050		0.17	T	T	36.3	37.1	X	5.8	910.0	14.8
1100									1280.0	12.4

[a] Analysis of columns marked with this symbol.
[b] Analysis of columns marked with this symbol.
[c] Analysis of columns marked with this symbol.
[d] Peak area cm^2.
[e] Denotes a trace amount.
[f] No product detected.

iso-Butanol

The products found with column 1 analysis and with katharometer detection in order of elution were: (a) 2-methylpropene, which merged at 700° with the second peak (b) consisting of the permanent gases beginning as a trace amount at 500°. Peak (c) was acetaldehyde, (d) iso-butyraldehyde, (e) acetone, (f) propionaldehyde, (g) methanol, (h) benzene, (i) water, and (j) undecomposed iso-butanol below 800°. Above 800° some toluene and cyclohexane were also produced.

Thermal degradation of iso-butanol between 300°–1050° with column 2 analysis confirmed the presence of propionaldehyde, iso-butyraldehyde, water, acetaldehyde, acetone, methanol, a trace of ethanol, and benzene. 1,3-Butadiene, a decomposition product of 2-methylpropene was also detected at temperatures above 800°. A trace of allene was also produced at 850° and above. Propylene was detected and its production followed. Confirmation of propylene together with a small amount of propane, 2-methylpropene and 1,3-butadiene were obtained by separate pyrolysis of iso-butanol at 600° and 700° with column 5 analysis.

The products found with column 3 analysis in order of elution were carbon monoxide, methane, ethane, and ethylene. Hydrogen was determined separately using a nitrogen carrier gas. The production of acetylene was followed with column 4 analysis.

The relevant results of the degradation of iso-butanol are collectively shown in the pyrometron, Table 2A and 2B.

sec-Butanol

The products found with column 1 analysis and with katharometer detection in order of elution were: (a) a peak representing 1-butene which merged at 750° with a peak (b) of the unresolved permanent gases commencing as a trace amount at 350°. Peak (c) was acetaldehyde, (d) cyclohexane, (e) a trace amount of acetone between 600° and 700°, (f) propionaldehyde, (g) a peak representing unresolved methyl ethyl ketone and methanol, (h) benzene, (i) residual sec-butanol up to a temperature of 800°, (j) a small amount of toluene, and (k) water.

Thermal degradation of the alcohol between 300° and 1100° with column 2 analysis confirmed the production of propionaldehyde, acetaldehyde, methyl ethyl ketone, the small amounts of acetone, methanol and ethanol, benzene and water. Propylene was identified and its production was followed together with 1,3-butadiene. Confirmation of propylene together with a small quantity of propane, 1-butene and 1,3-butadiene was obtained by pyrolysis of the isomer at 600° and 700° with column 5 analysis.

TABLE 2A

PYROMETRON OF ISO-BUTANOL: COLUMN 1 ANALYSIS[a]

T°	iso-Butanol	Water	Methanol	Acetone	iso-Butyraldehyde	Propionaldehyde	Acetaldehyde	2-Methylpropene	Benzene	Toluene	Cyclohexane
350	108.0										
400	121.4										
450	119.3										
500	123.2		T[b]	0.1	0.15	T		T			
550	122.1		T	0.36	0.39	0.36	T	0.74			
600	115.5		0.24	1.3	1.68	1.3	T	2.55			
650	90.3	1.0	0.96	2.59	4.09	T	0.2	10.1			
700	44.1	7.0	2.1	4.67	10.3		0.33		0.81		
750	7.0	10.1	2.01	0.6	1.08		0.62		1.8		
800	X[c]	10.1	1.5	0.22	X		0.27		3.82	0.77	1.08
850		11.2	0.85				T		6.5	1.21	1.61
900		12.1	T				T		8.93	0.68	0.54
950		11.3	X				T		10.3	T	0.27
1000		11.1							10.2	T	0.1
1050		10.8							8.83	T	X

[a] Peak area cm^2.

[b] Denotes a trace amount.

[c] No product detected.

TABLE 2B

PYROMETRON OF ISO-BUTANOL: COLUMN 2[a], 3[b], AND 4[c] ANALYSIS[d]

T°	iso-Butanol[a]	Ethanol[a]	Propylene/ propane[a]	1,3-Butadiene[a]	Allene[a]	Carbon monoxide[b]	Methane[b]	Ethane[b]	Ethylene[b]	Hydrogen[b]	Acetylene[c]
350	126.8										
400	126.5										
450	123.5										
500	116.3						T[e]			T	
550	114.7		T			T	0.25			1.8	
600	106.0	T	15.7			0.11	0.84			6.6	
650	27.2	1.2	46.9			1.64	4.18	T	T	16.4	
700	4.5	0.7	34.5			11.8	14.7	1.4	8.3	54.6	
750	T	T	19.2			22.4	23.6	2.4	18.4	131.2	
800	X[f]		2.88			23.4	28.8	2.1	22.1	156.0	
850			0.81	T	T	20.6	28.3	0.8	21.5	187.0	8.1
900			0.46	0.33	T	21.6	31.2	0.55	18.9	210.0	3.4
950			0.35	0.21	T	19.8	30.3	T	14.4	241.0	6.56
1000			0.29	T		22.1	32.6	T	6.7	270.5	8.88
1050			T			28.5	35.9	T	3.6	317.0	12.0
1100										463.0	11.02

[a] Analysis of columns marked with this symbol.
[b] Analysis of columns marked with this symbol.
[c] Analysis of columns marked with this symbol.
[d] Peak area cm^2.
[e] Denotes a trace amount.
[f] No product detected.

The products found with column 3 analysis over the temperature range of 350°–1100° in order of elution were carbon monoxide, methane, ethane, and ethylene. Hydrogen production was followed separately, and acetylene production was followed using column 4.

Relevant results of the degradation of sec-butanol are given in the pyrometron, Table 3A and 3B.

tert-Butanol

The products found with column 1 analysis with katharometer detection were in order of elution: (a) 2-methylpropene which commenced at 350° and reached a maximal concentration at 650°, (b) the permanent gases, (c) acetaldehyde in small amounts which commenced at 800°, (d) cyclohexane, (e) acetone, (f) residual tert-butanol up to 750°, (g) benzene, (h) toluene, and (i) water.

Examination of tert-butanol at temperatures between 300° and 1050° with column 2 analysis confirmed the production of acetone, ethanol between 650° and 850°, 2-methylpropene and the production of 1,3-butadiene from it. Some allene was also produced, forming at 700° and increasing up to 800° before decreasing to a trace amount at 1000°. Propylene was detected and its production followed. Because the large peak of 2-methylpropene obtained on the chromatograms overshadowed the acetaldehyde peak, the presence of the aldehyde could not be confirmed.

The products found with column 3 analysis were carbon monoxide, methane, ethane, and ethylene. Hydrogen production was followed separately using nitrogen as the carrier gas. Acetylene production was followed with column 4 analysis.

The results of the degradation of tert-butanol are given in the pyrometron, Table 4A and 4B.

DISCUSSION

The pyrograms obtained in this study of the butanols were more difficult to interpret than those from the previous studies (*4,9*) of the C_1–C_3 homologues of the aliphatic alcohols. The four-carbon structure offers additional thermal reaction possibilities resulting in the formation of a greater number of volatile products. Confirmation of the identity of almost all the products from each alcohol was obtained by the use of the Carbowax 1540 and Porapak T columns, and the silica gel and Porasil B columns for the permanent gases and hydrocarbons. A few products present in trace proportions and considered to be insignificant were not identified. Any difference between our observations and those of Wolf and Rosie (*16*) was not due to the inability of the above columns to separate any of the products. Any such discrepancy was checked by the

TABLE 3A

PYROMETRON OF SEC-BUTANOL: COLUMN 1 ANALYSIS[a]

T°	sec-Butanol	Water	Acetone	Propional-dehyde	Acetal-dehyde	1-Butene	Benzene	Toluene	Cyclo-hexane
350	113.4								
400	120.4					T[b]			
450	124.6					T			
500	127.9			T	T	0.12			
550	126.6			0.36	0.12	0.46			
600	114.4		T	1.84	0.88	2.94			
650	76.5	13.6	0.28	9.28	4.09	17.0			
700	21.1	13.2	T	15.7	4.98		1.5		
750	0.8	12.6		9.52	0.01		2.52		0.95
800	X[c]	15.7		2.95			5.8	0.9	1.57
850		15.3		0.12			8.15	1.0	0.73
900		15.0					10.2	0.85	0.51
950		15.0					10.0	T	0.22
1000		14.7					10.7	T	0.12
1050		15.3					8.55		T

[a] Peak area cm^2.

[b] Denotes a trace amount.

[c] No product detected.

TABLE 3B

PYROMETRON OF SEC-BUTANOL: COLUMN 2[a], 3[b], AND 4[c] ANALYSIS[d]

T°	sec-Butanol[a]	Methanol[a]	Ethanol[a]	Methyl ethyl ketone[a]	Propylene/ propane[a]	1,3-Butadiene[a]	Allene[a]	Carbon monoxide[b]	Methane[b]	Ethane[b]	Ethylene[b]	Hydrogen[b]	Acetylene[c]
350	138.0												
400	135.7												
450	129.5			T[e]									
500	127.7			0.6	T				T			T	
550	125.0			1.28	0.12				0.21			5.01	
600	109.5			1.12	0.36			T	1.06			30.4	
650	77.2		0.17	5.4	1.48			1.12	6.65	0.54	3.3	41.2	
700	29.8	0.9	0.7	7.8	7.9			7.1	21.8	1.89	13.0	80.8	
750	3.6	0.64	0.48	3.24	17.9	14.4	T	14.9	32.9	2.31	20.0	105.0	T
800	X[f]	0.12	0.30	1.08	13.3	6.8	T	19.0	36.8	2.1	21.7	138.5	0.7
850		T	T	X	4.2	2.7	T	18.1	35.9	1.49	20.2	160.4	2.05
900					1.5	1.5	T	17.5	34.8	0.4	18.5	209.0	5.04
950					0.76	1.1		17.7	32.9	T	13.9	254.8	6.08
1000					0.50	T		18.3	32.5	X	6.8	401.6	10.56
1050					0.18			27.0	39.5		3.7	478.4	11.3
1100					0.1							508.7	18.7

[a] Analysis of columns marked with this symbol.
[b] Analysis of columns marked with this symbol.
[c] Analysis of columns marked with this symbol.
[d] Peak area cm^2.
[e] Denotes a trace amount.
[f] No product detected.

TABLE 4A
PYROMETRON OF TERT-BUTANOL: COLUMN 1 ANALYSIS[a]

T°	tert-Butanol	Water	Acetone	Acetaldehyde	2-Methylpropene	Benzene	Toluene	Cyclohexane
300								
350	122.0	1.04			3.60			
400	115.9	1.96			9.92			
450	116.8	3.36			19.0			
500	101.3	5.73			24.5			
550	83.7	10.7			55.6			
600	57.8	16.7			86.0			
650	24.2	27.7	1.24		94.5			
700	3.15	35.7	5.06			0.99	T[b]	
750	X[c]	35.3	3.42			3.18	T	0.32
800		36.9	1.85	T		12.0	2.56	0.92
850		41.3	T	0.4		18.5	5.07	1.12
900		39.6		0.24		17.1	7.52	0.2
950		33.9		0.08		17.5	0.9	0.12
1000		32.8		X		13.9	T	X
1050		31.9				10.7	T	

[a] Peak area cm^2.
[b] Denotes a trace amount.
[c] No product detected.

determination of retention times by injection of samples of the suspected products into the pyrolysis tube and the column at their operating temperatures.

Our results show that the four isomers undergo dehydration with formation of their characteristic C_4 hydrocarbon. Normal, iso- and secondary butanols also undergo the dehydrogenation reaction, and evidence was obtained that a third reaction involving elimination of methanol also occurred with *n*- and iso-butanol. tert-Butanol appeared to degrade thermally by two additional, but minor routes. Acetone was obtained suggesting that demethylation occurred, and ethanol was another product accountable for by the introduction of a third decomposition route consisting of a demethylation and rearrangement mechanism.

n-Butanol

We propose that *n*-butanol thermally degrades through the following main routes:

$$CH_3CH_2CH_2CH_2OH \rightarrow CH_3CH_2CH_2CHO + H_2 \quad (1)$$
$$CH_3CH_2CH_2CH_2OH \rightarrow CH_3CH_2CH{:}CH_2 + H_2O \quad (2)$$
$$CH_3CH_2CH_2CH_2OH \rightarrow CH_3CH{:}CH_2 + CH_3OH \quad (3)$$

The principal volatile products found and recorded in the pyrometron (Table 1A and 1B) are more extensive in number than those found by Barnard (*1*), but they are comparable with those identified by Wolf and Rosie (*16*). Barnard pyrolyzed *n*-butanol in a static system between

TABLE 4B

PYROMETRON OF TERT-BUTANOL: COLUMN 2[a], 3[b], AND 4[c] ANALYSIS[d]

T°	tert-Butanol[a]	Ethanol[a]	Propylene/propane[a]	1,3-Butadiene[a]	Allene[a]	Carbon monoxide[b]	Methane[b]	Ethane[b]	Ethylene[b]	Hydrogen[b]	Acetylene[c]
300	143.0										
350	138.2										
400	131.9										
450	132.2										
500	110.4										
550	65.0										
600	28.1		T[e]				T				
650	9.6	T	0.22				0.57			T	
700	8.8	0.6	0.63		0.7		2.4		T	1.4	
750	X[f]	2.5	4.82		2.25	0.84	12.3	T	2.2	8.7	2.64
800		1.44	15.4	4.32	3.78	1.7	23.6	0.4	3.6	31.7	11.2
850		0.03	6.48	2.59	0.72	3.12	31.2	0.6	6.76	70.5	7.2
900		X	1.8	0.86	0.18	3.25	28.8	0.24	6.72	110.0	3.0
950			0.59	0.29	0.07	3.68	29.8	T	5.46	132.0	4.2
1000			T	T	T	4.51	34.9	T	3.31	226.5	4.65
1050						7.37	39.0	T	1.35	640.0	4.06
1100										986.5	5.55

[a] Analysis of columns marked with this symbol.
[b] Analysis of columns marked with this symbol.
[c] Analysis of columns marked with this symbol.
[d] Peak area cm^2.
[e] Denotes a trace amount.
[f] No product detected.

573°–629° in which the decomposition was followed by both a pressure change and analysis. The major products found by Barnard were formaldehyde, carbon monoxide, methane, and hydrogen. In addition, small amounts of water, ethane, ethylene, propane, and propylene were also obtained. Formaldehyde was the only aldehyde identified and determined, and lower alcohols were absent. On this evidence Barnard concluded that the $C_3H_7-CH_2OH$ bond is broken and formulated a mechanism involving the formation of the radical initiating step:

$$CH_3CH_2CH_2CH_2OH \rightarrow CH_3CH_2\dot{C}H_2 + \dot{C}H_2OH \quad (1)$$

followed by,

$$\dot{C}H_2OH \rightarrow \dot{H} + HCHO \quad (2)$$
$$HCHO \rightarrow H_2 + CO \quad (3)$$

and,

$$CH_3CH_2\dot{C}H_2 \rightarrow \dot{C}H_3 + CH_2CH_2 \quad (4)$$

The methyl radical then participates in a chain reaction:

$$CH_3CH_2CH_2CH_2OH + \dot{C}H_3 \rightarrow CH_3CH_2CH_2\dot{C}HOH + CH_4 \quad (5)$$
$$CH_3CH_2CH_2\dot{C}HOH \rightarrow CH_3CH_2\dot{C}H_2 + HCHO \quad (6)$$
$$CH_3CH_2\dot{C}H_2 \rightarrow \dot{C}H_3 + CH_2CH_2 \quad (7)$$

Our results cannot be fitted into the mechanism proposed by Barnard without modification in order to account for the various oxygenated products we have found. The marked differences in the results could be due to the fact that we employed a dynamic flow system and a more sophisticated analytical technique.

The pyrometron results (Table 1A and 1B) indicate that *n*-butanol begins to decompose at about 400° and is complete at about 750°. Trace amounts of hydrogen and 1-butene were detected at 400°, and water was obtained in a measurable quantity at 450°. This suggests that dehydrogenation (route 1) and dehydration (route 2) proceed simultaneously. Trace amounts of methanol, *n*-butyraldehyde and propylene were detectable at 500° and increased in measurable quantities as the temperature of pyrolysis was increased. Methane appeared at 550° and increased in quantity fairly rapidly until 850°. Carbon monoxide and ethylene appeared at 600° and followed a similar production pattern as methane. In order to account for the relatively small concentration build-up of *n*-butyraldehyde from *n*-butanol, route 1 must consist of a number of competitive reactions that are temperature dependent.

We propose that route 1 consists of a radical mechanism involving the initiation reaction:

$$CH_3CH_2CH_2CH_2OH \rightarrow CH_3CH_2CH_2\dot{C}HOH + \dot{H} \quad (A)$$

followed by the propagation reactions:

$$CH_3CH_2CH_2CH_2OH + \dot{H} \rightarrow CH_3CH_2CH_2\dot{C}HOH + H_2 \quad (B)$$
$$CH_3CH_2CH_2\dot{C}HOH \rightarrow CH_3CH_2CH_2CHO + \dot{H} \quad (C)$$
$$CH_3CH_2CH_2\dot{C}HOH \rightarrow CH_3CH_2CHO + \dot{C}H_3 \quad (D)$$
$$CH_3CH_2CH_2CH_2OH + \dot{C}H_3 \rightarrow CH_3CH_2CH_2\dot{C}HOH + CH_4 \quad (E)$$

The termination reactions are:

$$\dot{H} + \dot{H} \rightarrow H_2 \quad (F)$$
$$\dot{C}H_3 + \dot{H} \rightarrow CH_4 \quad (G)$$
$$\dot{C}H_3 + \dot{C}H_3 \rightarrow CH_2CH_2 + H_2 \quad (H)$$

The mechanism does not account for all the products identified such as carbon monoxide, methane, ethylene, and acetaldehyde. We could not confirm the production of formaldehyde because it had the same retention time as propylene on the Porapak T column. Comparable peak area measurements were obtained with katharometer and flame ionization detection. The possibility of formaldehyde formation cannot be overlooked in view of the fact that its production was reported by Barnard (*1*) as a major constituent of *n*-butanol thermolysis. The production of formaldehyde may be accounted for by incorporating step (6) of Barnard's propagation scheme into our proposed mechanism. Thus the radical (A) of route 1 may also eliminate formaldehyde as an additional step:

$$CH_3CH_2CH_2\dot{C}HOH \rightarrow CH_3CH_2\dot{C}H_2 + HCHO \quad (A1)$$
$$CH_3CH_2\dot{C}H_2 \rightarrow \dot{C}H_3 + CH_2CH_2 \quad (A2)$$

The methyl radical then participates in the same way as step (E) of the route 1 mechanism. Formaldehyde will decompose in the recognized manner into carbon monoxide and hydrogen.

When a 1 μl sample of *n*-butyraldehyde was pyrolyzed at 750° and at 960° with column 2 analysis, the permanent gases were formed together with propylene, water, propionaldehyde, acetaldehyde, and benzene. Some *n*-butyraldehyde also remained even at the higher temperature in a trace amount. The pyrolysis of 1 μl samples of propionaldehyde at 700° with column 2 analysis revealed that the permanent gases were formed together with propylene, water, acetaldehyde, a trace amount of benzene, and a small quantity of methanol. Some propionaldehyde also remained. The amount of methanol formed was much less than that obtained from 1 μl of *n*-butanol at the same temperature. Examination of acetaldehyde (1 μl samples) at 760° with column 2 analysis yielded information that the aldehyde decomposes into formaldehyde, water and the permanent gases. Wolf and Rosie (*16*) reported that crotonaldehyde

was formed from *n*-butanol, and Wolf (*17*) reported that acetaldehyde also produced crotonaldehyde at temperatures between 200°–700°. Under our conditions no crotonaldehyde could be detected in the pyrolysates with column 1 and 2 analysis. At 770° crotonaldehyde was found to decompose into the permanent gases, propylene, acetaldehyde, propionaldehyde, and benzene. Some crotonaldehyde also remained. On this evidence it is possible to suggest reactions that might account for the decomposition of the aldehydes.

n-Butyraldehyde produced in (C) of route 1 may decompose as follows:

$$CH_3CH_2CH_2CHO \rightarrow CH_3CH{:}CH_2 + HCHO \quad (I)$$
$$CH_3CH_2CH_2CHO \rightarrow CH_3CH_2CH_3 + CO \quad (J)$$
$$CH_3CH_2CH_2CHO \rightarrow CH_2CH_2 + CH_3CHO \quad (K)$$
$$CH_3CH_2CH_2CHO \rightarrow CH_2{:}CHCH{:}CH_2 + H_2O \quad (L)$$
$$CH_3CH_2CH_2CHO \rightarrow CH_3CH_2CHO + \ddot{C}H_2 \quad (M)$$

and,

$$\ddot{C}H_2 + \ddot{C}H_2 \rightarrow CH_2CH_2 \quad (N)$$

Propionaldehyde produced in (D) and (M) may decompose:

$$CH_3CH_2CHO \rightarrow CH_2CH_2 + HCHO \quad (O)$$
$$CH_3CH_2CHO \rightarrow CH_3CH_3 + CO \quad (P)$$
$$CH_3CH_2CHO \rightarrow CH_2{:}C{:}CH_2 + H_2O \quad (Q)$$
$$CH_3CH_2CHO \rightarrow CH_3CHO + \ddot{C}H_2 \quad (R)$$

and,

$$\ddot{C}H_2 + \ddot{C}H_2 \rightarrow CH_2CH_2 \quad (S)$$

Acetaldehyde produced in (K) and (R) may decompose:

$$CH_3CHO \rightarrow HCHO + \ddot{C}H_2 \quad (T)$$

and,

$$\ddot{C}H_2 + \ddot{C}H_2 \rightarrow CH_2CH_2 \quad (U)$$
$$CH_3CHO \rightarrow CH_4 + CO \quad (V)$$
$$CH_3CHO \rightarrow CH{:}{:}CH + H_2O \quad (W)$$

Finally, formaldehyde produced in (A1), (I), (O), and (T) of the scheme will decompose into carbon monoxide and hydrogen.

It would appear from the pattern of results (Table 1A and 1B) that step (C) of route 1 proceeds at fairly low temperatures up to 700°, and that above 750° step (A1) predominates. Formaldehyde, and eventually carbon monoxide, hydrogen, ethylene, and methane are formed, as shown by the large increase in their concentration at about 750°. *n*-Bu-

tyraldehyde was found to be fairly resistant to thermal decomposition as it was detectable in the pyrolysate after pyrolysis at 960°. Since none of the aldehyde was detected in the *n*-butanol pyrolysates at temperatures above 800° supports the proposition that the alcohol degrades preferentially via steps (A1) and (A2) in the higher range of temperatures.

Propionaldehyde is a minor constituent of *n*-butanol as shown in Table 1A, and is derived from the alcohol according to step (D) of route 1 and as a decomposition product of *n*-butyraldehyde according to step (M). Acetaldehyde on the other hand was produced, and increased in concentration, at the same time as the concentration of *n*-butyraldehyde was increasing with the rise in pyrolysis temperature. When *n*-butyraldehyde dropped to zero concentration acetaldehyde also ceased to be formed in measurable amounts. This suggests that acetaldehyde is a major decomposition product of *n*-butyraldehyde according to step (K). Acetaldehyde is also resistant to temperatures up to 950° and if *n*-butyraldehyde had still been formed via step (C) of route 1 at temperatures above 800°, then some acetaldehyde would have been detected. This supports the view that *n*-butanol undergoes dehydrogenation at temperatures between 500° and 800°.

Route 2 is an elimination reaction:

$$CH_3CH_2CH_2CH_2OH \rightarrow CH_3CH_2CH_2\dot{C}H_2 + \dot{O}H \quad (A)$$

$$CH_3CH_2CH_2\dot{C}H_2 \rightarrow CH_3CH_2CH{:}CH_2 + \dot{H} \quad (B)$$

and,

$$\dot{H} + \dot{O}H \rightarrow H_2O \quad (C)$$

In Table 1A it is seen that water production arises evenly between 450° and 750° and then the rate of formation falls considerably to reach a maximal at 900°. The amount of water produced at 900° was calculated to be about 24% of the possible yield from *n*-butanol via route 2. This value is an approximation only, since the amount of water formed will include some water produced from decomposition of the aldehydes obtained via route 1.

Benzene, toluene, and cyclohexane, as secondary products, are presumed to be formed by rearrangement following thermal breakdown of 1-butene and 1,3-butadiene. The pyrolysis of 1-butene has been studied (*3,7,15*). When samples of 1-butene were pyrolyzed (column 2 analysis) decomposition occurred readily at temperatures above 500° and was complete at 700°. Benzene, toluene, propylene, cyclohexane, and other hydrocarbons were formed, consistent with previous investigators' findings. 1,3-Butadiene pyrolyzed at 860° yielded a considerable quantity of benzene and other lower hydrocarbons. A proportion of the propylene obtained from *n*-butanol is derived from 1-butene produced according to

the route 2 reaction. Decomposition products of the cyclic and larger chain hydrocarbons also contribute to the amount of methane, ethane, ethylene, acetylene, and hydrogen formed at higher pyrolysis temperatures. However, the hydrocarbons will also decompose as indicated by the decrease in their concentration and rise in the production of hydrogen revealed in Table 1B.

In order to explain the considerable yield of methanol formed we have introduced route 3 as an additional decomposition reaction. This we propose is an elimination reaction:

$$CH_3CH_2CH_2CH_2OH \rightarrow CH_3CH_2\dot{C}H_2 + \dot{C}H_2OH \quad (A)$$
$$CH_3CH_2\dot{C}H_2 \rightarrow CH_3CH{:}CH_2 + \dot{H} \quad (B)$$

and,

$$\dot{C}H_2OH + \dot{H} \rightarrow CH_3OH \quad (C)$$

Methanol produced according to step (C) will decompose mainly into formaldehyde and hydrogen, and the aldehyde then decompose into carbon monoxide and hydrogen. A mechanism for the thermal degradation of methanol has been proposed by Ingram and Rizvi (*9*). The route 3 mechanism agrees partly with a scheme proposed by Barnard (*1*) in so far as step (A) is concerned. In the pyrometron, methanol and propylene are seen to commence their production at about 500° and reach their maximal concentration at about 700° and 750°, respectively. The amount of methanol produced at 700° was calculated to be of the order of 10% of the possible yield from 1 μl of *n*-butanol.

iso-Butanol

On evidence obtained from the pyrometron (Table 2A and 2B) we present mechanism reactions for the decomposition of iso-butanol under the pyrolysis conditions employed. The alcohol undergoes dehydrogenation, dehydration, and also degrades by an elimination reaction yielding methanol and propylene. The reaction routes are as follows:

$$(CH_3)_2CHCH_2OH \rightarrow (CH_3)_2CHCHO + H_2 \quad (1)$$
$$(CH_3)_2CHCH_2OH \rightarrow (CH_3)_2C{:}CH_2 + H_2O \quad (2)$$
$$(CH_3)_2CHCH_2OH \rightarrow CH_3CH{:}CH_2 + CH_3OH \quad (3)$$

The pyrometron tables show that appreciable decomposition of iso-butanol occurred at 550° and was complete at 800°. Trace amounts of hydrogen, 2-methylpropene, methanol, and a measurable quantity of isobutyraldehyde were obtained at 500°. This indicates that all three routes become involved in the degradation of the alcohol at the same starting temperature. For the route 1 reaction we propose that the alcohol decomposes via a radical mechanism involving the initiation reaction:

$$(CH_3)_2CHCH_2OH \rightarrow (CH_3)_2CHCHOH + \dot{H} \quad (A)$$

followed by the propagation reactions:

$$(CH_3)_2CHCH_2OH + \dot{H} \rightarrow (CH_3)_2CH\dot{C}HOH + H_2 \quad (B)$$
$$(CH_3)_2CH\dot{C}HOH \rightarrow (CH_3)_2CHCHO + \dot{H} \quad (C)$$
$$(CH_3)_2CH\dot{C}HOH \rightarrow CH_3CH_2CHO + \dot{C}H_3 \quad (D)$$
$$(CH_3)_2CHCH_2OH + \dot{C}H_3 \rightarrow (CH_3)_2CH\dot{C}HOH + CH_4 \quad (E)$$

The termination reactions are the same as those written for the route 1 scheme for *n*-butanol.

The propagation reactions (D) and (E) are justified by the fact that trace quantities of propionaldehyde and methane were obtained at 500°. The degradation pattern is complicated by the fact that iso-butyraldehyde also decomposes at higher temperatures, and that acetone is produced and follows the same formation and decomposition pattern as the aldehyde. It was considered unlikely that acetone was a pyrolysis product of iso-butanol because a mechanism could not be formulated to account for its production from the alcohol. When 1 μl samples of iso-butyraldehyde were pyrolyzed at 750° with column 1 analysis, the permanent gases were obtained together with measurable quantities of acetone, water, and benzene. Acetaldehyde was produced in a measurable amount and some iso-butyraldehyde remained. The evidence suggests that iso-butyraldehyde is decomposed by an elimination–rearrangement type of reaction into acetone, and by other routes similar to those suggested for *n*-butyraldehyde. The following routes may be involved in the decomposition of iso-butyraldehyde:

$$(CH_3)_2CHCHO \rightarrow CH_3CH{:}CH_2 + HCHO \quad (F)$$
$$(CH_3)_2CHCHO \rightarrow CH_3CH_2CH_3 + CO \quad (G)$$
$$(CH_3)_2CHCHO \rightarrow CH_2CH_2 + CH_3CHO \quad (H)$$
$$(CH_3)_2CHCHO \rightarrow CH_3C{\vdots}CCH_3 + H_2O \quad (I)$$
$$(CH_3)_2CHCHO \rightarrow CH_3COCH_3 + \ddot{C}H_2 \quad (J)$$

and,

$$\ddot{C}H_2 + \ddot{C}H_2 \rightarrow CH_2CH_2 \quad (K)$$

The decomposition of propionaldehyde produced in (D) of route 1, acetaldehyde from (H), and formaldehyde from (F) account for some of the carbon monoxide, methane, ethylene, propylene, and hydrogen produced according to the reactions proposed in the case of *n*-butanol. The decomposition of acetone also complicates the pyrolysis pattern of iso-butanol. A mechanism for the thermal degradation of acetone has been proposed in a previous publication (*4*). Ketene, methane, ethylene, carbon monoxide, and hydrogen were the major products. Ketene decomposes into carbon monoxide and ethylene.

The pattern of carbon monoxide and water production between 800° and 1000° (Table 2A and 2B) show that competition may occur between route 1 and 2. The yield of carbon monoxide decreased and water production increased steadily after 800°, until at 900° water reached a maximal concentration. Thereafter, the water concentration decreased and the carbon monoxide yield increased as the temperature of pyrolysis was raised.

Route 2 is an elimination reaction:

$$(CH_3)_2CHCH_2OH \rightarrow (CH_3)_2CH\dot{C}H_2 + \dot{O}H \quad (A)$$
$$(CH_3)_2CH\dot{C}H_2 \rightarrow (CH_3)_2C{:}CH_2 + \dot{H} \quad (B)$$

and,

$$\dot{H} + \dot{O}H \rightarrow H_2O \quad (C)$$

The first appearance of 2-methylpropene occurred at 500° in a trace amount, and increased in measurable quantities up to 650°. At higher temperatures the production of the hydrocarbon could not be followed because the peak on the chromatogram merged with the peak of the permanent gases. Water production was not measurable until 650° and then increased with each rise in temperature to reach its maximal concentration at 900°. At this temperature, the amount of water produced was calculated to be about 26% of the possible yield from iso-butanol via route 2. A proportion of the water is produced from the aldehydes so that the amount of the alcohol degraded through route 2 is less than 26%.

The 2-methylpropene formed in the reaction decomposes into propylene and 1,3-butadiene resulting in the production of benzene, toluene, and cyclohexane. Other hydrocarbons such as methane and hydrogen are also formed. The very high yield of propylene at 650° suggests that considerable iso-butanol is decomposed through the dehydrogenation route, and that the resulting iso-butyraldehyde decomposed fairly rapidly according to reaction (F). According to Molera and Stubbs (*13*), 2-methylpropene decomposes fairly rapidly into propylene as we have found.

The production of methanol through route 3 can be explained by means of an elimination reaction:

$$(CH_3)_2CHCH_2OH \rightarrow (CH_3)_2\dot{C}H + \dot{C}H_2OH \quad (A)$$
$$(CH_3)_2\dot{C}H \rightarrow CH_3CH{:}CH_2 + \dot{H} \quad (B)$$

and,

$$\dot{C}H_2OH + \dot{H} \rightarrow CH_3OH \quad (C)$$

The amount of methanol produced at 700° was calculated to be about 7% of the possible yield from 1 μl of iso-butanol.

The formation of ethanol obtained at temperatures between 600° and 750° may be explained by a further breakdown of the $(CH_3)_2\dot{C}H$ radical produced in step (A) of route 3.

$$(CH_3)_2\dot{C}H \rightarrow CH_2CH_2 + \dot{C}H_3 \qquad (D)$$

followed by,

$$\dot{C}H_2OH + \dot{C}H_3 \rightarrow CH_3CH_2OH \qquad (E)$$

The pyrolysis products found in our examination of iso-butanol were fairly consistent with those identified by Wolf and Rosie (*16*). These investigators did not identify methanol, but found in addition that diethyl ether, small amounts of *n*-butanol, *n*-butyraldehyde, methyl ethyl ketone, hexane, hexene-1, and methyl octane were also produced.

sec-Butanol

The pyrometron results (Table 3A and 3B) suggest that sec-butanol is decomposed mainly through the dehydrogenation and dehydration routes:

$$CH_3CH_2CHOHCH_3 \rightarrow CH_3CH_2COCH_3 + H_2 \qquad (1)$$
$$CH_3CH_2CHOHCH_3 \rightarrow CH_3CH_2CH{:}CH_2 + H_2O \qquad (2)$$

The pyrometron tables show that sec-butanol starts to decompose at about 400° with the production of 1-butene, and that decomposition is complete at about 800°. This suggests that the dehydration reaction proceeds first. The dehydrogenation reaction is seen to begin at about 450° by the production of methyl ethyl ketone. This product had a maximal concentration at 700° and then steadily decomposed to completion at 850°.

We propose that route 1 proceeds through a radical mechanism initiated by:

$$CH_3CH_2CHOHCH_3 \rightarrow CH_3CH_2\dot{C}OHCH_3 + \dot{H} \qquad (A)$$

followed by the propagation reactions:

$$CH_3CH_2CHOHCH_3 + \dot{H} \rightarrow CH_3CH_2\dot{C}OHCH_3 + H_2 \qquad (B)$$
$$CH_3CH_2\dot{C}OHCH_3 \rightarrow CH_3CH_2COCH_3 + \dot{H} \qquad (C)$$
$$CH_3CH_2\dot{C}OHCH_3 \rightarrow CH_3CH_2CHO + \dot{C}H_3 \qquad (D)$$
$$CH_3CH_2CHOHCH_3 + \dot{C}H_3 \rightarrow CH_3CH_2\dot{C}OHCH_3 + CH_4 \qquad (E)$$

The termination reactions are the same as those written for the *n*-butanol scheme.

The inclusion of steps (D) and (E) into the scheme is justified by the fact that propionaldehyde and methane are both formed at the same pyrolysis temperature of 500° in trace quantities. Thereafter, the two

products increased in their concentration at approximately the same rate until the maximal amount of propionaldehyde is reached at 700°.

Thermal decomposition of propionaldehyde, acetaldehyde, and methyl ethyl ketone complicate the thermolysis pattern by the production of their breakdown products. Possible reactions for the decomposition of the aldehydes have been proposed in the route 1 scheme for *n*-butanol. The pyrolysis of 1 μl samples of methyl ethyl ketone at 700° with column 1 analysis yielded a measurable quantity of acetaldehyde, propionaldehyde, and water, a small amount of benzene or ethanol and some undecomposed ketone. Examination of the ketone at 700° with column 2 analysis confirmed the production of propionaldehyde and ethanol. The amount of the aldehyde was considerably less than that produced from 1 μl of sec-butanol, showing that the bulk of propionaldehyde is derived from the alcohol according to step (D) of the route 1 scheme. Propylene was also produced in a considerable yield (6.12 cm^2 peak area). At 990°, methyl ethyl ketone yielded hydrogen, carbon monoxide, methane, ethylene, and a very small amount of ethane, obtained with column 3 analysis.

According to Hurd (*8*) methyl ethyl ketone is decomposed into ketene and methyl ketene. As we have not followed the full decomposition of the ketone we cannot provide confirmation. It would be extremely difficult to detect the ketenes under our pyrolytic conditions. Hurd proposed that methyl ethyl ketone could decompose in the following manner:

$$CH_3CH_2COCH_3 \rightarrow CH_2{:}C{:}O + CH_3CH_3$$
$$CH_3CH_2COCH_3 \rightarrow CH_3CH{:}C{:}O + CH_4$$

and that methyl ketene could decompose into ketene and ethylene:

$$2CH_3CH{:}C{:}O \rightarrow 2CH_2{:}C{:}O + CH_2CH_2$$

As we found that very little ethane was obtained from either sec-butanol or methyl ethyl ketone it is unlikely that the ketone decomposes in such a simple manner. It would appear that ethanol produced at temperatures between 650° and 850° is a product of the degradation of the ketone. The small amount of methanol produced between 700° and 850° is a breakdown product of sec-butanol.

For route 2 we propose the dehydration reaction:

$$CH_3CH_2CHOHCH_3 \rightarrow CH_3CH_2\dot{C}HCH_3 + \dot{O}H \quad (A)$$
$$CH_3CH_2\dot{C}HCH_3 \rightarrow CH_3CH_2CH{:}CH_2 + \dot{H} \quad (B)$$

and,

$$\dot{H} + \dot{O}H \rightarrow H_2O \quad (C)$$

Water had a maximal concentration at 800°, equivalent to a conversion of 33% of the alcohol into water via route 2. The amount of water formed includes that produced from decomposition of the aldehydes and ketone, and other oxygenated products, so that the percentage decomposition via route 2 will be less than 33%. The fact that the yield of 1-butene increased rapidly between 600° and 650°, and that 86% of the total yield of water was produced at 650° suggests that the dehydration reaction proceeds at a much faster rate than the dehydrogenation reaction.

Considerably more of 1,3-butadiene was obtained from 1-butene than from the 1-butene produced from *n*-butanol, or from 2-methylpropene produced from the two other butanols. The amount of 1,3-butadiene from sec-butanol at a pyrolysis temperature of 750°, is therefore, a significant feature of the thermal degradation of the alcohol. Benzene, toluene, cyclohexane, and some of the propylene are derived from 1-butene, and are not significant products. The small quantity of acetone produced between 600° and 700° was not a significant product. No attempt was made to elucidate a reaction for its formation.

tert-Butanol

The pyrometron (Table 4A and 4B) indicated that dehydration was the main thermal reaction involved in the decomposition of tert-butanol. The formation of acetone and ethanol above 600° suggests that demethylation reactions also occur at higher temperatures. Barnard (*2*) previously reported that tert-butanol decomposed mainly through its dehydration giving water and 2-methylpropene. A small quantity of carbon monoxide was also obtained, supposedly to come from decomposition of acetone, which was not, however, detected by Barnard. In their investigation of the thermal degradation of tert-butanol, Wolf and Rosie (*16*) also found some acetone and ethanol in the pyrolysates.

The reactions involved are as follows:

$$(CH_3)_3COH \rightarrow (CH_3)_2C{:}CH_2 + H_2O \quad (1)$$

$$(CH_3)_3COH \rightarrow CH_3COCH_3 + CH_4 \quad (2)$$

$$(CH_3)_3COH \rightarrow CH_3CH_2OH + CH_2CH_2 \quad (3)$$

Route 1 will proceed through an elimination reaction:

$$(CH_3)_3COH \rightarrow (CH_3)_3\dot{C} + \dot{O}H \quad (A)$$

$$(CH_3)_3\dot{C} \rightarrow (CH_3)_2C{:}CH_2 + \dot{H} \quad (B)$$

and

$$\dot{H} + \dot{O}H \rightarrow H_2O \quad (C)$$

The degradation pattern of tert-butanol was distinctly different from the patterns of the other isomers. Decomposition of the alcohol started at about 350° with the production of water and 2-methylpropene, and decomposition was complete at 700°. Water production reached a maximal concentration at 850°, which represented an 88% conversion via route 1. Above 850°, the water concentration began to decrease with the rise in pyrolysis temperature and the concentration of carbon monoxide increased correspondingly. At temperatures between 350° and 650°, therefore, it appears that the $>C-OH$ bond is readily broken according to reaction (A) of the route 1 scheme. Above 650° demethylation occurs by the fission of one or more $C-CH_3$ bond leading to the formation of acetone and ethanol. These products had maximal concentrations at 700° and 750°, respectively, and at higher temperatures began to decompose until at 850° they reached their complete decomposition point. At temperatures up to 850°, carbon monoxide, the decomposition product of acetone and ethanol, increased steadily and reached a leveling off point between 850° and 900°. Acetaldehyde, a decomposition product of ethanol, also reached its highest production value. This sequence of events, coupled with the fact that water production fell and carbon monoxide increased at temperatures above 850°, suggests that demethylation occurs more readily than dehydration at the higher temperature. Both acetone and ethanol are being formed, but are decomposed immediately.

Route 2 may be written as an elimination reaction with a breaking of the $O-H$ bond of the hydroxyl group:

$$(CH_3)_3COH \rightarrow (CH_3)_2\dot{C}OH + \dot{C}H_3 \quad (A)$$
$$(CH_3)_2\dot{C}OH \rightarrow CH_3COCH_3 + \dot{H} \quad (B)$$

and,

$$\dot{C}H_3 + \dot{H} \rightarrow CH_4 \quad (C)$$

Route 3 may be written as follows:

$$(CH_3)_3COH \rightarrow CH_3\ddot{C}OH + 2\dot{C}H_3 \quad (A)$$
$$2\dot{C}H_3 \rightarrow CH_2CH_2 + 2\dot{H} \quad (B)$$

and

$$CH_3\ddot{C}OH + 2\dot{H} \rightarrow CH_3CH_2OH \quad (C)$$

The quantities of benzene, toluene, and allene produced from tert-butanol were considerably greater than the amounts obtained from the other butanol isomers. For example, only traces of allene were obtained from the other three isomers. This difference is, therefore, a significant feature of the pyrolysis of tert-butanol. It is due to the availability of an

increased quantity of 2-methylpropene from which the products are derived.

Identification of the Butanols

The pyrometron results show that the butanol isomers can be distinguished from each other, by comparison of the peak area values of the oxygenated decomposition products, formed at a selected pyrolysis temperature. It is obvious that the type of product, i.e., aldehyde or ketone, is also useful in determining the identity of the alcohols. The combinations of the different aldehydes, ketones, and lower homologue alcohols produced from each butanol characterize each isomer alcohol. It was found in previous investigations (*4,9*) that the degradation reactions involved in the pyrolysis of organic compounds are reproducible under the same pyrolysis conditions. The carbon monoxide and water production ratio at a given pyrolysis temperature is, therefore, an important factor in determining the identity of the butanols.

Table 5 records the significant identifying products and their peak area

TABLE 5
SIGNIFICANT IDENTIFYING PRODUCTS OF THE BUTANOL ISOMERS: PEAK AREA cm^2 AT 750°

Product	n-BuOH	iso-BuOH	sec-BuOH	tert-BuOH
Column 1 analysis				
n-Butyraldehyde	1.4			
iso-Butyraldehyde		1.08		
Propionaldehyde	0.22		9.52	
Acetaldehyde	4.0	0.62	0.01	
Water	10.7	10.1	12.6	35.3
Acetone		0.6		3.42
Column 2 analysis				
Residual alcohol	T[a]	T	3.6	
Ethanol			0.48	2.5
Methanol	2.5	2.01	0.64	
Methyl ethyl ketone			3.24	
1,3-Butadiene			14.4	
Propylene/propane	28.6	19.2	17.9	4.82
Column 3 analysis				
Carbon monoxide	28.8	22.4	14.9	0.84
Ethane	4.22	2.4	2.31	T
Ethylene	29.4	18.4	20.0	2.2
Methane	22.9	23.6	32.9	12.3
Hydrogen	202.4	131.2	105.0	8.7

[a] Denotes a trace amount.

values obtained with a pyrolysis temperature of 750°. The values were taken from the relevant pyrometrons. In the present pyrolysis gas chromatographic analysis system used, three separate determinations necessary for each butanol were examined. Columns 1 and 2 were employed because not all the polar type products were separable on one or the other column, and column 3 was required for the separation and determination of the permanent gases and the light gaseous hydrocarbons. Other pyrolysis gas chromatographic systems have been described (*5,12,14*), but these are complex in design and are costly. It is believed that satisfactory results can be obtained with a more simple design of system similar to the one used in this investigation. Some modification is envisaged in which the pyrolysate will be divided by a splitter to pass through two separate chromatographic columns. One column will contain Carbowax 1540 or other suitable material necessary for analysis of polar products, and the other, silica gel, for the permanent gases, etc. The detection of the separated products will be achieved by means of katharometers. In this way, a single pyrolysis may be carried out at any selected temperature to yield a pyrogram "finger-print" similar to the one produced in Table 5.

CONCLUSIONS

This study of the pyrolysis of the butanols has shown that isomeric compounds may be identified using a relatively simple analysis system. Preliminary investigations have revealed that in many cases compounds in a mixture may be identified, and their concentration determined within limits from the pyrogram obtained at a single pyrolysis temperature.

One drawback of a pyrolysis gas chromatographic system is the inability of any one particular column to separate all the degradation products in a pyrolysate. The nature of both the functional group and its parent substrate, whether aliphatic or aromatic, will determine which kind of column material should be used. Carbowax 1540 in combination with silica gel was an ideal system for the analysis of C_1–C_4 aliphatic alcohols and some simple aliphatic esters. However, the Carbowax column was not suitable for the analysis of aromatic alcohols and esters. It was found that production of all the relevant oxygenated degradation products of some aliphatic acids and esters could not be followed using the Carbowax column. For example, acetic acid was not separated, and an additional column of FFAP material had to be used to follow the degradation of the acid (*10*) and also to follow the production of acids from esters (*11*). The degradation of some aromatic esters could only be followed by means of Antarox CO 990 column analysis. Similarity in retention times of products on a particular column also present a problem

in their identification. A case in point was methanol and methyl ethyl ketone. These products had the same retention times on Carbowax 1540, but had different retention values on Porapak T. However, the column could not be employed solely for analyzing products from the pyrolysis of the butanols because the retention time of water was close to those of acetaldehyde and 1-butene.

In spite of the inherent difficulties, pyrolysis combined with gas chromatography is proving to be a useful analytical tool. By selecting the appropriate temperature of pyrolysis and combination of columns, the technique can be employed to identify, differentiate, and determine the structure of a wide range of organic compounds. The pyrolysis method is, therefore, as versatile and informative as infrared and mass spectrometric analysis. The "finger-print" (pyrogram) of a compound resembles the infrared spectrum and is analogous to a mass spectrum in the way it is formed. It has the advantage of being less costly in its instrumentation than infrared or mass spectrometric techniques.

SUMMARY

A study of the pyrolytic behavior of the butanol isomers has been made in order to establish a basis for their identification from the thermolysis pattern of each butanol. The identity of the volatile products was established by gas chromatographic analysis. Each isomer was found to decompose via degradation reactions characteristic of the structural arrangement of each alcohol molecule for which mechanisms are proposed.

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The Determination of Organic Substances by the Oxidation with Permanganate

XVIII. The Oxidation of Malonic Acid[1]

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INTRODUCTION

For oxidimetric determination of malonic acid, compounds of hexavalent chromium (*13*) and tetravalent cerium (*11,12,16*) and periodic acid (*6*) have been used as reagents. The oxidation is usually effected by prolonged action of the reagent at an elevated temperature. The stoichiometry of the reaction varies according to the reagent used and according to the reaction conditions. Potassium permanganate was used for the oxidation of malonic acid in a direct titrimetric determination (*1*), performed in acidic solutions at 80°C. This titration cannot be recommended because of oxidation of the reaction products. In another work (*4*), the oxidation is carried out in an acidic and then in an alkaline medium, and the remaining permanganate is again determined in an acidic medium. In Imhoff's work (*7*), malonic acid was oxidized with permanganate, first in alkaline and then in an acidic medium, consuming 6.06 equivalents of the oxidant per mol of the studied substance. Drummond and Waters (*5*) observed a consumption of 5.99 equivalents per mol during oxidation in a potassium hydroxide medium and identified oxalate as the reaction product. In the present paper, the oxidation of malonic acid with permanganate was studied under various reaction conditions and new analytical procedures were proposed, based on the oxidation of the studied substance in a sodium carbonate medium or on successive oxidation in sodium carbonate and sulfuric acid media.

EXPERIMENTAL

Reagents

Potassium permanganate, 0.15, 0.1, and 0.015 *N* (eq.=mole/3).
Potassium bichromate, 0.1 and 0.025 *N*.

[1] Part XVII: The determination of organic compounds by their oxidation with permanganate. *Anal. Letters* **6**, 1113–1123 (1973).

Ferrous sulfate, 0.1 and 0.025 *N* in 0.5 *N* sulfuric acid.

The solution was standardized daily by potentiometric titration of a standard solution of potassium bichromate.

For thallium(I) sulfate, 0.1 *N*. The solution titer was determined by potentiometric titration of 0.1 *N* ceric sulfate (*15*).

Malonic acid, $1.875 \times 10^{-2}M$ and $1.25 \times 10^{-2}M$ solutions were prepared by dissolving accurately weighed amounts of the pure substance in distilled water and diluting to 1 l.

Sodium carbonate, 2M.

Sulphuric acid, 8N and 4N.

Telluric acid, solid.

Formic acid, concentrated and 0.5 *M* solution.

All the substances employed were of p.a. or puriss. purity.

PROCEDURES AND RESULTS

The Oxidation in an Acidic Medium and Successive Oxidation in Acidic and Alkaline Media

During potentiometric titration of malonic acid with permanganate in an acidic medium, it was found (*9*) that the oxidation proceeds slowly even at an elevated temperature, but further than would correspond to the formation of formic acid (*1*). When we monitored the reaction of 0.1 *N* solutions of the substances in solutions acidified with sulfuric or perchloric acid at laboratory temperature, using a twofold excess of the reagent, the reaction rate increased with increasing acidity of the reaction mixture, the reagent consumptions after a sufficiently long time (of the order of hours) corresponding approximately to the oxidation to carbon dioxide and water. However, precise measurements could not be carried out because of the instability of the reagent under the given conditions, which could not be compensated by the blank. During the study of the reaction in weakly acidic solutions (pH 3.5—6.0), we found (similar to Fleury and Courtois in oxidation with periodic acid (*6*)) that the oxidation is fastest at pH 4.2. With a twofold excess of the reagent at 40°C, the consumption corresponded to the oxidation to carbon dioxide and water in a time shorter than 3 hr, but even under these conditions, reagent decomposition during the reaction cannot be satisfactorily compensated by the blank. Detailed study of the successive oxidation of malonic acid with permanganate in an acidic and then an alkaline medium also did not yield analytically applicable results (*9*).

The Oxidation in an Alkaline Medium and Successive Oxidation in Alkaline and Acidic Media

Assuming that oxalate is formed by the oxidation of malonic acid with permanganate in an alkaline medium (*5*), it can be expected that, during

successive oxidation in an alkaline and then an acidic medium, it will be possible to oxidize malonic acid with permanganate to carbon dioxide and water. With regard to the stability of permanganate in sodium carbonate solutions even at elevated temperatures (*3*), the following procedure was employed to verify the above assumption. To 20 ml 2 *M* sodium carbonate, 10.00 ml of 0.1 *N* potassium permanganate (equiv. = mol/3) and 5.00 ml of $1.25 \times 10^{-2} M$ malonic acid were added. The mixture was stirred and heated under reflux on a boiling water bath. It was then cooled with water to laboratory temperature, and 25 ml 8 *N* sulfuric acid were added. After 15 min, the unreacted permanganate was converted by the addition of 5 ml 0.5 M manganese(II) sulfate to hydrated manganese dioxide, and the latter was converted into the pyrophosphate complex of tervalent manganese by adding 50 ml of a fresh mixture of a saturated solution of sodium pyrophosphate, 4 *N* sulfuric acid, and 0.5 *M* manganese(II) sulfate (in the ratio, 6:1:2). The complex formed was titrated with a 0.1 *N* standard solution of ferrous sulfate, using diphenylamine indicator (*2*). The blank was determined in parallel and the reagent consumption for the oxidation of the test substance was calculated from the difference. One milliliter of the 0.1 *N* ferrous sulfate solution corresponds to 3.1608 mg potassium permanganate and, with complete oxidation to carbon dioxide and water, to 1.3008 mg malonic acid.

The measuring results are given in Table 1; each value is the average of three measurements, the results of which did not differ by more than ±5%. The results indicate that, under the given conditions, malonic acid is quantitatively oxidized to carbon dioxide and water after 4 hr of the action of excess permanganate in the sodium carbonate medium and after completion of the oxidation in the acidic medium. When lower concentrations of the test substance are oxidized, longer oxidation with permanganate in sodium carbonate is required for the reaction to be complete. For example, 8 hr is required for the oxidation of 5 ml of $6.25 \times 10^{-3} M$ malonic acid.

The detection of oxalate formed during the oxidation of malonic acid with permanganate in the sodium carbonate medium. To 500 ml of

TABLE 1

THE OXIDATION OF MALONIC ACID WITH PERMANGANATE IN SODIUM CARBONATE AND SULFURIC ACID MEDIA. T IS THE TIME OF HEATING IN THE SODIUM CARBONATE MEDIUM; THE REACTION TIME IN THE SULFURIC ACID MEDIUM IS 15 MIN AT LABORATORY TEMPERATURE

Time T, (hours)	0.25	0.5	1	2	3	4	4.5	5	5.5	6
Reagent consumption, equiv./mol	4.61	5.52	6.59	7.71	7.76	8.00	7.95	7.91	7.98	8.09

0.15 *N* potassium permanganate were added 80 g of solid sodium carbonate and 250 ml $1.875 \times 10^{-2}M$ malonic acid. The mixture was refluxed on a boiling water bath for 4 hr. Then the unreacted permanganate was reduced with 3 ml of concentrated formic acid and the manganese dioxide filtered off, using an S4 glass filtration crucible. The filtrate was acidified with acetic acid, carbon dioxide was removed by boiling, and the hot solution was precipitated with a saturated solution of calcium chloride. The white precipitate was filtered off, washed with water, and used for microscopic and infrared spectrometric identification. Crystals of $CaC_2O_4.CaCl_2.7H_2O$ and $CaC_2O_4.CaCl_2.2H_2O$ were prepared by the procedure of Jones and White (*8*) and were photographed under a microscope (*9*). The shape and characteristic angles of the crystals were identical with the data given in the cited paper. The infrared spectrum (KBr pellet) of the calcium oxalate obtained by the above procedure was also identical with that of the authentic substance.

The determination of oxalic acid formed by the oxidation of malonic acid with permanganate in the sodium carbonate medium. To 20 ml 2 *M* sodium carbonate and 10.00 ml 0.1 *N* potassium permanganate (equiv. = mol/3), 5.00 ml of a solution containing 3 – 10 mg malonic acid were added. The reaction mixture was refluxed for 4 or 8 hr on a boiling water bath. Then the unreacted permanganate was reduced in the hot solution by 15 ml 0.5 *M* formic acid, which, under the given conditions, is oxidized to carbon dioxide and water. The manganese dioxide formed was filtered off with an S4 glass filtration crucible and was washed ten times with 10 ml portions of distilled water. The filtrate containing oxalate was collected in a titration vessel containing a solution of the pyrophosphate complex of tervalent manganese prepared previously. (To 10.00 ml of 0.015 *N* potassium permanganate were added 2.0 ml 0.5 *M* manganese(II) sulfate, and the hydrated manganese dioxide formed was dissolved in 30 ml of a fresh mixture of a saturated sodium pyrophosphate solution, 4 *N* sulfuric acid and 0.5 *M* manganese(II) sulfate in the ratio, 6 : 1 : 2). After adding 70 ml of 8 *N* sulfuric acid, the reaction mixture was 2 *N* in sulfuric acid. The pyrophosphate complex of tervalent manganese oxidizes oxalic acid quantitatively to carbon dioxide and water; the formic acid present does not interfere (*3*). After 1 hr the unreacted pyrophosphate complex of tervalent manganese was titrated potentiometrically with 0.025 *N* ferrous sulfate. The blank determination was carried out, and the consumption for oxalic acid was calculated from the difference. One milliliter of 0.025 *N* ferrous sulfate corresponds to 1.1255 mg of oxalic acid, or 1.3008 mg of malonic acid. The measuring results are given in Table 2; the standard deviation was calculated from seven measurements. The results show that the reaction proceeds according to the equation

TABLE 2
THE DETERMINATION OF OXALIC ACID, FORMED BY THE OXIDATION OF MALONIC ACID

Malonic acid taken (mg)	9.756	6.504	3.252
Corresponding theoretical amount of oxalic acid (mg)	8.442	5.628	2.814
Oxalic acid found (mg)	8.464	5.672	2.852
SD oxalic acid (mg)	0.039	0.015	0.015
Malonic acid found (mg)	9.781	6.555	3.296
SD (mg) malonic acid	0.045	0.017	0.017

$$HOOC—CH_2—COOH + 2\ MnO_4^- \rightarrow (COO)_2^{2-} + CO_2 + 2\ MnO(OH)_2 \quad (1)$$

and that the above procedure can be utilized for analytical purposes.

In the determination of manganese dioxide, formed according to equation (1), the oxidation was performed by the above procedure. After cooling the reaction mixture, the manganese dioxide corresponding to the reduction of permanganate by malonic acid was filtered off, using an S4 glass filtration crucible and was washed 10 times with 10 ml portions of distilled water. Then it was dissolved directly in the crucible in 50 ml of a fresh mixture of saturated sodium pyrophosphate, 4 *N* sulfuric acid and 0.5 *M* manganese(II) sulfate (in the ratio, 6:1:2). The pyrophosphate complex of tervalent manganese formed was titrated with a 0.1 *N* ferrous sulfate solution to a pinkish color, 2 drops of diphenylamine were added and the titration was completed to the disappearance of the blue-purple color. The amount of manganese dioxide was calculated from the difference of the consumptions in the determination itself and the blank, in which manganese dioxide formed by decomposition of the reagent during boiling, was determined. One milliliter of 0.1 *N* ferrous sulfate corresponds to 5.2470 mg of hydrated manganese dioxide or 2.6015 mg of malonic acid. The results confirm the reaction described by equation (1) and the analytical applicability of the given procedure (see Table 3).

TABLE 3
THE DETERMINATION OF MANGANESE DIOXIDE, FORMED BY THE REDUCTION OF PERMANGANATE DURING THE OXIDATION OF MALONIC ACID

Malonic acid taken (mg)	9.756	6.504	3.252
Corresponding theoretical amount of manganese dioxide (mg)	19.674	13.116	6.558
Manganese dioxide found (mg)	19.371	12.906	6.380
SD, manganese dioxide (mg)	0.218	0.108	0.307
Malonic acid found (mg)	9.606	6.400	3.164
SD, malonic acid (mg)	0.108	0.054	0.152

During the determination of the unreacted permanganate, the oxidation of malonic acid was carried out by the above-described procedure. After cooling the reaction mixture, 2 g telluric acid were added, and potentiometric titration (Pt—SCE electrodes) with a 0.1 *N* thallium(I) sulfate standard solution was performed. The amount of permanganate consumed in the oxidation and, consequently, also the amount of malonic acid was calculated from the difference in the consumptions for the blank and for the determination itself. 1 ml 0.1 *N* thallium(I) sulfate corresponds to 5.2679 mg of potassium permanganate, or 1.7343 mg of malonic acid. Using this procedure, 6.504 mg malonic acid were determined with a standard deviation of 0.047 mg malonic acid, calculated from seven measurements; the amount found was equal to that taken.

Since the titration of permanganate with a thallium(I) salt in a medium of an alkali carbonate and in the presence of telluric acid has not yet been described, we studied in greater detail (*9*) this reaction, which is suitable for the determination of permanganate in solutions of alkali hydroxide (*14*). We found that the titration proceeds best in 1 *M* sodium or potassium carbonate, when 1 g telluric acid is contained in 50 ml of the reaction mixture. The inflexion potential in the titration is located around 450 mV (vs. SCE), and the potential break at the equivalence point amounts to 100 mV per 0.02 ml 0.1 *N* thallium(I) sulfate. A partially soluble precipitate is formed during the titration. By comparing the absorbance curves of the soluble portion and of the solution obtained by dissolving the precipitate in 2 *N* sulphuric acid or in 2 *N* potassium hydroxide, we verified the fact that this is tris-telluratopermanganite, the formation of which was described by Lister (*10*).

DISCUSSION

The heretofore described procedures for the determination of malonic acid, based on its oxidation with permanganate, cannot be recommended, especially because of the nonstoichiometric reaction. During the relatively slow oxidation of malonic acid, the reagent instability also has an unfavorable effect and prevents analytical use of the reaction in strongly acidic and strongly alkaline solutions. In weakly acidic buffered solutions, in which permanganate solutions are relatively stable, there is an unfavorable effect, probably from reaction intermediates. For these reasons, the procedures proposed in the present paper are based on the oxidation in a sodium carbonate medium, where permanganate is stable. The formation of a defined amount of oxalate was verified in this medium. This fact led to the development of a method for the determination of mixtures of malonic and formic and of malonic and oxalic acids in the same solution, in addition to the above-discussed procedures; this application will be discussed in the next communication.

The titration of permanganate with thallium(I) sulfate in the presence of telluric acid and in a medium of an alkali carbonate will probably find broader application in indirect oxidimetric determinations with permanganate.

SUMMARY

The oxidation of malonic acid with permanganate was studied under various acidity conditions. Analytical procedures, based on oxidation with excess reagent in a sodium carbonate medium and titration of the oxalate or manganese dioxide formed or of unconsumed permanganate, are proposed. On the basis of successive oxidation in sodium carbonate and sulfuric acid media, a titration determination, involving complete oxidation of malonic acid to carbon dioxide and water, was developed.

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Determination of Sulfate in Waters

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INTRODUCTION

Determination of sulfate in waters is the concern of analytical chemists for a long time. Many gravimetric, titrimetric, potentiometric, conductometric, polarographic, radiometric, spectrophotometric, and nephelometric methods have been developed [for the list of most important methods see the references (*1,4–9*)], however, a fast, reliable, and sufficiently accurate method is still missing.

Nowadays, since it was proved independently several times (*3, 7, 10*) that the Sulfonazo III [3,6-bis(*o*-sulfophenylazo)-4,5-dihydroxy-2,7-naphthalenedisulfonic acid] family of indicators is far the best for indication of barium perchlorate titration of sulfate, that fact also offers a considerable improvement of sulfate determination in waters.

MATERIALS AND METHODS

Reagents

Aqueous 0.01 *M* barium perchlorate and 0.1% (w/v) Dimethylsulfonazo III, 4,5-dihydroxy-3,6-bis[(2-sulfo-*p*-tolyl)azo]-2,7-naphthalenedisulfonic acid, tetrasodium salt, were prepared in usual way. Since many commercial products of Dimethylsulfonazo III contain 1%–5% of calcium, it is advisable to shake the solutions of that indicator with Dowex 50-X8 (see below; 1 g of the wet resin per 100 ml of indicator solution) for about 15 min. The solution of barium perchlorate was standardized by means of potassium sulfate in a similar procedure (without ion exchanger) as described below. All chemicals used were Baker's analyzed reagents (J. T. Baker Chemical Co., Phillipsburg, N. J.).

Procedure

Place about 50 ml of water to be analyzed and 1 g of wet Dowex 50-X8 (20–50 mesh) into a 250-ml conical flask. Shake efficiently (shaker Model S 1070, Eberbach & Co., Ann Arbor, Mich.) for 5 min, then pipet 10 ml of the supernatant solution (or of the filtrate after removing the resin by filtration) into a 50 ml titration flask. Add 10 ml of analytical grade acetone, 3 drops of 0.1% Dimethylsulfonazo III and ti-

trate until the color of the solution changes from red-violet to bluish green; 1 ml of 0.01 *M* barium perchlorate corresponds to 0.9607 mg of SO_4^{2-}. If the solution contains less than 50/μg of SO_4^{2-} per ml, its preliminary concentration by evaporation to reach that value is advisable.

RESULTS AND DISCUSSION

The results of sulfate determination in waters compared with classical gravimetric barium sulfate determination are presented in Table 1. The effects of resin amount and time are described in Table 2.

The titration indicated by Dimethylsulfonazo III can be performed in the pH range 1–7. The interferences of that titration were investigated earlier (*2*). Since practically all metal ions are removed by cation exchanger, the only interferences from common ions are PO_4^{3-} (>100 μg/ml) and AsO_4^{3-} (>140 μg/ml).

All members of the Sulfonazo III family of indicators [for the list of them see the references (*2,3*)] exhibit a reaction with barium ions of approximately the same quality. Considerable differences among them reported sometimes are mainly due to a different quality of commercial products. Calcium and the corresponding mono azo derivative are the most frequent impurities. The advantages of Dimethylsulfonazo III in comparison with other members of the family are the convenient bathochromic effect of the methyl group and easy preparation of the *p*-toluidine-*m*-sulfonic acid.

The spectrophotometric sulfate determination (*1*, *4*, *7*) should be classified as inferior to the described method because of higher sensitivity towards a series of factors (interfering substances, concentration of organic solvent, the effect of temperature), long time that is necessary for quantitative precipitation of barium sulfate and the necessity of its removing either by filtration or centrifugation. Even the potentiometric

TABLE 1
SULFATE DETERMINATION IN WATERS[a]

Sample No.	μg SO_4^{2-}/ml	
	This method	Gravimetry $BaSO_4$
7	497, 498	496
12	492, 494	499
15	419, 419	418
1R[b]	14, 16	14
2TTH	1830, 1830	1840
3RW	1670, 1671	1670

[a] The samples were industrial waters from a copper plant.
[b] After preliminary evaporation.

TABLE 2
THE EFFECT OF RESIN AMOUNT (A) AND SHAKING TIME (B)

(A)	Resin amount (g)	0.1	0.5	0.75	1.0	1.5	2.0	3.0
	μg SO_4^{2-}/ml	1520	1840	1995	1990	1995	2000	1990
(B)	Time of shaking (min)	1	3	5	10	15	20	
	μg SO_4^{2-}/ml	1890	1992	1990	1995	1990	1990	

(A) Shaking time 5 min. (B) Resin amount 1 g. (AB) The primary solution contained 2000 μg SO_4^{2-}/ml and 1323 μg Cu^{2+}/ml. Copper was selected since it seriously interferes with Dimethylsulfonaze III indicated titration.

determination by means of selective electrodes is lacking a substantial advantage since a suitable electrode sensitive towards barium ions was not found yet.

SUMMARY

Determination of sulfate in industrial waters is performed by shaking with Dowex 50-X8 resin (removing of metal ion interferences) and titration of aqueous acetone (1/1, v/v) solution with barium perchlorate employing Dimethylsulfonazo III as indicator. Only phosphate and arsenate interfere.

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Volumetric Determination of Pt(IV) in the Presence of Ir, Pd, and Rh with Ferrous Ammonium Sulfate in Alkaline Mannitol Medium

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INTRODUCTION

The shift of the redox potential of Fe(II)/Fe(III) in the presence of complexing agents was used in our previous studies for the determination of numerous inorganic ions and organic compounds, using a standard $FeSO_4$ solution as titrant and triethanolamine and mannitol or glycerol in the presence of KOH as the titration medium (*1-8*). The oxidized Fe(III) is bound in a very stable complex, and thus the reducing power of Fe(II) is increased to approach that of the strongest reducing agents, such as amalgams, Cr(II), V(II), etc. In this paper we have extended this principle for the volumetric determination of Pt(IV) in the presence of Pd(II) and Rh(III).

EXPERIMENTAL

A 0.1 *N* ferrous ammonium sulfate solution was prepared by dissolving $Fe(NH_4)_2(SO_4)_2.6H_2O$ (Lachema p.a.) in 1 l of 0.1–0.5 *N* H_2SO_4 and standardized with $K_2Cr_2O_7$. — 0.02 *N* and 0.01 *N* solutions were prepared by accurate dilution. The mannitol solution was prepared by dissolving the appropriate amount of recrystallized mannitol in distilled water. A 0.01 *M* sodium chloroplatinate solution was prepared by dissolving 454 mg Na_2PtCl_6 (Johnson, Matthey & Co., Ltd., London) in 100 ml of distilled water. An ammonium chloroiridate solution was prepared by dissolving 1.1050 g of $(NH_4)_2IrCl_6$ (Johnson, Matthey & Co., Ltd., London) in 250 ml of water. A 0.05 *M* palladium chloride solution was prepared by diluting a 10% solution of $PdCl_2$ (Safina—Czechoslovakia) in water and was standardized gravimetrically.

Potentiometric determinations were performed with a Multoscope V (Laboratorní Přístroje, Prague) with a platinum foil as indicator elec-

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trode and a saturated calomel reference electrode. All titrations were carried out in an inert atmosphere using a suitable titration vessel.

PROCEDURE

Thirty milliters of approximately 1 *M* mannitol solution was transferred to a 150 ml titrating vessel, to which was added 5 ml of 5 *M* KOH. Nitrogen was passed for about 5 min. Then an accurately measured aliquot of the Pt(IV) solution was added and the solution was diluted to 50 ml with distilled water. Nitrogen was passed again for about 5 min. The titration was carried out with a 0.02 or 0.01 *M* Fe(II) solution, depending upon the concentration of the Pt(IV) solution, under an inert atmosphere, and the solution was stirred with a magnetic stirrer. Near the equivalence point, the titrant was added in portions of 0.01–0.02 ml from a microburette. The potential stabilizes quickly during the titration as well as in the vicinity of the end point. A blank experiment was carried out simultaneously.

RESULTS AND DISCUSSION

The most suitable medium was found to be the solution of 0.5 *M* mannitol and 0.5 *M* KOH (Table 1). In this medium, the potential stabilizes practically instantaneously during the titration, and the marked change in potential corresponding to the reduction of Pt(IV) to Pt(II) is given by a slope of $\Delta mV/\Delta ml = 3500$ for 0.02 ml of 0.02 *M* Fe(II) solution.

The sensitivity and accuracy of this method is well suited for the volumetric determination of platinum salts. Amounts down to 0.39 mg of Pt(IV) in a volume of 50 ml were titrated with an error not exceeding 2% (Table 2).

It has been found that platinum can also be determined by adding a

TABLE 1
THE INFLUENCE OF MANNITOL AND KOH CONCENTRATIONS ON THE REDUCTIMETRIC TITRATION OF PT(IV) COMPLEX WITH 0.02 *M* FE(II) SOLUTION[a]

Mannitol (*M*)	KOH *M*	Pt found (mg)	Error (%)
0.10	0.50	4.02(3)	+3.05
0.20	0.50	3.98(5)	+2.07
0.40	0.50	3.93(2)	+0.71
0.50	0.50	3.93(2)	+0.71
0.50	0.05	4.10(9)	+5.25
0.50	0.10	4.06(1)	+4.04
0.50	0.20	3.99(6)	+2.35
0.50	0.50	3.93(2)	+0.71
0.50	1.00	3.93(2)	+0.71

[a] 50 ml of the solution contained 3.904 mg Pt.

TABLE 2
THE DETERMINATION OF PT(IV) IN A MEDIUM OF 0.5 *M* MANNITOL AND 0.5 *M* KOH BY TITRATION WITH 0.02 *M* FE(II) SOLUTION

Pt taken (mg)	Pt found[a] (mg)	Error (%)
0.39(0)	0.39(5)	+1.28[a]
0.78(1)	0.79(1)	+1.28[a]
1.95(2)	1.99(3)	+2.10
3.90(4)	3.93(2)	+0.71
9.76(0)	9.98(1)	+0.80
13.66(4)	13.85(8)	+1.41
19.52(0)	19.73(2)	+1.08

[a] Each result is the average of three determinations.
[b] Titrated with 0.01 *M* Fe(II) solution.

known excess of a standard Fe(II) solution and back-titrating potentiometrically with a standard dichromate solution. Pt(IV) is then reduced to metallic Pt. This procedure requires a very carefully maintained inert atmosphere, and the errors varied within ±5%, probably due to deposition of reduced Pt on the indicator electrode. The direct titration is thus preferable.

In further experiments, the behavior and interference of some other platinum metals were studied. No reduction of iridium(IV) was observed under the conditions described above for the determination of Pt(IV). This behavior can be explained in two ways: either Ir forms a very stable complex with mannitol in alkaline medium which is resistant against reduction with Fe(II), or it is reduced in the presence of mannitol alone. The latter possibility was found to be most probable and was tested by setting aside an Ir(IV) solution with mannitol for various time periods, acidifying, and titrating oxidimetrically (in 0.5 *N* H_2SO_4) with a $KMnO_4$ solution. The results showed that after 5–10 min (corresponding to the time needed for deaeration with a stream of N_2 when adjusting the medium for titration with Fe(II) in alkaline mannitol medium) Ir(IV) is quantitatively reduced to Ir(III). It is thus possible to determine Pt(IV) in the presence of Ir(IV) up to a Pt(IV):Ir(IV) ratio of 1:5.

Palladium(II) is not reduced to metallic Pd with Fe(II) in an alkaline mannitol medium unless Pt(IV) is quantitatively reduced to Pt(II), and it is possible to determine Pt(IV) in the presence of Pd(II) up to a Pt(IV):Pd(II) ratio of 1:1. The same holds for Rh(III). Nevertheless, when using excess Fe(II) solution in alkaline mannitol medium, Pd(II) is quantitatively reduced to Pd, and the excess unreacted reagent can be determined with a standard dichromate solution. It is thus possible to determine, e.g., the sum of Pt(IV) and Pd(II) by using excess Fe(II) and then, in another aliquot of the sample, to determine Pt(IV) by direct titration.

SUMMARY

A potentiometric reductimetric method for the determination of platinum (Pt(IV)–Pt(II)) with a standard Fe(II) solution in an alkaline medium of mannitol is described. The method, the error of which does not exceed 2%, can be used in the presence of palladium, iridium, and rhodium.

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The Oxidation of Aminophenazone and Phenazone with Ceric Sulfate

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INTRODUCTION

In our previous communications in this journal we dealt with the oxidation of aminophenazone (AP) and phenazone (P) with ferricyanide (*1*) and hydrogen peroxide (*2*); we studied the reaction course and the products in strongly alkaline media (ferricyanide) and in weakly acidic, neutral, and weakly alkaline media (hydrogen peroxide). In the present paper, this study is supplemented with investigation of the oxidation with ceric sulfate in strongly acidic and carbonate media.

EXPERIMENTAL

Aminophenazone (VCHZ Synthesia, Pardubice-Semtín, Czechoslovakia) and phenazone (VEB-Chem. Co., GDR) met the Czechoslovak Pharmacopoeia-ČsL 3-purity requirements. Solutions of 0.1 *N* $Ce(SO_4)_2$ and 0.1 *N* $Na_2S_2O_3$ were prepared and standardized using common methods. The other reagents used, a 0.4% starch solution, a 10% $CaCl_2$ solution, 50% H_2SO_4, a 20% K_2CO_3 solution, a 0.04% methanolic solution of bromocresol green, KI, methanol, chloroform, butanol, benzene, anhydrous acetic acid, formic acid, CCl_4, isopropylamine, and ethyl acetate were products of Lachema (Czechoslovakia) or were prepared from products of this company and were of p.a. purity. The following systems were employed for chromatographic separations: S 1—chloroform : methanol (90 : 10); S 2—chloroform : CCl_4 : benzene : ethanol : ethyl acetate (8 : 8 : 20 : 2 : 2); S 3 : chloroform : isopropylamin (97 : 3); S 4—ethyl acetate : water : formic acid (50 : 15 : 10). For thin-layer chromatography, Silufol UV 254 plates from Kavalier (15 : 15) and DC-Fertigplatten Kieselgel 60 F 254 plates (the layer thickness, 0.25 mm) were used.

PROCEDURES

(*a*) *1* N *Sulfuric Acid Medium*

Aqueous solutions of AP (2×10^{-4} mole) and P (2×10^{-4} mole) were oxidized with a 20 times molar excess of ceric sulfate in 1 *N* sulfuric acid at laboratory temperature and in daylight, in ground-glass stoppered flasks. The degree of oxidation was determined after 24, 48, 72, and 96 hr. The unconsumed amount of ceric sulfate was determined iodometrically. The oxidized solution was acidified with 50% sulfuric acid before the titration to a resulting concentration of about 2 *N*, cooled to laboratory temperature, potassium iodide was added, and the solution was titrated with 0.1 *N* sodium thiosulfate using starch indicator.

(*b*) *0.1* N *Sulfuric Acid Medium*

Aqueous solutions of AP (2×10^{-4} mol) and P (2×10^{-4} mole) were oxidized with excess ceric sulfate in 0.1 *N* sulfuric acid in the same way as in the previous paragraph.

Oxidation of AP and P in an Acid Medium at an Elevated Temperature

Aqueous solutions of AP and P, containing 2×10^{-4} mol of the substances, were oxidized with a 20 times molar excess of ceric sulfate in 1 *N* sulfuric acid at 45°C, 60°C, and 80°C in ground-glass stoppered flasks. The degree of oxidation of AP and P was determined at 1-hour intervals over 6 hr. The unconsumed ceric sulfate was determined iodometrically as in paragraph (a).

Thin-layer Chromatography, Extraction

Three milliliters of the oxidized solutions of AP and P were extracted with 3 ml chloroform, and 30 to 50 μl of the extracts were placed on Silufol UV 254 or DC-Fertigplatten plates, simultaneously with fresh 0.1% solutions of AP and P in methanol, containing 5–10 μg of the substances. The substances to be chromatographed were applied to the chromatogram in a stream of cool air and were developed in the usual manner in saturated chambers, until they had traveled a distance of 12–15 cm, employing the S 1, S 2, and S 3 systems and detection in UV light at 254 nm.

Detection of Oxalic Acid by Thin-Layer Chromatography

To about 20 ml of aqueous AP and P solutions oxidized with excess ceric sulfate in 1 *N* and 0.1 *N* sulfuric acid at laboratory temperature, a 10% calcium chloride and methyl red indicator were added, and the solution was made alkaline with 10% ammonia. The solution with the

precipitate was heated to about 70°C, cooled, the calcium oxalate was filtered off, washed with water made weakly alkaline with dilute ammonia, and dried. Oxalic acid was liberated from calcium oxalate using Werner's procedure (*5*) and detected by thin-layer chromatography. A 1% oxalic acid standard solution in methanol was applied to the chromatogram simultaneously with the sample, the amount of oxalic acid being 50 μg, and the chromatogram was developed in the usual manner in the S 4 system.

The Oxidation of AP and P in a Potassium Carbonate Medium at Laboratory Temperature

Aqueous solutions of AP and P containing 2×10^{-4} mole of the substances were oxidized with a 20 times molar excess of ceric sulfate in 15% and 20% potassium carbonate at laboratory temperature and in daylight. The degree of oxidation was determined after 24 hr. The unconsumed ceric sulfate was determined in the same way as described in paragraph (a).

Thin-Layer Chromatography of the Oxidation Products

Aqueous AP and P solutions containing 5×10^{-4} mole of the substances were oxidized with excess ceric sulfate in 20% potassium carbonate under the above experimental conditions. Five milliliters of the oxidized solutions were extracted with chloroform (5 ml for AP and 2 ml for P), and the extracts were filtered through anhydrous sodium sulfate. The extracts were applied to Silufol UV 254 and Silufol plates without an indicator in an amount of 0.1–0.2 ml; fresh solutions of AP and P containing 10 μg of the substances were applied to the chromatogram simultaneously. The chromatograms were developed upwards in the usual manner, using the S 1 system, and the detection was performed in UV light at 254 and 366 nm.

RESULTS AND DISCUSSION

The long-term oxidation of aminophenazone and phenazone aqeous solutions containing 2×10^{-4} mole of the substances with excess ceric sulfate in an acidic medium (1 *N* and 0.1 *N* sulfuric acid) at laboratory temperature is illustrated in Tables 1 and 2 and Figs. 1 and 2.

It can be seen from the figures that the oxidation of AP and P proceeds much more rapidly during the first few hours than during the following time. The rate and the character of the oxidation of the two substances are similar, in contrast to the oxidation of aqueous AP and P solutions with excess potassium ferricyanide in an alkaline medium (*1*), where their behavior was different: P was oxidized more slowly, and the character of its oxidation was different from that for AP. The strength of

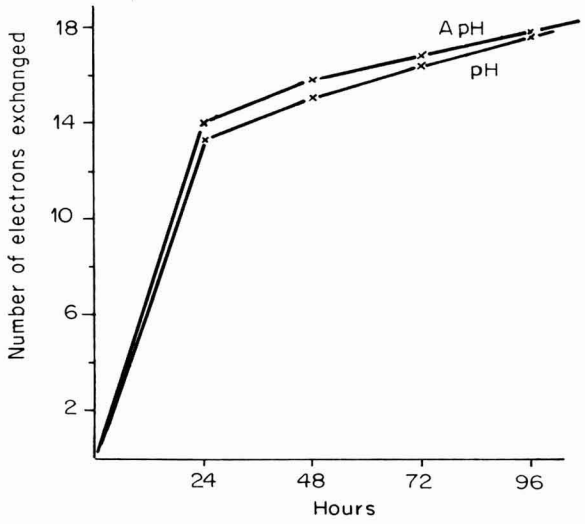

FIG. 1. The time dependence of the oxidation of aqueous AP and P solutions (2×10^{-4} mol) with 0.1 *N* ceric sulfate in 1 *N* sulfuric acid at laboratory temperature.

ceric sulfate as an oxidant for AP and P depends little on the acidity of the medium; the number of electrons exchanged is similar in 1 *N* and 0.1 *N* sulfuric acid (Tables 1 and 2). The character of the oxidation is similar in the two mediums (Figs. 1 and 2).

The oxidation of AP and P with excess ceric sulfate at an elevated temperature in 1 *N* sulfuric acid is depicted in Figs. 3 and 4 and Table 3.

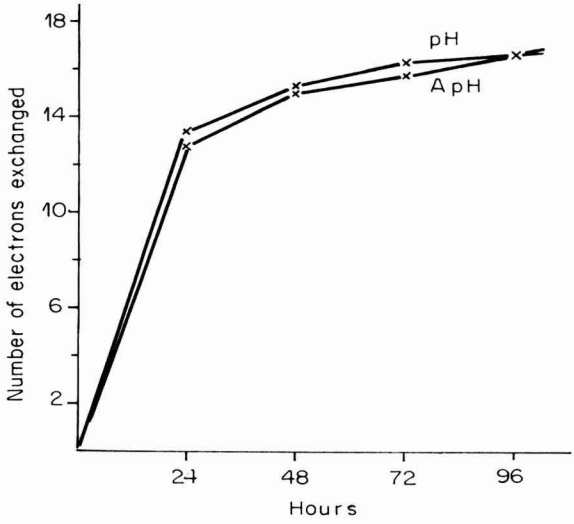

FIG. 2. The time dependence of the oxidation of aqueous AP and P solutions (2×10^{-4} mol) with 0.1 *N* ceric sulfate in 0.1 *N* sulfuric acid at laboratory temperature.

TABLE 1
THE TIME DEPENDENCE OF THE OXIDATION OF AP AND P AQUEOUS SOLUTIONS (2×10^{-4} MOL) WITH 0.1 *N* CERIC SULFATE IN 1 *N* SULFURIC ACID AT LABORATORY TEMPERATURE

	The number of electrons exchanged	
Hours	AP	P
24	14	13.3

TABLE 2
THE TIME DEPENDENCE OF THE OXIDATION OF AP AND P AQUEOUS SOLUTIONS (2×10^{-4} MOL) WITH 0.1 *N* CERIC SULFATE IN 0.1 *N* SULFURIC ACID AT LABORATORY TEMPERATURE

	The number of electrons exchanged	
Hours	AP	P
24	12.7	13.4
48	15	15.3
72	15.7	16.3
96	16.6	16.6

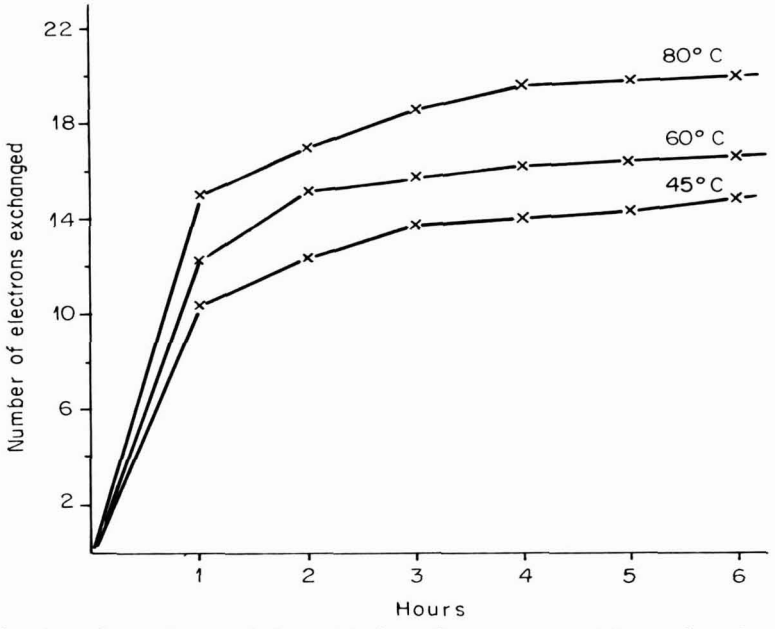

FIG. 3. The time dependence of the oxidation of an aqueous AP solution (2×10^{-4} mol) with 0.1 *N* ceric sulfate in 1 *N* sulfuric acid at an elevated temperature (45°C, 60°C, 80°C).

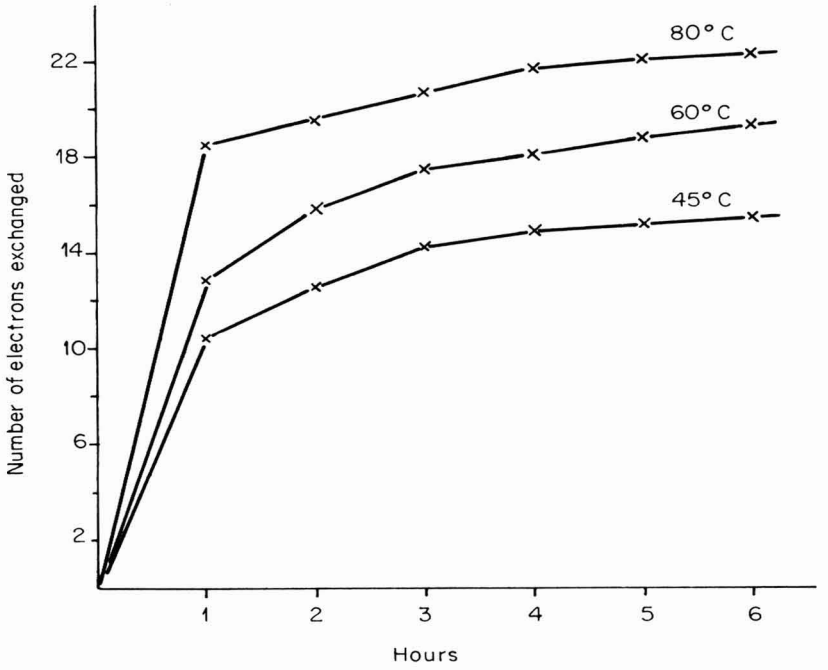

FIG. 4. The time dependence of the oxidation of an aqueous P solution (2×10^{-4} mol) with 0.1 *N* ceric sulfate in 1 *N* sulfuric acid at an elevated temperature (45°C, 60°C, 80°C).

Comparison of the results given in Tables 1 and 2 with those summarized in Table 3 shows the effect of temperature on the oxidation of AP and P. While 14 electrons are exchanged after 24 hr of the oxidation of AP at laboratory temperature, 10 electrons are exchanged after 1 hr at 45°C, and 12 and 15 electrons after 1 hr at 60°C and 80°C, respectively. The oxidation of P is hastened analogously by increasing the temperature; 13.3 electrons are exchanged after 24 hr at laboratory temperature, while the numbers of electrons exchanged after 1 hr at 45°C, 60°C, and 80°C are 10.4, 13, and 18.5, respectively.

The character of the oxidation of the two substances is not changed by an increase in the temperature: the oxidation is relatively rapid during the first few hours and then the reaction rate decreases. In an acidic medium, P is oxidized somewhat faster than AP at an elevated temperature (Table 3); at 60°C, 19 electrons are exchanged during the oxidation of P in 1 *N* sulfuric acid, while only 17 electrons are exchanged during the oxidation of AP under these conditions. At 80°C, 22 electrons are exchanged during the 6-hr oxidation of P and 20 electrons during the 6-hr oxidation of AP; hence the difference in the number of electrons exchanged in the oxidation of P and AP under identical experimental conditions was 2 both at 60 and 80°C (Table 3).

TABLE 3
THE TIME DEPENDENCE OF THE OXIDATION OF AP AND P AQUEOUS SOLUTIONS (2×10^{-4} MOL) WITH 0.1 *N* CERIC SULFATE IN 1 *N* SULFURIC ACID AT AN ELEVATED TEMPERATURE

Temperature	Hours	The number of electrons exchanged	
		AP	P
45°C	1	10.45	10.45
	2	12.4	12.6
	3	13.8	14.2
	4	14.0	14.8
	5	14.3	15.1
	6	14.9	15.4
60°C	1	12.3	12.95
	2	15.2	15.85
	3	15.8	17.5
	4	16.2	18.0
	5	16.4	18.8
	6	16.8	19.2
80°C	1	15.1	18.5
	2	17.0	19.5
	3	18.6	20.7
	4	19.6	21.7
	5	19.8	22.0
	6	19.9	22.2

During the oxidation of the aqueous AP solution with excess ceric sulfate in an acidic medium, the purple-blue coloration of the solution, observed during the oxidation of AP with potassium ferricyanide (*1*) immediately after the addition of the oxidant and during the oxidation of AP with hydrogen peroxide (*2*), when the coloration lasted 24 hr, did not appear.

The degree of oxidation of aqueous AP and P solutions containing 2×10^{-4} mole of the substances with excess ceric sulfate in 20% potassium carbonate is shown in Table 4. While 13 electrons are exchanged in

TABLE 4
THE OXIDATION OF AP AND P AQUEOUS SOLUTIONS (2×10^{-4} MOL) WITH 0.1 *N* CERIC SULFATE IN 20% POTASSIUM CARBONATE AT LABORATORY TEMPERATURE

Hours	The number of electrons exchanged	
	AP	P
24	2.16	2.55

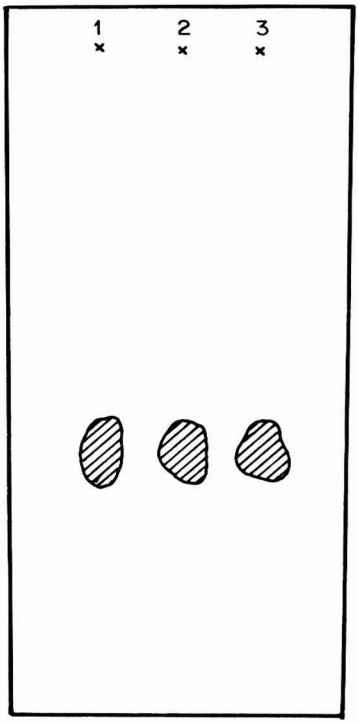

FIG. 5. Thin-layer chromatographic detection of oxalic acid. (1) Standard oxalic acid solution (50 μg). (2) Oxalic acid isolated from the AP solution oxidized with ceric sulfate in 1 *N* sulfuric acid at laboratory temperature. (3) Oxalic acid isolated from the P solution oxidized with ceric sulfate in 1 *N* sulfuric acid at laboratory temperature. System: S 4. Detection: 0.04% aqueous solution of bromocresol green.

0.1 *N* sulfuric acid after 24 hr of the oxidation of AP and P (Table 2), only 2 and 2.5 electrons are exchanged for AP and P in potassium carbonate, respectively, under the same experimental conditions.

During oxidation of AP and P in potassium carbonate of 30% or higher concentration, potassium carbonate separated from the solution and at 15% or lower concentration basic salts of ceric sulfate were formed. Hence a 20% potassium carbonate medium appeared to be optimal for this purpose.

During the long-term oxidation of AP and P in this medium, a white, finely crystalline substance appeared in the solution. After 5-day oxidation, the crystalline substance was filtered off, washed with cold water, and dried. It was readily soluble in dilute mineral acids (with evolution of a gas) and in 30% acetic acid and insoluble in solutions of alkali hydroxides and in dilute ammonia. Under the given conditions it was assumed that the substance is cerous carbonate. Tervalent cerium was detected using common reactions, namely, reactions with oxalic acid, quinine chloride, and hydrogen peroxide, and 8-hydroxyquinoline.

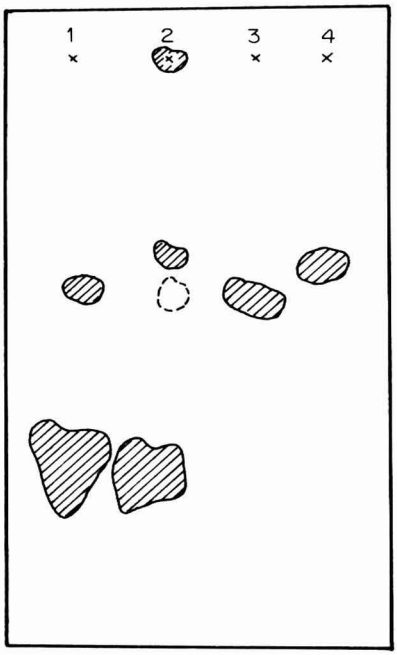

FIG. 6. Thin-layer chromatographic study of aqueous AP and P solutions oxidized with 0.1 *N* ceric sulfate in 20% potassium carbonate. (1) AP (5×10^{-4} mol) after 24 hr of the oxidation. (2) P (5×10^{-4} mol) after 24 hr of the oxidation. (3) an AP standard solution (10 μg). (4) a P standard solution (10 μg). System: S 1.

CHROMATOGRAPHIC STUDY

(*a*) *The Oxidation Products of AP and P Formed in Acidic Media*

The oxidation products of AP and P were studied using thin-layer chromatography in the slowest stage of the oxidation, namely, after 96 hr of oxidation at laboratory temperature and after 6 hr at an elevated temperature. Various separating systems were employed (see experimental) that enabled sufficiently effective separation of the substances studied. It was found that neither AP nor P are present in the oxidized solutions and that the brown-yellow substance formed during the oxidation of AP and P with ceric sulfate under the given experimental conditions is not identical with 1-pheynl-3-methyl-4-(phenylazo)-5-pyrazolone, which is formed during the oxidation of AP with hydrogen peroxide in an acidic medium (*2*). The substance exhibited an R_F value of 0.76 in system S 3 and was detectable in UV light at 254 nm as well as visually. The substance was extracted into methanol and its spectrum was recorded in the range from 44 to 22 kc; no pronounced absorption maximum was found.

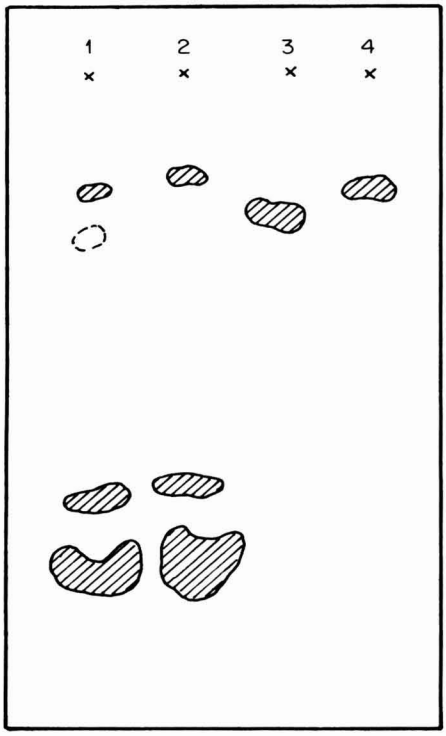

FIG. 7. Thin-layer chromatographic study of aqueous AP and P solutions oxidized with 0.1 *N* ceric sulfate in 20% potassium carbonate. (1) AP (5×10^{-4} mol) after 24-hr oxidation. (2) P (5×10^{-4} mol) after 24-hour oxidation. (3) an AP standard solution (10 μg). (4) a P standard solution (10 μg).

The oxidation of AP and P with ceric sulfate under these conditions probably causes deeper changes on the pyrazolone ring; this assumption is supported by the formation of oxalic acid, which was detected in the oxidized solutions by thin-layer chromatography (Fig. 5).

(*b*) *The Oxidation Products of AP and P Formed in Potassium Carbonate Medium*

Systems S 1 and S 2 (Figs. 6 and 7) were employed. A substance with an R_F value of 0.73 was found in systems S 1; it was detectable in UV light at 254 nm and 366 nm. This substance was also detected in a small amount in solutions of AP and P in 20% potassium carbonate, which were stored for 7 days under the same conditions as the solutions oxidized with ceric sulfate (Fig. 8). This phenomenon can be explained by the oxidation of AP and P with atmospheric oxygen in the alkaline medium.

Employing system S 2 it was found that the substance detected in system S 1 ($R_F = 0.73$) is a mixture of two substances. One substance

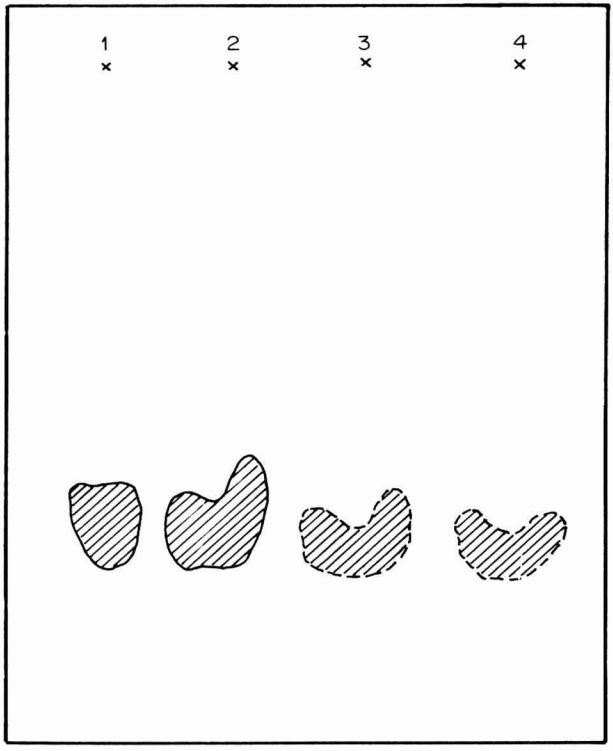

FIG. 8. Thin-layer chromatographic study of aqueous AP and P solutions oxidized with 0.1 *N* ceric sulfate in an alkaline medium. (1) AP (5×10^{-4} mol) in 20% potassium carbonate after 24-hr oxidation. (2) P (5×10^{-4} mol) in 20% potassium carbonate after 24-hr oxidation. (3) AP (5×10^{-4} mol) solution in 20% potassium carbonate after 7 days of storing. (4) P (5×10^{-4} mol) solution in 20% potassium carbonate after 7 days of storing. System: S 1. ◌ spots of low intensity.

with $R_F = 0.77$ could be detected in UV light only at 254 nm, while the other with $R_F = 0.95$ was detectable both at 254 and 366 nm. Phenol, as one of the assumed oxidation products, or the oxidation products of phenol, which were detected during the oxidation of AP with potassium ferricyanide (*1*), were not detected in the AP and P solutions oxidized under the present conditions.

The following conditions can be drawn on the basis of the above results and discussion:

The monitoring of the long-term oxidation of aqueous AP and P solutions with ceric sulfate in acidic media (1 *N* and 0.1 *N* sulfuric acid) has shown that the oxidation proceeds much more rapidly during the first few hours than later (Tables 1–3 and Figs. 1–4).

In the alkaline medium (20% potassium carbonate) these oxidations are slow. At laboratory temperature, 2 electrons are exchanged during the 24-hr oxidation of AP (Table 4), while in an acid medium (1 *N* sul-

furic acid) 14 electrons are exchanged under identical experimental conditions (Table 1).

The dependence of the degree of oxidation of AP and P on temperature is given in Table 3. The character of the oxidation of aqueous solutions of AP and P is similar under these conditions (Figs. 1 and 2).

The chromatographic study of the products of the oxidation of AP and P with ceric sulfate in acidic media revealed that the pyrazolone ring is decomposed—see the detection of oxalic acid in the oxidized solutions of AP and P (Fig. 5). Oxalic acid was not detected in the alkaline solutions after oxidation with ceric sulfate.

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Guanosine Tetra- and Pentaphosphate Analysis

PEI-Cellulose Thin-Layer Purification and Luciferin–Luciferase Liquid Scintillation Quantitation

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INTRODUCTION

Delayed luminescence analysis (DLA) is a delayed light-emission phenomenon that occurs when crude firefly enzyme preparation comes in contact with various nucleoside triphosphates (*5*) and adenosine tetraphosphate (*6*). The system is very useful to quantitate micro amounts of these compounds that occur in living things which are either of plant or animal origin. This report demonstrates the extension of the system to certain guanosine tetra- and pentaphosphates which includes guanosine 5′triphosphate, 3′ diphosphate (pppGpp), guanosine 5′ tetraphosphate (ppppG) and diguanosine 5′,5′′ tetraphosphate (GppppG).

Certain guanosine tetra- and pentaphosphates (ppGpp) and pppGpp have been thought to control polymerase reactions in stringent strains of *Escherichia coli* (*2*) or possibly inhibition of the synthesis of selected proteins (*3*). The diguanosine tetraphosphate (GppppG) functions as an ATP generating system for egg maturation of *Artemia salina* (brine shrimp) (*13*). The function and significance of linear guanosine tetraphosphate (ppppG) is unknown to the author but has been reported previously (*12*). Most recently it was reported that ppGpp is synthesized by mouse embryonic ribosomes (*4*) and therefore a regulatory function at the mammalian level may also be possible.

MATERIALS

GTP and linear guanosine tetraphosphate (ppppG) were purchased from Sigma Chemical Co., St. Louis, MO. The various guanosine tetra- and pentaphosphates (ppGpp, pppGpp, pGppG, and pppGp) were kind gifts from Drs. Hamel and Cashel (Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, Bethesda,

Md. The diguanosine tetraphosphate (GppppG) was a kind gift of Dr. A. H. Warner, Department of Biology, University of Windsor, Ontario, Canada. Plastic thin layer plates (polyethyleneimine cellulose with 254 indicator) were purchased from Brinkmann. All chromatography was done in a Kodak sandwich apparatus and spots viewed under 260 mμ light. All counting (light detection) was accomplished on a Packard Liquid Scintillation Counter Model 3320 equipped with RCA 4501-V3 bialkali photomultipliers. Photomultiplier coincidence was shut off. The window of the first channel A-B was adjusted to interval 60–65 with 100% gain.

METHODS

The various compounds were purified using PEI-cellulose thin layer chromatography and 1.6 *M* LiCl as the competitor. The plastic thin layer plates (Brinkmann) were prewashed with water (ascending), dried, and the various nucleotides (1 mg/1 ml) dissolved in Krebs Henseleit buffer (pH 7.4) were spotted (5 or 10 μl) and dried. Water was migrated in an ascending fashion for the whole length of the plate and the plates were dried. In the same ascending direction 1.6 *M* LiCl was migrated for the whole length of the plate (approximately 8 in). The plates were dried and nucleotides viewed and cut out and finally eluted with 0.01 *M* sodium hydroxide. The solution was neutralized with sulfuric acid and diluted to appropriate concentration for the production of the standard curve. A new standard curve should be used with each enzyme preparation because of variability in the light-producing system, namely luciferin–luciferase. The preparation of the enzyme (*10*) and the assay via liquid scintillation counter have been described previously (*5*).

A good review (*11*) of all aspects of the assay can be found in a recent Packard Technical Bulletin (No. 20) obtainable from Packard Instrument Co., Inc., Downers Grove, Ill. 60515.

RESULTS

In Fig. 1 is depicted absorption of the various nucleotides and contaminants viewed under ultraviolet light at 260 mμ. The guanosine tetraphosphate (linear) appears to be contaminated with GTP and possibly GDP as well. The pppGpp and ppGpp appear to be essentially pure with pCppG and GTP showing only one spot. On the second part of the chromatogram GppppG appears to show little contamination with a migration rate similar to GTP and pppGp appearing to be grossly contaminated (a fact confirmed by Drs. Hamel and Cashel). Because the mixture could not be resolved further, data on its use are not presented.

In Fig. 2 the time-delay curve ppppG is indicated with peak light emission shown at approximately 7 min. With this delay held constant, a

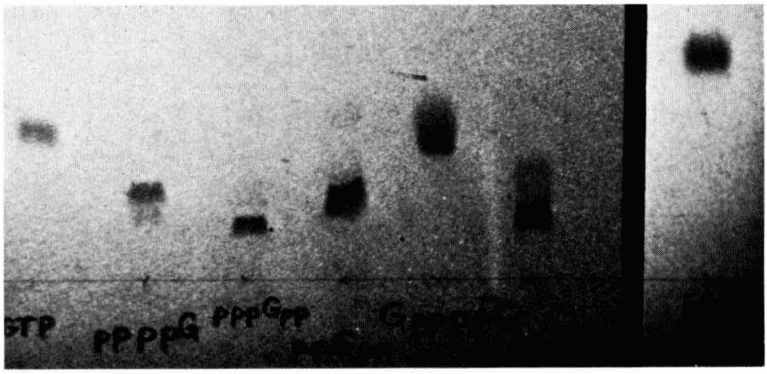

FIG. 1. Polyethyleneimine cellulose thin layer chromatography of the various guanosine tri-, tetra-, and pentaphosphates. Five microliters of 1 mg/ml solution was spotted and chromatography accomplished in an ascending mode twice (1) H_2O and dried, (2) 1.6 *M* LiCl. The compounds spotted left to right were GTP, ppppG, pppGpp, ppGpp, GppppG, pppGp, and pCppG. The line appearing below the spots is the origin where the various compounds were spotted. The spots appear dark on a lighter background because they are photographed under ultraviolet (260mμ) light which they absorb and the PEI cellulose contains a fluorescent indicator which appears bright under 260 mμ light.

standard curve (Fig. 3, log-log plot) is linear form 20 to 200 μmoles/ml versus light emission (counts per 0.1 min).

As seen in Fig. 4 the peak of light emission from pppGpp occurred in approximately 11 min. The initial short increase in light may be due to

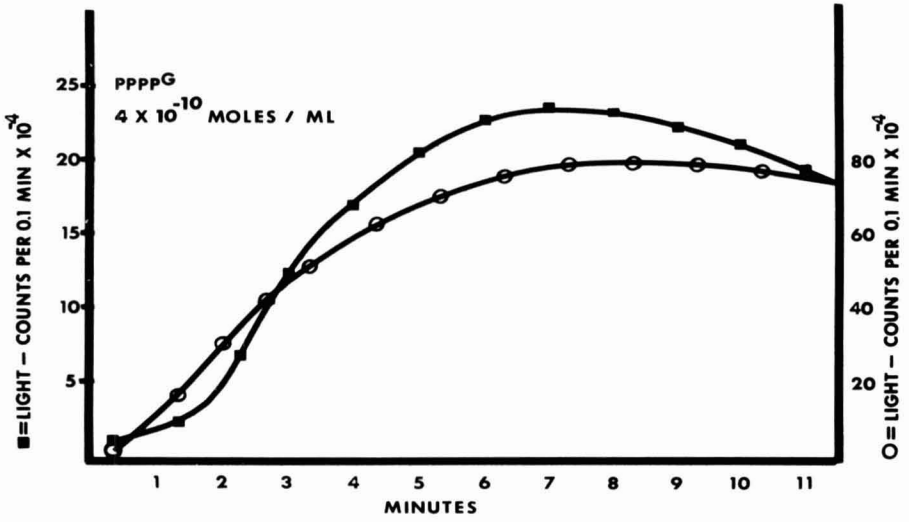

FIG. 2. Plot of delayed light emission kinetics of linear guanosine 5'tetraphosphate (ppppG). The concentration of ppppG used was 4×10^{-10} moles per ml of solution. Reaction was started by addition of firefly extract to the substrate in phosphate buffer (10 mM KH_2PO_4 and 4 n*M* $MgSO_4$, pH 7.4) and assayed at 6°C in the spectrometer.

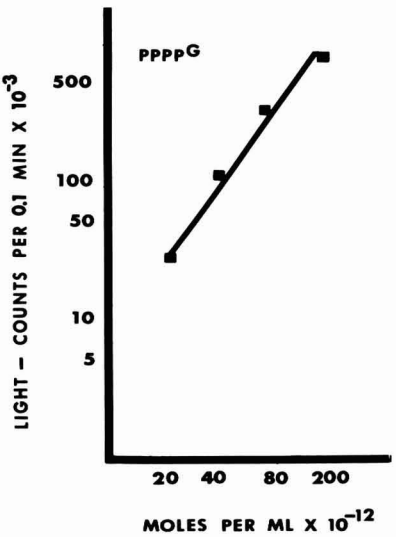

FIG. 3. Log × log relationship between concentration of guanosine 5'tetraphosphate (ppppG) and counts per 0.1 min. Reaction was started by addition of firefly extract to ppppG in phosphate buffer and assayed at 6°C and 7 min after addition of enzyme.

slight contamination of the preparation with GTP. In Fig. 5 linearity is displayed from 15 to 150 μmol/ml versus light emission (counts per 0.1 min) plotted in log-log mode.

In Fig. 6 it is seen that a 3-min delay occurs in the peak emission of

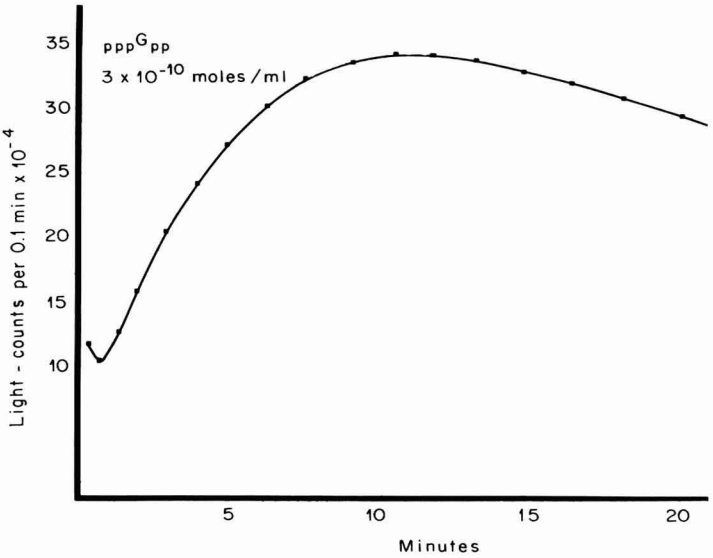

FIG. 4. Plot of delayed light emission kinetics of guanosine 5′ triphosphate 3′ diphosphate (pppGpp). The concentration of pppGpp was 3×10^{-10} moles/ml of solution. Reaction was started by addition of firefly extract to the substrate in phosphate buffer (10 m*M* KH_2PO_4 and 4 m*M* $MgSO_4$, pH 7.4) and assayed at 6°C in the spectrometer.

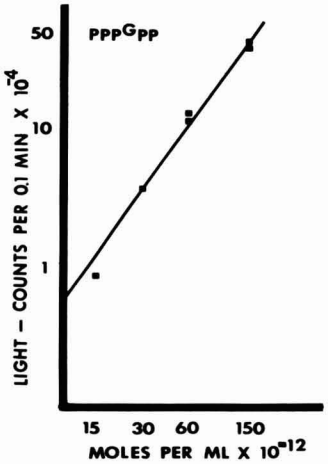

FIG. 5. Log × log relationship between concentration of guanosine 5′ triphosphate 3′ diphosphate (pppGpp) and counts per 0.1 min. Reaction was started by addition of firefly extract to pppGpp in phosphate buffer and assayed at 6°C and 12 min after addition of enzyme.

light using GppppG. Using the 3-min delay it is seen in Fig. 7 that a linear relationship between concentration of nucleotide from 21×10^{-12} to 110×10^{-12} moles per ml and emission of light exists when plotted log-log.

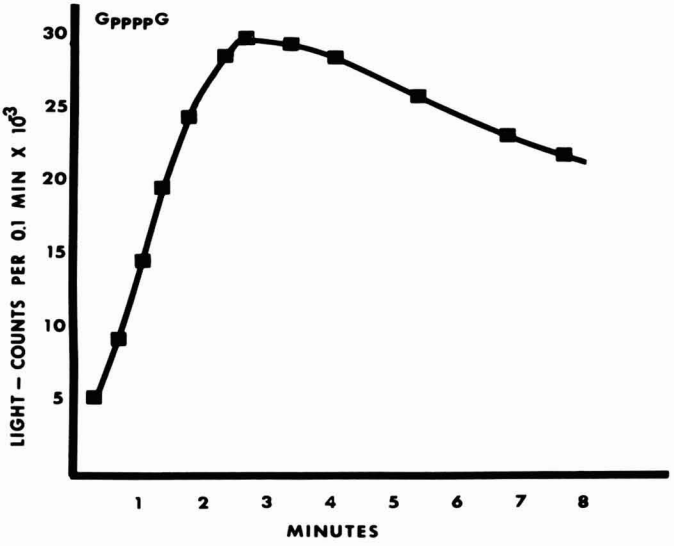

FIG. 6. Plot of delayed light emission kinetics of diguanosine 5′5″ tetraphosphate (GppppG). The concentration of GppppG was 4×10^{-10} moles/ml of solution. Reaction was started by addition of firefly extract to the substrate in phosphate buffer (10 m*M* KH_2PO_4 and 4 m*M* $MgSO_4$, pH 7.4) and assayed at 6°C in the spectrometer.

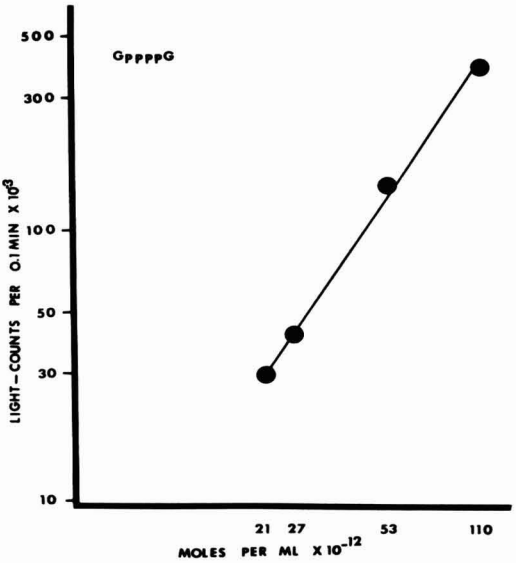

FIG. 7. Log × log relationship between concentration of diguanosine 5′5′′ tetraphosphate (GppppG) and counts per 0.1 min. Reaction was started by addition of firefly extract to GppppG in phosphate buffer and assayed at 6°C and 3 min after addition of enzyme.

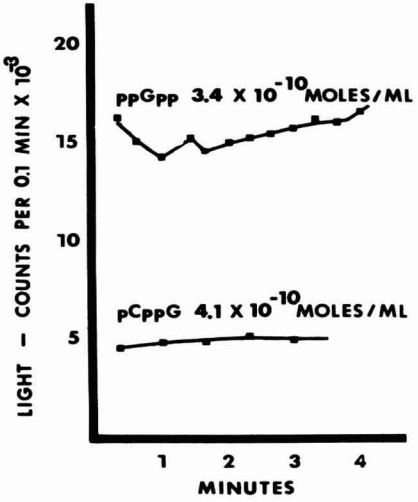

FIG. 8. Plot of delayed light emission kinetics of guanosine 5′ diphosphate 3′ diphosphate (ppGpp) and (pCppG). The concentration of ppGpp was 3.4×10^{-10} moles/ml of solution and pCppG was 4.1×10^{-10} moles/ml of solution. Reaction was started by addition of firefly extract to the substrate in phosphate buffer (10 m*M* KH_2PO_4 and 4 m*M* $MgSO_4$, pH 7.4) and assayed at 6°C in the spectrometer.

In Fig. 8 a light emission versus time experiment was run with ppGpp and pCppG. At the concentrations $3–4 \times 10^{-12}$ moles/ml that were used no real emission of light was apparent. The variability with ppGpp indicates some light producing contaminants but these were not identified nor were further experiments performed.

DISCUSSION

A perusal of the data developed in this work leads one to certain conclusions that appear to be valid.

(1) Many guanosine tetra-and pentaphosphates do react with a crude luciferin–luciferase system and produce light. The purity of the compounds becomes quite important for quantitative assessment of a given compound because many of these compounds produce light and this is not a good qualitative indicator of which compound might be present. The system is quite sensitive in its limits of detection (generally in the picomole per milliliter range) so it should be excellent for detection of small amounts of the endogenous material that may be present in bacterial or mammalian systems.

(2) There seems to be a consistent theme present throughout this work in the materials that produce light. The particular nucleotide to be tested must have a minimal GTP structure or the potential to produce GTP directly from simple-bond cleavage without rearrangement. The compounds that are reactive in this system are ppppG, pppGpp, GppppG, and GTP and the compounds that are nonreactive are ppGpp and pCppG. Both of the latter inactive compounds lack the GTP structure, and they do not produce GTP directly from a simple-bond cleavage.

It is unfortunate that guanosine 5′ diphosphate 3′ diphosphate (ppGpp) does not react with the system directly because this is an important control compound in stringent strains of *E. coli* and, possibly mammalian systems as well, and an assay for the substance is needed, other than the insensitive ultraviolet-absorption-detection system. Clearly if an enzyme that converts nucleoside diphosphates (e.g., ppGpp) to the corresponding triphosphate state were available (e.g., myokinase causing ADP + ADP → ATP + AMP) there would be the possibility of getting a coupled reaction. Another possibility would be to isolate the enzyme that causes the conversion of ppGpp → pppGpp and add that to the system (*1*).

It should be noted that the conversion to a GTP-like molecule based on the time of maximal-light emission (Figs. 2, 4, 6) must be accomplished in the following order (quickest one first): GppppG, ppppG, and finally pppGpp. Logically this would follow the ease of chemical and/or enzymatic hydrolysis to produce GTP.

The mechanism of how the crude firefly enzyme system (luciferin–

luciferase) produces light with such diverse structures apparently lies in a transphosphorylation reaction that involves multiple reactions. It is known that the crude preparation contains nucleoside diphosphate kinase (*8,9*) which using a variety of purine and pyrimidine triphosphates can phosphorylate ADP to produce the ATP that can be utilized in the luciferin–luciferase–Mg-reaction to produce light. The production of the nucleoside triphosphate from nucleoside tetra- or pentaphosphates would present an additional complication where the nucleotides could either directly phosphorylate ADP to ATP or a nucleoside triphosphate would have to be generated from the parent structure via phosphatases. Most probably we have an ATP-generating system that via a series of steps eventually produces emitted light. The time in which a maximum output of light is produced probably represents the various interactions that must take place before maximal light output can be reached. We know from the work of McElroy that the luciferin–luciferase reaction is essentially specific only for ATP (*7*).

I hope that this assay system stimulates research in the quest for an explanation of the regulatory activities of guanosine tetra- and pentaphosphates.

SUMMARY

A system is presented that can separate and quantitate in picomole amounts various guanosine tetra and penta phosphates namely guanosine 5′ triphosphate, 3′ diphosphate (pppGpp), guanosine 5′ tetraphosphate (ppppG), and diguanosine 5′ tetraphosphate (GppppG). It was found to be inactive with guanosine 5′ diphosphate, 3′ diphosphate (ppGpp), and a synthetic compound pCppG.

The analytical detection system uses a crude firefly luciferin–luciferase system in which the various derivatives probably transphosphorylate ADP to produce the ATP necessary to emit light with the luciferin–luciferase system.

The system should be useful in quantitating reactions in which guanosine tetraphosphates and pentaphosphates are involved. Their role is apparently one of control at either RNA polymerase or ribosomal levels and should be important in further research in molecular biology.

ACKNOWLEDGMENTS

The author gratefully acknowledges the technical help of Crystal Newcomer and Kathy Van Dyke for help in preparation of the manuscript. The work was supported financially by grants from the World Health Organization, West Virginia Affiliate of the American Heart Association, and an NIH institutional grant to the Medical Center at West Virginia University.

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MICROCHEMICAL JOURNAL 20, 388–391 (1975)

Comments on the Potentiometric Titration of Halides Including Fluoride with a Mixed Titrant[1]

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INTRODUCTION

Chou and Sams (*2*) recently described in this Journal the potentiometric titration of the first four halides using a mixed titrant containing silver nitrate and thorium nitrate. The electrode system used to monitor the titration consisted of a platinum billet indicator electrode and a saturated calomel reference electrode, isolated by a salt bridge from the solution. The authors concluded that titrations were best carried out at 0°C and pH 7.2. No buffers were used.

Owing to our continuing interest in the analysis of halides and fluoride, this method seemed of interest. Our attempts to duplicate this work yielded good results for chloride and bromide. However, even with a variety of modifications, no satisfactory endpoint breaks were obtained for fluoride. Indeed, this is not surprising, since there is no reason to expect the platinum electrode to respond to changes in fluoride ion concentration. That this electrode responds to changes in bromide and/or chloride ion concentration is probably due to adsorption of halide ion on the platinum electrode which then acts as a halide-sensing electrode.

EXPERIMENTAL METHODS

Reagent grade chemicals were used throughout. The mixed titrant contained 0.025 *N* silver nitrate and 0.05 *N* thorium nitrate. The titration system was controlled by a conventional PDP-8/I minicomputer processor. A summary of its operation and a schematic of the equipment were published elsewhere (*6,7*). The emf was monitored by means of a platinum electrode and a double-junction reference electrode (salt bridge filled with 1 *N* potassium nitrate).

Titration endpoints were calculated according to Savitsky and Golay (*5*). A convolute was used for a third-order second derivative using 25

[1] This work was performed under the auspices of the U. S. Atomic Energy Commission.

points. The zero-crossing was found by interpolating linearly near the sign change.

Stirring was provided by a magnetic stirrer. The stirring motor was separated from the titration vessel by a water-cooling plate and an aluminum plate connected to ground.

RESULTS AND DISCUSSION

We first attempted to verify the results reported by Chou and Sams (*2*) on the potentiometric titration of halides including fluoride with a mixed titrant containing silver nitrate and thorium nitrate. Titrations were carried out at 23 ± 1°C and pH 7.2. Chou and Sams recommend titration at 0°C for best reproducibility; however, for verification of their method ambient temperature was deemed adequate. To lower the solubilities of the silver halides some experiments were carried out in a partially nonaqueous medium (methanol).

For chloride and bromide, the breaks in potential near the endpoint were considerably less than those commonly obtained with a silver electrode or the various silver-sensing ion-selective electrodes. A nonaqueous or partially nonaqueous medium, which reduces the solubility of the silver halides, generally increases the potential breaks of this titration. However, when the platinum electrode was used no such effect was evident. For fluoride in a medium containing 80% methanol, the break was minimal (on the order of 10 mV). This break is too small to be analytically useful and, moreover, was not reproducible.

Lingane (*4*) has stated that mixtures of halides cannot be accurately titrated since silver halides readily form mixed crystals and solid solutions, leading to a tendency for coprecipitation of the more soluble halide. Therefore, in the titration of bromide and chloride, e.g., the results for bromide will be considerably larger than expected. The sum of the halides, however, can usually be determined with fair accuracy.

Various means have been employed to reduce this coprecipitation phenomenon. Shiner and Smith (*8*) recommend an acetate buffer containing a few drops of a nonionic detergent. We have found this medium not as effective as the one recommended by Bowers *et al.* (*1*), in which coprecipitation effects are reduced by adding various electrolytes to flocculate the silver bromide as it is formed. Of the various electrolytes suggested, we have found 0.1 *M* potassium nitrate to be satisfactory.

Only a limited number of experiments were performed with the platinum/calomel electrode system. Satisfactory breaks were obtained for bromide or chloride in any of the following media: (a) aqueous, pH 7.2, (b) 0.1 *N* potassium nitrate, (c) 60% or 80% methanol, and reproducible results were obtained. Some typical titration curves are shown in Fig. 1. For equimolar amounts of bromide and chloride at the conditions recom-

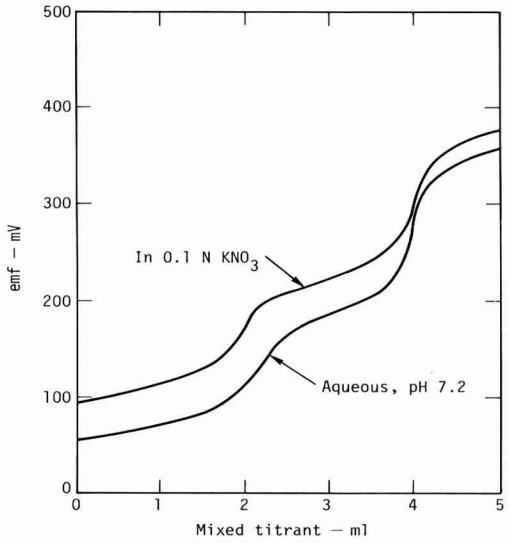

FIG. 1. Potentiometric titration of 0.05 mmol bromide and 0.05 mmol chloride with mixed titrant (0.025 *N* $AgNO_3$, 0.05 N $Th(NO_3)_4$) and platinum/calomel electrode system.

mended by Chou and Sams (*2*) the recovery for bromide was high, as expected. A medium of 0.1 *N* potassium nitrate greatly improved the bromide recoveries. Likewise, in 80% methanol without potassium nitrate, the results were improved but to a lesser extent. These results are summarized in Table 1. No attempt was made to determine the accuracy and precision of this method. However, the platinum indicator electrode can indeed be used in the determination of halides by potentiometric titration with silver nitrate. The coprecipitation usually found in the titration of bromide and chloride also occurs but can be reduced by titration in the presence of a flocculant such as 0.1 *N* potassium nitrate. No useable endpoint breaks were obtained in the titration fluoride by this method. We have thus been unable to verify the results reported by Chou and Sams even in 80% methanol.

As a possible alternative method for determining halides including

TABLE 1
RECOVERY OF BROMIDE AND CHLORIDE BY POTENTIOMETRIC TITRATION

Titration medium	Bromide recovery (%)	Bromide plus chloride recovery (%)	Number of replicates
Aqueous, pH 7.2	113.4	99.4	2
0.1 *N* KNO_3	100.6	99.6	2
80% methanol	103.6	100.4	2

fluoride with a single titrant, we recommend reinvestigation of the work reported by Jay *et al.* (*3*). These workers determined fluoride by potentiometric titration with acetonic silver perchlorate in acetic anhydride. The other halides can be determined in solvents such as acetone, N, N-dimethylformamide, and dimethyl sulfoxide. The work reported was rather preliminary in nature and no data are given on accuracy and precision. A serious disadvantage is that acetic anhydride must be used as solvent for fluoride. However, in this solvent all four halides can be differentiated.

ACKNOWLEDGMENT

The author wishes to express his deep appreciation to Marienne Stark.

SUMMARY

An attempt was made to verify work reported by others on the potentiometric titration of halides including fluoride with a mixed titrant of silver nitrate and thorium nitrate. The platinum indicator electrode can indeed be used to monitor the titration of bromide and chloride. However, we could not verify the results reported for fluoride. There seems to be no theoretical basis on which to expect the platinum electrode to respond to changes in fluoride ion concentration.

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Polarographic Investigation of the Zimmermann Reaction Mechanism

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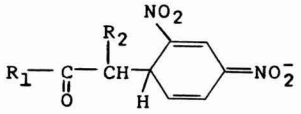
INTRODUCTION

The Zimmermann reaction, in which ketosteroids react with *m*-dinitrobenzene in the presence of base, is widely used for the colorimetric determination of 17-ketosteroids occurring in human urine, as well as for 17-hydroxy corticosteroids after their conversion to 17-ketosteroids. The mechanism of this reaction has been examined by a number of researchers (*3–5,7,8,10–13*). Experimental evidence for the structure proposed by Zimmermann and supported by other researchers is incomplete.

Previous researchers (*3*) proposed that the main product should have structure I if the reaction were carried out with a large excess of ketone. However, in the presence of a large excess of *m*-dinitrobenzene, Zimmermann's conditions, the main product was to have structure II.



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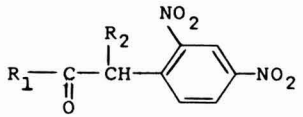

II

Polarographic examination of the Zimmermann reaction was undertaken to add further experimental evidence to assist in elucidating the mechanism of this reaction.

MATERIALS AND METHODS

The 3β-hydroxyandrost-5-en-17-one (dehydroepiandrosterone), m.p. 150–152°C, was purchased from British Drug Houses Ltd., Toronto, Ontario, Canada. Absolute ethanol was obtained from Consolidated Alcohols, Ltd., Toronto, Ontario, Canada. Reagent grade potassium hydroxide and *m*-dinitrobenzene were from Fisher Scientific Company, Don Mills, Ontario, Canada. Nitrogen 99.996% pure was from Liquid Carbonic of Canada Ltd. Triple distilled mercury was obtained from Engelhard Industries of Canada, Ltd.

A Sargent (Sargent-Welch Scientific Company) Model XVI Polarograph was employed for this investigation. The concentration study was carried out in a 5-ml Heyrovsky cell. The characteristics of the capillary used were: $m = 1.767$ mg s^{-1}; $t = 4.68$ sec; $m^{2/3}t^{1/6} = 1.890$ $mg^{2/3}$ $s^{-1/2}$. The height of the mercury column was 71.5 cm. The cell was placed in a water bath maintained at 25 ± 0.1°C with a Haake Model ED "Unitherm" constant temperature circulator.

A stock solution of *m*-dinitrobenzene was prepared by dissolving 0.50 g in a 500-ml volumetric flask which was filled to volume with absolute ethanol. A stock solution of potassium hydroxide was prepared by dissolving 4.5 g in a 10-ml volumetric flask which was filled to volume with distilled water. The blank was prepared by adding 1.0 ml of absolute ethanol, 0.1 ml of stock potassium hydroxide solution, and 0.1 ml of *m*-dinitrobenzene stock solution to a 10-ml volumetric flask. The flask was placed in an ice bath in the cold room in the dark for 60 min. After incubation, the flask was filled to volume with absolute ethanol and mixed by inversion. A 5-ml aliquot was added to a 10-ml Heyrovsky cell. The cell was placed in a 25°C water bath and deaerated for 15 min with nitrogen just prior to polarographic analysis. Test solutions containing 3β-hydroxyandrost-5-en-17-one were prepared at three concentrations. For the low concentrations, a standard solution of 3β-hydroxyandrost-5-en-17-one was prepared by adding 0.030 g to a 10-ml volumetric flask which was filled to volume with absolute ethanol. The most dilute test solution was prepared similar to the blank, but in place of the 1.0 ml of absolute ethanol in the blank, 0.1 ml of the standard ketosteroid solution and 0.9 ml of absolute ethanol were added. Similarly, the intermediate concentration used 1.0 ml of the standard ketosteroid solution in place of the 1.0 ml absolute ethanol used in the blank. The high concentration

TABLE 1
EFFECT OF 3β-HYDROXYANDROST-5-EN-17-ONE ON THE REDUCTION WAVES OF *m*-DINITROBENZENE

3β-Hydroxyandrost-5-en-17-one concentration[a] (mg/ml) in Heyrovsky cell	Diffusion current[b] (μA)		Half-wave potential[b] (V vs Hg Pool)		Color
	Wave 1	Wave 2	Wave 1	Wave 2	
0	1.155	1.120	−0.540	−0.786	—
0.30	0.995	0.926	−0.524	−0.781	Purple
3.0	0.478	0.490	−0.511[c]	−0.765[c]	Purple

[a] Refer to text for complete details.

[b] The values reported represent an average of two samples at each concentration studied, with three polarograms run for each sample.

[c] Variations in $E_{1/2}$ values were observed between the two samples.

solution was prepared by adding 0.030 g of 3β-hydroxyandrost-5-en-17-one to a 10-ml volumetric flask, and the remaining reagents were added as in the blank solutions. After 60 min in an ice bath in the cold room (in the dark), all solutions were deaerated for a period of 15 min with nitrogen prior to polarographic analysis. Duplicate analyses were performed for each concentration studied, with three polarograms obtained for each run.

In another experiment, both blank and test solutions were filled to volume with distilled water (instead of absolute ethanol) after the 60-min incubation period. The test solutions contained 0.5 ml of a standard solution of 3β-hydroxyandrost-5-en-17-one (0.030 g/10 ml absolute ethanol). In all other respects, these solutions were prepared and analyzed as in the previous section.

RESULTS

All test solutions containing 3β-hydroxyandrost-5-en-17-one had turned purple after the 60-min cold room incubation (Tables 1 and 2). A slight decrease of the diffusion current (I_d) was observed for the test solution containing the low 17-ketosteroid concentration (0.1 ml ketosteroid standard). The test solution containing the high concentration of 17-ketosteroid (0.030 g) resulted in a greater than 50% reduction in I_d for both the first and second nitro group reduction waves of *m*-dinitrobenzene.

The water diluted system containing 3β-hydroxyandrost-5-en-17-one showed a marked change in half-wave potential ($E_{1/2}$) values (Table 2). The $E_{1/2}$ of the second reduction wave shifted to a greater extent than the $E_{1/2}$ of the first nitro group reduction wave of *m*-dinitrobenzene. A blank solution under similar experimental conditions did not show this disproportionate shift. In the experiments where water instead of eth-

TABLE 2
EFFECT OF 3β-HYDROXYANDROST-5-EN-17-ONE ON THE REDUCTION WAVES OF *m*-DINITROBENZENE

3β-Hydroxyandrost-5-en-17-one concentration[a] (mg/ml) in Heyrovsky cell	Diffusion current[b] (μA)		Half-wave potential[b] (V vs Hg Pool)		Color
	Wave 1	Wave 2	Wave 1	Wave 2	
0	0.800	0.761	−0.509	−0.754	—
0.150	0.577	0.574	−0.474	−1.039	Purple

[a] Refer to text for complete details.

[b] The values reported represent an average of two samples at each concentration studied, with three polarograms run for each sample.

anol was used to dilute the solutions, a slight turbidity was observed. The decrease in I_d is very likely due to decreased solubility of the 17-ketosteroid-*m*-dinitrobenzene compound.

DISCUSSION

All test solutions which contained 3β-hydroxyandrost-5-en-17-one turned purple after incubation for 60 min in the cold room. Since the I_d of both nitro reduction waves of *m*-dinitrobenzene in the presence of a low 17-ketosteroid concentration did not greatly decrease, it is concluded that a nitro anion is not required for purple color formation. Only at highly elevated concentrations of 3β-hydroxyandrost-5-en-17-one is a decrease observed for both nitro reduction waves. To show that this observed decrease is due to a reaction taking place and not to other effects of this large amount of 17-ketosteroid, 0.030 g of the steroid was added to a blank solution which was mixed by deaeration with nitrogen. The results of this experiment showed only a slight decrease which was due to reaction taking place during the mixing-deaeration procedure prior to polarographic examination. A pale purple color was observed, also attesting to a reaction taking place. Blanks were run with all solutions in order to eliminate Meisenheimer complex formation (*1,6,9*) between the hydroxide ion and the *m*-dinitrobenzene. The I_d values obtained for a blank solution prepared fresh at room temperature and examined immediately were about 3% higher than the I_d values for a cold room blank which was stored for 60 min in an ice bath in the cold room. From these results, it is concluded that the amount of nitro anion formation due to the presence of hydroxide ion is small under the conditions of this experiment. Since comparison is made between blank and test solutions, any hydroxide interference would be minimized.

A very significant shift of $E_{1/2}$ was observed in the test solutions which were diluted to volume with water. The first reduction wave shifted 0.035 V to a less negative potential, while the second wave shifted 0.285 V to a more negative potential. Blank solutions not containing 17-ketosteroid did not show this shift between the two nitro reduction waves. This is additional evidence that attachment is not symmetrical, thus supporting previous theories (*3,7,11,12*). The decrease in I_d was not significant because the solution was turbid due to a decreased solubility of the Zimmermann reaction product.

The decreased I_d at high concentrations of 3β-hydroxyandrost-5-en-17-one compared to *m*-dinitrobenzene is believed to indicate the formation of a nitro anion, which is not present at low concentrations of the steroid. Similar formation of nitro anions has recently been proposed in the mechanism of the Jaffé reaction (*2*). Furthermore, nitro anion formation has also been suggested for the Zimmermann reaction (*3,14*).

Regeneration of the nitro reduction waves of *m*-dinitrobenzene was attempted by adding acid and then base to the purple colored Zimmermann reaction solution. To a control purple colored solution a premixed acid and base solution was added. Although both test and blank solutions showed decreases in the I_d values, no conclusions could be made because all solutions had turned turbid and a white precipitate settled in the tubes.

The formation of *m*-nitroaniline during the Zimmermann reaction has been reported by Reissert (*13*) and confirmed by King and Newall (*7*). Our polarography results indicate that the formation of *m*-nitroaniline cannot take place to any appreciable extent under conditions commonly employed in clinical chemistry laboratories (low 17-ketosteroid concentration compared to *m*-dinitrobenzene). However, the decrease in the I_d of the two nitro reduction waves in the presence of excess 17-ketosteroid could be explained by the formation of *m*-nitroaniline.

The polarography findings support the presently accepted theories of attack at the less sterically hindered side of *m*-dinitrobenzene. Although the mechanism probably goes through an intermediate nitro anion formation, the final spectrophotometrically measured product does not contain a nitro anion under conditions commonly employed in hospital laboratories (because the 17-ketosteroid levels are usually low compared to *m*-dinitrobenzene). The evidence presented supports the theory proposed in the publication by Corker *et al.* (*3*), in which he describes that under the conditions employed by Zimmermann (large excess *m*-dinitrobenzene), nitro anion formation is unlikely, while under an excess 17-ketosteroid concentration (Canbäck's conditions) the final measured product would contain a nitro anion. However, our findings indicate that a very large excess of 17-ketosteroid must be present in order for the final product to contain a nitro anion.

SUMMARY

Polarographic examination of the Zimmermann reaction adds further experimental evidence to the proposed theory of methylene group attack to the para position of *m*-dinitrobenzene. It also supports the theory that nitro anion formation can occur at high concentrations of 3β-hydroxyandrost-5-en-17-one compared to *m*-dinitrobenzene. Furthermore, polarographic analysis shows that the purple chromogen measured in the determination of steroids by the Zimmermann reaction does not contain a nitro anion.

ACKNOWLEDGMENTS

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BOOK REVIEWS

Current Topics in Microbiology and Immunology. Vol. 64. Edited by W. ARBER, R. HAAS, W. HENLE, P. H. HOFSCHNEIDER, J. H. HUMPHREY, N. K. JERNE, P. KOLDOVSKY, H. KOPROWSKI, O. MAALOE, R. ROTT, H. G. SCHWEIGER, M. SELA, L. SYRUCEK, P. K. VOGT, E. WECKER. Springer-Verlag, Berlin, 1974. 137 pp. $27.90.

This is volume 64 of a continuing series. The four articles are: Suppressor Mutations in Yeast (D. C. Hawthorne and U. Leupold), Cell Interaction in Conjugation of Ciliates (A. Miyake), The Pathogenesis of Autoimmunity in New Zealand Mice (N. Talal and A. D. Steinberg), and Nucleic Acid Reassociation as a Guide to Genetic Relatedness among Bacteria (R. L. Moore). The papers are well written and contain much useful information. The book is badly overpriced.

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The Analysis of Organic Materials. An International Series of Monographs. Edited by R. BELCHER and D. M. W. ANDERSON. **The Determination of Nitro and Related Functions.** By Y. A. GAWARGIOUS. Academic Press, London, 1973. 154 pp. L3.50.

The book is divided into six chapters—The Determination of Nitro, Nitroso, and Azo Groups, Recommended Methods of Analysis for Nitro, Nitroso, and Azo Groups, Determination of the Azide Group, The Determination of Nitrates and Nitramines, Recommended Methods of Analysis for Nitrate Groups, and Appendix, the last named gives a brief account of microdeterminations which became available after the completion of the manuscript.

Following the scheme of the other numbers in this international series, the book reviews the available methods for the determination of these various functions and then proceeds to recommend some of these methods for use. Included are titrimetric, gasometric, gravimetric, colorimetric, spectroscopic, polarographic, chromatographic, electrometric, enzymatic, etc., methods. The real value of the book is that it has gathered a great deal of information, previously widely scattered, into compact well-organized form that will save a tremendous amount of time for those involved in these determinations. The information included should also be of value to organic chemists not engaged in analytical work since it contains many functional group reactions which have synthetic value.

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Advances in Heterocyclic Chemistry, Vol. 16. Edited by A. R. KATRITZKY AND A. J. BOULTON. Academic Press, New York, 1974. ix + 349 pp. $35.00.

This is the new installment of a series of review articles in heterocyclic chemistry. This volume has six highly authoritative reviews all of which are concerned with a topic

of intense interest and all of which are extensively documented with up to date (many into 1973) literature references.

The following topics are included:

1. Base catalyzed hydrogen exchange by Elvidge, Jones, O'Brien, Evans and Sheppard. This topic is of great importance since it can serve as a method for deuterium and tritium labeling of nucleotides and peptides (specifically histidines). The recent findings in the elucidation of the reaction mechanism in five- and six-membered heterocyclic rings is presented. 2. 1,2,3-Triazoles, by Gilchrist and Gymer. The synthesis, spectroscopic properties and reactions of these molecules are discussed. Interest in these molecules is primarily due to their function as precursors for azapurines and potential anticancer agents. 3. Nitrogen-bridged six-membered ring systems by Kricka and Vernon. Sections include syntheses and reactions of 7-azabicyclo (2.2.1) heptanes, naphthalen-1,4-imines and anthracen-9,10-imines. 4. Homolytic substitution of heteroaromatic compounds by Minisci and Porta. Topics include alkylation, acylation, α-oxyalkylation, amidation, arylation reactions. Products, mechanism of reactions as well as the sources of the radicals are discussed. 5. Dibenzothiophenes by Ashby and Cook. Naturally occurring as well as synthetic dibenzothiophenes are discussed with respect to both synthesis and derivatization. 6. Cationic polar cycloaddition by Bradsher. This chapter discusses primarily Diels-Alder type reactions with positively charged heteroatomic systems.

As were the previous volumes in this series, volume 16 is also a worthy addition to any chemical library.

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Ascorbinometric Titrations. By L. ERDEY AND G. SVEHLA. Akademiai Kiado, Budapest, 1973. 183 pp. $8.50.

The late Professor Erdey introduced the use of ascorbinometric titrations based on the use of ascorbic acid as a reducing agent in 1950. This book attempts to give a systematic account of both principles and details of the methods which evolved during the ensuing 15 years. The principles are straightforward and simple and only a few pages are required to set these forth, the remainder of the book is a how-to manual. Each of the determinations presented includes a discussion, procedure and statement of the error potential. There is a 170-item bibliography and separate author and subject indices.

No wild claims are made for the superiority of this method. The user is given more than adequate information to determine whether the method is advantageous for any given application.

The book is well printed and bound.

BILL ELPERN, *9 Surrey Way, White Plains, New York 10607*

Microbial Metabolism. Edited by H. W. DOELLE. Dowden, Hutchinson & Ross, Stroudsburg, PA, 1974. xiii + 424 pp. $25.00.

This series of "benchmark" books has been criticized for merely reprinting old papers and charging a good price for them. This criticism is unjustified as it misunderstands the purpose—to present in one place papers of historical importance in a given discipline. Every paper in every book of this series that this reviewer has seen, has been a landmark,

representing a step forward—in many cases, a giant step. Furthermore, there can be little fault found with the selection of papers.

This volume is separated into six divisions of microbial metabolism: early stages; carbohydrates; inorganic compounds; aromatic carbon; and anaerobic fermentation. In the first division, classics by such greats as Pasteur, Liebig, and Buchner are presented in the original language and in excellent translations. In the other sections, the dates of some of the papers—real benchmarks—make one realize that microbiology is, indeed, a young discipline.

This book should be in every research library if for no other reason than the convenience, in one place, of reference to these basic papers. Those with an abiding historical bent will want their personal copies.

DAVID B. SABINE, *484 Hawthorne Avenue, Yonkers, New York 10705*

Microprobe Analysis as Applied to Cells and Tissues. Edited by T. HALL, P. ECHLIN, AND R. KAUFMANN. Academic Press, New York, 1974. xix + 435 pp. $20.25 (£7.80).

This book is the result of a conference held at Seattle, Washington, in May of 1973, on the subject of the title of this text.

The text concerns itself with methods of analysis for the contents of cells by three types of probes. One is the irradiation of an area of approximately 0.2 μm^2 with the electron beam, another is the irradiation of an area of approximately 0.5 μm^2 with a laser beam and the third is the irradiation of an area of 1.0 μm^2 with a collimated X-ray beam.

In the case of fresh tissue the electron beam and laser beam will punch a hole out of the cell, vaporizing and exciting the elements of the punched out portion. The X-ray beam is nondestructive. For the electron beam the electron scanning microscope may be used with prepared specimens embedded in plastic to retain the original location of the elements so as to locate the distribution of elements within the cell.

The general trend is to read the output of the emitted X-rays from the electron or X-ray probes by nondispersive techniques using a S_i (L_i) solid state detector. Assay for the biologically important elements Na, K, Cl, Mg, Ca and Fe are practicable with this technique.

Of greatest interest is the application of the mass spectrograph to the resolution of ion emission generated by the electron or laser probes. A mass spectrum of the elements is obtained including the isotopes of the elements, if desired. As little as 10^{-9} pg of an element can be detected. In place of the electron or laser probes other atoms such as argon or hydrogen can be used to bombard the specimen. The eventual possibility of fragmented organic molecules analyzed by the mass spectrograph also exists.

The subject matter above is discussed in the text in four sections. The first section discusses the techniques. The second section concerns the preparation of the specimen and the fourth section is on the application of the techniques to specific problems such as tissue culture media, calcium transport and electrolyte distribution in biological materials. The third section describes quantitation with various special techniques.

The book is uniformly well written and is illustrated with numerous drawings and half tones. It achieves its objective of presenting the present state of the science of microprobe analysis. It would make an excellent adjunct text to an advanced course in analytical chemistry or instrumental analysis. It is of particular value to those engaged in microprobe analysis as an idea book for improving their techniques.

It is recommended that this book be added to the library of the analytical, microchemical and biochemical laboratories. Chemists and biologists in general would do well to read the text in order to obtain some idea as to the potential of this field.

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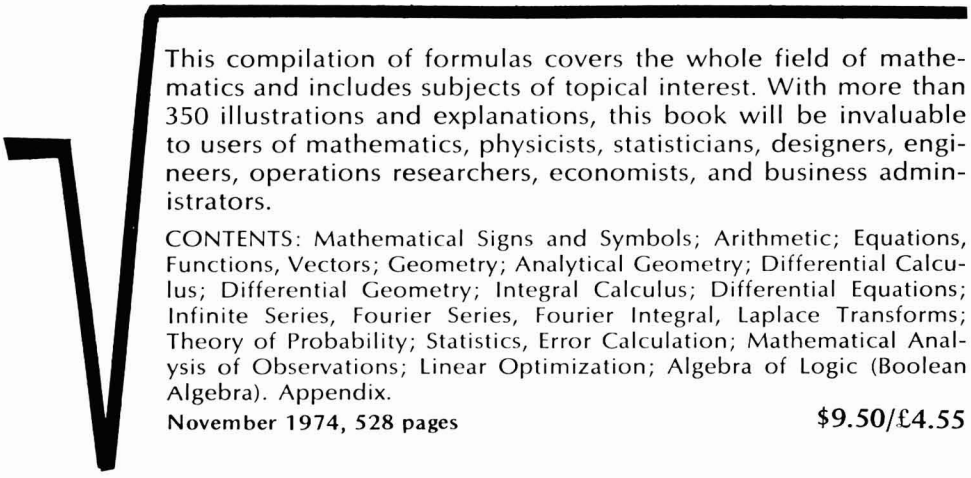

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