REVIEW ARTICLE

L'ANALYSE POTENTIOMETRIQUE APPLIQUEE AU CONTROLE DES MEDICAMENTS

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Dans cet exposé nous ne décrirons pas en détail la théorie de la potentiométrie. Il existe sur ce sujet des ouvrages spécialisés généraux dans lesquels le lecteur trouvera tous les détails désirés. Parmi ceux de langue anglaise, nous citerons ceux de Britton,[1] de Clark[2] et de Kolthoff.[3,4] Comme l'indique Morton dans ce Journal[5] il est indispensable de consulter les exposés annuels faits par Furman dans *Analytical Chemistry*. Nous ne décrirons pas les potentiomètres utilisés qui ont fait l'objet de l'article de Morton. Notons seulement qu'il importe d'opérer avec des instruments tels que la pile, dont on mesure la force électromotrice, ne débite pas de courant.

Principes de la Potentiometrie

Pour la compréhension de ce qui va suivre nous allons rappeler rapidement les bases de la potentiométrie et décrire brièvement les électrodes utilisées, qu'elles soient indicatrices ou qu'elles soient de référence. Lorsqu'on plonge un métal dans la solution d'un de ses sels, l'expérience montre qu'il existe une différence de potentiel entre le métal et la solution. Cette différence de potentiel est donnée par la formule de Nernst $E = -\frac{RT}{nF} \ln \frac{P}{p}$ dans laquelle R est la constante des gaz, n la valence du métal, F le Faraday, ln le logarithme népérien, P la pression de dissolution du métal, p la pression osmotique de la solution. La pression osmotique étant proportionnelle à la concentration c du sel, on peut aussi écrire:

$$E = Cte + \frac{RT}{nF} \ln c$$ en confondant la concentration avec l'activité.

Le potentiel correspondant à la concentration $c = 1$ de la solution normale s'appelle le potentiel normal du métal, E_0, par suite:

$$E = E_0 + \frac{RT}{nF} \ln c$$

L'ensemble métal-solution forme une demi-pile ou ce que l'on appelle, improprement d'ailleurs, une "électrode." L'électrode correspondant à la solution normale est "l'électrode normale."

Lorsqu'un barreau *d'argent* plonge dans une solution renfermant des ions Ag^+ à la concentration $[Ag^+]$ le barreau prend, par rapport à la solution, le potentiel $E = E_0 + \frac{RT}{F} \ln [Ag^+]$.

L'hydrogène se comporte comme un métal. Une lame de platine, baignée par de l'hydrogène gazeux, joue le rôle d'une lame d'hydrogène moléculaire H_2 et prend, par rapport à une solution acide, un potentiel défini par la concentration en ions H^+ de cette solution. Ce potentiel est donné par la formule :

$$(I)\quad E = -\frac{RT}{F} \ln \frac{[H_2]^{\frac{1}{2}}}{[H^-]}$$

$[H_2]$ étant la concentration en hydrogène sur l'électrode et $[H^+]$ la concentration en ions H^+ de la solution.

L'électrode normale à hydrogène est obtenue en faisant arriver l'hydrogène sous la pression atmosphérique dans une solution acide normale, N. E_0 est le potentiel normal.

Par suite : $E = E_0 + \frac{RT}{F} \ln [H^+]$

La formule (I) peut s'écrire :

$$E = \frac{RT}{F} \ln [H^+] - \frac{RT}{F} \ln [H_2]^{\frac{1}{2}}$$

ou en logarithmes décimaux :

$$E = -2{,}302 \frac{RT}{F} pH + 2{,}302 \frac{RT}{2F} r\, H_2$$

E dépend donc de deux variables $[H^+]$ et $[H_2]$.

(1°) Si $[H_2]$ est constant, la mesure de E permet la détermination de la concentration en ions $[H^+]$ c'est-à-dire du *p*H.

(2°) Pour une certaine acidité $[H^+]$ la mesure de E donnera la valeur de la concentration $[H_2]$ en hydrogène moléculaire, c'est-à-dire le potentiel d'oxydo-réduction.

Apparaissent donc immédiatement deux des utilisations analytiques de ces forces électromotrices, d'une part l'étude de la neutralisation des acides et des bases jointe à celle de tout phénomène mettant en jeu des ions H^+, d'autre part l'étude des réactions d'oxydation ou de réduction. En ajoutant l'observation du potentiel pris par un barreau d'argent au cours de la précipitation de l'ion argent en argentimétrie, on a l'ensemble de la titrimétrie potentiométrique.

Comment l'étude de ces potentiels peut-elle permettre à l'analyste de déceler la fin d'un titrage ? Considérons le potentiel pris par un fil d'argent plongeant dans une solution de nitrate d'argent dans laquelle on verse de l'iodure de potassium. Au fur et à mesure que l'on verse de l'iodure la concentration c en ions argent diminue. Il en est donc de même du potentiel du fil d'argent. Les variations du potentiel, lentes au début, sont extrêmement rapides près de la précipitation totale. La tangente à la courbe est sensiblement perpendiculaire à l'axe des abcisses. Lorsqu'on ajoute un excès d'iodure la concentration des ions Ag^+ diminue encore. En effet le produit de solubilité de l'iodure $[I^-]\ [Ag^+] = s$ montre que l'addition d'ions I^- diminue la concentration des ions $Ag.^+$

L'ensemble de la courbe présente une inflexion à tangente très relevée au point d'équivalence (Fig. 1). Le potentiel obtenu lors de la précipitation d'un iodure par le nitrate d'argent présente de même une variation brutale à la fin de la précipitation (Fig. 2). L'abcisse de l'inflexion représente le volume v de réactif qui correspond à la fin de la précipitation.

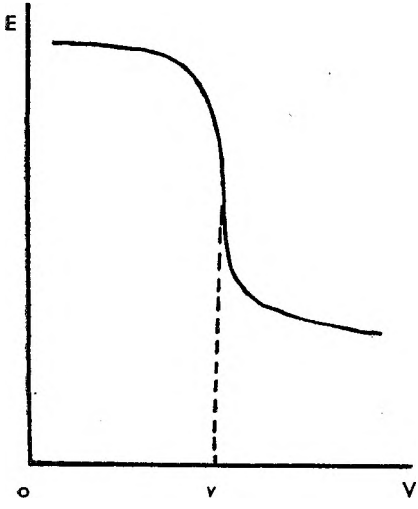


FIG. 1

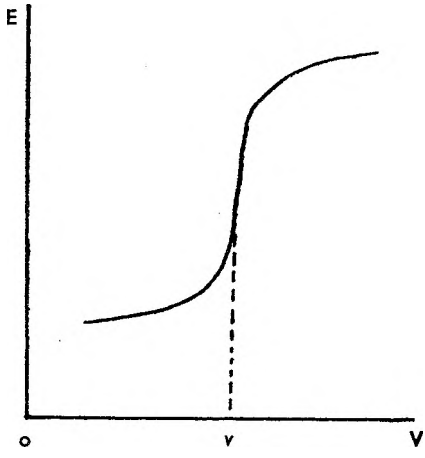


FIG. 2

Nous renvoyons le lecteur aux ouvrages spécialisés pour l'exposé des relations qui existent entre la valeur du produit de solubilité et la forme de l'inflexion.

Si l'on envisage la neutralisation d'un acide par une base, on a des variations du même genre au cours de la disparition progressive des ions H^+, avec point d'inflexion permettant de repérer le volume de base versé à la fin du titrage. Il faut noter que le point d'inflexion est d'autant plus net que la constante d'ionisation k de l'acide est plus grande, et que la concentration est plus élevée. Cela explique la difficulté rencontrée lors du titrage des acides faibles surtout s'ils sont en solution diluée. Dans ce cas l'inflexion est à peine visible et le dosage n'est pas possible. Si le produit k.c de la constante d'ionisation par la concentration c est inférieur à $27.K_w$, K_w étant la constante de l'eau, le point d'inflexion n'est pas visible. Pratiquement[6] k.c doit être supérieur à 10^{-10} La même remarque peut être faite pour le titrage des bases par les acides.

Enfin l'observation d'une variation brusque du potentiel d'oxydo-réduction permet, d'une facon analogue, la mise en évidence de la fin d'une réduction ou d'une oxydation. Sans entrer dans des détails techniques, remarquons qu'au lieu de construire toute la courbe, il suffit, ajoutant de petites portions de réactifs Δv, de noter les *variations de potentiel* ΔE qui en résultent. Le point final de titrage se manifeste par un maximum sur la courbe qui représente $\frac{\Delta E}{\Delta v}$ en fonction de v.

En argentimétrie on peut encore opérer par titrage à *potentiel repéré* ou *potentiel défini*. Dans une opération préliminaire effectuée dans les *mêmes* conditions que le dosage final, on détermine le potentiel de fin de précipitation. Lors du dosage final il suffit de verser du nitrate d'argent jusqu'à ce que le potentiomètre indique la valeur précédemment repérée. Cette technique excellente tient compte de l'influence des autres ions présents dans la solution. Elle est, de plus, extrêmement rapide.[7,8,9,10] Elle convient particulièrement bien au dosage des halogènes après destruction de la matière organique par le peroxyde de sodium dans une microbombe.

Le métal et la solution ne constituent qu'une demi-pile. Pour effectuer une mesure du potentiel il est nécessaire d'adjoindre une autre demi-pile, de potentiel *constant*, appelée "électrode de référence." L'ensemble donne la force électromotrice que l'on mesure au potentionmètre. En général on prend l'électrode classique au calomel saturée, parfois l'électrode de verre. Dans les mémoires cités plus loin les auteurs décrivent les électrodes utilisées. Les électrodes telles que le fil d'argent, l'électrode à hydrogène sont dites "électrodes indicatrices." En acidimétrie et alcalimétrie, on emploie peu l'électrode à hydrogène délicate à réaliser, on se sert quelquefois d'un barreau d'antimoine, de l'électrode à quinhydrone, mais surtout de l'électrode de verre maintenant universellement connue. Pour les dosages oxydimétriques on emploie presqu'uniquement le platine.

Enfin nous ne terminerons pas ces généralités sans décrire sommairement la méthode si rapide du "dead stop end point"[11] utilisée pour déceler la présence d'un réactif oxydant.[12] En voici le principe. Dans la solution à titrer plongent deux fils de platine. Une faible force électromotrice est appliquée entre ces deux électrodes. Celles-ci se polarisent et la force contre électromotrice de polarisation équilibre la force électromotrice appliquée. Lorsqu'on ajoute un excès de réactif, les électrodes se dépolarisent et le courant passe, provoquant, dans les appareils actuellement en service, la fermeture d'un oeil cathodique. Cette méthode est utilisable avec l'iode et spécialement avec le réactif de Fischer pour le dosage de l'eau.

Avantages de la Potentiometrie

La potentiométrie est peut-être moins rapide que l'observation directe d'un changement de coloration, car elle demande un assez grand nombre de mesures, sauf cependant si l'on opère à potentiel repéré, mais par contre elle élimine le facteur personnel d'appréciation de fin de réaction. Ce fait à lui seul suffit à justifier la méthode. Tout analyste connaît en effet les difficultés d'observation du changement de teinte d'un réactif coloré et l'impossibilité de déceler ce changement de coloration dans un milieu trouble ou très fortement coloré lui-même. D'autre part les méthodes habituelles deviennent souvent inutilisables dans des solutions très diluées. La potentiométrie permet au contraire d'opérer avec facilité. C'est ainsi que l'argentimétrie en solution 0,01 N ou 0,005 N s'effectue aisément. Enfin il est possible de réaliser des dosages automatiques[13] fort utiles dans les laboratoires où doivent se faire des

dosages en série. Très souvent enfin la potentiométrie évite les extractions ou les séparations. On réalise ainsi des gains de temps appréciables.

Signalons de plus que la potentiométrie se prête bien à l'analyse semi-micro et microchimique.[14] La précision est fort acceptable. On peut l'estimer à 0,5 pour cent. dans des conditions normales et a 5 pour cent. si l'inflexion de la courbe de potentiel est molle.

Milieux Non Aqueux. La réalisation des titrages dans les milieux non aqueux prend chaque jour de plus en plus d'importance et spécialement, semble-t-il, en potentiométrie.[15,16]

Il convient de signaler particulièrement une étude très utile sur un grand nombre de produits pharmaceutiques, alcaloïdes, vitamines, etc.[17] Dans ces milieux en effet l'ionisation est différente de celle produite dans l'eau. Certains acides, aux courbes de potentiel inutilisables dans l'eau, donnent au contraire dans ces milieux des inflexions bien marquées. Le lecteur rencontrera de nombreux exemples de dosages ainsi réalisés. Les mélanges d'eau et d'alcool sont fort employés. Les courbes que nous reproduisons (Fig. 3) montrent particulièrement bien l'action favorable de l'alcool lors du dosage des sels d'alcaloïdes par de la soude.[18]

De la même facon a été étudié[17] le titrage de sels de bases organiques dans des mélanges d'acide acétique glacial et d'eau.

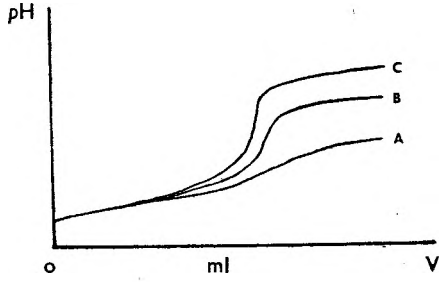


FIG. 3. Chlorhydrate de cocaïne titré avec de l'hydroxyde de sodium 0,01N.
A. Eau
B. Ethanol 50 pour cent.
C. Ethanol 75 pour cent.

APPLICATIONS AUX PRODUITS PHARMACEUTIQUES

Les dosages que nous allons décrire maintenant sont relatifs d'une part à des produits spécifiquement médicamenteux et d'autre part à des éléments, corps composés ou groupements, souvent rencontrés au cours de l'analyse des médicaments. Nous nous sommes parfois permis de signaler des dosages de produits non pharmaceutiques afin de faire connaître des méthodes vraisemblablement utilisables pour l'analyse des médicaments.

I. DOSAGES D'INTERET GENERAL

Groupements Fonctionnels

L'étude des groupements fonctionnels par potentiométrie a été effectuée en 1950 dans une revue de Lykken[19]; nous en retiendrons les faits essentiels suivants:—

Acides Carboxyliques. Le choix du solvant[20] est d'une importance considérable. Le mélange alcool isopropylique-benzène[16] est utilisé pour les acides forts et faibles, l'éthylènediamine[21] pour les acides faibles.

Diacides et Hydroxyacides. On peut utiliser l'oxydation par les composés cériques suivie d'un dosage en retour à l'aide d'un réducteur.[22]

Phénols. Le titrage se fait dans l'éthylènediamine avec l'amino-éthylate de sodium comme réactif titrant.[23] La méthode permet le dosage des phénols en présence des acides carboxyliques. On peut titrer, à l'aide du mélange bromure-bromate, les phénols et les crésols par la méthode du "dead stop."[19]

Amines et Bases Azotées. On opère en milieu aqueux ou alcoolique pour les amines et les bases plus fortes que l'aniline. Avec les bases plus faibles on opère dans l'acide acétique anhydre en titrant par l'acide perchlorique.[24,25,26] Il est possible de différencier les amines primaires, secondaires et tertiaires.[27]

Aldéhydes. Par mesure du *p*H atteint après addition de bisulfite on peut effectuer le dosage des aldéhydes.[28]

Cétones. Une méthode analogue à la précédente permettrait le dosage de l'acétone par mesure de l'acidité libérée après addition de chlorhydrate d'hydroxylamine.[29,30]

Les *Quinones* peuvent être titrées avec le thiosulfate.[31]

Alcools. La méthode de Fischer est plus facile si l'on observe le point final par la méthode du "dead stop."[32]

Peroxydes. Les peroxydes organiques peuvent se titrer en suivant leur réduction potentiométriquement.

Utilisation de l'hydrure Li Al H_4. Un excès d'une solution de cet hydrure dans le tétrahydrofurane[33] ajouté au corps à doser, mis lui-même dans le tétrahydrofurane, permet de titrer les groupements fonctionnels suivants: — OH, — NH_2, R_2CO, RCHO, RCO_2R'. L'excès d'hydrure est déterminé potentiométriquement par une solution titrée d'alcool éthylique ou propylique dans le benzène[34] anhydre. Le tout doit être fait à l'abri de l'air et de l'humidité.[35]

Indice de Non-Saturation

Cet indice se mesurant par l'action d'un oxydant, iode ou brome, la potentiométrie est toute indiquée pour son évaluation. Pour la bromuration on emploie, soit du brome en solution dans le tétrachlorure de carbone,[36] soit une solution stable de bromure-bromate.[37] Le dosage est conduit en présence de chlorure mercurique comme catalyseur. La fin du dosage est déterminée par la méthode du "dead stop."

Indice de Saponification

Il a été décrit une méthode dans laquelle l'essai à blanc devient inutile, en déterminant la quantité d'acide utilisée entre deux inflexions de la courbe potentiométrique après saponification.[38]

Signalons une étude critique des méthodes potentiométriques.[39] La saponification a été faite en suivant les techniques officielles. Divers milieux hydro-alcooliques ont été étudiés.

Une mesure à l'échelle semi-micrométrique permet des dosages sur 50 mg d'échantillon[14,40] à l'aide d'acide chlorhydrique 0,02 N.

Dérivés Nitro. Dérivés Nitroso. On réduit par un excès d'une solution de sel titaneux et on dose en retour avec de l'alun ferrique.[41]

II. Dosages d'Elements ou Composes Mineraux

Les éléments ou composés minéraux dont nous allons nous occuper maintenant interviennent soit comme des médicaments d'origine minérale, soit dans des médicaments organo-métalliques ou arsénicaux, soit comme sels minéraux organiques. Nous suivrons dans cet exposé la classification périodique. Un certain nombre des méthodes que nous allons décrire ont été proposées pour des études métallurgiques. Il nous a paru cependant intéressant de les signaler ici étant donné la variété de dosages que l'on doit effectuer dans l'analyse des médicaments.

Cuivre. En mettant en jeu des phénomènes d'oxydo-réduction utilisant comme réactif le sulfate cérique on peut doser le cuivre, amené à l'état cuivreux, par le chlorure chromeux.[42] Un dosage est possible à l'aide d'une solution titrée d'iodure de potassium.[43] On peut également doser le cuivre en ajoutant une solution titrée d'argent[44] avant de doser le cuivre par le cyanure de potassium en milieu ammoniacal. Ce procédé a été proposé pour des alliages. Le titrage par un cyanure peut être réalisé.[45] Il convient de signaler l'influence de la gélatine lors du dosage du cuivre par un cyanure.[46] De bons résultats ont été obtenus en complexant le cuivre avec des sels de l'acide nitrilotriacétique et dosant les ions hydrogènes libérés.[47]

Argent. Comme nous l'avons exposé dans les généralités, le dosage de l'argent se fait remarquablement bien à l'aide d'une électrode d'argent et d'une solution titrée d'halogénure. Le titrage peut également être fait à l'aide d'un cyanure.[48] Une méthode indirecte a été proposée.[49] Elle est utilisable à l'échelle micro ou semi-micro. On ajoute un excès d'iode pour effectuer la réaction $2Ag + I_2 = 2IAg$. L'excès d'iode est titré par de l'arsénite en utilisant le "dead stop." Le protargol et le collargol sont titrables en milieu ammoniacal, soit à l'aide d'une solution connue de thiocyanate, soit avec une solution connue d'iodure.[50] Comme pour le cuivre l'influence de la gélatine a été étudiée.[46] En solution extrêmement diluée 0,002 N, l'argent peut être titré avec une précision de 0,5 pour cent. avec du 1-nitroso-2-naphtol à *p*H 8.[51]

Calcium. Les dosages de calcium interviennent fréquemment lors de l'analyse des médicaments et l'emploi des méthodes potentiométriques peut rendre des services.

On peut doser le calcium[52] potentiométriquement en utilisant une réaction indiquée par Treadwell. Les ions ferriques, mais non les ions ferreux, donnent avec les ions fluor un complexe. Si une solution de chlorure de calcium contenant un sel ferreux et un peu de sel ferrique est titrée par du fluorure de potassium, ce dernier précipite d'abord le calcium. L'excès de fluorure ajouté ensuite complexe des ions ferriques en produisant une brusque variation du potentiel d'oxydo-réduction ferreux/ferrique. On opère en solution alcoolique à 50 pour cent. saturée de chlorure de sodium. Il faut noter que la méthode permet d'obtenir la somme calcium + magnésium lorsque ces métaux sont présents tous deux, ce qui arrive très fréquemment.

Le calcium peut être titré en présence du magnésium par précipitation

à l'aide d'une solution d'oxalate, filtration et détermination de l'excès d'oxalate par du nitrate d'argent.[53] Avec une électrode d'antimoine on peut déceler la fin de la précipitation du calcium par une solution titrée de palmitate de sodium.[54]

Baryum. La méthode au palmitate décrite précédemment est utilisable pour le baryum.

Zinc. Une méthode indiquée pour les alliages peut nous être utile. On précipite le zinc par le ferrocyanure en suivant le dosage avec une électrode de platine.[55] Il est intéressant de signaler que le magnésium, le manganèse et l'aluminium ne gênent pas. Si le fer est présent on doit le complexer à l'aide d'un oxalate. La méthode de formation de complexe indiquée pour le cuivre permet, en mesurant l'acidité libérée par les sels de l'acide nitrilotriacétique, de déterminer le zinc.[47]

Mercure. Le mercure peut être dosé à l'aide d'un iodure. L'influence de la gélatine a été examinée.[46] L'utilisation des "complexants" conduit, par mesure de l'acidité libérée, à de bons résultats.[47]

Acide Borique. Complexé avec du mannitol, l'acide borique peut être titré directement.[56] L'utilisation des milieux non aqueux glycol + alcool divers permet le dosage de l'acide borique et du borax.[57,58] Il serait d'autre part possible de doser l'acide borique dans l'éthylènediamine.[59]

Aluminium. Les métallurgistes ont indiqué un dosage de l'aluminium en mesurant les variations de potentiel du système ferreux/ferrique lorsqu'on ajoute un excès de liqueur titrée de fluorure.[60,61,62,63] Ces études sont intéressantes car elles tiennent compte des autres métaux qui peuvent être présents en même temps que l'aluminium.

Carbonate. On a dosé l'anhydride carbonique, CO_2, par de la baryte dans de l'eau distillée.[64]

Cyanure. Outre l'argentimétrie,[65] un cyanure libre peut être titré par le chlorure de zinc.[66]

Etain. L'étain à l'état de chlorure stannique peut être titré potentiométriquement par une base telle que la quinoléine, l'isoquinoléine ou la pyridine dans l'oxychlorure de sélénium anhydre.[67]

Plomb. On utilise une méthode voisine de celle indiquée pour le calcium et l'aluminium faisant intervenir les variations du potentiel ferreux/ferrique lorsqu'on complexe les ions ferriques avec un fluorure. Le plomb est déterminé à l'aide d'une solution titrée de fluorure alcalin en présence d'un chlorure ou bromure alcalin. Il se précipite un fluochlorure ou fluobromure de plomb et l'excès de fluorure produit une brusque variation du potentiel ferreux/ferrique.[68] On peut effectuer un dosage indirect. Pour cela on ajoute de l'iodate de potassium dont l'excès est titré par du nitrate d'argent.[69] Il est possible de titrer le plomb avec du ferrocyanure; l'influence de divers facteurs a été mise en évidence,[46,70] en particulier celle de la gélatine. Enfin en complexant[47] avec les sels de l'acide nitrilotriacétique, l'acidité libérée peut conduire à une mesure du plomb.

Titane. On rencontre parfois le titane dans les médicaments. Amené à la valence IV, il peut être titré par l'ion chromeux avec une

électrode de mercure. Le dosage peut être conduit automatiquement.[71] Le permanganate a été employé après réduction par du zinc. On peut opérer en présence de fer[72,73] Il est également possible de déterminer le titane à l'aide du système ferreux/ferrique.[74]

Nitrites. On effectue un dosage potentiométrique par l'eau oxygénée en suivant le potentiel d'oxydo-réduction à l'aide d'une électrode de platine.[75]

Ammoniaque. Le dosage de l'ammoniaque serait facilité en opérant en milieu non aqueux et en utilisant une électrode de verre.[58]

Phosphate. Le dosage se fait par argentimétrie. On précipite par un excès de nitrate dans un tampon boraté à *p*H 9. On ajuste ensuite à *p*H 7 ou 8. On filtre et on titre l'excès d'argent avec une solution connue de bromure de potassium.[76]

Arsenic. L'arsenic trivalent est titrable par le bromate de potassium avec des électrodes polarisées.[77]

L'arsenic trivalent et l'antimoine trivalent sont titrés l'un en présence de l'autre[78,79] à l'aide du sulfate cérique. Après titrage de l'antimoine dans des conditions convenables, on ajoute du monochlorure d'iode et on dose l'arsenic.

Antimoine. Après réduction par le chlorure chromeux on peut faire un dosage potentiométrique avec le sulfate cérique.[42] Un dosage identique à celui de l'arsenic peut être effectué avec du bromate de potassium.[77] Comme indiqué précédemment on peut titrer l'arsenic et l'antimoine l'un en présence de l'autre.

Eau. La détermination de l'eau s'effectue très souvent par la méthode de Fischer en observant la fin du dosage par la technique du "dead stop." Dans le cas de médicaments tels que des extraits ou des poudres végétales pour lesquels cette méthode n'est pas encore devenue officielle, il convient d'être prudent et de comparer les résultats donnés par le Fischer avec ceux suivis habituellement par chauffage au bain-marie ou à l'étuve. La durée de contact avec le réactif doit également faire l'objet d'études particulières. Si très souvent les résultats sont identiques à ceux donnés par le chauffage on ne peut généraliser cette observation. Des remarques intéressantes ont été faites.[6,12,80,81,82,83] Il est, de plus, indispensable de consulter l'ouvrage de Mitchell et Smith intitulé "Aquametry" (Interscience Publishers) qui renferme un grand nombre de renseignements tant pratiques que théoriques. Dans les huiles et les graisses il est recommandé[84] d'entraîner l'eau avec de la pyridine sèche. Cette pyridine, recueillie à l'abri de l'air, est titrée au Fischer. Le benzène sec a été utilisé pour d'autres produits. Une autre méthode pour doser l'eau dans des liquides organiques consiste[85] à extraire l'eau par de l'éthylène glycol. Disons pour terminer que certaines substances qui réagissent avec l'iode ne peuvent être titrées par le réactif de Fischer.

Sulfates. Une méthode potentiométrique de dosage des sulfates a été proposée récement.[86] On ajoute du méthanol, du persulfate d'ammonium, puis du chlorure de baryum.

Fluorures. Les fluorures ayant été introduits dans un certain nombre de dentifrices, on peut être amené à effectuer leur dosage. La technique

déjà signalée de formation de complexe avec les ions ferriques, entraînant une variation du potentiel ferreux/ferrique, peut être employée pour déterminer les fluorures par précipitation à l'état de fluochlorure de plomb insoluble.[162]

Halogènes. Le dosage des halogènes, chlorure, bromure et iodure est extrêmement fréquent. On opère par argentimétrie. La potentiométrie est certainement la méthode de choix pour permettre d'apprécier le point d'équivalence. L'électrode d'argent est, comme nous l'avons vu, une électrode simple qui fonctionne parfaitement. Le dosage des halogènes peut être assez facilement effectué à l'échelle micrométrique, après combustion d'un produit organique,[8,87] à l'aide d'une solution de nitrate d'argent 0,01 N en milieu nitrique. Les ions halogènes peuvent être titrés simultanément s'ils sont en quantités sensiblement égales comme l'indique la courbe ci-jointe.[88] (Fig. 4).

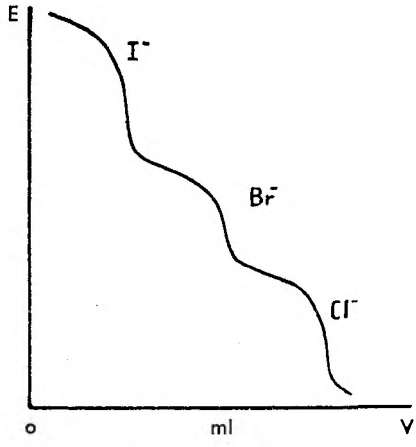


FIG. 4. Halogenures titrés avec le nitrate d'argent 0,01N

On a également proposé le titrage des trois halogènes[89] en présence les uns des autres en milieu ammoniacal. Trois cassures sont visibles: iodure, bromure, complexe chlorure d'argent. Signalons le dosage de Cl^- dans l'eau.[90] La courbe d'étalonnage est une droite de 1 à 10.000 parties par 1,000,000. D'autres auteurs[91,92,93,94,95,96] ont également étudié le dosage des chlorures en solution très diluée. Comme nous l'avons dit dans les généralités on a conseillé le titrage à potentiel défini.[5,97] Cette méthode a été utilisée en présence de cuivre ainsi que pour évaluer des traces de chlore dans l'eau potable.[7] Il est possible de titrer de petites quantités de bromure de l'ordre de 0,1 mg. dans un volume de moins de 1ml.[98] On a pu évaluer les chlorures en présence de l'anhydride sulfureux[99] et doser à la fois un oxalate et un chlorure en présence l'un de l'autre.[100] Signalons pour l'iode une détermination catalytique indirecte,[101] mettant en jeu des réactions d'oxydo-réduction du système As trivalent/Ce tetravalent. Il semble important de signaler la possibilité du dosage simultané des bromures et des thiocyanates (SCN^-)[102] à l'aide d'une électrode d'argent en milieu eau-acétone à 80 pour cent. La technique du "bottled end point" a été utilisée entre autres pour le titrage de l'ion Cl^-[103] Certains auteurs[104] proposent une méthode rapide de dosage des chlorures utilisant deux électrodes métalliques, l'une de cuivre, l'autre d'argent, immergées dans la solution du chlorure à titrer dans laquelle on a ajouté du sulfate de cuivre. Enfin abandonnant l'argentimétrie certains chimistes ont repris potentiométriquement le titrage des chlorures par le nitrate mercurique.[105]

Chlorites. La réduction des chlorites en milieu légèrement alcalin par une solution connue de sulfate ferreux peut conduire à un titrage potentiométrique.[106]

Manganèse. En raison de son aptitude à changer de valence, le manganèse peut se prêter au dosage potentiométrique. Les sels manganeux peuvent être oxydés en pyrophosphate de manganèse trivalent par le permanganate de potassium à *p*H sensiblement neutre 6 à 7. Un grand nombre de métaux ne gênent pas. Le titrage inverse de pyrophosphate de manganèse en sel manganeux peut être réalisé à l'aide d'un sel ferreux.[107] On peut également se servir du passage de Mn divalent à Mn trivalent à l'aide du permanganate en milieu acide en présence de fluorure.[108,109] Il est possible d'utiliser du ferricyanure comme réactif en opérant sous benzène à l'abri de l'air.[110]

Fer. Le facile passage de l'état ferreux à l'état ferrique et inversement a conduit à la réalisation de dosages potentiométriques. C'est ainsi que l'on peut réduire le fer par les ions chromeux.[71] Après réduction à l'état ferreux par un réactif à base d'argent, on peut titrer, en microméthode, par un composé cérique.[111] En milieu non aqueux (oxychlorure de sélénium anhydre) le chlorure ferrique peut être dosé.[67] Une méthode a été indiquée pour titrer les sels ferreux et ferriques les uns en présence des autres. Le sel ferrique est évalué par addition d'un excès de thiosulfate suivi d'un dosage en retour avec une solution connue de chlorure ferrique. Quant au sel ferreux il est déterminé par titration au bromate et la somme totale ferreux + ferrique est donnée par potentiométrie.[112]

Cobalt. Par suite de sa présence dans la vitamine B_{12}, il peut être utile de connaître des dosages potentiométriques du cobalt. Le réactif utilisé en excès est le ferricyanure. On titre en retour par une solution connue de cobalt[113,114,115,116]. Il convient de citer un dosage en présence de "Versene" avec du sulfate cérique.[117]

III. Produits Organiques

Acides. Etant donné que les acides organiques sont le plus souvent des acides faibles, les variations de *p*H ou de potentiel au point final de la réaction sont faibles et peu utilisables. Pour cette raison on opère très fréquemment en milieu non aqueux, soit pour les acides eux-mêmes, soit pour leurs sels. C'est ainsi par exemple que l'on peut titrer dans l'éthylènediamine des acides carboxyliques par le sel de sodium de l'éthanolamine.[118] Ce milieu permet de différencier les acides carboxyliques des phénols. Des acides gras sont déterminés en solution dans le butanol,[119] dans des mélanges glycol, butanol, alcool isopropylique, etc.[120] L'acide acétique glacial est fréquemment employé pour titrer les sels d'acides faibles à l'aide d'acide perchlorique en milieu acétique.[121] Deux acides faibles en mélange seraient titrables en milieu non aqueux.[122] Il faut noter l'utilisation de la coulométrie en acidimétrie. Ce procédé consiste à produire électrolytiquement le réactif titrant, oxydant ou alcali. Ce procédé paraît avantageux dans certains cas. La potentiométrie intervient pour fixer la fin du titrage. On peut citer les travaux suivants[123,124,125]. Cette technique se prête bien à l'analyse automatique.

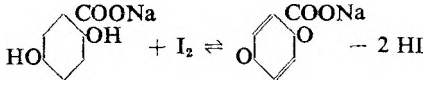
Nous allons maintenant passer en revue quelques acides particuliers.

Acide formique, acide acétique. La potentiométrie permet de titrer en mélange des acides de force différente comme l'acide acétique et l'acide chlorhydrique.[6] Pour les acétates de sodium et de potassium on obtient de meilleurs résultats en milieu alcoolique que dans l'eau.[126]

Acide benzoïque. L'acide benzoïque peut être déterminé en milieu aqueux mais dans l'éthylènediamine cet acide devenant acide fort il est possible alors de le différencier du phénol.[59]

Acide glutarique. La titration s'effectue dans des mélanges aqueux de dioxane et d'eau à 50—65 pour cent. de dioxane.[127]

Acide gentisique. Le dosage par l'iode est impossible à effectuer visuellement par suite de la teinte du milieu. Au contraire ce dosage se conduit facilement par potentiométrie[128] avec une électrode de platine, par de l'iode 0,1 N.



L'acide iodhydrique est fixé par du bicarbonate de sodium. Les courbes potentiométriques sont parfaites.

Acide malonique, acide oxalique, acide succinique. On peut effectuer un titrage de ces acides dans un milieu formé de dioxane (50 à 65 pour cent.) et d'eau.[127] L'argentimétrie permet d'autre part de doser successivement un oxalate et un chlorure l'un en présence de l'autre.[100]

Acide salicylique. L'acide salicylique donne une courbe très convenable dans l'éthylènediamine anhydre comme solvant.[59] La fonction phénol et la fonction acide sont toutes deux bien marquées comme avec les mélanges de phénol et d'acide benzoïque dans ce même milieu.

Les acides gras suivants: acide laurique, myristique, palmitique et stéarique sont titrables avec du nitrate d'argent à des concentrations allant de 0,1 à 0,02 N.[129]

Il convient en terminant de signaler les perturbations apportées par la *présence de charbon adsorbant.* Etant donné qu'un certain nombre de médicaments spécialisés renferment du charbon, il est utile de noter son action.

Il a été démontré[130] que les courbes de titrage des acides forts ont, en présence de charbon adsorbant, les caractères des courbes d'acides faibles. Les dosages sont donc beaucoup plus difficiles. L'étude a été faite pour l'acide picrique, l'acide maléique, l'acide phosphorique, etc. Nous reproduisons quelques courbes. (Figs. 5 and 6.)

Il faut noter d'autre part que, lorsqu'un sel est en solution en présence de charbon, l'expérience montre que la partie acide est plus adsorbée que la partie basique.[131]

Bases. Des faits analogues à ceux rencontrés chez les acides se produisent chez les bases et l'utilisation de solvants non aqueux est fréquente. C'est ainsi que les sels de bases organiques,[121] ou les bases elles-mêmes se titrent bien dans l'acide acétique glacial à l'aide de l'acide perchlorique.[132] L'étude du titrage des sels halogénés de bases organiques dans l'acide

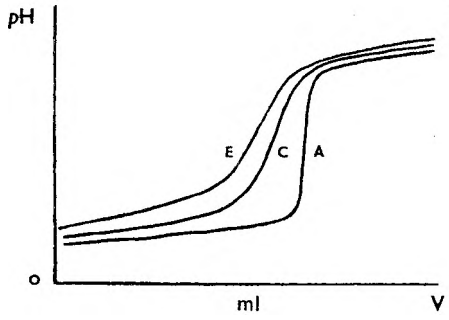


FIG. 5. Acide picrique 0,0078 N titré avec de l'hydroxyde de sodium 0,252 N.

A. Noir Norit 0
C. „ 500 mg.
E. „ 1000 mg.

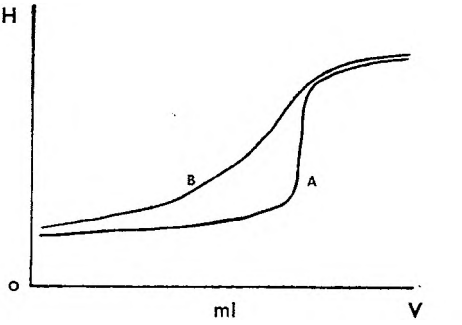


FIG. 6. Acide salicylique 0,008 N titré avec de l'hydroxyde de sodium 0,2535 N.

A sans charbon
B avec charbon

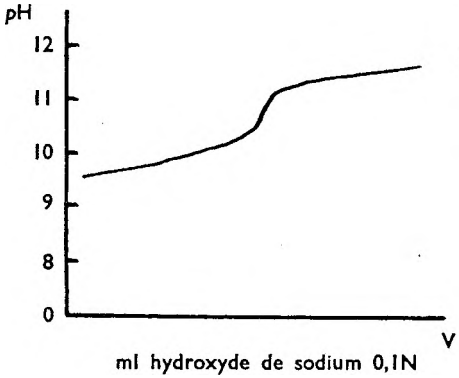


FIG. 7. Sulfate d'éphédrine.

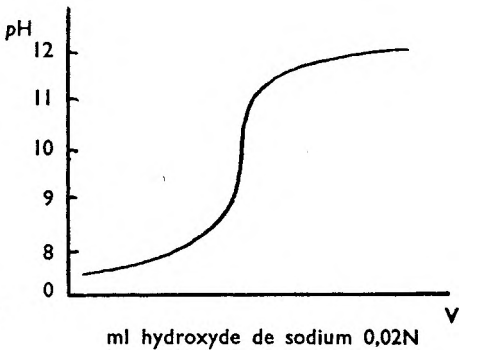


FIG. 8. Sulfate de codéine.

acétique glacial a été faite à l'aide de l'acide perchlorique dissous dans du dioxane en présence d'acétate mercurique et à l'aide d'une électrode de verre.[133] Les auteurs font remarquer que les solvants habituels des préparations pharmaceutiques, alcool éthylique, alcool isopropylique, glycérol, propylèneglycol, carbowax n'interfèrent pas. Signalons en dehors de l'acidimétrie et de l'alcalimétrie une méthode d'oxydation des amines aromatiques par l'iodate de potassium.[134]

Aniline. Un dosage acidimétrique a été proposé en milieu non aqueux, glycol + butanol, etc.,[120] à l'aide d'une électrode de verre.

Alcaloïdes. Un grand nombre d'alcaloïdes ou leurs sels peuvent être titrés par potentiométrie.[135,136,137] Une étude très importante des sels d'alcaloïdes a été exécutée sur une vingtaine d'entre eux[18] en milieu hydro-alcoolique à l'aide d'une solution de soude 0,01 N avec une électrode de verre. Il faut de même attirer l'attention sur toute une série de dosages réalisés dans l'acide acétique anhydre.[138] Nous reproduisons ci-dessous quelques courbes publiées dans un article de Herd[135]. (Figs. 7, 8.)

Nous allons donner les références relatives à un certain nombre de sels de bases organiques. Nous les énumérerons par ordre alphabétique.

Amphétamine sulfate.[138]

Arécoline bromhydrate.[138]

Astérol, 2-diméthylamino-6-(β-diéthylamino éthoxy)-benzothiazol bichlorhydrate.[138]

Atropine nitrate.[138]

Atropine sulfate.[18]

Homatropine bromhydrate.[18]

Brucine.[138]

Caféine: Il a été proposé une méthode de dosage en periodure à l'aide d'iodate de potassium.[139] Par acidimétrie le dosage est possible également.[138]

Cocaïne chlorhydrate.[18]

Dromoran, *dl*-3-hydroxy-N-méthyl morphinan bromhydrate.[138]

Emétine chlorhydrate.[18]

Désoxyéphédrine chlorhydrate.[138]

Ephédrine chlorhydrate. Pseudo-éphédrine chlorhydrate.[18]

Ephédrine sulfate.[135] Le dosage s'effectue en milieu hydro-alcoolique. 100 ml. d'alcool à 95° sont ajoutés à 50 ml. d'une solution de sulfate d'éphédrine à 1 pour cent. On dose avec de la soude 0,1 N. On peut opérer en milieu acétique.[138]

Guanidine chlorhydrate.[138]

Phénylbiguanidine.[138]

Hyoscine bromhydrate.[18]

Nisentil, *dl*-α-1,3-diméthyl-4-phényl-4-propionoxypipéridine chlorhydrate.[138]

Opium, alcaloïdes et dérivés:

Codéine phosphate.[18,138]

Codéine sulfate.[135,138]

Morphine sulfate, chlorhydrate.[18,138,140]

Apomorphine chlorhydrate.[18]

Diamorphine chlorhydrate.[18]

Papavérine chlorhydrate.[18,138]

Pavatrine, β-diéthylaminoéthylfluorène-9-carboxylate chlorhydrate.[138]

Pilocarpine nitrate.[18]

Quinacrine chlorhydrate (Atébrine).[138]

Quinine chlorhydrate.[18]

Quinine sulfate.[13,140]

Quinidine sulfate.[18]

Roniacol, 3-pyridylcarbinol tartrate.[138]

Strychnine chlorhydrate.[18,140]

Syntropan, tropic acid-2,2-diméthyl-3-diéthylaminopropanol phosphate.[138]

Théophylline. Aminophylline. Le dosage potentiométrique se fait par de la soude 0,1 N en milieu aqueux. Le dosage est possible en

presence de phénobarbital. On observe nettement deux points d'inflexion distincts.[141,142]

Thephorin, 2-méthyl-9-phényl-2, 3, 4, 9-tétrahydro-1-pyridindène tartrate.[138]

Enfin signalons les dosages en milieu acétique de bromure de néostigmine, du chlorhydrate, du citrate et du tartrate de choline.[138] Plusieurs des produits précédents ont été dosés dans des formes médicamenteuses telles que des tablettes.[138]

Amino-acides. Etant des acides faibles, les amino-acides peuvent se doser en milieu non aqueux dans l'éthylènediamine anhydre[59] à l'aide du sel de sodium de l'éthanolamine.

Dans l'acide acétique glacial on peut titrer l'histamine et l'histidine sous forme de sels par une solution 0,1 N d'acide perchlorique dans le dioxane en présence d'acétate mercurique.[138] Les sels de ces acides sont difficilement solubles dans l'acide acétique, il est nécessaire de chauffer et de prolonger l'agitation. On opère à l'aide d'une électrode de verre.

Amino-alcools. Il a été décrit un dosage de la *procaïne*[143] par bromométrie par le mélange bromure-bromate avec une solution à 0,001 N de bromate de potassium. La *larocaïne* se titre en milieu alcoolique.[138]

Hydrazine. Il convient de signaler une étude potentiométrique systématique de l'oxydation quantitative de l'hydrazine par l'iodate de potassium. Il s'agissait de tenter un dosage de l'azote hydrazinique.[144] D'après les auteurs la méthode qui est excellente pour le sulfate d'hydrazine pourrait, peut-être, être généralisée pour des dérivés de l'hydrazine. Il faut choisir convenablement le mode opératoire, c'est-à-dire le point final de réduction de l'iodate qui est le chlorure d'iode, l'iode ou l'iodure selon que l'on opére en milieu chlorhydrique, sulfurique ou alcalin. La phénylhydrazine a enfin été titrée à l'aide d'un réactif cuprique.[145]

Alcools. Nous ne reviendrons pas sur la méthode de Fischer dont nous avons parlé au chapitre des groupements fonctionnels. La méthode à l'hydrure d'aluminium et de lithium, déjà signalée, permet le dosage de l'alcool.[33,146] Le *glycérol* se dose à l'aide de bichromate de potassium.[147]

Esters. Après saponification il est possible en titrant l'acide salifié d'utiliser les techniques décrites pour les acides et pour leurs sels.[148]

Sucres. Certains sucres peuvent être titrés potentiométriquement à l'aide de ferricyanure alcalin.[149]

Phénols. Comme nous l'avons dit le solvant est l'éthylènediamine.[19,59,118]

La bromuration du phénol a pu être suivie par le couple d'électrodes platine-noir de platine.[150] Le phénol, le thymol sont oxydés par un excès d'hypobromite et titrés ensuite en retour potentiométriquement.[151]

Amides. L'acétanilide et l'acétamide ont été titrés dans le nitrobenzène avec une électrode à quinhydrone.[152]

Vitamines

Acide ascorbique. Par ses propriétés réductrices l'acide ascorbique

se prête bien à un dosage potentiométrique. Il a été décrit un titrage par l'iode.[153] L'iodate a été également utilisé.[154]

La réaction classique avec le 2,6-dichlorophénolindophénol peut être utilisée en milieu coloré. Ce dosage est très pratique surtout si l'on utilise la technique du "dead stop."[155]

Un dosage tout différent a été proposé. On opère en présence de sel de Mohr, de bromure et on utilise une solution titrée de bromate 0,1 N. La méthode a été employée avec succès pour des tablettes médicamenteuses.[156]

La *thiamine* (B_1) la *pyridoxine* (B_6) ainsi que l'*acide nicotinique* et l'*amide nicotinique* sont titrables, dans l'acide acétique anhydre, par une solution d'acide perchlorique dans le dioxane.[138] (Fig. 9.)

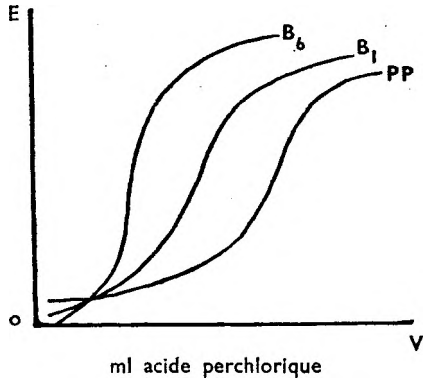


FIG. 9. Vitamines titrés par l'acide perchlorique.
B_1 thiamine
B_6 pyridoxine
PP amide nicotinique

Barbituriques

On titre le phénobarbital dans l'alcool à 95° et on verse de la soude 0,1 N.[135] Le dosage est possible avec des tablettes colorées. (Figs. 10, 11.)

Le *pentobarbital sodique* donne dans l'alcool éthylique d'excellentes courbes de titration avec l'acide sulfurique 0·1N. Les dosages sont suivis avec une électrode de verre.

Des dosages convenables ont pu être réalisés dans des mélanges complexes, tels que phénobarbital, sous-nitrate de bismuth, oxalate de cérium, colorant, ou encore barbiturique, éphédrine.[135]

Rappelons pour mémoire l'analyse des mélanges aminophylline-phénobarbital[141] par la soude 0,1 N en milieu aqueux.

A signaler encore un dosage des barbituriques.[137]

Sulfamides

La réaction au nitrite de sodium se prête bien à un dosage potentiométrique. La courbe des potentiels possède une forme excellente.[157,158]

Les composés suivants ont été titrés avec succès: sulfanilamide, sulfathiazol, sulfaguanidine, sulfadiazine, sulfasuxidine, sulfamérazine sodé, sulfadiazine sodé, sulfathiazol sodé. Les essais effectués sur des tablettes sont satisfaisants.

En milieu non aqueux on peut également titrer les sulfamides dans l'anhydride acétique.[159]

Chloramine

L'action de la chloramine sur une solution titrée d'iodure de potassium peut être utilisée pour réaliser un dosage potentiométrique.[160]

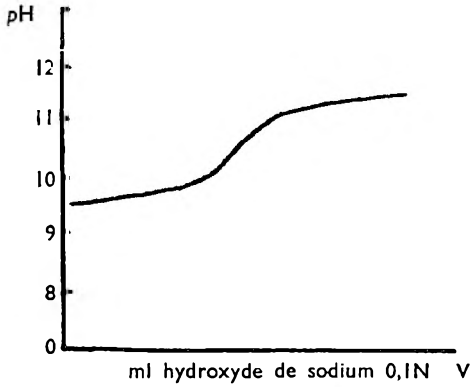


FIG. 10. Phénobarbital.

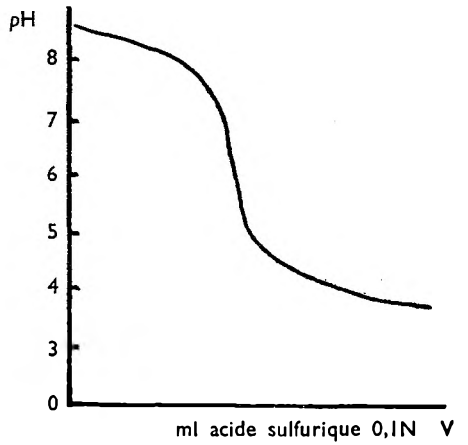


FIG. 11. Pentobarbital sodé.

Sulfure de Carbone

On transforme le sulfure de carbone en xanthate par la soude et l'alcool. On titre ensuite à l'iode soit en tracant la courbe potentiométrique, soit par la technique du "dead stop."[161]

CONCLUSIONS

Il n'était pas possible, dans une revue comme celle-ci, de donner au lecteur des renseignements suffisamment détaillés pour permettre la réalisation d'un dosage. Notre ambition a été uniquement de rassembler les indications bibliographiques indispensables pour conduire à la lecture des mémoires originaux.

Le nombre des publications citées montre que la potentiométrie a rendu de grands services dans l'analyse des médicaments. Elle en rendra certainement beaucoup de nouveaux, spécialement dans les milieux non aqueux, en permettant de s'affranchir de la sujétion des indicateurs colorés. Il semble enfin que, par l'utilisation des "complexants," s'ouvre un champ nouveau de recherche à l'analyste qui voit ainsi s'accroître de jour en jour les moyens mis à sa disposition.

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RESEARCH PAPERS

DERIVATIVES OF DIPHENYL ETHER AS ANTITUBERCULOUS COMPOUNDS

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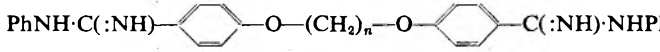
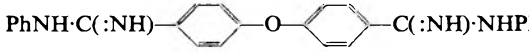
Received May 19, 1952

Derivatives of diphenyl ether exhibit a remarkable range of biological activities; appropriate examples are afforded by thyroxine, alkaloids of the *Menispermaceæ* and *Berberidaceæ* and phenamidine. Appreciable activity *in vitro* against *Mycobacterium tuberculosis* has been observed in the depsidone, physodic acid,[1] in diploicin derivatives[2] and in a series of simple derivatives of diphenyl ether.[3]

Aromatic ethers containing the *N*-arylamidino group exhibit a highly specific antituberculous activity *in vitro* which is maintained in the presence of serum.[4,5] In the di-(4-*N*-arylamidinophenoxy)-alkanes[4] only compounds containing an odd number of atoms in the chain uniting the two phenyl nuclei (A; n = odd number) are active. Accordingly it appeared not unlikely that di-(4-*N*-arylamidino)-diphenyl ethers (B) would possess similar activity.



(A)



(B)

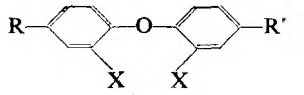
The *N*-arylamidino- and di-(*N*-arylamidino)-diphenyl ethers II, III, IV, V, VI, VIII and IX (Table I) were prepared by interaction of the appropriate arylammonium benzenesulphonate and nitrile.[6] No homogeneous product could be isolated in attempts to prepare 4:4′-di-(*N*-2-pyridylamidino)-diphenyl ether (VII) by the aluminium chloride method[7] but this compound was readily obtained by Pinner's method. 4:4′-Di-(2-benzimidazolyl)-diphenyl ether (X), a cyclic analogue of the diamidines, was prepared by fusion of 4:4′-dicyanodiphenyl ether with 2-aminophenylammonium toluene-4-sulphonate. When 4:4′-dicyanodiphenyl ether was brought into reaction with four equivalents of hydroxylamine, the main product was the corresponding diamidoxime (XI); the monoamidoxime (XII), formed with one equivalent of hydroxylamine, afforded the carbamidoamidoxime when treated with a further equivalent of hydroxylamine in aqueous ethanol. For the formation of the oxygen isosteres of the diamidines, 4:4′-di(*N*-phenylcarbamido)-diphenyl ether (XIV) and 4:4′-di-(*N*-2-pyridylcarbamido)-diphenyl ether (XV), aniline and 2-aminopyridine respectively were acylated with 4:4′-dichloroformyldiphenyl ether.

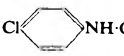
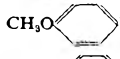
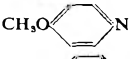
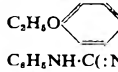
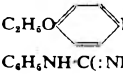
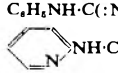
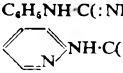
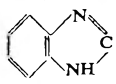
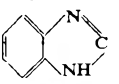
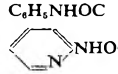
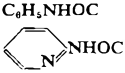
Antituberculous Activity

The activities of the compounds tested are listed in Table I. As was anticipated, the di-(4-*N*-arylamidino)-diphenyl ethers were highly active in the presence of serum. The effects of substituents in the *N*-aryl group in causing a decrease in activity were similar to those observed in the di-(4-*N*-arylamidinophenoxy)alkanes[4] except in the case of the 4-chloro compound (III); in the present series the decrease in activity was considerably less marked: Isosteric replacement of the *N*-phenyl-amidino groups of compound II by *N*-2-pyridylamidino (VII) or 2-benzimidazolyl (X) groups produced a considerable fall in activity; this effect was even more evident in the oxygen isosteres XIV and XV.

TABLE I

Antituberculous activities of diphenyl ether derivatives



No.	R	R'	X	Activity*
I	$NH_2{\cdot}C(:NH)$	$NH_2{\cdot}C(:NH)$	H	5†
II	$C_6H_5NH{\cdot}C(:NH)$	$C_6H_5NH{\cdot}C(:NH)$	H	100
III			H	50
IV			H	50–100
V			H	50
VI	$C_6H_5NH{\cdot}C(:NH)$	$C_6H_5NH{\cdot}C(:NH)$	I	10
VII			H	10
VIII	$C_6H_4NH{\cdot}C(:NH)$	H	H	50–100
IX	$C_6H_3NH{\cdot}C(:NH)$	CH_3O	H	81
X			H	5
XI	$HONH{\cdot}C(:NH)$	$HONH{\cdot}C(:NH)$	H	9
XII	$HONH{\cdot}C(:NH)$	CN	H	5
XIII	COOH	COOH	H	<1
XIV	C_6H_5NHOC	C_6H_5NHOC	H	<1
XV			H	1
XVI	NH_2	NH_2	H	<1

* Dilution in thousands at which complete inhibition of the growth of *M. tuberculosis* (human virulent strain) was maintained for 4 weeks in modified Long's medium (by the floating pellicle method) in the presence of 10 per cent. of serum.
† Tested in the absence of serum.

The foregoing and other structural variations recorded in Table I, all of which led to a decrease in activity, bear little resemblance, where comparison is possible, to the relationships between structure and activity observed by Barry *et al.*[3] in other derivatives of diphenyl ether. Further evidence on the specific effect of the *N*-aryl group, additional to that illustrated by the relative activities of compounds I and II, is afforded

by a comparison of the activity of compound VIII with that of the corresponding unsubstituted amidine (VIII; R = $NH_2 \cdot C(:NH)$; R′ = X = H) prepared by Barry *et al.*[3]; employing a similar method of testing they found this compound to be active at 1 to 10,000.

With compound II some evidence of activity *in vivo* in guinea-pigs was obtained but its toxicity was relatively high. Although compound III had a considerably lower acute toxicity, no activity *in vivo* could be demonstrated because of its high chronic toxicity.

Experimental

4:4′-*Di*-(N-*phenylamidino*)-*diphenyl ether* (II). 4:4′-Dicyanodiphenyl ether[8] (10 g.) and phenylammonium benzenesulphonate (22·6 g., 2 mols.) were heated together, with occasional stirring, in a refluxing nitrobenzene bath (210° C.) for 1 hour. A solution of the cooled melt in ethanol (250 ml.) on pouring into 5 N sodium hydroxide (30 ml.) and crushed ice (100 g.) afforded 4:4′-di-(*N*-phenylamidino)-diphenyl ether which crystallised as leaflets, m.pt. 207° to 208° C., from ethanol; yield 14 g. (69 per cent.). Found: N, 13·8; $C_{26}H_{22}ON_4$ requires N, 13·8 per cent. The dibenzenesulphonate crystallised from methanol in prisms, m.pt. 186° to 188° C. Found: N, 7·5; $C_{38}H_{34}O_7N_4S_2$ requires N, 7·75 per cent.

The following diamidines were similarly prepared from 4:4′-dicyanodiphenyl ether and the appropriate arylammonium benzenesulphonate.[4]

4:4′-*Di*-N-4-*chlorophenylamidino*)-*diphenyl ether* (III). Needles, m.pt. 190° to 190·5° C. with slight decomposition, from ethanol; yield 91 per cent. Found: N, 11·8; $C_{26}H_{20}ON_4Cl_2$ requires N, 11·8 per cent. The dibenzenesulphonate, rosettes of needles from ethanol, had m.pt. 284° to 285° C. Found: N, 6·8; $C_{38}H_{32}O_7N_4S_2Cl_2$ requires N, 7·1 per cent.

4:4′-*Di*-(N-4-*methoxyphenylamidino*)-*diphenyl ether* (IV). Leaflets, m.pt. 231° to 232° C., from ethanol; yield 50 per cent. Found: N, 11·7; $C_{28}H_{26}O_3N_4$ requires N, 12·0 per cent. The dipicrate crystallised as needles, m.pt. 181° to 183° C. with decomposition, from acetic acid. Found in material dried at 110° C. *in vacuo*: C, 51·8; H, 3·8; $C_{40}H_{32}O_{17}N_{10}$ requires C, 51·9; H, 3·5 per cent.

4:4′-*Di*-(N-4-*ethoxyphenylamidino*)-*diphenyl ether* (V). Leaflets, m.pt. 238° to 239° C., from ethanol. Found: N, 11·2; $C_{30}H_{30}O_3N_4$ requires N, 11·3 per cent. The monopicrate crystallised as orange plates, m.pt. 208° to 210° C., from ethanol. Found, in material dried at 110° C. *in vacuo*: N, 13·7; $C_{36}H_{33}O_{10}N_7$ requires N, 13·6 per cent.

2:2′-*Diiodo*-4:4′-*di*-(N-*phenylamidino*)-*diphenyl ether* (VI). The product obtained when 2:2′-diiodo-4:4′-dicyanodiphenyl ether[9] (3·7 g.) and phenylammonium benzenesulphonate (4 g.; 2 mols.) were heated together at 210° C. for 2 hours was dissolved in ethanol (50 ml.); basic material was extracted with aqueous lactic acid from the precipitate formed on adding the ethanol solution to aqueous ammonia, and purified by crystallisation from ethanol. Yield 2·8 g. (54 per cent.). Prisms, m.pt. 246° to 247° C. Found: C, 47·6; H, 3·2; N, 8·5; $C_{26}H_{20}ON_4I_2$ requires C, 47·4; H, 3·0; N, 8·5 per cent.

4:4′-*Di*-(N-2-*pyridylamidino*)-*diphenyl ether* (VII). A suspension of finely powdered 4:4′-dicyanodiphenyl ether (10 g.) in absolute ethanol (70 ml.) was saturated with dry hydrogen chloride at 0° C. and shaken for 2 days. After keeping a further 7 days most of the solid had dissolved. The solvent was removed *in vacuo* and 2-aminopyridine (25·6 g.) dissolved in absolute ethanol (50 ml.) was added. The semi-solid mass, obtained after keeping for 3 days, on crystallisation from a mixture of *iso*propanol and dilute hydrochloric acid, afforded the dihydrochloride trihydrate as prisms, m.pt. 196° to 198° C. after sintering at 180° C. Yield 20 g. (82 per cent.). Found: C, 53·7; H, 5·1; H_2O (Karl Fischer), 10·6; $C_{24}H_{20}ON_6,2HCl,3H_2O$ requires C, 53·8; H, 5·2; H_2O, 10·1 per cent. 4:4′-Di-(*N*-2-pyridylamidino)-diphenyl ether crystallised from ethanol as fine needles, m.pt. 207° to 208° C. with decomposition. Found: C, 70·7; H, 4·8; N, 20·8; $C_{24}H_{20}ON_6$ requires C, 70·6; H, 4·9; N, 20·6 per cent. The dipicrate monohydrate crystallised as needles, m.pt. 213° to 214° C. after sintering at 147° to 150° C., from aqueous acetic acid. Found: N, 19·0; loss at 110° C. *in vacuo*, 2·5; $C_{36}H_{26}O_{15}N_{12},H_2O$ requires N, 19·0; H_2O, 2·0 per cent.

4-N-*Phenylamidinodiphenyl ether* (VIII). This was prepared in the usual way from 4-cyanodiphenyl ether[9] and phenylammonium benzenesulphonate and purified as its benzenesulphonate which crystallised as prisms, m.pt. 157° to 158° C., from water. Yield 84 per cent. Found in material dried at 110° C. *in vacuo*: N, 6·4; $C_{25}H_{22}O_4N_2S$ requires N, 6·3 per cent. The base crystallised as leaflets, m.pt. 139° to 140° C., from light petroleum (b.pt. 100° to 120° C.). Found: N, 9·9; $C_{19}H_{16}ON_2$ requires N, 9·7 per cent.

4-(4-*Methoxyphenoxy*)-N-*phenylbenzamidine* (IX). This was prepared from 4-(4-methoxyphenoxy)-benzonitrile[10] and phenylammonium benezenesulphonate in the manner described for compound VI and obtained as leaflets, m.pt. 130° to 131° C., from light petroleum (b.pt. 100° to 120° C.). Found: N, 9·1; $C_{20}H_{18}O_2N_2$ requires N, 8·8 per cent. The benzenesulphonate crystallised as prisms, m.pt. 158° to 159° C., from *iso*propanol. Found: N, 6·0; $C_{26}H_{24}O_5N_2S$ requires N, 5·9 per cent.

4:4′-*Di*(2-*benzimidazolyl*)-*diphenyl ether* (X). When 4:4′-dicyanodiphenyl ether (4·4 g.) and 2-aminophenylammonium toluene-4-sulphonate (11·2 g.; 2 mols.) were heated together at 210° C. an exothermic reaction occurred; after this had subsided (20 minutes) the resulting solid was heated at 210° C. for 15 minutes. The cooled product, after extraction with hot water to remove ammonium toluene-4-sulphonate, was dissolved in aqueous lactic acid and the crude base, m.pt. 350° to 354° C., liberated by ammonia, was crystallised as leaflets from nitrobenzene. 4:4′-Di-(2-benzimidazolyl)-diphenyl ether had m.pt. above 360° C. Yield 5 g. (62 per cent.). Found: N, 14·0; $C_{26}H_{18}ON_4$ requires N, 13·9 per cent. Its monopicrate crystallised from aqueous cellosolve as needles, m.pt. 285° to 287° C. with decomposition. Found in material dried at 110° C. *in vacuo*: N, 15·7; 15·6; $C_{32}H_{21}O_8N_7$ requires N, 15·5 per cent.

4:4′-*Diamidoximinodiphenyl ether* (XI). (*a*) 4:4′-Dicyanodiphenyl

ether (11 g.) was dissolved in ethanol (500 ml.) and a solution of hydroxylamine in water (30 ml.) prepared from the hydrochloride (7 g.; 2 mols.) and sodium carbonate (5·3 g.; 1 mol.) was added. The mixture was refluxed for 28 hours, filtered and evaporated to about 50 ml. The solid which separated was repeatedly crystallised from aqueous ethanol and afforded 4-(4-cyanophenoxy)-benzamidoxime (XII) as prisms, m.pt. 196° to 197° C., with decomposition. Yield 5·3 g. (42 per cent.). Found: C, 66·5; H, 4·5; N, 16·3; $C_{14}H_{11}O_2N_3$ requires C, 66·4; H, 4·4; N, 16·6 per cent. 4-(4-Cyanophenoxy)-benzamidoxime on refluxing for 24 hours with aqueous ethanolic hydroxylamine (2 mols.) gave 4-(4-carbamidophenoxy)-benzamidoxime which crystallised from aqueous *iso*propanol as plates, m.pt. 172° to 173° C., with decomposition. Found: N, 15·4; $C_{14}H_{13}O_3N_3$ requires N, 15·5 per cent.

(*b*) The dinitrile (11 g.) and hydroxylamine (8 mols.) were heated together in aqueous ethanol at 65° to 70° C. for 24 hours. The crude product, obtained as described above, after repeated crystallisation from aqueous *iso*propanol yielded 4:4′-diamidoximinodiphenyl ether as prisms, m.pt. 193° to 194° C., with decomposition, depressed to 178° to 180° C. on admixture with 4-(4-cyanophenoxy)-benzamidoxime. Yield 3 g. (21 per cent.). Found: C, 58·5; H, 4·9; N, 19·7; $C_{14}H_{14}O_3N_4$ requires C, 58·7; H, 4·9; N, 19·6 per cent.

No improvement in the yield was effected by carrying out the reaction in absolute ethanol using a solution of hydroxylamine prepared from the hydrochloride and sodium ethoxide or triethylamine.

4:4′-*Dichloroformyldiphenyl ether.* 4:4′-Dicyanodiphenyl ether on boiling with sulphuric acid (70 per cent.) for 3 hours afforded 4:4′-dicarboxydiphenyl ether (XIII) which crystallised as prisms, m.pt. 328° to 330° C., from acetic acid. Yield 85 per cent. Found: Eq. wt., 132; required, 129. Schickh[11] records no melting point for this acid. The dicarboxylic acid (21·5 g.) and thionyl chloride (41 ml.) were refluxed together for 4 hours. The excess of thionyl chloride was removed under reduced pressure and the residue was crystallised from light petroleum (b.pt. 100° to 120° C.); prisms, m.pt. 82° to 83° C. Found: C, 57·3; H, 2·9; $C_{14}H_8O_3Cl_2$ requires C, 57·0; H, 2·7 per cent.

4:4′-*Di*-(N-*phenylcarbamido*)-*diphenyl ether* (XIV). This was prepared from the diacyl chloride described above and aniline under Schotten–Baumann conditions and crystallised from acetic acid as leaflets, m.pt. 297° to 299° C. Found: N, 6·9; $C_{26}H_{20}O_3N_2$ requires N, 6·9 per cent.

4:4′-*Di*-(N-2-*pyridylcarbamido*)-*diphenyl ether* (XV). 4:4′-Dichloroformyldiphenyl ether (7·7 g.) and 2-aminopyridine (7·5 g.) were boiled together in dry benzene (60 ml.) for 30 minutes; the solvent was evaporated and the residue was extracted first with water and then with dilute hydrochloric acid. The base liberated by ammonia crystallised from ethanol as prisms, m.pt. 181° to 182° C. Yield 4 g. (32 per cent.). Found: N, 13·4; $C_{24}H_{18}O_3N_4$ requires N, 13·7 per cent.

4:4′-*Diaminodiphenyl ether* (XVI). 4:4′-Dinitrodiphenyl ether, reduced in acetic acid with stannous chloride and hydrochloric acid, afforded

the diamine in 85 per cent. yield. This by interaction with benzenesulphonic acid in water gave the dibenzenesulphonate which crystallised from methanol as needles, m.pt. 286° to 288° C., with decomposition. Found: N, 5·5; $C_{24}H_{24}O_7N_2S_2$ requires N, 5·4 per cent.

The author gratefully acknowledges his indebtedness to Mr. C. E. Coulthard, Dr. L. Dickinson and Miss B. Croshaw, Boots Pure Drug Co., Ltd., for the biological tests.

Summary

1. A number of derivatives of diphenyl ether have been prepared for examination of their antituberculous activity.

2. Some evidence of activity *in vivo* was obtained with 4:4′-di-(*N*-phenylamidino)-diphenyl ether but the compound was toxic.

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THE SYNTHESIS OF TWO ISOMERIC α-HYDROJUGLONE GLUCOSIDES

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Received May 12, 1952

ATTEMPTS to synthesise α-hydrojuglone-5-β-D glucoside isolated[1] from the walnut (*Juglans regia*) and shown[2] to be responsible for the "apparent vitamin C" activity,[3] gave rise to two isomeric tetra-acetylated glucosides[4] which could be further acetylated, apparently, to the same hexa-acetate. It was assumed that these compounds were the 5-acetylated glucosides of α- and β-hydrojuglones, because both these isomers had proved suitable starting material. This work has been expanded, and it would now appear that whereas the first compound is α-hydrojuglone-5-tetra-acetyl-β-D glucoside, which by hydrolysis of its acetyl groups becomes identical with the compound isolated from the walnut, the second has the glucose moiety attached to the naphthalene nucleus through the hydroxyl in the 1 position. Details of the work leading to these conclusions are given below.

EXPERIMENTAL

Tetra-acetyl-α-D-glucosyl bromide was prepared from glucose penta-acetate[5] in 84·5 per cent. yield.

α-Hydrojuglone (1:4:5-trihydroxynaphthalene) was prepared from juglone by the method of Willstatter and Wheeler.[6] The ethereal solution (of α-hydrojuglone) was filtered through a column of dried magnesium sulphate into light petroleum (b.pt. 60° to 80° C.) and the α-hydrojuglone rapidly separated as colourless needles m.pt. 148° C. Yield 89·7 per cent.

β-Hydrojuglone (5-hydroxy-2:3-dihydro-1:4-naphthoquinone) was prepared by vacuum distillation of α-hydrojuglone from an oil bath at 170° C. and recrystallising the distillate from ethanol to give pale yellow needles m.pt. 96° to 97° C. Yield (from juglone) 65 per cent.

Tetra-acetylated glucosides. (*a*) Juglone 6·96 g. (0·04 mole) and tetra-acetylglucosyl bromide 16·44 g. (0·04 mole) were dissolved in dry acetone 600 ml. 1·5N ethanolic potassium hydroxide 26·5 ml. (0·04 mole) was added under nitrogen, then the flask was stoppered and left for 16 hours at room temperature in the dark. The dark violet reaction mixture was filtered, the filtrate was evaporated to dryness under reduced pressure and the residue extracted with ethanol, 600 ml. The extract showed (spectrophotometrically) the presence of 1·06 g. of α-hydrojuglone-5-tetra-acetyl-β-D glucoside (5·25 per cent. of theory). This could not be separated from the tarry side-reaction products by the normal methods of crystallisation, so the extract was chromatographed on alumina in the manner described for the natural glucoside.[1] This gave 0·84 g. (4·2 per cent.) of α-hydrojuglone-5-tetra-acetyl-β-D glucoside.

(*b*) α-Hydrojuglone 5·27 g. (0·03 mole) and tetra-acetylglucosyl bromide 12·33 g. (0·03 mole) were dissolved in acetone 70 ml. and 1·5N

ethanolic potassium hydroxide 20 ml. (0·03 mole) added under nitrogen. After 16 hours, glacial acetic acid 2 ml. was added and the mixture filtered from the sandy precipitate of potassium bromide which had first separated after 15 minutes. The filtrate was evaporated to dryness under reduced pressure and the yellow-brown crumbly residue stirred with ether 20, 10 and 10 ml., transferred to a filter and sucked dry. The almost white residue was stirred with ethanol (95 per cent.) 20 ml. refiltered and washed with a further 10 ml. This gave 7·78 g. of mixed tetra-acetylated glucosides. Taken up in boiling ethanol 75 ml. α-hydrojuglone-1-tetra-acetyl-β-D glucoside crystallised immediately on cooling (6·34 g.).

The mother liquors, together with the ethereal and ethanolic washes, were evaporated to dryness under reduced pressure. The residue, extracted with chloroform, precipitated with light petroleum (b.pt. 60° to 80° C.) and recrystallised from benzene, gave α-hydrojuglone-5-tetra-acetyl-β-D glucoside 3·59 g. Overall yield 65·4 per cent.

(*c*) β-Hydrojuglone 5·28 g. (0·03 mole) treated in the same manner gave α-hydrojuglone-1-tetra-acetyl-β-D glucoside 5·27 g. and α-hydrojuglone-5-tetra-acetyl-β-D glucoside 3·00 g. Overall yield 54·6 per cent.

α-*Hydrojuglone-5-tetra-acetyl-β-D-glucoside.* Readily soluble in ethanol, acetone, ether, chloroform, ethyl acetate, benzene and acetic acid; less so in light petroleum and insoluble in water; it crystallised from benzene as white microcrystals m.pt. 179° C. (decomp.). Found: C, 55·8; H, 5·79; CH_3CO, 33·7 per cent.; $C_{24}H_{26}O_{12}$ requires, C, 56·9; H, 5·14; CH_3CO, 34·0 per cent. It slowly decomposed in ethanol solution but was quite stable in acid ethanol, in which it showed (Fig. 1)

$\lambda_{max.}$	*225*	*308*	*326*	*341* mμ.
$E^{1\ per\ cent.}_{1\ cm.}$	791	123·3	122·3	117·6

and $[\alpha]_D^{20°\ C.}$ — 53·5° C. 0·04 per cent.

1·15 g. was refluxed for 10 minutes with acetic anhydride 9 ml. and anhydrous sodium acetate 1 g., the mixture was cooled and diluted with water and the precipitated solid recrystallised from ethanol then ether, gave the hexa-acetate of α-hydrojuglone-5-β-D glucoside, 0·85 g. (63·5 per cent.) as tiny white needles m.pt. 140° to 141° C. Found: C, 56·9; H, 5·22; CH_3CO, 41·0 per cent. $C_{28}H_{30}O_{14}$ requires, C, 57·0; H, 5·08; CH_3CO, 43·7 per cent.

0·25 g. in methanol, 10 ml., treated with 3 per cent. w/v ethereal diazomethane solution, 20 ml., for 3 hours, gave on evaporation and recrystallisation of the residue from methanol 4 ml., 0·177 g. of 1:4-dimethoxy-5-hydroxynaphthalene-5-tetra-acetyl-β-D glucoside m.pt. 174° to 175° C. Found: C, 58·2; H, 5·58; CH_3O, 11·65 per cent. $C_{26}H_{30}O_{12}$ requires, C, 58·4; H, 5·6; CH_3O, 11·6 per cent.

The dimethoxy derivative, 0·15 g., in methanol, 10 ml., refluxed for 30 minutes with 2N hydrochloric acid, 7·5 ml., gave 5-hydroxy-1:4-dimethoxynaphthalene 0·03 g. (55 per cent.) m.pt. 155° to 156° C. The melting point was not depressed by admixture with an authentic sample prepared from juglone acetate by the method of Ruelius and Gauhe.[7] Found: C, 69·7; H, 5·85 per cent. $C_{12}H_{12}O_3$ requires C, 70·6; H, 5·88 per cent.

α-Hydrojuglone-5-β-D-glucoside. (*a*) A solution of α-hydrojuglone-5-tetra-acetyl-β-D glucoside, 1 g., in methanol, 25 ml., from which the air had been displaced by a stream of nitrogen, was treated for 1 hour with 2N ethanolic potassium hydroxide, 2 ml. The colour became deep purple but this was discharged on adding 2N sulphuric acid, 2 ml. The mixture was

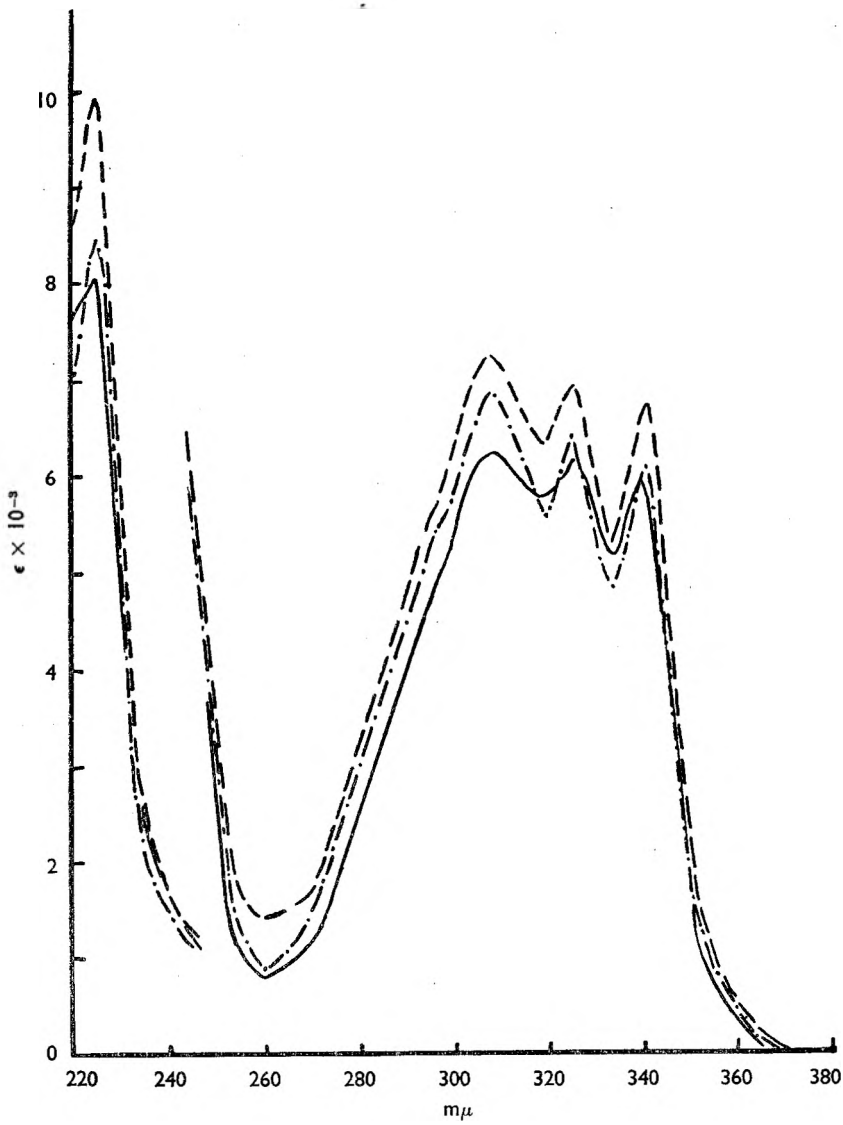


FIG. 1. Absorption curves in 0·1N ethanolic hydrochloric acid of
——— α-hydrojuglone-5-tetra-acetyl-β-D glucoside.
– – – α-hydrojuglone-5-β-D glucoside, synthetic.
–·–·–· α-hydrojuglone-5-β-D glucoside, natural.
Left-hand curve is 1–5 dilution of that on right.

centrifuged to remove precipitated potassium sulphate, and the supernatant liquid diluted with water, 25 ml., and evaporated at reduced pressure till all the methanol had been removed. The aqueous residue was extracted with ethyl acetate to give α-hydrojuglone-5-β-D glucoside 0·32 g. (48·2 per cent.).

(*b*) A solution of the hexa-acetate of α-hydrojuglone-5-β-D glucoside, 1·59 g., in methanol, 25 ml., was hydrolysed in the same manner, giving 0·55 g. (59 per cent.) of α-hydrojuglone-5-β-D glucoside.

The glucoside was a light fawn coloured microcrystalline powder. Found: C, 57·7; H, 5·87 per cent. $C_{16}H_{18}O_8$ requires C, 56·8; H, 5·32 per cent. The melting point 178° C. (decomp.) was not depressed by admixture with the glucoside isolated from the walnut.

α-*Hydrojuglone*-1-*tetra-acetyl*-β-D *glucoside.* Readily soluble in chloroform, acetone, ethyl acetate and acetic acid; less so in ether, ethanol and light petroleum; it crystallised from ethanol as white needles m.pt. 196° to 197° C. Found: C, 56·5; H, 5·14; CH_3CO, 33·1 per cent. $C_{24}H_{26}O_{12}$ requires C, 56·9; H, 5·14; CH_3CO, 34·0 per cent. Solutions in acid ethanol were quite stable and showed (Fig. 2)

$\lambda_{max.}$	*238*	*315*	*333*	*343*	mμ
$E^{1\ per\ cent.}_{1\ cm.}$	337	130	157·4	155·2	

and $[\alpha]_D^{20°\ C.}$ — 75·5° C. 0·1 per cent.

1 g. refluxed for 10 minutes with acetic anhydride, 5 ml., and anhydrous sodium acetate, 1 g., cooled, added to water, 70 ml., and the precipitated solid recrystallised from ethanol then ether, gave the hexa-acetate 0·8 g. (68·5 per cent.) m.pt. 140° to 141° C. as tiny white needles. Found: C, 57·2; H, 5·3; CH_3CO, 43 per cent. $C_{28}H_{30}O_{14}$ requires C, 57·0; H, 5·1; CH_3CO, 43·7 per cent. The melting point did not appear to be depressed by admixture with the hexa-acetate prepared from the natural glucoside nor with that from α-hydrojuglone-5-tetra-acetyl-β-D glucoside, and like these it showed in acid ethanol

$\lambda_{max.}$	*226*	*288*	*295*	*325*	mμ
$E^{1\ per\ cent.}_{1\ cm.}$	1092	126·8	144	41·8	

$[\alpha]_D^{20°\ C.}$ — 83·7° C. 1 per cent.

0·5 g. in methanol, 40 ml., treated with 3 per cent. w/v ethereal diazomethane solution, 50 ml., for 1 hour at room temperature, the solvent removed at the pump and the residue recrystallised from 4 ml. of methanol, gave the monomethoxy derivative, 0·222 g., as microneedles, m.pt. 148° to 149° C. Found: C, 57·3; H, 5·3; CH_3O, 6·26 per cent. $C_{25}H_{28}O_{12}$ requires C, 57·7; H, 5·38; CH_3O, 6·0 per cent.

α-*Hydrojuglone*-1-β-D *glucoside.* (*a*) A solution of α-hydrojuglone-1-tetra-acetyl-β-D glucoside, 1 g., in methanol, 25 ml., was hydrolysed for 1 hour at room temperature with 2N ethanolic potassium hydroxide, 2 ml., under nitrogen and the free glucoside extracted with ethyl acetate as described above. Yield 0·5 g. (75 per cent.) m.pt. 179° to 180° C.

(*b*) The hexa-acetate of α-hydrojuglone-1-β-D glucoside, 1·9 g., in

methanol, 25 ml., treated in the same manner gave 0·77 g. of glucoside (70·3 per cent.) m.pt. 179° to 180° C. Readily soluble in water, ethanol and acetone, less so in ethyl acetate and *iso*amyl alcohol and insoluble in ether, chloroform and light petroleum, it crystallised from ethyl acetate as white microcrystals, m.pt. 179° to 180° C. Found: C, 55·5; H, 5·41

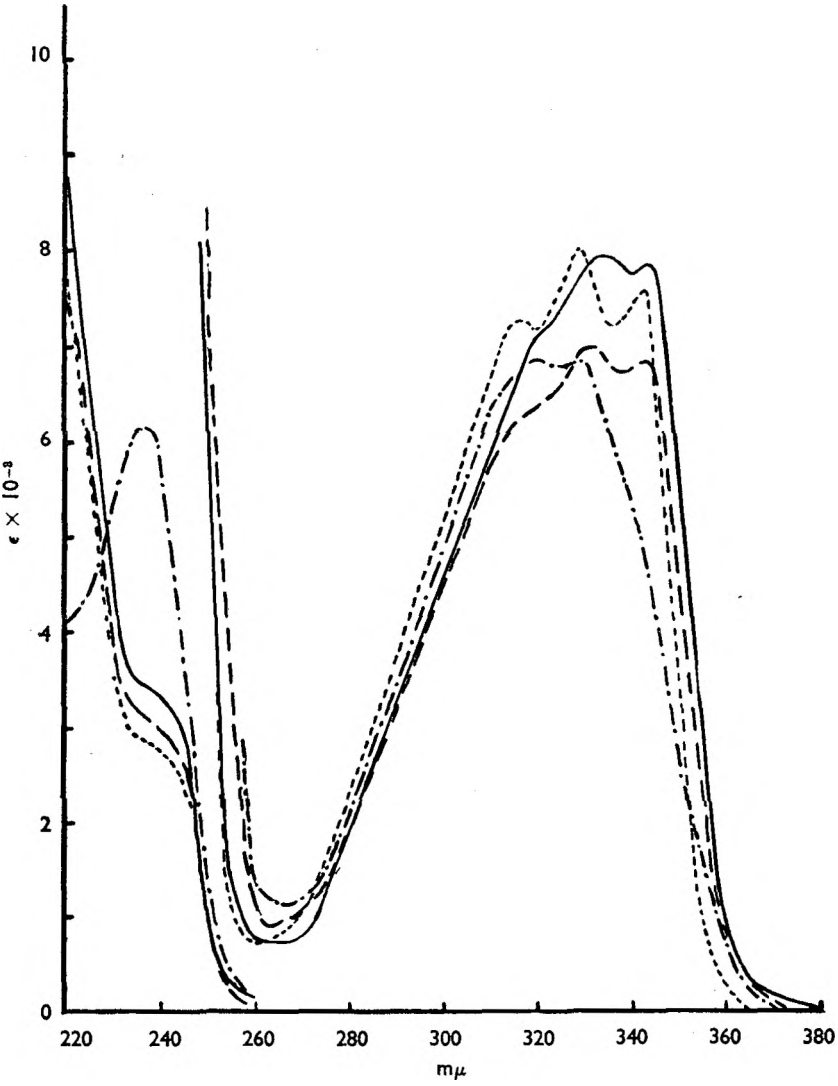


FIG. 2. Absorption curves in 0·1 N ethanolic hydrochloric acid.

———— α-hydrojuglone-1-tetra-acetyl-β-D glucoside.
— — — α-hydrojuglone-1-β-D glucoside.
- - - - - α-hydrojuglone-1-β-D glucoside methoxy derivative.
- · - · - · 1 : 8-dihydroxynaphthalene-4-sulphonic acid.

Left-hand curve is 1–5 dilution of that on right.

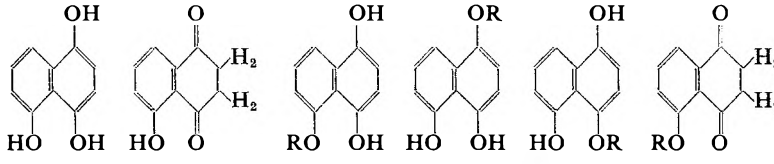
per cent. $C_{16}H_{18}O_8$ requires C, 56·8; H, 5·32 per cent. Ethanolic solutions were unstable, but in acid ethanol it showed (Fig. 2)

$\lambda_{max.}$	*240*	*320*	*333*	*344*
$E^{1\text{ per cent.}}_{1\text{ cm.}}$	444	187·8	207·5	203·5

and $[\alpha]_D^{20°\,C.}$ — 93° C. 0·1 per cent.

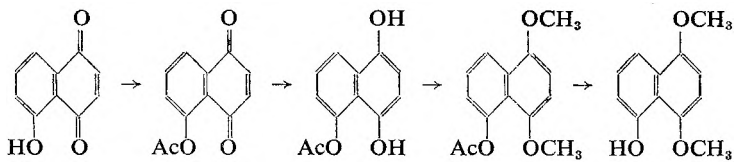
Discussion

The formation of two acetylated glucosides in a constant proportion of 1:1·75 from both α- and β-hydrojuglone (I and II) by the action of tetra-acetylglucosyl bromide in the presence of one equivalent of potassium hydroxide, suggests that one of these has structure III, whilst the other is IV, V or VI.



where R is $C_{14}H_{19}O_9$.

The isomer more readily soluble in ether, on hydrolysis of the acetyl groups gives rise to a glucoside identical with that found in acid ethanol extracts of the walnut. That this has structure III is shown by its reaction with diazomethane to give a dimethoxy derivative, which on heating with acid is hydrolysed to 5-hydroxy-1:4-dimethoxynaphthalene. This latter compound has been synthesised by the method of Ruelius and Gauhe[7] as follows



It has been suggested[4] that the second tetra-acetylated glucoside has structure VI. The evidence for this was based upon the facts that it was readily prepared from β-hydrojuglone, and that its hexa-acetate appeared in melting point and ultra-violet absorption data (Fig. 3) identical with that obtained from III. It showed, however, no ketonic properties, and attempts to prepare derivatives with *p*-nitrophenylhydrazine, phenylhydrazine and phenylsemicarbazide resulted only in the formation of coloured solutions from which the bulk of the glucoside was recovered unchanged. Infra-red examination of this compound revealed that instead of the carbonyl groups expected, there was absorption due to two hydrogen bonded hydroxyl groups.

Of the alternative structures IV and V, the former, with a *peri*naphthalene structure would be expected to show some hydrogen bonding, whereas the latter would not, unless the glucose portion of the molecule

were involved. It is probably due to this bonding that with diazomethane only a monomethoxy derivative is formed by the glucoside. Since the same result is obtained with 1 : 8-dihydroxynaphthalene[8,9] it appears that IV is the more likely structure for the second glucoside. Some further slight evidence for this is shown (Fig. 2) in the similarity of its ultra-violet absorption curve with that of 1 : 8-dihydroxynaphthalene-4-sulphonic acid.

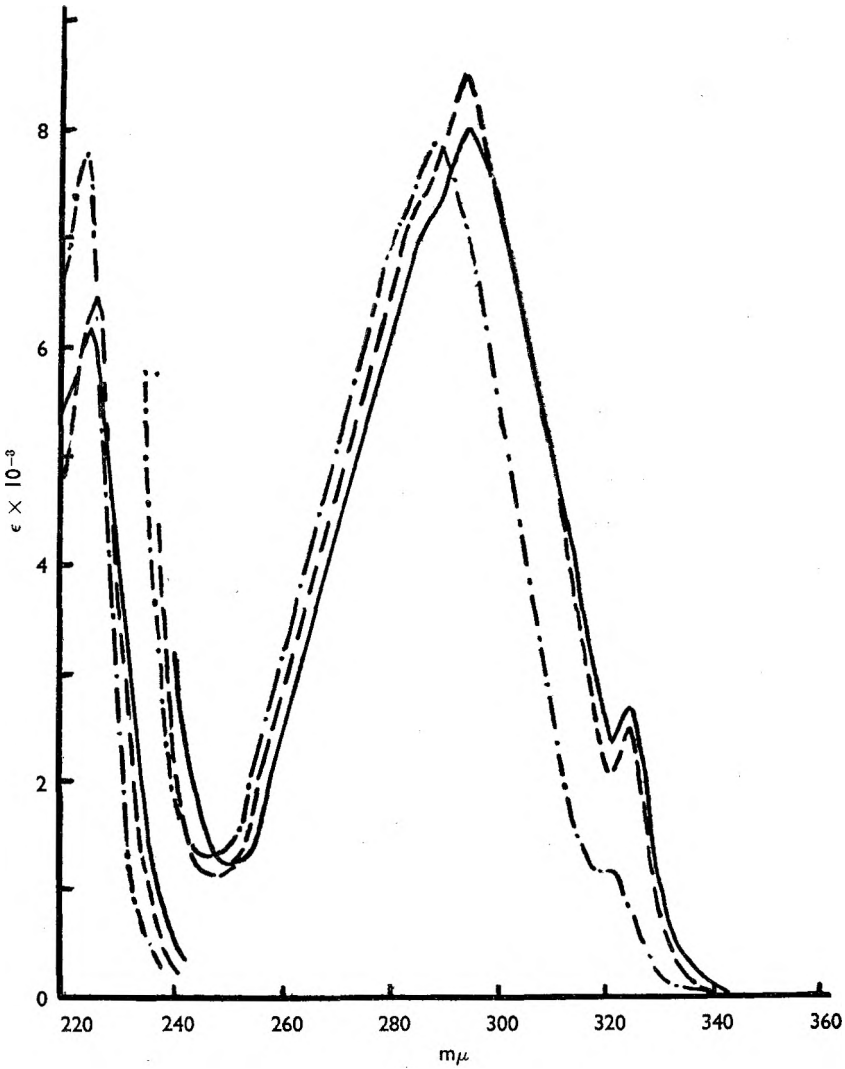


FIG. 3. Absorption curves in 0·1N ethanolic hydrochloric acid of
——— Hexa-acetate of α-hydrojuglone-5-β-D glucoside.
– – – – Hexa-acetate of α-hydrojuglone-1-β-D glucoside.
– · – · – · 1 : 4 : 5-triacetoxynaphthalene.
Left-hand curve is 1–10 dilution of that on right.

SUMMARY

1. Two tetra-acetylated glucosides have been prepared in 55 to 65 per cent. yield from both α- and β-hydrojuglone.

2. Removal of the acetyl groups from the more ether-soluble of these gives α-hydrojuglone-5-β-D glucoside identical with that isolated from the walnut.

3. It has been shown that the second glucoside is not β-hydrojuglone-5-β-D glucoside as previously reported, but probably α-hydrojuglone-1-β-D glucoside.

I wish to thank Dr. F. Wokes, the Director of the Laboratories, for affording the opportunity to publish this work (part of which was included in the thesis for a Ph.D. degree of the University of London), Miss Nora Baxter for technical help and Dr. R. H. Thomson of Aberdeen for a gift of 1 : 8-dihydroxynaphthalene-4-sulphonic acid. Carbon, hydrogen, acetyl and methoxyl determinations were made by Drs. Weiler and Strauss of Oxford.

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THE SPECTROPHOTOMETRIC IDENTIFICATION AND ESTIMATION OF STRYCHNINE, BRUCINE AND MORPHINE IN VISCERA EXTRACTS

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Received May 9, 1952

INTRODUCTION

THE extraction of substances such as alkaloids from toxicological specimens and their identification when present in small amounts has been the subject of much study, ably summarised by Bamford,[1] Authenreith[2] and Turfitt.[3] In the case of alkaloidal extracts the detection proceeds by a series of colour reactions, each of which uses, without possibility of recovery, an appreciable aliquot of a sample often submitted in small quantity. Many of the tests yield negative results with further waste of valuable material and some are by no means specific: thus yohimbine and the alkaloids of *Gelsemium elegans* give blue colours with Fröhde's reagent so that an investigator may well be put on the wrong track in the initial stages. Furthermore impurities often interfere with colour reactions; a purification process may be required and the amount of extract reduced even more.

Turfitt (*loc. cit.*) has noted the use which may be made of spectrophotometric analysis in toxicological work. Elvidge[4] and Brustier[5] have studied the absorption spectra of many pure alkaloids and found not only that most have pronounced absorption bands in the far ultra-violet region but that these absorption bands are sufficiently specific to be used for identification.

We have applied spectrophotometry to the detection and estimation of the alkaloids, particularly strychnine, brucine and morphine, in Stas-Otto extracts from viscera and some natural products. We have standardised the procedure by measuring the absorption spectra of the pure alkaloids in ethanolic solutions and tested the method with viscera extracts containing known amounts of strychnine, brucine, and morphine. We have had in view the possibility of detecting strychnine and brucine in both purified and crude Stas-Otto extracts, the crude extracts being those obtained by the normal Stas-Otto process without subsequent purification. We have also studied the quantitative estimation of these alkaloids, both singly and when present as a mixture. Experiments have been made with Stas-Otto extracts of viscera free from alkaloids to determine the extent to which other materials may be extracted and interfere with the spectrophotometric determination.

It is not proposed to describe the methods of determining absorption spectra or the analysis of a spectral curve because these have already been dealt with adequately, e.g., by Twyman and Lothian[6] and by Brode.[7] The usual precautions were taken against solvent impurities, instrumental aberration and in the use of cells. All extinction coefficients are expressed

in the usual form of $E_{1\ cm.}^{1\ per\ cent.}$, i.e., by log I_0/I where I_0 is the intensity of the incident light and I that of the light emerging from a 1 cm. cell containing a 1 per cent. solution of the alkaloid in question, it being assumed that Beer's Law can be used to express the factor between a 1 per cent. solution and that used.

Experimental

I. General

All absorption spectra were measured with a Beckmann Spectrophotometer, Model DU, using a hydrogen discharge tube as an ultra-violet radiation source. Cells were of quartz, of accurately known cell length and the optical faces were kept perfectly clean. The spectral range examined lay between 2100Å and 3500Å. Extinction coefficients were measured at intervals of not more than 20Å, but at critical points, i.e., turning points, inflexions, etc., measurements were made at distances 5Å apart. Extinction values for many solutions were repeated several times, and the instrumental error, in the density range 0·4 to 1·0, was found to be 1 per cent. or less. In some cases solutions were prepared in duplicate and it was found that their absorption curves were practically identical, the differences not exceeding 1 per cent. except for readings taken at the lower range of the "density" drum, which were of no great significance in this work.

The chemicals used in this work were of B.P. quality and their purity was checked by a series of melting point determinations. Ethanol, used as a solvent, was allowed to stand over silver nitrate for several days with periodic shaking and was finally distilled over caustic potash. A spectrophotometric examination of the ethanol did not reveal the presence of any impurities.

The concentrations of the experimental solutions were arranged so that maximum "density" readings would not exceed 1·0. This precaution was taken to ensure accuracy, since all readings above 1·0 and below 0·4 on the "density" drum have a greater degree of error than those between 0·4 and 1·0.

II. The Absorption Spectra of Strychnine, Brucine and Morphine

Strychnine, brucine and morphine were dissolved in ethanol (95 per cent. v/v) to give solutions of 0·0025 per cent. w/v, 0·0025 per cent. w/v and 0·005 per cent. w/v respectively. The ultra-violet absorption spectra were determined and the curves are shown in Figure 1. Those for strychnine and brucine indicate pronounced absorption bands whilst the curve for morphine shows one less pronounced absorption band in the same region. The wavelengths at which maxima and minima occur for these solutions and the extinction coefficients at these wavelengths are given in Table I.

III. The Absorption Spectra of Strychnine, Brucine and Morphine Extracted from Specimens of Viscera

The Stas-Otto process which was used to extract the alkaloids from specimens of viscera has been described in many texts[1,2] and is still recog-

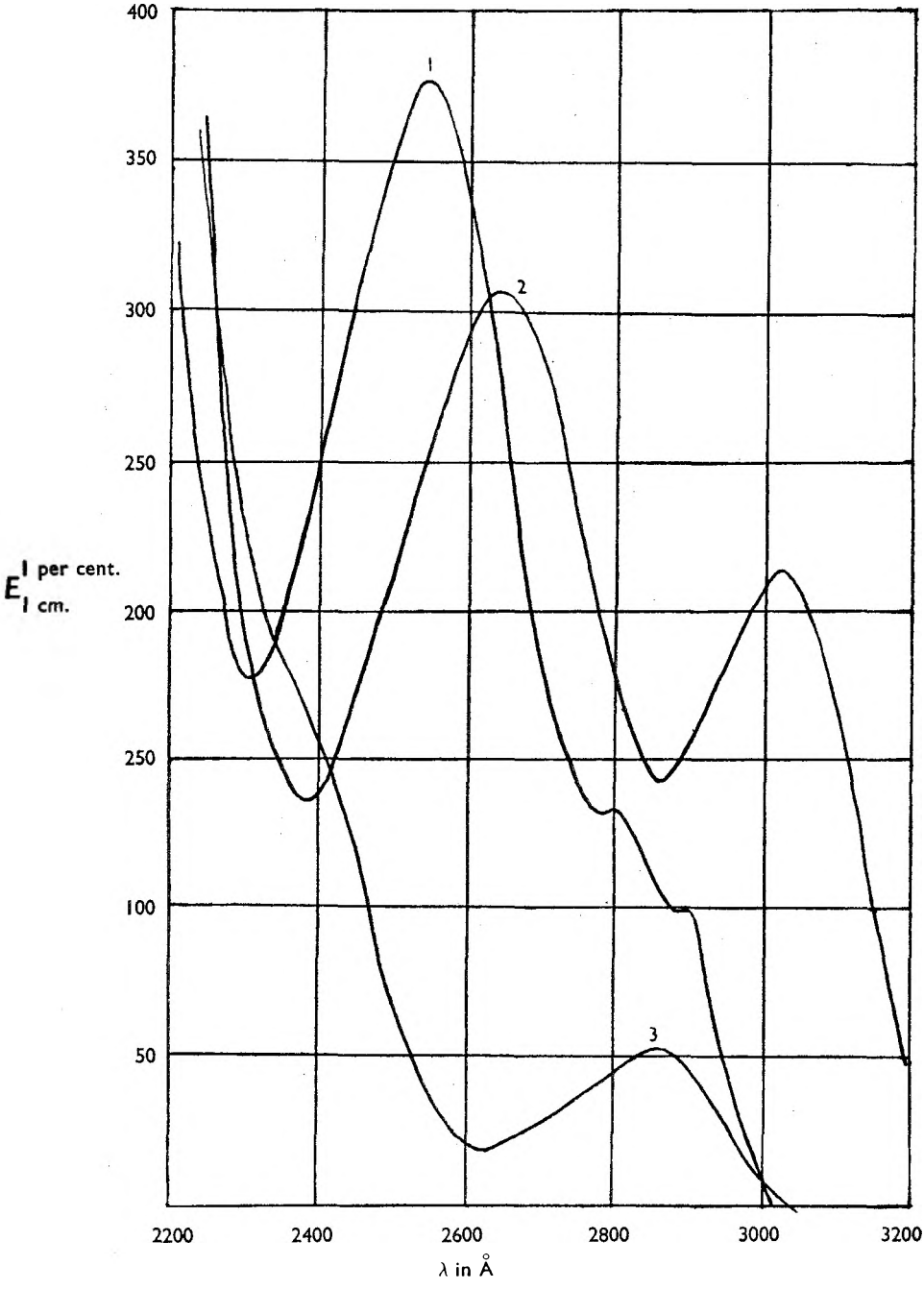

FIG. 1. Strychnine, brucine and morphine in ethanol (95 per cent.).

1. Strychnine.
2. Brucine.
3. Morphine.

TABLE I

Alkaloid	Maxima	$E_{1\text{ cm.}}^{1\text{ per cent.}}$	Minima	$E_{1\text{ cm.}}^{1\text{ per cent.}}$
Strychnine	2550Å	377	2320Å	180
	2800Å	130	2780Å	130
	2900Å	101	2880Å	100
Brucine	2640Å	307	2380Å	138
	3025Å	216	2870Å	143
Morphine	2870Å	55	2630Å	22

nised as the most satisfactory method. A brief outline of the Stas-Otto process is given below.

(*a*) The specimen of viscera is reduced to a fine state, made acid with tartaric acid and extracted with warm ethanol (90 per cent.)

(*b*) The ethanolic extract is filtered and carefully evaporated to dryness.

(*c*) The residue is extracted with absolute ethanol, cooled and filtered, and the filtrate carefully evaporated to dryness. If the residue is "dirty," the extraction with absolute ethanol is repeated.

(*d*) The final residue is taken up with water and filtered.

(*e*) The acidified aqueous solution is extracted with ether and the residual aqueous solution, made alkaline with sodium hydroxide, is ex-extracted with either chloroform or ether. This extract normally contains any strychnine and brucine present. The aqueous residue is neutralised with acid, make alkaline with ammonia and extracted with ethanolic chloroform (10 per cent. of ethanol and 90 per cent. of chloroform). This final extract contains any morphine which may be present.

The extracts obtained by alkaline chloroform and alkaline ethanolic chloroform are described in this work as "crude" extracts, i.e., extracts not subjected to further purification processes such as those described by Bamford (*loc. cit.*). The term "blank" Stas-Otto extract is used here to refer to any extract from viscera which has been examined and found to be free from alkaloidal substances.

IV. Morphine in Viscera Extracts

In the first experiments extracts were examined from routine toxicological cases which were shown to contain morphine. The extracts, both purified and crude, were dissolved in ethanol (95 per cent.) to give a solution of 0·005 per cent. w/v and the absorption curves are shown in Figure 2. It will be seen that all these curves have a pronounced $E_{max.}$ at 2870Å and $E_{min.}$ at 2630Å and the general shape of the curve gives a satisfactory qualitative test for morphine. The nature of the extract, i.e., whether purified or crude, has a profound effect on the value of the $E_{max.}$ and in some cases tends to flatten the curves at the turning points. The amount of morphine present in the extracts was determined from the values of the $E_{max.}$ and $E_{min.}$ and compared with the amount of morphine determined on the same extract by the method of the British Pharmacopœia, 1948. In general the amounts determined by the British Pharmacopœia method and by the spectroscopic method on purified morphine extracts were in good agreement but the results from "crude" extracts were open to considerable error.

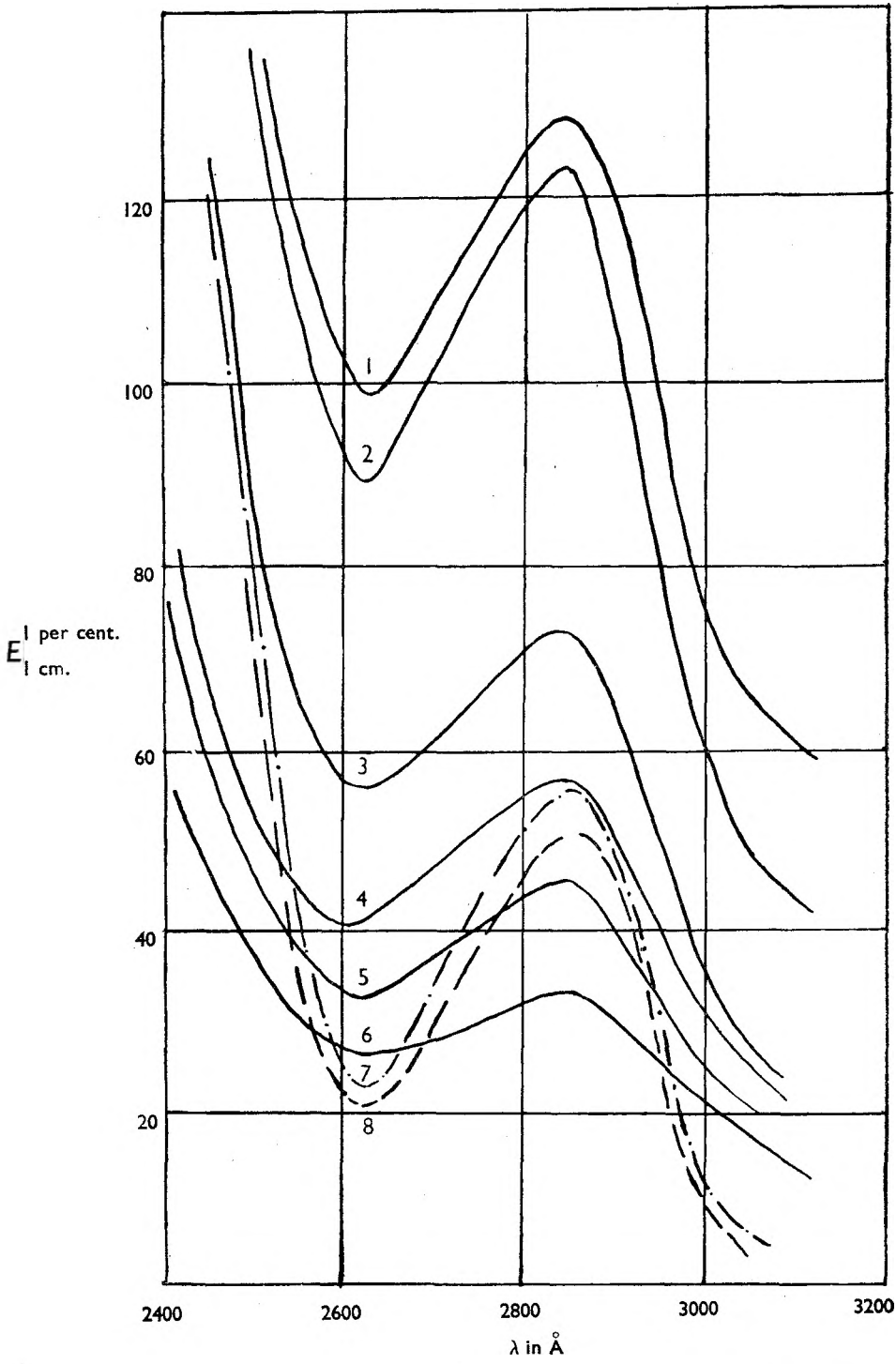

FIG. 2. Morphine in viscera extracts from toxicological cases.
1, 2, 3, 4, 5, 6 and 8. Morphine in viscera extracts both "purified" and "crude."
7. Morphine in ethanol (95 per cent.).

In the second series of experiments known amounts of morphine were added to aliquot parts of a "blank" Stas-Otto extract and the mixture dissolved in ethanol (95 per cent.) to give 0·005 per cent. w/v solutions. From the absorption spectra curves (Fig. 3) and especially from the $E_{max.}$ and $E_{min.}$ values at 2870 and 2630Å the amounts of morphine in the mixtures were calculated and are shown in Table II.

This experiment shows that the shape of the curve can be used for the qualitative detection of morphine unless the extract contains considerable amounts of extraneous matter as in curve 4: in such a case the extract

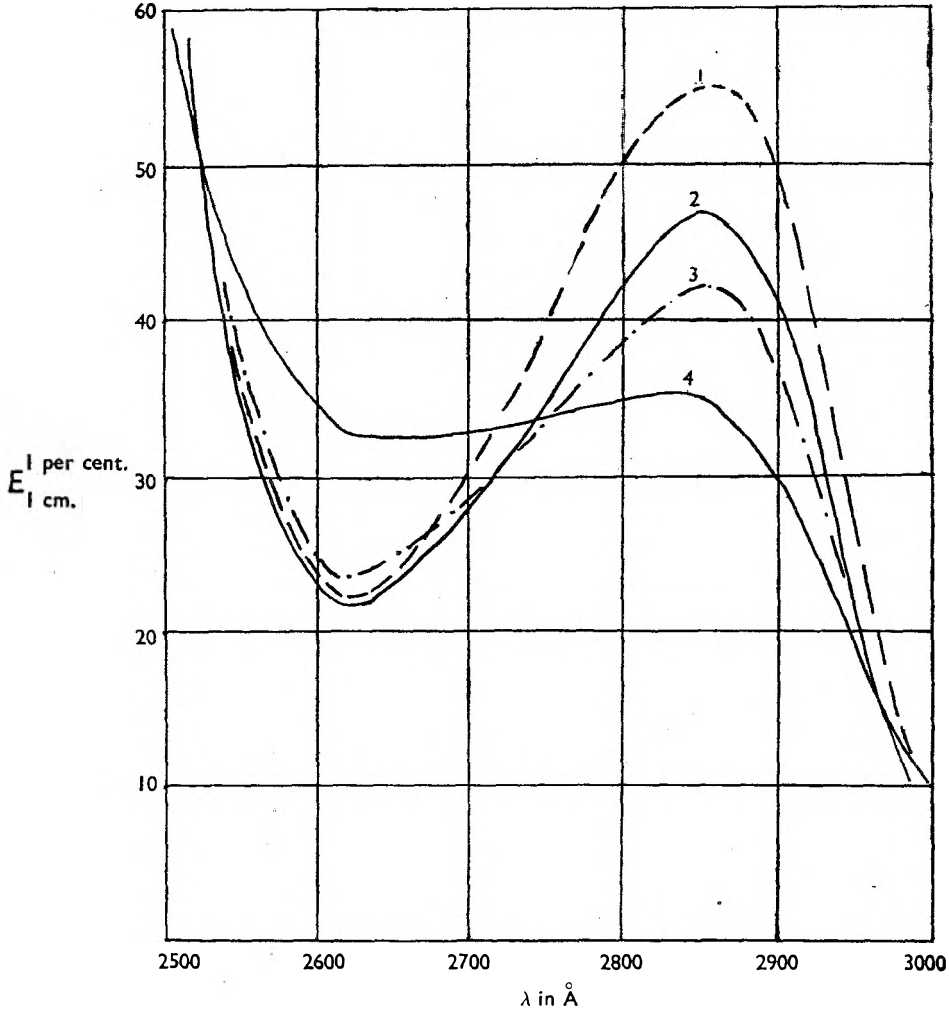

FIG. 3. Known amounts of morphine added to "blank" viscera extracts.
1. Morphine in ethanol (95 per cent.)
2. 5 mg. morphine and 1 mg. viscera extract in ethanol (95 per cent.).
3. 3 mg. morphine and 1 mg. viscera extract in ethanol (95 per cent.).
4. 1 mg. morphine and 1 mg. viscera extract in ethanol (95 per cent.).

should be cleaned up by further purification. Furthermore, the value of $E_{max.}$ at 2870Å gives a good quantitative measure of the amount of morphine present if the extract is not too impure; again it is clear that if, as with curve 4, the extract is only 50 per cent. pure, a further purification is required unless the agreement between 1 mg. of "known" morphine and 1·24 mg. "found" is sufficient for the purpose of the analysis. The $E_{min.}$ at 2630Å is of little use for quantitative work because the impurity in the extract must also contribute to the value of E at this wavelength.

TABLE II

Curve	Morphine added to 1 mg. of extract	Morphine found at 2630Å	Morphine found at 2870Å
2	5 mg.	5·7 mg.	5·0 mg.
3	3 mg.	4·2 mg.	3·0 mg.
4	1 mg.	2·9 mg.	1·24 mg.

TABLE III

Curve	Strychnine added mg.	Strychnine plus extract mg.	Strychnine found at 2320Å mg.	Strychnine found at 2550Å mg.	Strychnine found at 2800Å mg.
1	3	4·9	6·5	3·3	4·5
2	3	5·0	3·7	2·9	3·4
3	3	4·5	3·0	2·8	2·8
4	3	4·5	2·8	2·6	2·6
5	3·5	5·5	3·0	3·2	3·0

The general conclusion from these two sets of experiments is that morphine can be estimated with accuracy even when only small amounts are available, making use of the $E_{max.}$ of a purified extract.

V. Strychnine in Viscera Extracts

In the third series of experiments known amounts of strychnine were added to alkaloid-free viscera extracts and the mixture dissolved in ethanol (95 per cent.). In one case strychnine was added to liver known to be alkaloid-free and the mixture extracted by the Stas-Otto process. The absorption curves for these solutions, shown in Figure 4, exhibit the characteristic features of the curve for pure strychnine, in particular, the pronounced minimum at 2320Å and maximum at 2550Å. The subsidiary features of the strychnine curve in the region of 2800Å and of 2900Å can also be seen in the curves for the extracts. Ample qualitative evidence for the presence of strychnine is afforded and it is clear that any other substances extracted from the viscera did not affect the shape of the absorption curve to an extent sufficient to interfere with its value for qualitative detection.

The extinction values are, of course, less than those for pure strychnine because the extracts contained diluent material. A comparison of the observed values of E with those for pure strychnine at the same wave length should give the amount of strychnine in the extract, and in Table III the amount known to be in each extract is compared with that found from the values of E at 2320Å, 2550Å and 2800Å. This table shows that

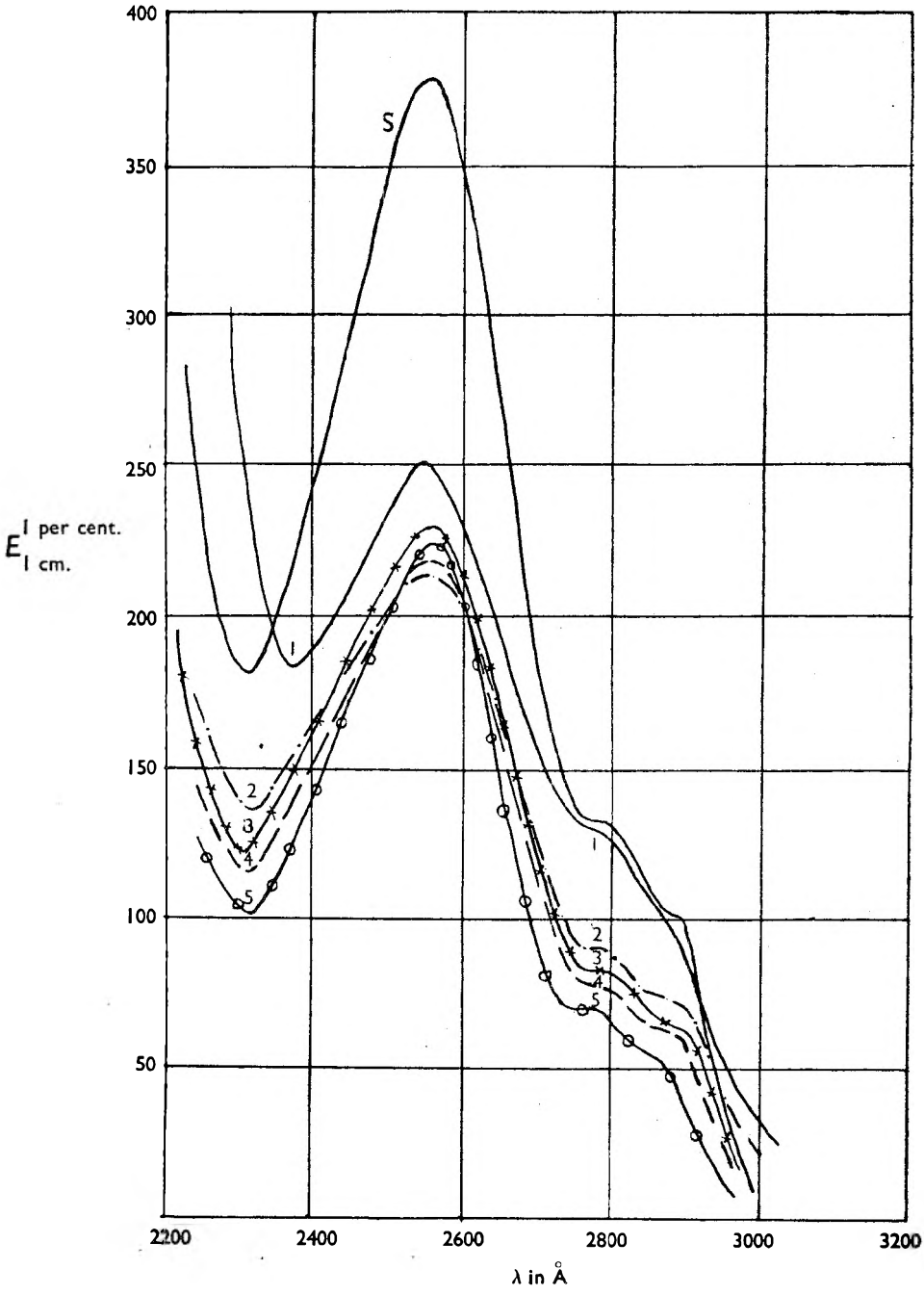

FIG. 4. Strychnine in viscera extracts.

S. Strychnine in ethanol (95 per cent.).

1–5. Strychnine in viscera extracts: dissolved in ethanol (95 per cent.).

the other material present in the extract does interfere with the quantitative estimation if the value of 2320Å is used whereas the $E_{max.}$ at 2550Å gives a good quantitative estimate of the strychnine present.

The conclusions drawn from these experiments are therefore similar to those from the experiments on morphine.

VI. The Analysis of Dilute Solutions Containing Small Amounts of Both Strychnine and Brucine

It was considered that the spectrophotometric technique would be ideally suited for the analysis of solutions containing mixtures of alkaloids in very small amounts. With this in view ethanolic solutions were prepared containing both strychnine and brucine, the ratio of strychnine to brucine ranging from 3:1 to 1:3. The solutions were diluted to contain 0·0025 per cent. of total alkaloids and were examined spectrophotometrically. The absorption curves obtained are shown in Figure 5.

These absorption curves were analysed as follows:—

(1) An approximate percentage of brucine was determined by making use of the experimentally determined extinction coefficient at 3025Å and the known extinction coefficient at 3025Å for pure brucine.

(2) The extinction coefficient for this approximate amount of brucine was calculated for a wavelength of 2550Å and subtracted from the experimental extinction coefficient at this wavelength: the residual extinction was treated as belonging to strychnine and used to calculate the amount of strychnine in the mixture.

(3) If the amount of strychnine was found to be appreciable, the brucine was recalculated, due allowance being made for the absorption due to strychnine at wavelength 3025Å.

(4) As a check, the brucine was recalculated using the $E_{max.}$ at 2640Å after allowance had been made for the absorption due to strychnine at this wavelength.

Example: Analysis of curve 3 *in Figure* 5.

Experimental extinction at 3025Å	= 156
Extinction for pure brucine at 3025Å	= 216
∴ brucine in the mixture	= 72·0 per cent.
Extinction for 72·0 per cent. of brucine at 2550Å	= 181
Experimental extinction at 2550Å	= 270
∴ Extinction due to strychnine at 2550Å	= (270–181) = 89
Extinction for pure strychnine	= 376
∴ strychnine in the mixture	= 24·0 per cent.

Check.

Experimental extinction at 2640Å	= 295
Extinction due to 24·0 per cent. of strychnine at 2640Å	= 74
∴ Extinction due to brucine at 2640Å	= (295–74) = 221
Extinction due to pure brucine at 2640Å	= 308
∴ brucine	= 72 per cent.

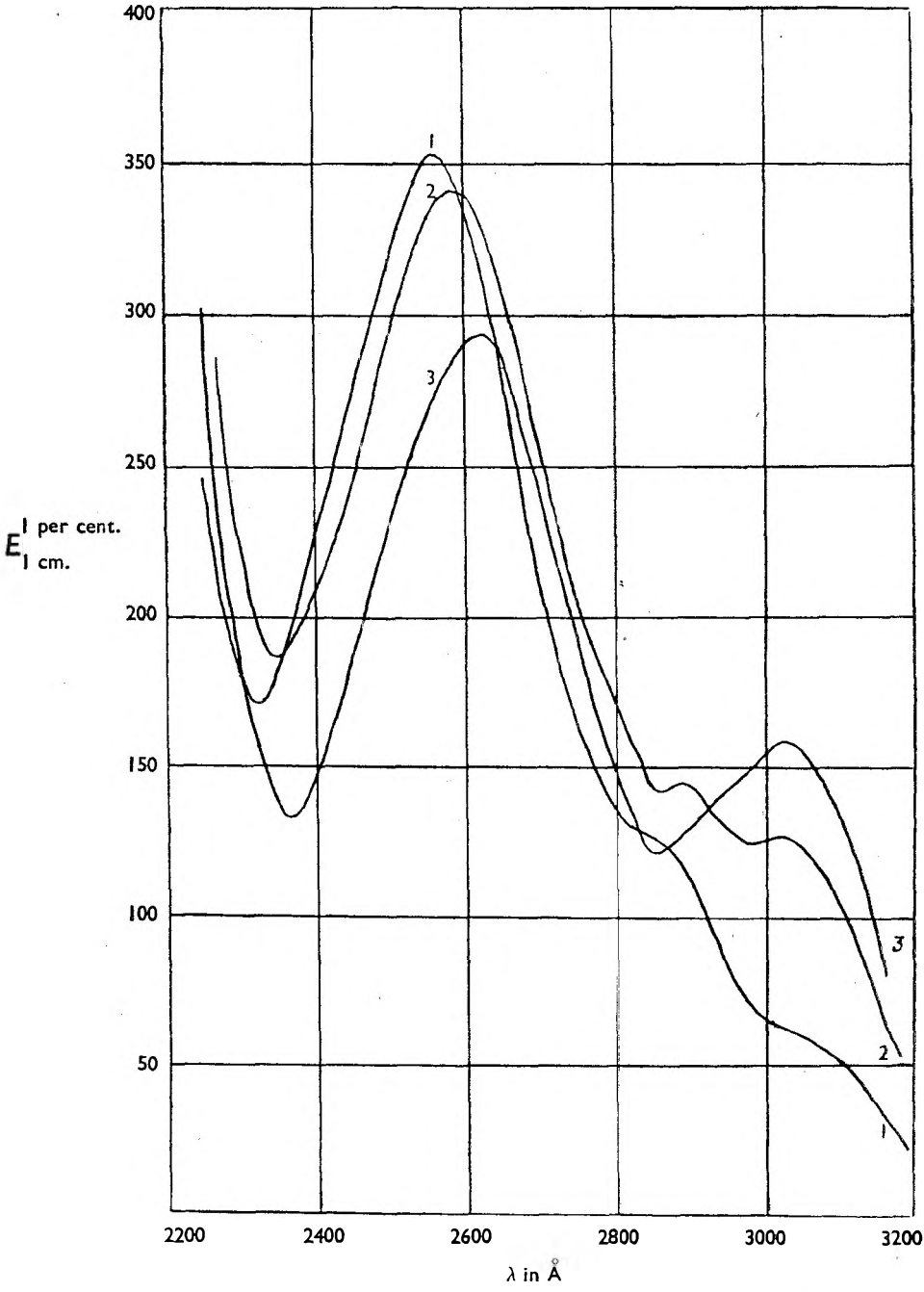

FIG. 5. Mixtures of strychnine and brucine in ethanol (95 per cent.).
1. 3·75 mg. of strychnine and 1·25 mg. of brucine in 100 ml. of ethanol.
2. 2·5 mg. of strychnine and 2·5 mg. of brucine in 100 ml. of ethanol.
3. 1·25 mg. of strychnine and 3·75 mg. of brucine in 100 ml. of ethanol.

The known and experimentally determined quantities of strychnine and brucine in the solutions for which graphs are given in Figure 5 are shown in Table IV and the values suggest that the method should be a valuable one for the analysis of this difficult mixture.

TABLE IV

ANALYTICAL RESULTS ON SOLUTIONS CONTAINING 5 MG. OF MIXED ALKALOIDS IN 100 ML. OF ETHANOL

Curve		Known amounts mg.	Found mg.
1	Strychnine	3·75	3·82
	Brucine	1·25	1·27
2	Strychnine	2·5	2·65
	Brucine	2·5	2·65
3	Strychnine	1·25	1·20
	Brucine	3·75	3·60

VII. Analysis of Stas-Otto Extracts Containing Both Strychnine and Brucine

As an extension of the above, known amounts of both strychnine and brucine were added to aliquot parts of a "blank" Stas-Otto extract. The alkaloids and extract material were dissolved in ethanol (95 per cent.), diluted to a suitable concentration and the solutions examined spectrophotometrically. The absorption curves are shown in Figure 6.

These absorption curves were analysed by the same method as that used for strychnine and brucine in pure solution and the known and experimentally determined quantities of strychnine and brucine are shown in Table V.

TABLE V

MIXTURES OF STRYCHNINE AND BRUCINE IN STAS-OTTO EXTRACTS

Curve		Known amounts mg.	Calculated mg.
1	Strychnine	3·3	3·9
	Brucine	2·0	2·0
2	Strychnine	6·6	6·2
	Brucine	4·0	4·3

DISCUSSION

These experiments do not pretend to be an exhaustive investigation; rather are they intended as an inspection of the potentialities of the spectrophotometric method in an analytical field where the nature of the sample and the amounts available have been most unfavourable to the analyst. It is hoped that by drawing attention to this method, interest may be stimulated to further experimental work in this field.

SUMMARY

1. Spectrophotometric measurements have been made on the absorption spectra of morphine, strychnine and brucine and of strychnine-brucine mixtures.

2. The work has been extended to Stas-Otto viscera extracts containing these alkaloids.

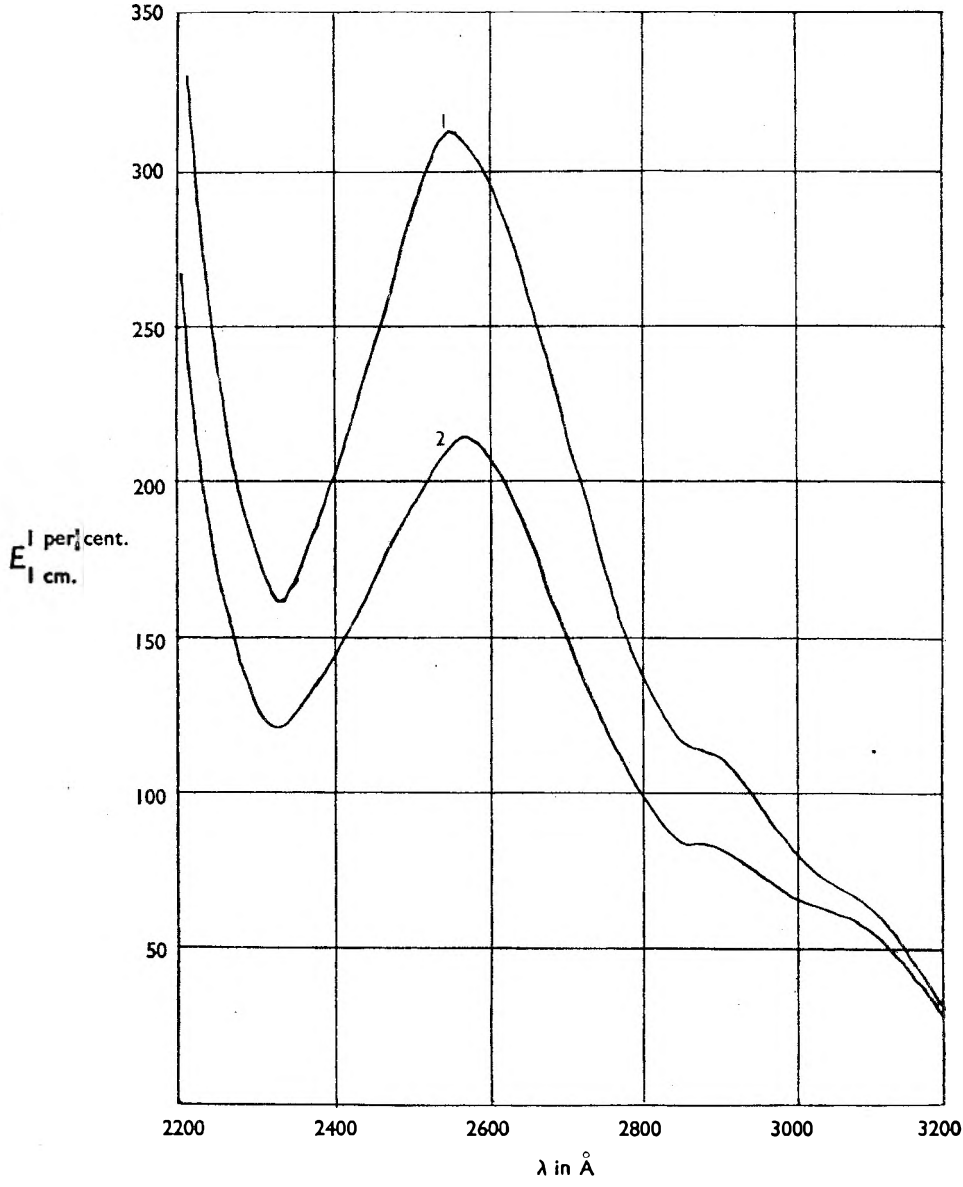

FIG. 6. Mixtures of strychnine and brucine in viscera extracts.

1. 0·0066 g. of extract containing 3·3 mg. of strychnine and 2·0 mg. of brucine.
2. 0·0168 g. of extract containing 6·6 mg. of strychnine and 4·0 mg. of brucine.

3. The use of such measurements for both qualitative and quantitative work on these substances is discussed and sufficient data have been presented to suggest that the method is one with considerable potentialities.

The author is indebted to Professor R. A. Robinson for his interest and assistance in this work and also wishes to thank Mr. A. W. Burtt, Chief Chemist, Singapore, for his help.

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THE STABILITY OF NORADRENALINE SOLUTIONS

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Received May 21, 1952

Noradrenaline is rapidly becoming the drug of choice for the treatment of acute hypotension following splanchnicectomy and lumbar sympathectomy.[1,2] When phæochromocytoma are removed, infusions of noradrenaline have been administered over many hours to maintain the blood pressure.[3] It is important, therefore, to be sure that the activity of such infusions is maintained when they are used in medicine. The object of this investigation was to determine (*a*) the optimal conditions for stability of strong solutions of noradrenaline, and (*b*) if any deterioration occurs when dilutions of noradrenaline are stored under conditions which simulate those in the operating theatre.

Methods

One sample of pure synthetic *l*-noradrenaline bitartrate (2 mg. = 1 mg. of base) was used for preparing solutions of noradrenaline of a strength of 1 in 1000 in freshly prepared distilled water containing 0·8 per cent. of sodium chloride (Analar). They were divided into two equal volumes and to one was added 0·1 per cent. of sodium metabisulphite (B.P.). The *p*H values of these solutions were determined with a Cambridge *p*H meter. Solutions of other *p*H values were prepared by the addition of the minimal quantity of hydrochloric acid or sodium bicarbonate. The solutions were transferred to freshly washed and dried 1-ml., 5-ml. and 10-ml. ampoules, which complied with the official tests for limit of alkalinity of glass. Some ampoules were filled with the solutions whilst others were left half full. Batches of the sealed ampoules were maintained at 115° C. for 30 minutes or 6 hours. The solutions were then assayed biologically by their effects on the blood pressure of a spinal cat and the pendular movements of an isolated strip of rabbit ileum. The standard reference solution used throughout was an unheated solution of noradrenaline bitartrate (1 in 1000 of base) containing 0·1 per cent. of sodium metabisulphite. This was stored in the refrigerator (4° C.) when not in use.

For the tests for stability of dilutions, ampoules of the standard reference solution and of noradrenaline supplied by Bayer Products Ltd. ("Levophed") were used. A known volume was first added to 100 ml. of isotonic sodium chloride solution, 5 per cent. dextrose solution in distilled water, or distilled water to give final concentrations of noradrenaline of 10^{-6}, 4×10^{-6} and 10^{-5}. The *p*H values of these solutions were determined. In some experiments, this was adjusted to *p*H 7·9 with solid sodium bicarbonate. Solutions were then stored at 37°, 18° and 4° C. for 6, 12 and 48 hours before being assayed. In the case of the

alkaline solutions, comparisons were made after storage for 6 hours at 18° C. and 30 minutes and 1 hour at 37° C. In the second series of experiments, solutions of noradrenaline of similar strengths were prepared using as diluents isotonic sodium chloride solution alone and with ascorbic acid (0·001 per cent.), 5 per cent. dextrose solution in distilled water, or rabbit, cat or human plasma and whole blood. The dilutions were stored for 6, 9, 12 and 24 hours at room temperature (18° C.) before being assayed. Control injections of the diluents into the cat were ineffective.

Results

The *p*H values of solutions of noradrenaline (1 in 1000) in distilled water containing 0·8 per cent. of sodium chloride with and without 0·1 per cent. of sodium metabisulphite were 3·5 and 3·9 respectively. There was little alteration in these values following the heat treatment. The results of the assays on these heated solutions are shown in Table I.

TABLE I

THE EFFECT OF METABISULPHITE ON THE COLOUR AND PHYSIOLOGICAL ACTIVITY OF SOLUTIONS OF NORADRENALINE BITARTRATE (1 IN 1000 OF BASE) WHEN HEATED IN AMPOULES. STANDARD REFERENCE SOLUTION IS AN UNHEATED SOLUTION CONTAINING 0·1 PER CENT. OF SODIUM METABISULPHITE

Size of ampoule (ml.)	Volume contained (ml.)	30 minutes at 115° C.				6 hours at 115° C.			
		Plain solutions		Solutions with metabisulphite		Plain solutions		Solutions with metabisulphite	
		Colour	Activity	Colour	Activity	Colour	Activity	Colour	Activity
1	1·2	Grey-brown	85	Colourless	100	Dark brown	40	Light brown	60
	0·6	Grey-brown	75	Colourless	95	Dark brown	20	Light brown	50
5	6·4	Brown	80	Colourless	100	Dark brown	20	Colourless	89
	3·2	Dark brown	75	Colourless	95	Dark brown	20	Light brown	82
10	11·0	Brown	70	Colourless	95	Dark brown	10	Colourless	70
	5·5	Dark brown	50	Colourless	91	Dark brown	5	Light brown	65

It will be noted that the presence of metabisulphite is essential for stability. In its presence, solutions of noradrenaline bitartrate may be autoclaved without a significant loss in activity. In the longer heating experiments, the colour of the solution is no longer a guide to activity. Solutions in half-filled ampoules deteriorate more than those in full ampoules.

Solutions of noradrenaline bitartrate at various *p*H values were then heated for 6 hours at 115° C. in full 5-ml. ampoules to determine the optimal *p*H value for stability. The results shown in Figure I clearly indicate that this value lies around 3·5. The reason why noradrenaline without metabisulphite should be more stable at more acid *p*H values is not clear, although a different inactivation process is suggested by the yellow-brown colour of these solutions.

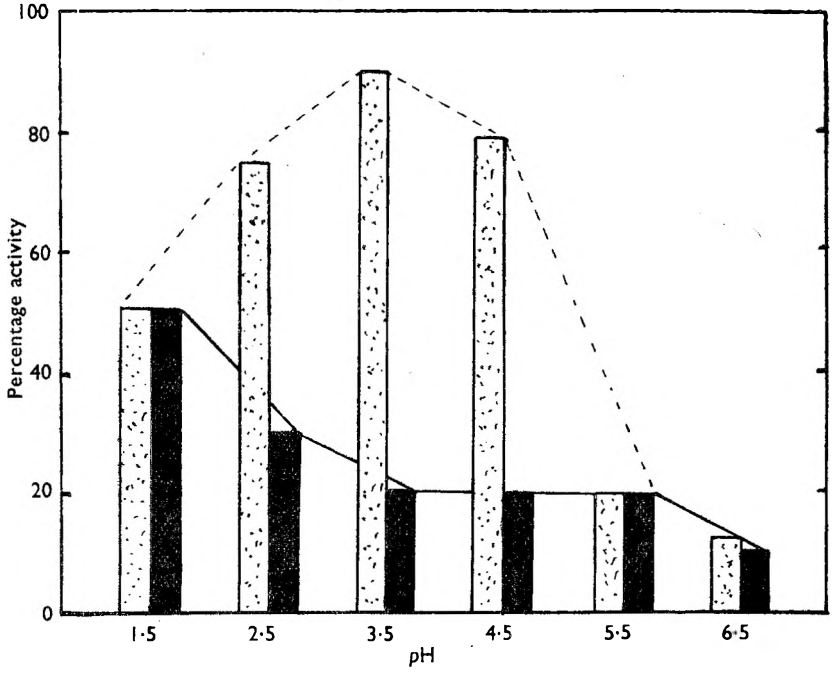

FIG. 1. The influence of metabisulphite (0·1 per cent.) on the physiological activities of solutions of noradrenaline bitartrate (1 in 1000 of base) of varying *p*H values, when heated for 6 hours at 115° C. in full 5-ml. ampoules. Black areas represent plain solutions; stippled areas are solutions with metabisulphite. Standard reference solution of noradrenaline (1 in 1000) = 100 per cent.

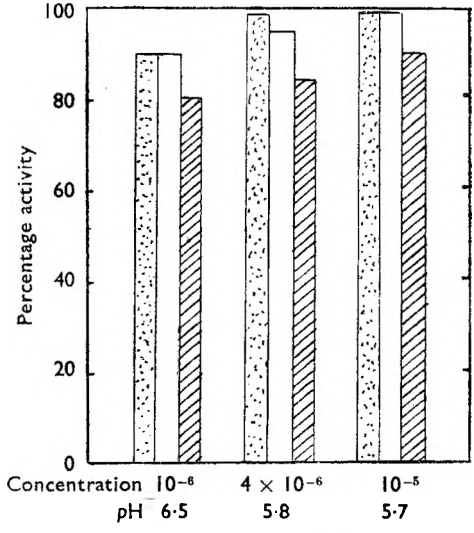

FIG. 2. The effect of storage for 6 hours at room temperature on the activities of dilutions of noradrenaline bitartrate (containing metabisulphite) in 5 per cent. dextrose solution, distilled water, and normal saline. Plain areas, distilled water; stippled areas, dextrose solution; shaded areas, saline solution.
Note that dilutions in saline solution lose activity first.

In the first series of dilution experiments, storage of solutions of noradrenaline at 3 concentrations showed that even at room temperature dilutions in saline solution tend to lose activity whilst those in dextrose solution and water do so at a much slower rate (Fig. 2). A similar picture was seen when dilutions at 10^{-5} were stored for 48 hours at 3 different temperatures (Fig. 3A). When the diluted solutions were made alkaline and stored, the results shown in Figure 3B were obtained. There was a significent loss of activity when such dilutions in saline were stored at room temperature for 6 hours.

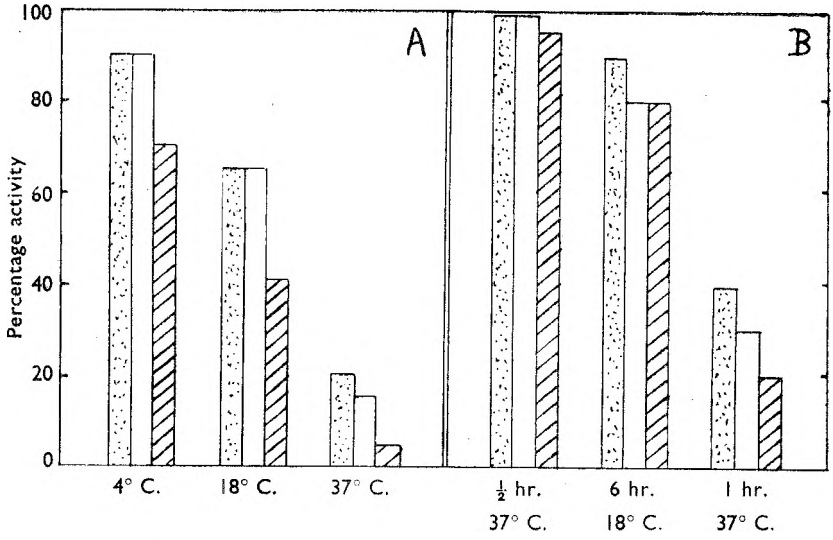

FIG. 3A. The effect of temperature on the activities of noradrenaline dilutions (10^{-5}) when stored for 48 hours. Initial *p*H 5·6. Dilutions in saline solution again show greatest loss of activity.
FIG. 3B. The effect of storage on the activities of noradrenaline dilutions (4×10^{-6}) adjusted to *p*H 7·9. Dilutions in saline solution and water show some loss when stored for 6 hours at 18° C. Plain areas, distilled water; stippled areas, dextrose solution; shaded areas, saline solution.

Similar dilutions of noradrenaline in blood, plasma and saline solution with ascorbic acid (10^{-5}) were next tested and the results of the assays are shown in Figure 4. After 6 hours storage at room temperature, some loss of activity was shown in the dilutions in saline solution and in whole blood. However, even after 12 or 24 hours, the relative activities of dilutions in plasma, dextrose solution and saline solution containing ascorbic acid were very high.

DISCUSSION

The effect of *p*H on the stability of noradrenaline in solution (1 in 1000) has been studied at a high temperature (115° C.) for short periods since it would be necessary to keep the solutions for many months or even years to obtain any considerable destruction at low temperatures. It is

possible, however, to use the results to form an opinion of the effects of low temperatures and long periods, i.e. the stability during storage. The optimum conditions for stability have been found to be possessed by solutions of noradrenaline bitartrate (1 in 1000 of base) containing 0·1 per cent. of sodium metabisulphite. Such solutions (*p*H 3·5 to 3·9) may be autoclaved (115° C. for 30 minutes) without loss of activity, provided the ampoules are more than half-full. These conditions are very similar to those found in corresponding experiments carried out to test the stability of adrenaline tartrate in solution.[4]

In the dilution experiments carried out under conditions similar to those used in clinical practice, saline solution appears to be the diluent allowing greatest loss of activity. No significant loss of activity, however, was found

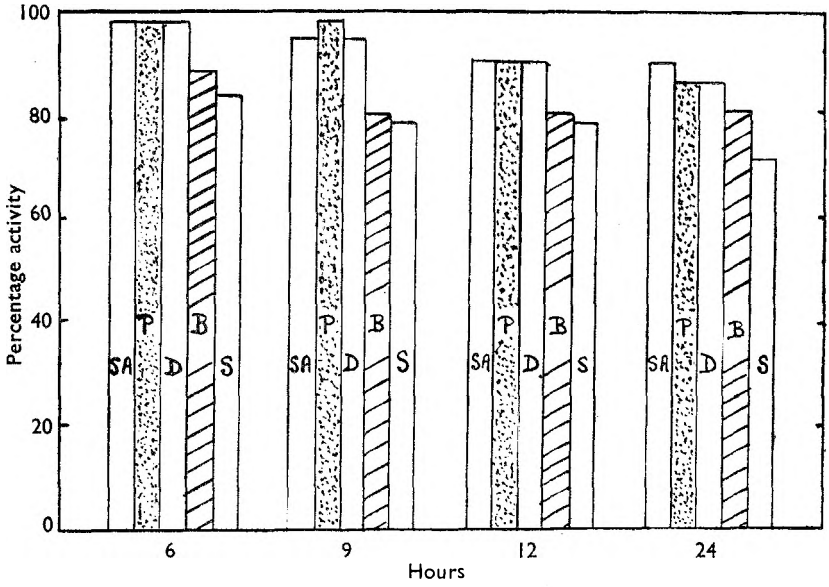

FIG. 4. The effect of storage at room temperature on the activities of dilutions of noradrenaline bitartrate (10^{-6} to 10^{-5}) in saline solution with 10^{-6} ascorbic acid (SA), plasma (P), dextrose 5 per cent. in water (D), whole blood (B), and saline solution (S). Initial *p*H values 5·9 to 6·7. Dilutions in dextrose solution or plasma are fairly stable.

when dilutions in plasma, dextrose solution or saline solution containing ascorbic acid (10^{-5}) were stored for up to 9 hours at room temperature. Corresponding experiments using adrenaline acid tartrate also showed a similar pattern of results. Dilutions of adrenaline or noradrenaline in a mixture of equal volumes of normal saline solution and dextrose solution again showed loss of activity but it was not as great as that found in dilution experiments in saline solution alone. Further work showed that dilutions of noradrenaline in an isotonic mixture of 4 per cent. dextrose solution and 0·18 per cent. sodium chloride solution in distilled water are as stable as those in 5 per cent. dextrose solution in distilled water.

SUMMARY

(1) It is suggested that the optimum conditions for stability and storage of solutions of noradrenaline when enclosed in well-filled ampoules are (*a*) an initial *p*H of approximately 3·6, and (*b*) the presence of 0·1 per cent. of sodium metabisulphite.

(2) A solution of noradrenaline bitartrate with metabisulphite satisfies these conditions and can be sterilised by autoclaving (115° C. for 30 minutes), for the loss of activity would be negligible.

(3) Dilutions of noradrenaline bitartrate solutions with metabisulphite are more stable in 5 per cent. dextrose solution, in distilled water, and in plasma than in saline solution and whole blood.

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THE USE OF ION EXCHANGERS IN THE ANALYSIS OF SALTS OF WEAK ORGANIC BASES AND WEAK ORGANIC ACIDS

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Received April 2, 1952

The determination of the amount of alkaloid in alkaloidal salts is often undertaken by supersaturating a solution with a base and shaking out the alkaloid with ether, chloroform or other suitable organic solvents. After evaporation of the latter, the amount of the free base is determined by weighing or titration. The many manipulations necessary make this a troublesome method and, as the evaporation of the solvent may cause loss of alkaloid, a better method would be desirable.

In 1936 Franck[1] made the following observation. When an ethanolic extract of an alkaloidal drug was passed through a column of a specially prepared aluminium oxide (Merck, purissimum anhydricum), not only the colouring matters, but also the anions were adsorbed by the column, so that the resulting liquid was an almost colourless solution of the alkaloidal bases, which could be determined by titration after evaporation of the ethanol.

In recent years this promising method has been studied in many laboratories, and Reimers[2] and collaborators have worked out a method using chromatographic analysis for alkaloidal salts. This method is used to a great extent in the Danish Pharmacopœia, 1948. Bjørling[3] has also used aluminium oxide for analysis of alkaloidal salts and pointed out that some difficulties may arise from adsorption in the column. Although the use of aluminium oxide has given good results, we were interested in investigating whether the use of modern ion exchangers would not give just as good results and also extend the field of use, so that with the aid of these one could determine, not only the amount of base in salts of organic bases but also the amount of acid in salts of organic acids. Good results with this method have been obtained during recent years, and this principle has been applied to a great extent in different fields of organic analysis.

Ion Exchangers

Certain compounds possess the ability to exchange ions with a solution, in which they are themselves insoluble. Because of this property they are called *ion exchangers*. The ion exchangers are denoted *cation* or *anion* exchangers, according to whether the ions which they exchange with the solution, are cations or anions. An ion exchanger can be defined as a high molecular, polyvalent, insoluble and indiffusible ion, of which the polyvalent charge is in equilibrium with relatively small ions of opposite charge. The ion-exchanging groups in anion-exchangers are usually amino groups. In cation exchangers they can be, for example,

sulphonic acid groups, carboxyl groups or phenol groups. The following reaction schemes are given as an illustration.

When a solution of a salt of a weak organic base is passed through a column of an anion exchanger the process indicated in Figure 1 takes place:—

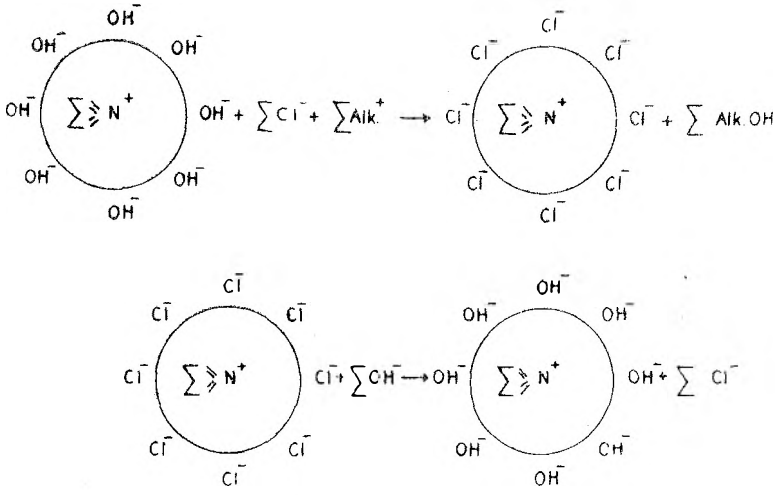

FIG. 1

The base will remain in solution and may be determined in that state.

The anion exchanger is regenerated by passing a more concentrated solution of sodium hydroxide through the column and subsequent washing with water.

The corresponding processes for cation exchangers are indicated in Figure 2.

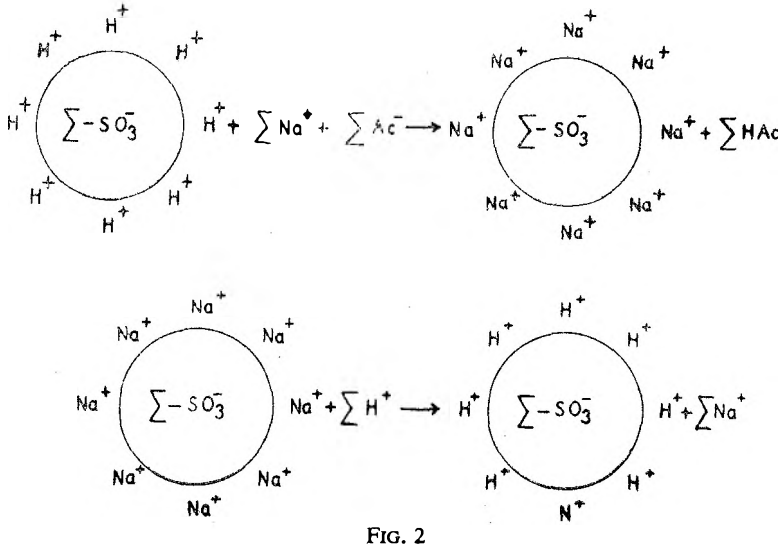

FIG. 2

The cation exchanger is regenerated by passing 4 N hydrochloric acid through the column and subsequent washing with water.

The use of ion exchange for analytical purposes was suggested as early as 1909 at the International Congress for Applied Chemistry in London,[4] and it has occasionally been brought forward in the literature during the succeeding years. An example is Folin and Bell's[5] use of ion exchange for the determination of ammonia in urine; but it was not until the nineteen thirties, when Adams and Holmes[6,7] published their work on organic ion exchangers, that these were extended for use in analytical chemistry. The modern ion exchangers, on a synthetic resin basis, are prepared both as cation exchangers (sulphonic acids, carboxylic acids and phenols) and as anion exchangers (strong or weak bases containing acyclic or aromatic amino groups).

Ion exchange resins have a field of application which extends over practically the whole *p*H scale. Since they can be obtained in a pure state for analytical purposes, and can be easily repurified after use, one has an implement for analytical chemistry having great possibilities.

Our investigations are of a preliminary nature as we have not investigated as many compounds and groups as we would have liked, but we have primarily aimed at the analysis of alkaloidal salts and salts of simple organic acids.

The Analysis of Salts of Natural Alkaloids and Other Weak Organic Bases

For the analysis of alkaloidal salts we have preferably used the strongly basic ion exchanger, Amberlite IRA-400, as we found it more suitable for this purpose than Amberlite IR-4B.

A glass tube 8 mm. in diameter and about 90 mm. long was used for the column, the bottom part of the tube was drawn out to a narrow tube and to the top was fused another glass tube 20 mm. in diameter and having a capacity of 15 ml. (Fig. 3).

Fig. 3

Before a column was prepared, the ion exchanger, which was used in the commercially available particle size, was made to swell by immersion overnight in the solvent concerned. It was then suspended in water and poured into the glass tubing, in which had been previously placed a little glass wool to act as a filter. The top of the column was finally covered with a layer of glass wool. The column was thus formed by sedimentation, and had a height of about 75 mm. Purification of the ion exchanger was carried out by passing 50 ml. of N sodium hydroxide through the column. After subsequent washing with water until the washings were colourless on the addition of phenolphthalein, the column was ready for use. Between experiments, or when not in use, the column was always filled with water by placing a stopper in the upper end of the tubing before the rinsing water had reached the top of the column.

Kunin[8] gives the following values for the capacity of the ion

exchangers which we used: Anion exchanger Amberlite IR–4B, weak base, 10 milli-equivalents/g. or 2·5 milli-equivalents/ml. Anion exchanger Amberlite IRA-400, strong base, 2·3 milli-equivalents/g. or 1·0 milli-equivalent/ml. Cation exchanger Amberlite IR 100, of the phenol-methylene sulphonic acid type, 1·75 milli-equivalents/g. or 0·65 milli-equivalents/ml. Our experiments have, by and large, confirmed the given figures.

The solubility of the ion exchangers was determined in order to obtain some idea of the stability of the ion exchanger in relation to the solvent used for the experiments. 6 ml. of regenerated and washed Amberlite IRA–400 was suspended in 75 ml. of ethanol (75 per cent.) and allowed to stand for 24 hours at room temperature. The ethanol was then filtered off and after evaporation the residue was determined. Weight of the residue 0·002 g. The solubility was further determined under experimental conditions which had more similarity to those employed in the actual determination: 500 ml. of ethanol (75 per cent.) was filtered through a column (8 mm. diameter, 120 mm. height) of regenerated and washed ion exchanger, at room temperature and the residue from the evaporation was determined. Weight of the residue 0·0024 g. The solubility of the ion exchanger in the solvents used is so small that it can be ignored.

In order to obtain as great an accuracy as possible we have not used the commercial salts of the organic bases as they contain varying amounts of water of crystallisation. We dissolved the free base, the purity of which was controlled by titrating with 0·1 N hydrochloric acid, in ethanol or methanol (50 per cent.) and neutralised this solution with 0·1 N hydrochloric acid using bromophenol blue as indicator. This indicator is particularly suitable for the titration of bases having a *p*A* value about 7 to 8 in a solution in ethanol (about 50 per cent.).[9,10]

The solution of the salt of the organic base was then filtered through a column of Amberlite IRA-400. The flask and column were rinsed with 50 ml. of ethanol (50 per cent.). In order to finish the subsequent titration in a medium which contains about 50 per cent. of ethanol, about 9 ml. of ethanol (86 per cent.) was added, this being used to rinse the narrow part of the column and the neck of the flask. This solution was now titrated with 0·1 N hydrochloric acid after the addition of 2 drops of bromophenol blue indicator, as the indicator added in the first titration was retained by the ion exchanger. This method was used in the determination of the natural alkaloids and other organic bases listed in Table I.

The quantity of ion exchanger used for the experiments corresponds to a little more than twice the amount—expressed in milli-equivalents—of the substance which was used for the experiments. In a few cases, shown under "remarks" in the table, it was necessary to use ethanol of a higher concentration (75 per cent. v/v), and even to elute with warm ethanol. In some cases (ethylmorphine, hydrocodone, oxycodone)

* pA is defined from the following equation

$$pA = pH - \log. \frac{[base]}{[salt]}$$

TABLE I

DETERMINATION OF SALTS OF NATURAL ALKALOIDS AND OTHER ORGANIC BASES USING AMBERLITE IRA-400

0·5 to 1 milli-equivalent of alkaloidal salt
Column: 8 × 75 mm.
Unless otherwise specified the elution is carried out with 50 ml. of ethanol (50 per cent. v/v)

Base	Recovered, per cent.	Remarks
Amphetamine	99·5, 99·0, 98·9, 99·0	
Atropine	99·1, 98·8, 99·1, 99·1	
	99·4, 99·4	
Brucine	99·0, 100·2, 100·0	
Butacaine	99·1, 99·3, 99·6	
Cinchonine	100·0, 100·3*	
Cincaine	99·1, 98·9, 99·1	
Cocaine	98·8, 99·0, 99·0	
Codeine (chloride)	99·4, 99·1, 99·0, 98·5	
	98·6, 98·9	
Codeine (phosphate)	99·1, 99·5, 99·1	
Diphenhydramine	98·4, 98·2, 98·9, 98·0	
	99·1, 99·3	ethanol (75 per cent. v/v
Emetine	99·6, 99·5, 99·8	
Ephedrine	99·0, 99·8, 99·7	
Ethylmorphine	99·5, 99·1, 99·1	
	98·8, 99·4	methanol (75 per cent. v/v)
Homatropine	99·8, 100·0, 100·0	
Hydrocodone	96·5, 97·0	
	98·6	ethanol (75 per cent. v/v)
	99·6, 98·9, 98·8	ethanol (75 per cent. v/v), warm
	99·8	methanol (75 per cent. v/v)
Hyoscyamine	99·6, 99·4, 99·7, 100·0	
Methadone	99·1, 99·5, 99·5	
Oxycodone	95·4	
	95·2, 94·6	ethanol (50 per cent. v/v), warm
	97·6	methanol (50 per cent. v/v)
	98·4	methanol (50 per cent. v/v), warm
	100·0, 100·2	methanol (75 per cent. v/v), warm
Papaverine	99·2, 100·8	
Pethidine	99·4, 99·5	
Procaine	99·7, 99·4, 99·2, 99·1	
Quinidine	100·0, 100·2*	
Quinine	97·7, 100·0*	
Scopolamine	100·0, 99·8, 99·9	
Strychnine	100·3, 100·8*	

* Electrometric titration.

the use of methanol gave the best results. It was found necessary to carry out a blank determination on the reagents. The amount of 0·1 N hydrochloric acid used was 0·06 ml. Also a quantitative determination of atropine was attempted using Amberlite IR-4B, the method being the same as that described above. The results showed that there was only incomplete conversion in this ion exchanger. To prove that this was the case and not adsorption of the alkaloid base to the column, the following experiment was carried out. The filtrate from the column of Amberlite IR-4B was passed through a purified column of Amberlite IRA-400. On titration of the filtrate the same amount of 0·1 N hydrochloric acid was used as before the passage through the columns.

From strychnine base, which is very slightly soluble, a solution was prepared in absolute ethanol. This contained in 10 ml. strychnine base equivalent to about 0·6 ml. of 0·1 N hydrochloric acid. The titrations were therefore carried out using 0·01N hydrochloric acid, but as the end-point was not distinct, the solutions were titrated potentiometrically.

The method was as follows: 10 ml. of the solution was measured, and 6 ml. approx. of 0·01 N hydrochloric acid and 4 ml. of water were

added. The solution was passed through the column of Amberlite IRA–400 (diameter 0·7 mm., height 40 mm.) and this was washed with 40 ml. of warm ethanol (50 per cent.). After the addition of 9·5 ml. of ethanol (86 per cent.) the solution was titrated potentiometrically. The blank was determined by diluting 6 ml. approx. of 0·01 N hydrochloric acid with 4 ml. of water and 10 ml. of absolute ethanol and passing this solution through the ion exchanger. The ion exchanger was then washed with 40 ml. of ethanol (50 per cent.), 9·5 ml. of ethanol (86 per cent.) was added to the filtrate and the solution was titrated potentiometrically.

The alkaloids cinchonine, quinidine and quinine, which cannot be accurately titrated by the use of bromophenol blue as indicator, because of the buffer action at the end-point, were also titrated potentiometrically. The method was the same as for strychnine except that larger amounts of alkaloid were used for the determination, and therefore the column of the ion exchangers used was as described in the general method. The amount of ethanol (50 per cent.) used for washing was 50 ml. and the titration was carried out with 0·1 N hydrochloric acid.

Physostigmine salicylate was decomposed (strong red coloration) by Amberlite IRA-400 and only about 60 per cent. was recovered.

Histamine, carbacholine and homatropine methyl bromide were tested by the ordinary method, but have not given satisfactory results.

For morphine, due to the fact that this alkaloid contains phenol groups, IRA–400 could not be employed. An experiment was made with a large column of IR-4B, but the results were not satisfactory. Only 96·5 and 96·8 per cent. was recovered using methanol (75 per cent.) for elution.

The Analysis of Weak Organic Acids

Bjørling[11] used Amberite IR–100H for the determination of the amount of acid in some sodium and calcium salts of organic acids. The results were generally good for salts of lactic acid, tartaric acid, citric acid, gluconic acid, sulphosalicylic acid, glycerophosphoric acid, and amidopyrine methanesulphonic acid (novalgin), but the method could not be utilised for salts of propionic or benzoic acids. It should also be mentioned that Wiesenberg[12] has used the same method in the analysis of different inorganic salts and sodium acetate in the determination of *N*-acetyl compounds. We have thoroughly investigated the method and can confirm Bjørling's results but we have found that salts of propionic acid, butyric acid and benzoic acid can also be analysed in this way, when water containing 15 to 30 per cent. of ethanol is used for elution. The column was prepared as described on page 568 and the ion exchange resin Amberlite IR–100H was used. The solution of the salts were prepared from the pure, titrimetrically determined organic acids, of which a known amount was neutralised by sodium or calcium hydroxide. Formic acid, acetic acid and malonic acid could be directly eluted with water, whilst this was not the case with the other organic acids examined. By elution with dilute ethanol (15 to 30 per cent.) all the acids examined could be determined quantitatively, as shown in Table II.

An amount of acid was used which corresponded to about 15 ml.

TABLE II

ANALYSIS OF SODIUM AND CALCIUM SALTS OF ORGANIC ACIDS USING AMBERLITE IR-100H

1·5 milli-equivalents of acid
Column: 11 mm. × 65 mm.
Eluted with 70 ml. of water or ethanol

Acid	Eluted with	Recovered per cent.
Formic	water	99·0
	ethanol (15 per cent.)	99·5, 100·0
Acetic	water	99·4, 99·4
	ethanol (15 per cent.)	100·0
Propionic	water	99·7, 99·7
	ethanol (15 per cent.)	100·0, 100·0
Butyric	water	87·1
	ethanol (20 per cent.)	96·3
	ethanol (30 per cent.)	99·3
	ethanol (40 per cent.)	99·9
Malonic	water	99·7, 100·3
Lactic	water	98·6, 98·7, 99·1
	ethanol (15 per cent.)	99·2
Gluconic	water	98·2, 98·3, 99·6
	ethanol (15 per cent.)	99·9, 100·0
Lævulic	water	98·2, 98·3, 99·6
	ethanol (15 per cent.)	99·9, 100·0
Benzoic	ethanol (15 per cent.)	89·2, 90·1
	ethanol (30 per cent.)	100·0

of 0·1 N base. The solutions were filtered through a column of Amberlite IR–100H; the amount of eluate was about 70 ml. This was titrated with 0·1 N base (indicator phenolphthalein).

The analytical technique used is easy to carry out and gives good results for all the compounds examined; a more extensive investigation on the use of the method for pharmaceutical problems of an analytical nature would be highly desirable.

SUMMARY

1. The analysis of some salts of weak organic bases and weak organic acids using synthetic organic ion exchangers (Amberlites) is described.

2. The method has given good results and as the Amberlites are commercially available of a purity suitable for analytical purposes, may easily be regenerated and do not give rise to difficulties from adsorption, they are recommended for rapid analyses of the salts mentioned.

3. A disadvantage is that the presence of neutral salts, e.g. sodium chloride, affects the results.

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THE QUANTITATIVE DETERMINATION OF CINNAMON IN THE FORM OF POWDER

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Received March 17, 1952

Saber[1] has found: (1) For barks in which the fibres occur either isolated or arranged in single files, the area of fibres per g. of the powdered bark is an excellent criterion for determining the amount of these drugs in the form of powder. (2) This datum for cinnamon is 92·5 and for cassia 13·1 sq. cm. per g., figures which differ so widely that they can be used successfully in determining the amount of cassia in cinnamon or *vice versa*. (3) The area of fibres per g. of cinnamon is in direct relation to its quality and grade, for the best quality it is about 100 and for the lowest quality, viz. featherings and chips, it is 70 and 40 respectively. (4) Samples of cinnamon that give lower results than those of quills may be either adulterated or of inferior quality.

The method for the determination of the area of fibres in powdered cinnamon bark is given by Wallis in his excellent *Practical Pharmacognosy*.[2] Briefly, the method consists of preparing 10 ml. of a suspension of 0·1 g. of cinnamon powder No. 90 and 0·05 g. of lycopodium, cleared with 3 ml. of chloral hydrate, in a suspending fluid, consisting of mucilage of tragacanth, glycerin and water (1:2:2). A mount is prepared. With the aid of a camera lucida the outlines are traced for all the fibres and portions of fibres seen in 5 strips across the cover-glass each having a width equal to the diameter of field of view (about 0·5 mm.). The outlines are cut out and the paper included is weighed. Knowing the weight of a definite area of paper (about 1 sq. dm.) and the magnification, the actual area of the fibres seen in 5 strips is calculated. Then the weight of cinnamon in the 5 strips is calculated from the number of spores in 24 fields of view and the weight of 94,000 spores = 1 mg. From the area and the weight of fibres in 5 strips and from the moisture of the cinnamon the area of fibres per g. of dry cinnamon is calculated. The results of 4 mounts, which differ by no more than about 10 per cent. of the average, are averaged.

The method, summarised above, is long and tedious. Dr. T. E. Wallis suggested to me the possibility of measuring instead of drawing the fibres. Difficulty in calculating the area of fibres from measurements of length and breadth is found in the determination of the breadth of the fusiform fibres. At first I tried to measure the area of outlined fibres cut out of paper and the results were encouraging. Then, with the aid of an ocular micrometer, I tried to measure the length and breadth of fibres seen in the microscope, while the same were drawn with a camera lucida. The results obtained being satisfactory, I have repeated the determination with the measuring method alone.

Two samples of cinnamon (*C. zeylanicum*) have been examined: (1) A powder, No. 85, kindly given by Dr. Wallis (assays 1 to 10 of Table I). (2) A powder, No. 100, made by myself from a specimen of cinnamon supplied by Merck and prepared from 20 g. of the bark, taken out of different sticks and pulverised in a mortar with a minimum of waste (assay 11). In Table I are the results of this investigation.

TABLE I

Cinnamomum zeylanicum

Suspension and sample	Area of fibres in sq. cm. per g. of powder, dried at 100° C. Ranges and average for 4 preparations of each suspension	
	Drawing and weighing	Measuring and calculating
Wallis 1	93 to 96·7 to 108	—
,, 2	88 to 95·0 to 101	—
,, 3	79 to 88·0 to 95	79 to 86·2 to 95
,, 4	85 to 91·7 to 100	78 to 87·2 to 97
,, 5	—	92 to 96 to 101
,, 6	—	85 to 92 to 97
,, 7	—	81 to 86 to 89
,, 8	—	82 to 89·5 to 101
,, 9	—	84 to 91·0 to 94
,, 10	—	86 to 93·0 to 101
Merck 11		83 to 89·5 to 102

	Drawing	Measuring
Arithmetic grand average	92·85	90·0
Average deviation	3·00	2·62
Standard deviation	3·33	3·14
Idem in parts per cent.	3·53	3·49

It appears from Table I that the determination of the area of fibres in cinnamon can be made by the measuring method quite as accurately as with the drawing method. However, this method has little advantage because it does not economise time; in reality, the calculation of the product from length and breadth and the addition of all the products is a long operation, about as time-consuming as the cutting out and weighing of the outlines; further the estimation of the average breadth of the fibres is quite as tedious as the drawing of their outlines; finally, measuring with the aid of the ocular micrometer is not easy, because for certain of the fibres seen in the field of view it is impossible to bring the micrometer to coincide with them.

The idea that the total length per g. of the fibres might be as good a criterion as the area, led me to examine this possibility. Suspensions and preparations were made with the same powder of Merck's cinnamon, described above, and also with Merck's cassia prepared in the same way as the cinnamon powder. The microscopical images were projected on to a white glass plate at a magnification of about 385 and the projected fibres were measured for length with a plastic transparent metre, divided in mm. The projection method is very easy and not so tedious as the use of a camera lucida. I have noted the results of two sets of 5 strips (series 1 alternating with series 2, viz. 1, 3, 5, 7, 9 and 2, 4, 6, 8, 10, the interval between two strips being 1·5 mm.) and the results of the sum of 10 strips, with the object of gaining an idea of the accuracy of the results from 5 strips. Table II shows the results of this investigation.

TABLE II

Cinnamomum zeylanicum (MERCK'S SAMPLE)

Suspension	Length of fibres in mm. per g. of powder dried at 100° C. Ranges and average for 4 preparations of each suspension		
	First series of 5 strips	Second series of 5 strips	Total of 10 strips
1	253 to 292 to 372	239 to 275 to 317	252 to 284 to 322
2	240 to 286 to 336	253 to 283 to 341	271 to 285 to 305
3	297 to 329 to 351	218 to 245 to 273	285 to 287 to 294
4	232 to 281 to 303	235 to 305 to 384	270 to 293 to 307
Arithmetic average ..	297	277	287
Average deviation ..	16	17	2·75
Standard deviation ..	18·9	21·5	3·50
Idem in parts per cent. ..	6·4	7·8	1·22

Cinnamomum cassia (MERCK'S SAMPLE)

1	9 to 41 to 55	40 to 47 to 61	35 to 44 to 48
2	6 to 34 to 58	24 to 53 to 77	31 to 44 to 57
3	46 to 50 to 55	26 to 39 to 55	38 to 45 to 52
4	37 to 49 to 62	24 to 36 to 62	31 to 43 to 62
Arithmetic average ..	43·5	43·75	44
Average deviation ..	6	6·25	0·5
Standard deviation ..	6·5	6·68	0·707
Idem in parts per cent. ..	14·9	15·0	1·6

The results in Table II show that the length of fibres per g. is quite as good a criterion as is the area of fibres per g. The datum for cinnamon (287) and that for cassia (44) differ so widely that they can be used successfully in determining the amount of cassia in cinnamon or *vice versa*. The ratios are as follows:

$$\frac{C.\ zeylanicum}{C.\ cassia} \quad \text{Area } \frac{92{\cdot}5}{13{\cdot}1} = 7 \quad \text{Length } \frac{287}{44} = 6{\cdot}5$$

From Table II it can be seen that the results of 5 strips are more accurate for *C. zeylanicum* than for *C. cassia*. It is, however, advisable to count the results from 10 strips for both. The measurement of the length of fibres is much quicker and easier than the determination of the area by drawing or by measuring.

For these experiments it was important to have preparations which last longer than those with tragacanth–glycerin–water mixture. A clarified suspension in oil is excellent and can be prepared as follows. Weigh out 0·1 g. of powdered cinnamon and mix it on a plate (glass or porcelain) with 0·5 ml. of solution of chloral hydrate (5 in 2), clear by keeping overnight (18 hours) in a humid chamber at ordinary temperature, and dry the mixture at ordinary temperature. Add to the dry clarified powder 1 ml. of a solution of camphor in ether and liquid paraffin (3:3:1), mix and add to about 10 ml. of a suspending fluid, consisting of a 1 per cent. solution of aluminium monostearate in liquid paraffin. Weigh 0·05 g. of lycopodium and add this to the suspension of cinnamon. The homogeneity of the suspension can be improved by the use of a magnetic stirrer. The suspension prepared by this method shows well clarified cinnamon particles and shows hardly any tendency to settle when allowed to stand for several weeks. With such a suspension in

oil the preparations on microscope slides may be kept in a slide-box without danger of deterioration or desiccation.

Summary and Conclusions

1. The area of fibres per g. in cinnamon can be determined by calculation from measurements of length and breadth with the same accuracy as by drawing.

2. The length of fibres per g. from cinnamon and from cassia is also an excellent criterion for their quantitative determination.

3. The ratio of the length of fibres per g. of *C. zeylanicum* to that per g. of *C. cassia* is nearly the same as the ratio of the area of fibres from these two spices.

4. A quasi stable suspension of clarified cinnamon in liquid paraffin can be obtained by adding to it 1 per cent. of aluminium monostearate. A method is described for clarifying the cinnamon with solution of chloral hydrate previous to adding the oil. A magnetic stirrer improves the homogenity of the suspension in oil.

To Dr. T. E. Wallis I wish to renew here my grateful acknowledgment for suggesting the subject of this research, for kind encouragement, for following my work step by step and for the sample of cinnamon. I should like to express my sincere thanks to Prof. A. Hifny Saber for sending a copy of his method of determination, and to my assistants, Mrs. Ragula and Mrs. Schelesowska, and my students, Roosemont and Swinnen, for preparative work.

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SODIUM PROPIONATE AND ITS DERIVATIVES AS BACTERIOSTATICS AND FUNGISTATICS

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Received February 19, 1952

The microbiological and pharmacological properties of sodium propionate which have been reported in previous communications,[1,2] suggested the possibility of obtaining simple derivatives which may exhibit greater activity against micro-organisms, while retaining a low order of toxicity in animals. Further *in vitro* studies have now been made of the effects of sodium propionate and certain of its derivatives on bacteria and fungi in an attempt to gain some information regarding the mechanism of the inhibitory action and to achieve enhancement of efficiency.

General Microbiology

Minimal bacteriostatic concentrations of sodium propionate for common organisms after 48 hours at *p*H 7 are shown in Table I and it is apparent that the most susceptible bacteria are largely Gram-negative,

TABLE I

Inhibitory concentrations of sodium propionate for common micro-organisms

Inhibitory concentration, per cent.	Bacteria	Fungi
0·1 to 1·5	*Pseudomonas aeruginosa* *Salmonella typhosa* *N.gonorrhœae* *B. cereus* *Serratia marcescens*	*Candida albicans* *Aspergillus niger* *Aspergillus fumigatus* *Epidermophyton interdigitale*
1·6 to 3·0	*Staphylococcus aureus* (Heatley) *Staphylococcus aureus* (Webb) *Staphylococcus albus* *Proteus vulgaris* *Streptococcus dysgalactiae* *Streptococcus agalactiae* *Streptococcus uberis* *Diplococcus pneumoniae*	— — — — — — —
3·1 to 4·5	*Staphylococcus aureus* (S. 53) *Staphylococcus aureus* (F.D.A. 209)	— —
4·6 to 6·0	*Escherichia coli* *Streptococcus pyogenes*	— —

although no generalisation is possible. The growth of pathogenic fungi appears to be inhibited by relatively low concentrations, but it has been found that certain non-pathogenic organisms of the *Penicillium* type continue to multiply in the presence of 5 per cent. or more of the compound.

The technique previously adopted[1] in studying the development of resistance has been modified by subculturing various bacteria in media

containing progressively larger concentrations of sodium propionate or sulphacetamide sodium until each group failed to grow. For convenience in interpretation, the quotient obtained by dividing the final bacteriostatic concentration by the initial bacteriostatic concentration was termed the "resistance index" and it was found that the value for sodium propionate rarely exceeded 1·5, although that for sulphacetamide sodium was invariably greater.

Mechanism of Bacteriostasis

Concentrations of sodium propionate which are capable of inhibiting growth for prolonged periods are not necessarily bactericidal and since some species of *Penicillium* and *Cladosporium* grow in media containing 6 per cent. of the compound, it would seem that the effect is not one of direct cell-toxicity. This deduction is supported by the observation that the toxicity for animal tissues appears to be very slight. Tests with a number of organisms at *p*H ranging from 8 to 5 show that bacteriostatic and fungistatic activity increases as the media are made less alkaline or more acid; greater inhibition of growth is apparent at *p*H 4, but at this value, the effect of hydrogen ion concentration alone is considerable. In acid solutions, the compound is only feebly dissociated and thus the toxic action is apparently produced by the molecule rather than the ion.

The structural similarity between propionic acid, alanine and certain other amino-acids may appear to offer a possible explanation of the mechanism on the basis of substrate competition. To test this theory, *Pseudomonas aeruginosa* was cultured on nutrient agar containing varying amounts of sodium propionate and DL α-alanine but the results, which are shown at Table II, indicate that the amino-acid does not materially affect bacteriostatic action. It has also been found that the addition of urea and histamine produces no significant change in the inhibitory concentration of sodium propionate.

TABLE II

Effects of sodium propionate and alanine on *Pseudomonas aeruginosa* (N.C.T.C. 7244)

Sodium propionate, per cent.	Alanine, per cent.	Growth after:	
		24 hours	48 hours
0	0	+	++
0·7	0	–	–
0·5	0	–	+
0·4	0	tr	++
1·0	1·0	–	–
0·5	0·5	tr	+
0·4	0·4	+	++
0·5	2·5	–	–
0	2·5	++	++
0	1·0	++	++
0	0·5	+	++

Since it was previously observed[1] that some antagonism may exist between sodium propionate and sulphacetamide sodium when these agents are present in equal concentration, it was decided to investigate the effects of small amounts of the sulphonamide on the activity of sodium propionate and to ascertain the influence of *p*-aminobenzoic acid. The latter substance was neutralised with sodium bicarbonate before use and *p*H of the nutrient agar was adjusted to approximately 7 in all cases. As may be seen from Table III, 0·1 per cent. or less of sulphacetamide sodium had little effect, but 1 per cent. of sodium *p*-aminobenzoate markedly increased the concentration of sodium propionate required to achieve bacteriostasis.

TABLE III

EFFECTS OF SODIUM PROPIONATE, SULPHACETAMIDE SODIUM AND SODIUM PARA-AMINOBENZOATE ON *Pseudomonas aeruginosa* (N.C.T.C. 7244)

Sodium propionate, per cent.	Sulphacetamide sodium per cent.	Sodium *p*-aminobenzoate, per cent.	Growth after:	
			24 hours	48 hours
0	0	0	+	+++
0·4	0	0	trace	++
0·75	0	0	–	–
0	0·1	0	++	+++
0·6	0·05	0	trace	++
0·6	0·1	0	–	–
0	0	1·0	+	+++
0·4	0	1·0	+	+++
0·75	0	1·0	trace	+++
1·0	0	1·0	trace	+

Low concentrations of sodium propionate appear to be oxidised quite readily by *Pseudomonas aeruginosa* with the production of carbon dioxide, but respiration is progressively reduced as the bacteriostatic level is approached. Inhibition of growth is probably associated with accumulation of sodium propionate within the cell and interference with normal carbohydrate metabolism; the dehydrogenase system may be principally affected in this way. The derivatives selected for study were designed to reduce oxidation by micro-organisms and to increase the inhibition of normal respiration, in an endeavour to obtain indications of more active groupings and, if possible, to gain further data regarding the antibacterial mechanism of sodium propionate.

BACTERIOSTATIC ACTIVITY OF DERIVATIVES

Solutions of the sodium salts of the following compounds were prepared with *p*H of approximately 7·0 and these were tested for bacteriostatic and fungistatic activity in neutral media:—α-bromopropionic acid; β-bromopropionic acid; αβ-dibromopropionic acid; α-bromopropionamide; α-hydroxy-β-phenylpropionic acid; β-phenoxypropionic acid.

The results, which are summarised in Table IV, show that simple substitution products exhibit greater activity than sodium propionate especially against *Proteus vulgaris* and *Staphylococcus aureus*. Some slight

influence may, however, be exerted on the results by the tendency of solutions of these derivatives rapidly to become acid, *p*H changes being observed after about 24 hours.

Under the conditions of testing, the bacteriostatic concentrations are generally much lower than those of sulphacetamide sodium, but these substitution products are of no therapeutic interest on account of their unstable nature. It would appear, moreover, that the bromopropionates

TABLE IV

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF DERIVATIVES OF PROPIONIC ACID

Compound	Concentration, per cent.	Growth after 48 hours at 37° C.				
		Pseudomonas aeruginosa	*Proteus vulgaris*	*Staphylococcus aureus*	*Candida albicans*	*Epidermophyton interdigitale*
Sodium α-bromo-propionate	0·1	+	trace	+	+	+
	0·25	–	–	–	+	–
	0·5	–	–	–	+	–
	1·0	–	–	–	–	–
Sodium β-bromo-propionate	0·25		–	–	+	+
	0·5	–	–	–	+	
Sodium α, β-dibromo-propionate	0·5	+			trace	
Sodium α-bromopropion-amide	0·1	+		+	+	
	0·25	trace	–	+	trace	–
	0·5	–	–	–	–	–
Sodium α-hydroxy-β-phenyl-propionate	0·1	+			+	
	0·25	+	–	–	+	–
	0·5	–	–	–	–	–
Sodium β-phenoxy-propionate	0·1	+			+	
	0·25	–	–	–	trace	–

exert some toxic effects on animal tissues. More desirable properties may be possessed by certain derivatives of β-phenylpropionic acid, a compound which is related to substances occurring in normal metabolism, although it is less important than propionic acid in this respect. Tests with solutions of sodium β-phenylpropionate adjusted to *p*H 7 and *p*H 9 have shown that *Pseudomonas aeruginosa* (N.C.T.C. 7244) is not inhibited at 0·75 per cent. although sodium propionate at this concentration completely suppresses growth; the five organisms quoted in Table IV, moreover, continue to grow in neutral media containing 0·5 per cent. of sodium β-phenylpropionate. The latter compound is apparently metabolised by certain organisms, but it is considered that the union of halogen and possibly other radicals with carbon of the benzene nucleus is likely to overcome this effect and to enhance bacteriostatic and fungistatic activity.

INTERPRETATION OF RESULTS

Sodium propionate inhibits the growth of a considerable number of pathogenic bacteria and fungi, but it is interesting to find that species of

Penicillium and *Cladosporium* continue to multiply on media containing 6 per cent. of the compound, since propionates are frequently used as preservatives for bread and other foods, especially in America. The bacteriostatic and fungistatic effect is probably produced by the molecule, rather than the ion, and it is suggested that inhibition of growth is associated with interference with the normal carbohydrate metabolism of the micro-organisms. Streptomycin appears to interfere with the utilisation of pyruvate and although this antibiotic is a much more powerful bacteriostatic than sodium propionate, the types of bacteria susceptible to these two drugs exhibit similarities; Dubos[3] reported that low concentrations of sodium propionate inhibit growth of mammalian tubercle bacilli. One important difference, however, lies in the development of resistance to streptomycin within a short period.

The precise mechanism of the antibacterial action of sodium propionate may prove difficult to explain since activity *in vitro* does not necessarily parallel therapeutic efficiency. For example, various local infections in animals have often responded very rapidly to the compound in concentrations which represented the minimal bacteriostatic levels determined by culturing. The clinical efficiency of sodium propionate has now been demonstrated by Tucker[4] who employed this agent in the treatment of 188 animals suffering from various infections, 50 cases having previously resisted therapy with penicillin, sulphonamides and other drugs.

Sodium salts of various substitution products of propionic acid have been found to possess relatively high bacteriostatic and fungistatic activity, but certain of these substances have pharmacological and pharmaceutical disadvantages. Interesting properties may, however, be exhibited by certain derivatives of phenylpropionic acid and it is intended to investigate selected compounds in due course.

Summary and Conclusions

1. The bacteriostatic and fungistatic activity of sodium propionate against 21 micro-organisms is described briefly and it is suggested that the toxic effect is produced by the molecule rather than the ion.

2. Alanine, urea and small amounts of sulphacetamide sodium or histamine do not materially affect the bacteriostatic concentration for *Pseudomonas aeruginosa*, although *p*-aminobenzoic acid exhibits some antagonistic action. Low concentrations of sodium propionate are oxidised by this organism, but respiration is progressively reduced as the bacteriostatic level is approached, the inhibition of growth probably being associated with interference with normal carbohydrate metabolism. There is evidence that the compound is more effective *in vivo* than *in vitro*.

3. Certain substitution products possess relatively high antibacterial and antifungal activity and although none of those investigated is considered suitable for therapeutic use, the study of further derivatives is justified.

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A NOTE ON THE ACTION OF *N*-BROMOSUCCINIMIDE ON ALIPHATIC DICARBOXYLIC ACIDS

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Received February 4, 1952

The reaction mentioned in the title does not appear to have been previously investigated.

Saturated Aliphatic Dicarboxylic Acids. *N*-Bromosuccinimide reacts with oxalic, malonic and succinic acids in aqueous solution on heating at the temperature of a boiling water-bath, forming carbon dioxide hydrogen bromide and succinimide. With acids other than oxalic acid, an olefine is obtained. The reaction proceeds easily at room temperature on shaking an aqueous solution of oxalic acid and *N*-bromosuccinimide, but with homologues of oxalic acid, heat is necessary to effect the reaction. Compared with the fatty acids the dicarboxylic acids possess higher dissociation constants, and are therefore stronger acids. This is especially so in the case of oxalic acid. This fact illustrates why such a reaction takes place. Succinimide has been isolated in the reaction with oxalic and malonic acids, and the formation of ethylene with malonic and succinic acids has been proved.

Unsaturated Aliphatic Dicarboxylic Acids. Maleic acid is a stronger acid than its isomer. On the other hand the hydrogen atom of the second carboxyl group of fumaric acid is more easily dissociated than that of maleic acid (Ostwald). Of the two stereoisomeric acids, fumaric acid is the more stable. *N*-Bromosuccinimide reacts with maleic and fumaric acids in boiling aqueous solution to give succinimide, carbon dioxide, hydrogen bromide and acetaldehyde. No free acetylene could be detected, but the presence of acetaldehyde was proved by the isolation of acetaldehyde 2:4-dinitrophenylhydrazone. The addition of water to acetylene to form acetaldehyde is well known. The behaviour of the two stereoisomeric acids towards *N*-bromosuccinimide is very much the same, except for the fact that in the case of maleic acid, a portion (about 10 per cent.) is converted into the more stable fumaric acid.

Experimental

N-Bromosuccinimide and Oxalic Acid. To a cold solution of 1·26 g. (1 mol.) of crystalline oxalic acid in 25 ml. of distilled water, 1·78 g. (1 mol.) of powdered *N*-bromosuccinimide was added and the solution was shaken, vigorously. A reaction proceeded instantaneously at room temperature the solution assumed a brown-red colour, and carbon dioxide and hydrogen bromide were evolved. In about an hour nearly all the *N*-bromosuccinimide had gone into solution, The brown-red solution was filtered and the filtrate was concentrated to about 5 ml. Colourless crystals separated

out on cooling and were recrystallised from methanol, giving succinimide, m.pt 125° to 126° C.

N-*Bromosuccinimide and Malonic Acid.* Malonic acid 1·04 g. (1 mol.) and powdered *N*-bromosuccinimide 1·78 g. (1 mol.) were heated with 25 ml. of water in a boiling water bath. The *N*-bromosuccinimide gradually dissolved and the solution became yellow. The gases evolved were removed in a current of nitrogen and found to be carbon dioxide, hydrogen bromide and ethylene. After one hour's heating in the water bath, the reaction mixture was filtered and from the filtrate 0·6 g. of succinimide was obtained. With *N*-bromosuccinimide and succinic acid similar results were obtained.

N-*Bromosuccinimide and Maleic Acid.* Maleic acid 1·16 g. (1 mol.) and powdered *N*-bromosuccinimide 1·78 g. (1 mol.) were heated with 35 ml. of water on the electric plate for one hour. A vigorous reaction took place, carbon dioxide and hydrogen bromide being evolved. From the reaction mixture, which had a conspicuous paraldehyde odour, 0·12 g. of fumaric acid, m.pt. 302° to 304° C. was isolated. From the filtrate 0·6 g. of succinimide was isolated.

In a second experiment the evolving gases were removed by a current of nitrogen and were passed, after washing with sodium hydroxide solution into ammoniacal cuprous chloride solution, but no acetylene could be detected. The sodium hydroxide solution became turbid and on heating a portion of this solution a yellowish precipitate of aldehyde resin was observed.

In a third experiment maleic acid 2·32 g., and *N*-bromosuccinimide 3·56 g., with 50 ml. of water were refluxed in a boiling water bath for one hour. On cooling the solution the odour of paraldehyde was perceptible. Dilute sulphuric acid (5 ml.) was added, the reaction mixture was heated and the gases evolved were received in 2:4-dinitrophenylhydrazine sulphate solution in methanol. The orange crystals formed were identified as acetaldehyde 2:4-dinitrophenylhydrazone by m.pt. and mixed m.pt.

N-*Bromosuccinimide and Fumaric acid.* Equimolecular quantities of *N*-bromosuccinimide and fumaric acid were used. The same results as in the case of maleic acid were recorded; again no free acetylene could be detected.

Summary

1. *N*-Bromosuccinimide reacts with saturated aliphatic dicarboxylic acids such as oxalic acid in aqueous solution at room temperature evolving carbon dioxide with the formation of hydrogen bromide and succinimide. With homologues of oxalic acid heat is necessary to effect the reaction.

2. *N*-Bromosuccinimide reacts with unsaturated aliphatic dicarboxylic acids such as maleic and fumaric acids in boiling aqueous solution giving carbon dioxide, hydrogen bromide and succinimide. The acetylene which is expected in the reaction is converted into acetaldehyde.

The author wishes to thank Professor J. Mitchell and Dr. D. H. Marrian for allowing him to carry out this work at the Radiotherapeutics Department, University of Cambridge.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Desmethylcolchicine, a Constituent of U.S.P. Colchicine. R. M. Horowitz and G. E. Ullyot. (*Science*, 1952, **115**, 216.) A batch of U.S.P. colchicine was purified by adsorbing on alumina and then eluting the pure colchicine with chloroform. Upon further elution with methanol-chloroform (1 : 99), desmethylcolchicine was obtained (up to 4 per cent. based on the weight of impure colchicine). The evidence for the structure of this compound, of formula $C_{21}H_{23}O_6N$, which is similar to colchicine but with one of the methoxyl groups in ring A demethylated, is as follows: (*a*) contains three methoxyl groups, (*b*) yields colchicine on treatment with diazomethane, and (*c*) gives a colour with ferric chloride only after it has been heated in dilute hydrochloric acid (presence of an enol ether). It is obtained as yellow prisms from ethyl acetate-ether containing a trace of chloroform. It turns to a glass at *ca* 176° to 190° C., recrystallises at *ca* 200° to 210° C., and melts finally at 275·5° to 277° C. No chemical changes are involved in these transitions. A comparison is made of the physical constants of this desmethylcolchicine, and one obtained (designated "Substance C") from the seeds of *Colchicum autumnale* by Santavý and Reichstein (*Helv. chim. Acta.*, 1950, **33**, 1606), and the two compounds are assumed to be identical. A. H. B.

Ergot Alkaloids, Paper Chromatography of. L. Fuchs and M. Pöhm. (*Scientia pharm.*, 1951, **19**, 232.) The composition of the ergotoxine group of alkaloids was determined by paper chromatographic separation of the amino-acids obtained after hydrolysis with concentrated hydrochloric acid; ergocristine gives phenylalanine, ergocornine valine, and ergokryptine leucine, in addition to proline which is formed from all three. Suitable solvents with the corresponding *R* values, are given below:

	n-butanol 4 acetic acid 1 water 5	*n*-butanol 1 benzyl alcohol 1 water 2
Proline	0.34	0.10
Valine	0.50	0.15
Leucine	0.66	0.30
Phenylalanine ..	0.63	0.40

The composition of the ergotoxine group of alkaloids varies greatly. Any one of the three alkaloids mentioned above may predominate. In some cases (individual sclerotia) ergocristine or ergocornine were absent, in others all three were present in equal quantities. G. M.

Tropine, Paper Chromatographic Separation of, from Esters. P. Mathes and W. Klementschitz. (*Scientia pharm.*, 1951, **19**, 235.) Tropine derivatives may be separated and differentiated by paper chromatography, using as solvent a mixture of 70 parts of β-ethoxyethanol with 30 of water. The operation is carried out in an atmosphere of ammonia to avoid the formation of "tails," and either the free bases or their hydrochlorides may be used. *R* values

are as follows: tropine, 0·75; lactyltropine, 0·85; atropine, 0·90; *l*-hyoscyamine, 0·90; homatropine, 0·90. The spots are developed with a 0·5 per cent. ethanolic solution of diphenylcarbazone. Quantities may be estimated by measuring the area of the spots. G. M.

ANALYTICAL

Amidines, Volumetric Assay of. F. H. Stephan. (*Anal. Chem.*, 1951, **24**, 180.) Two new volumetric methods are given for the amidines lomidine dimethane sulphonate, lomidine diisethionate, hexamidine diisethionate, benzamidine hydrochloride, *p*-sulphamidobenzamidine, stilbamidine diisethionate, 2-hydroxystilbamidine diisethionate and *p*-arsonobenzamidine. In the first method the amidine is precipitated with an excess of iodine, rendered alkaline, and the residue is separated by filtration. The filtrate is acidified and the iodine not consumed by the amidine is liberated and titrated with standard thiosulphate; the equivalent weight is equal to the molecular weight of the product divided by double the number of amidine groups in the formula. A table is given of the precision and accuracy of various methods. The second method depends on the fact that at room temperature and at a *p*H between 5·2 and 7·0, mercuric acetate interacts with the amidines to form voluminous gelatinous white insoluble derivatives which are termed "mercuriamidines." Under these conditions the reaction is quantitative and can be applied to the volumetric determination of the amidines, the solution used being 0·1 N mercuric acetate buffered with crystalline sodium acetate. Most accurate results were obtained at 18° to 22° C.; below 12° C. results were low while above 28° C. results were high due to increased mercuration. The mercuriamidines were white, bulky and gelatinous, insoluble in water and ethanol, but soluble in dilute acids. R. E. S.

Belladonna Preparations, Assay of, by Partition Chromatography. G. Schill and A. Ågren. (*Svensk farm. Tidskr*, 1951, **55**, 781, 797, 825.) By using an organic solvent as mobile phase and an acid buffer solution as immobile phase it is possible to separate the alkaloids of belladonna completely from neutral ballast substances. The first stage of the assay varies with the preparation: *Extractum belladonnae*, 10 g. is mixed with 10 ml. of M sodium carbonate and 10 g. of kieselguhr: *Extractum belladonnae liquidum* (1 : 1), 10 g. is mixed with 10 ml. of M sodium carbonate and 20 g. of kieselguhr: *Belladonnae folium*, 30 g. of the powder is mixed with about 30 ml. of M sodium carbonate to give a homogenous and rather dry mixture. The mixture is packed in a column and eluted with 200 ml. of chloroform at about 2 ml. per minute. The eluate is allowed to flow directly on to a column containing 15 g. of kieselguhr previously mixed with 4 ml. of 0·5 M phosphoric acid, and packed by forming into a slurry with chloroform. This second column is washed with 100 ml. of chloroform and the alkaloids are then eluted with 250 ml. of chloroform which has previously been shaken with half its volume of concentrated ammonia, and filtered. The eluate is passed through 10 g. of alumina, concentrated to 10 ml. and the optical rotation is determined. It is then evaporated to dryness, the residue being re-evaporated with 10 ml. of chloroform twice, and the residue is titrated with 0·1N hydrochloric acid. For satisfactory results a suitable kieselguhr must be used. This should correspond to the United States Pharmacopoeia specification, and pass a number of other tests which are described. It is important that it should be free from chlorides and nitrates. G. M.

Diethylstilbœstrol and Related Œstrogens, Spectrophotometric Assay of. C. A. Kelly and A. E. James. (*J. Amer. pharm. Ass. Sci. Ed.*, 1952, **41**, 97.) The assay depends upon the formation of a stable red colour from stilbœstrol by diazotisation and coupling with *m*-nitraniline. The method is applicable to tablets and pharmaceutical preparations and to benzœstrol, hexœstrol, mestilbol and dienœstrol, with slight variations in the wavelength of maximum absorption. The diazotisation solution is prepared by dissolving 50 mg. of *m*-nitraniline in 2 ml. of dilute hydrochloric acid, cooling and adding 1 ml. of 5 per cent. w/v sodium nitrite solution followed after 2 minutes by 95 ml. of cold water, 1 ml. of a 5 per cent. w/v solution of sulphamic acid and sufficient water to produce 100 ml. A solution containing 20 to 80 μg. of stilbœstrol in 5 ml. of ethanol (50 per cent.) is mixed with 1 ml. of 5 per cent. sodium borate solution and 2 ml. of cold diazotisation solution and allowed to stand for 10 minutes. 1 ml. of 10 per cent. sodium hydroxide solution is added, and sufficient water to produce 10 ml. The transmission is measured at the absorption maximum of 510 mμ, and read against a standard curve. Beer's Law applies with quantities up to 80 μg. of stilbœstrol in 10 ml. G. B.

Ephedrine in Pharmaceutical Preparations, Colorimetric Assay of. L. G. Chatten and L. I. Pugsley. (*J. Amer. pharm. Ass. Sci. Ed.*, 1952, **41**, 108.) The determination depends upon the reaction of ephedrine with picryl chloride in benzene solution to produce a yellow colour which is measured in a colorimeter with a filter transmitting at 400 mμ, the wavelength of maximum absorption of the coloured compound. There is a linear relationship between light absorption and amount of ephedrine, when quantities between 0·1 and 0·6 mg. in 9 ml. of benzene are treated with 1 ml. of a 0·3 per cent. solution of picryl chloride in benzene. Heating the mixture at 75° to 77° C. for 20 minutes, while insufficient to complete the reaction, gives satisfactory sensitivity. The intensity of the colour tends to increase after removal of the solution from the hot water bath; consequently the procedure described must be followed in detail, the colour being read after 3 minutes at room temperature. Under such conditions, optical density = 0·0415 + 0·8667 × quantity of ephedrine in mg. Methods are described for the extraction of ephedrine from aqueous sprays, water-soluble jellies, syrups, capsules, tablets and ointments, for which the colorimetric method gives good results whereas results of gravimetric assays tend to be too high. G. B.

Gentisic and Salicylic Acids, Simultaneous Spectrophotometric Determination of. L. J. Kleckner and A. Osol. (*J. Amer. pharm. Ass. Sci. Ed.* 1952, **41**, 103.) Solutions adjusted to *p*H 5·0 to 8·0, containing a total of 15 to 35 mg. of the acids in 1000 ml. of water are examined in the spectrophotometer at 296 mμ (absorption maximum for salicylic acid) and 320 mμ (absorption maximum for gentisic acid). The result is calculated from the absorption at these wavelengths, by means of the given equations derived from absorption data for the acids. The total quantity of the acids is checked by measurement of the absorption at 306·5 mμ, at which wavelength salicylic and gentisic acids exhibit equal absorption. An assay may be considered satisfactory if the sum of the results for salicylic and gentisic acids is within 0·7 mg. of that for total acids calculated from the absorption at 306·5 mμ. Small amounts of lactose or sucrose do not interfere, but ether-insoluble interfering substances have to be removed by extracting the acid solution with ether, evaporating the extract and preparing the solution for assay from the residue. G. B.

Local Anæsthetics, Analytical Characters of. K. Steiger and E. Kühni. (*Acta Pharm. Internat.*, 1951, **2**, 1.) The reactions of a number of local anæsthetics towards halides, cyanide, thiocyanate, alkali, oxidising agents and alkaloidal reagents are described. The following values are given for the melting points (Thiele block) of the picrates, after drying for 48 hours over phosphorus pentoxide:—Procaine monopicrate, 131·5° to 132·5° C.; procaine dipicrate, 147° to 148° C.; larocaine picrate, 163° to 165° C.; monocaine picrate, 164° to 165·5° C.; pantocaine picrate, 115° to 118° C.; farmocaine picrate, 91° to 93° C.; amylocaine picrate, 111° to 114° C.; alypin monopicrate, 130° to 132° C.; alypin dipicrate, 187·5° to 190·5° C.; metycaine picrate, 119·5° to 121·5° C.; intracaine picrate, 114·5° to 116° C.; diocaine picrate, 115·5° to 117·5° C.; tropacocaine picrate, 223·5° to 234° C.; xylocaine picrate, 218° to 222° C.; surfacaine picrate, 89·5° to 92·5° C.; stadacaine picrate, 118° to 120° C.; cocaine picrate, 158° to 161° C. G. M.

Methanol, Assay of and Content in Brandy. M. St. Mokranjac and S. Radmić. (*Acta pharm. Jug.*, 1951, **1**, 97.) The best conditions for the quantitative determination of methanol by Denigés method are obtained when the solution contains 5 per cent. of ethanol; higher percentages reduce the sensitivity of the test. The test is most sensitive when carried out in aqueous solution, but the curves obtained do not then obey Beer's law. In carrying out the assay, the ethanol content should be adjusted to 5 per cent., the solution should be allowed to stand for 1 hour at 30° C. before the absorption is measured, and sulphuric acid diluted with an equal volume of water should be used. The methanol content of various brandies ranged from 0·108 to 1·455 per cent., expressed as ml. per 100 ml. of absolute ethanol. The lowest percentage was found in a plum brandy in the manufacture of which the first runnings of the distillation were rejected. A limit of 1 ml. of methanol per 100 ml. of absolute ethanol is suggested for all brandies. G. R. K.

Morphine in Opium, Determination of, by Paper Chromatography. A. B. Svendsen. (*Pharm. Acta Helvet.*, 1951, **26**, 323.) About 0·25 g. of morphine is rubbed down with 0·5 ml. of concentrated formic acid, transferred with the aid of 2·5 ml. of water to a 3G3 glass filter, and filtered, the residue being washed by rubbing with 0·5 ml. of formic acid (5 per cent.) for 2 minutes, this operation being repeated until there is, in all, 5 ml. of extract. Of this solution 0·01 ml. is chromatographed, using a mixture of ethyl acetate, formic acid and water (10:1:3). A further 0·01 ml. of solution is applied, so that the spot corresponds to 0·02 ml. It is necessary to have 6 spots on the paper: 5 for the quantitative series, and 1 in order to determine the position of the morphine. The latter is done by cutting off a strip of the paper, containing one spot, and developing it by spraying with 2 per cent. sodium nitrite solution: after 2 minutes the paper is placed in an ammoniacal atmosphere. Each spot is then cut out and placed in a test tube, where it is treated with 2 ml. of 1 per cent. hydrochloric acid and 2 ml. of sodium nitrite solution (0·5 per cent.). After exactly 10 minutes, 1 ml. of 5 per cent. ammonia is added. If necessary, the mixture is filtered on a sintered glass filter, and the extinction is determined at 450 mμ. The amount of morphine is then derived from a standardisation curve obtained with pure morphine. G. M.

Quinine and Quinidine, Identification of. D. Barković. (*Acta pharm. Jug.*, 1951, **1**, 73, 91.) Solutions of quinine or quinidine containing sodium acetate,

calcium carbonate, magnesium oxide, or similar substances, and warmed to 60° C. yield a red colour with bromine water. In the presence of sodium bicarbonate or an excess of sodium acetate, the red product is soluble in chloroform. On the addition of hydrochloric acid to the red solution, the colour becomes yellow but is restored to red by the further addition of sodium acetate. The yellow product yields the reactions of peroxides with potassium iodide. The red colour of the original solution also becomes yellow on the addition of zinc and sulphur dioxide or sodium pyrosulphite, and eventually disappears, but returns when the mixture is allowed to stand or is treated with an oxidising agent. If the yellow mixture is treated with dilute sulphuric acid, it gives with ferric chloride a colour similar to that given by pyrocatechol. To distinguish between the two alkaloids about 5 mg. is dissolved in 2 to 3 ml. of very dilute acetic acid (the salts are dissolved in water) by warming to 60° C. and treated with a drop of a 5 per cent. solution of sodium acetate, and, without mixing, 2 drops of bromine water. After 30 to 60 seconds, a red to violet-red colour develops on mixing. 2 to 3 minutes later, 3 ml. of 2N sodium hydroxide is added and after a further 1 to 2 minutes, the mixture is shaken with 1 to 2 ml. of chloroform. In the presence of quinidine the chloroform becomes red or violet-red, whereas in the presence of quinine it remains colourless or becomes only pale red. If the quinine solution is made slightly alkaline with sodium hydroxide and shaken with chloroform, the red or violet-red colour develops fully. In the presence of an excess of alkali the chloroform layer gradually becomes red or violet-red in the presence of quinidine but remains colourless or becomes only pale red in the presence of quinine. G. R. K.

Rutin, Determination of, in Presence of Quercitin. L. Hörhammer and R. Hänsell. (*Arch. Pharm. Berl.*, 1951, **284**, 276.) Both rutin and quercitin give colours with zirconium oxychloride, the maxima of absorption being at about 430 and 475 mμ respectively, but that with rutin is destroyed almost completely by citric acid. For the detection of quercitin in presence of rutin, 20 ml. of an absolute acetone extract is treated with 1 ml. of a 2 per cent. solution of aluminium chloride in methanol. Quercitin gives a strong green fluorescence, visible in daylight, and generally increased by dilution with methanol. It is possible to detect 0·5 mg. of quercitin in presence of 5 g. of rutin. For the determination of rutin in presence of quercitin, the preparation is extracted in a micro-Soxhlet extractor with methanol. Aliquots of the solution are transferred to two 50-ml. measuring flasks. To one is added 1 ml. of a 2 per cent. methanolic solution of zirconium oxychloride ($ZrO(Cl_2),8H_2O$). After making up to the mark with methanol and waiting for 20 minutes, the extinction coefficient is determined (filter S43). The other flask is treated with so much methanol that, after filling to the mark with water, the concentration of methanol will be 20 per cent. The contents are then treated with 1·0 ml. of 2 per cent. aqueous solution of citric acid, and 1 ml. of zirconium oxychloride solution. After making up with water, and waiting for 20 minutes, the absorption is again determined as before. A blank is used to compensate for any colour in the extract. The results are calculated according to the formulæ, which hold for the range of 5 to 90 per cent. of quercitin in presence of 95 to 10 per cent. rutin:

$$C_Q = \frac{E'_m - 0{\cdot}03}{0{\cdot}508}; \quad C_R = \frac{E_m - C_Q\,0{\cdot}481}{0{\cdot}384}$$

G. M.

Sulphanilamides in Mixture, Spectrographic Determination of. J. Thomas and G. Lagrange. (*J. Pharm. Belg.*, 1951, **6**, 355.) A spectrophotometric method for the identification and determination either alone or in mixture of sulphadiazine, sulphamerazine, sulphathiazole and sulpha-2-ethyl thiodiazol is described. The principle consists of first determining the absorption of the mixture at 440 mμ (sulphadiazine) and at 320 mμ (sulphamerazine) after extraction and dilution with acetone so that 2 ml. contains 0·8 mg. of the drug present in greatest quantity. Evaporate 2 ml. to dryness *in vacuo*, add 5 drops of ethanol and evaporate again. To the residue in a tube add 2 ml. of concentrated sulphuric acid and 1 ml. of a fresh, colourless, aqueous 5 per cent. solution of resorcinol drop by drop, shaking and cooling in iced water. Cover the tube with tinfoil and place for 30 minutes in a water-bath at 80°. Cool immediately and make up to 10 ml. with a mixture of equal parts of a 40 per cent. v/v sulphuric acid solution and glycerol (A). Cool in iced water, shake and make the determinations 10 minutes after leaving the bath in a 5-mm. cell for sulphadiazine and after 15 minutes in a 1-cm. cell for sulphamerazine. A blank of 2 ml. of concentrated sulphuric acid, 1 ml. of water and solution (A) to 10 ml. is used. The absorption of a 1 mg. per cent. solution in N hydrochloric acid in a 1-cm. cell is measured at 280 mμ for sulphathiazole and at 268 mμ for sulpha-2-ethyl thiodiazol. H. D.

Zinc Oxide in Ointments, Assay of. J. Deltombe. (*J. Pharm., Belg.*, 1952, **7**, 93.) The following method gives accurate and reproducible results in about 30 minutes on ointments containing 5 to 10 per cent. of zinc oxide. Dissolve 5 g. of ointment by shaking with 30 ml. of chloroform, add 30 ml. of dilute sulphuric acid and shake for 10 minutes. Add 3 drops of mixed methyl orange-bromocresol green indicator and 5 drops of phenolphthalein solution, add 9 ml. of a 20 per cent. solution of sodium hydroxide and titrate with 0·25N sodium hydroxide until the orange-red colour disappears (i.e., the solution is almost colourless when shaken and yellowish on standing). Titrate with 0·25N sodium hydroxide to the phenolphthalein end-point (purple-violet). Each ml. of 0·25N sodium hydroxide used between the two end-points is equivalent to 0·0101725 × 4/3 g. of ZnO. A long, but accurate, method is to weigh the zinc oxide on a sintered-glass filter after removal of the ointment base by repeated washing with chloroform. The U.S.P. XIII method gives low, and the B.P. 1948 variable, results. The method of titration as sulphate in chloroform gives high results in the presence of starch unless the technique is suitably modified. G. B.

ORGANIC CHEMISTRY

Rutin and Related Flavonols, Preparation of Water-soluble Metal Complexes of. C. F. Krewson and J. F. Couch. (*J. Amer. pharm. Ass. Sci. Ed.*, 1952, **41**, 83.) A number of metallic compounds were tested for their power to increase the solubility of rutin, which is soluble only in about 10,000 parts of cold water. Rutin (0·7 g.) was dissolved in 150 ml. of boiling water, the metallic compounds added and the mixture boiled, cooled and filtered. The residue of insoluble rutin was weighed, and the quantity in solution calculated. The following compounds were effective in solubilising the rutin:—ferrous ammonium sulphate, ferrous lactate, ferric chloride, ferrous gluconate, ferric pyrophosphate, saccharated iron oxide and cuprous chloride. With some of these substances stoichiometric complexes appeared to be formed, for example, 2 moles of rutin reacted with each mole of ferrous gluconate whereas equimolecular complexes were formed with ferric chloride and ferrous ammonium sulphate. A preparation made with ferrous gluconate contained 28 mg. of rutin and 1·16 mg. of

iron in 5 ml. and had the *p*H 4·2. Saccharated iron oxide preparations contained up to 550 mg. of rutin and 2 g. of iron in 5 ml. at *p*H 6·8 to 9·3, and could be diluted with water without the formation of a precipitate. Saccharated iron oxide, but not ferrous gluconate, increased the solubility of quercitin and quercitrin in addition to rutin. G. B.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Amylase and Maltase in Urine. P. F. Fleury, J. E. Courtois and D. Ramon. (*Bull. Soc. Chim. biol. Paris*, 1951, **33**, 1762.) Samples of human urine, preserved with chloroform were mixed with phosphate buffer at *p*H 7 and maltose solution and incubated for 22 hours at 37°. The solution was boiled to remove chloroform and to inactivate the enzyme, and glucose was estimated by the yeast fermentation method of Ramon. Control experiments were carried out with urine previously boiled for 10 minutes to inactivate the enzyme. Urine samples showed a faint maltasic activity, and the urinary maltase appeared to be identical with the α-glucosidase of certain tissues. Attempts were made to obtain urinary amylase free from maltase by fractionation with ammonium sulphate and by chromatography on cellite-alumina columns. A satisfactory purified amylase was prepared by precipitation with 40 to 70 per cent. of acetone, maltase being inactivated by acetone treatment. Reducing sugars produced by the action of this amylase contained about 2 per cent. of glucose. G. B.

Penicillin, Radioactive, Chromatography of. E. Lester Smith and D. Allison. (*Analyst*, 1952, **77**, 29.) Radioactive penicillin of high specific activity (up to 600 millicuries per g.) was prepared by fermentation of a synthetic medium containing sulphur-35 as sulphate. The resulting mixture of penicillins was then subjected to paper chromatography, being spotted out on buffered strips, which were developed with ether in the special apparatus described by Goodall and Levi (*Analyst*, 1947, **72**, 277; *Quart. J. Pharm. Pharmacol.*, 1948, **21**, 85). Quantitative assessment was made by the bio-autographic method on agar plates inoculated with *B. subtilis* and also by two radiometric methods; sections containing individual penicillins were cut from the paper chromatograms, radio-autographs being used as guides, and either they were "counted" directly under a thin end-window Geiger-Müller tube or aqueous extracts were evaporated on planchettes for counting. Details are given of modifications of the chromatographic procedure. Attempts to utilise the ^{35}S-penicillin in an isotope dilution assay specifically for benzylpenicillin were unsatisfactory since the *iso*propyl-ether complex method was inadequate as a means of purification. R. E. S.

Quinine and Quinidine, Action of on Cholinesterase. E. Bach, B. Robert and L. Robert. (*Bull. Soc. Chim. biol. Paris*, 1951, **33**, 1805.) Quinine or quinidine was dissolved in 9 ml. of recently boiled water and mixed with 1 ml. of 0·1M acetylcholine chloride or bromide, 1 drop of cresol red solution and 0·5 ml. of horse serum as a source of cholinesterase. Control solutions were prepared without the inhibitor. Experiments were carried out at 37° C., hydrolytic action being determined by a titrimetric method. Results were corrected to 40° C. (optimum for the reaction) and the rate of hydrolysis interpreted according to the equations of Goldstein and Strauss. Inhibition commenced at a

concentration of about 10^{-5} mol./l. for quinine and about one-tenth that concentration for quinidine. In a horse serum containing 4·76 per cent. of enzyme at 40° C. the competitive system with quinine was observed in the zone A_8B_I (substrate in great excess relative to enzyme-substrate complex; free enzyme and enzyme-inhibitor complex in comparable amounts) and quinidine in the zone A_8A_I (substrate and inhibitor in great excess relative to enzyme-substrate and enzyme-inhibitor complexes). The dissociation constant of the cholinesterase-quinine complex was $8{\cdot}55 \times 10^{-6}$ and of the cholinesterase-quinidine complex, $2{\cdot}23 \times 10^{-6}$. The concentration of non-specific (blood serum) cholinesterase in horse serum estimated from the maximum rate of hydrolysis was of the order of 10^{-8} mol./l. G. B.

Vitamin B_{12}, New Form of. U. J. Lewis, D. V. Tappan and C. A. Elvehjem. (*J. biol. Chem.*, 1952, **194**, 539). The isolation of a vitamin B_{12}-active material present in the fæcal matter of rats is reported. Dried fæces were extracted with boiling water, the resulting extract being filtered and evaporated to a syrup under reduced pressure; after precipitating proteins with ethanol, the active fraction was adsorbed on charcoal, from which it was eluted with hot ethanol (65 per cent.). The extract was then purified by alumina adsorption and chromatography on a silica column using *n*-butanol-water as the solvent system; a crystalline product was obtained. The compound had growth-promoting properties for *L. leichmannii* but was inactive for the rat; the growth response of the chick to the material was comparable to that of crystalline vitamin B_{12}. The feeding of inorganic cobalt to the rats brought about an increased production of the new factor although the amount of activity present in the liver, kidney, spleen, muscle tissue and urine remained essentially the same whether extra cobalt was present or absent. Details of the paper chromatography of the new vitamin B_{12}-active form are given using a bioautographic technique and *n*-butanol-water as the solvent; the behaviour differed from that of vitamin B_{12} itself. The absorption spectrum in water differed considerably from that of vitamin B_{12}, the peak at 278 mμ being absent. R. E. S.

BIOCHEMICAL ANALYSIS

Acetic Acid, Micro-analytical Estimation of. E. Ciaranfi and A. Fonnesu. (*Biochem. J.*, 1952, **50**, 698.) A method is presented for the micro-estimation of acetates and acetic acid in blood and tissues. The sample (1 g. of tissue, 5 ml. of blood) is mixed with excess of methanol and distilled under conditions such that the methyl esters of the fatty acids are produced; the acetate (b.pt. 57° C.) boils at a lower temperature than methanol (b.pt. 65° C.), whereas the methyl esters of the other members of the homologous series have a higher boiling point; thus by esterification of the fatty acids and subsequent distillation a considerable separation of acetic acid from the remainder can be effected. By repeated distillations the C_3 to C_7 fatty acid esters are removed from the resulting methyl acetate; the ester is then saponified and the resulting sodium acetate brought to dryness. Formic acid and hydrogen sulphide are removed by oxidation with potassium dichromate-sulphuric acid, the acetic acid remaining unchanged; the acetic acid is then oxidised by increasing the sulphuric acid concentration and by adding silver ions. The dichromate reduction is measured photometrically (450 mμ) and the acetic acid is estimated by the difference in the extinction. The method permits the estimation of acetic acid in blood and tissues (0·1 to 2·3 mg.) with sufficient accuracy for biological

purposes, the error being less than ± 10 per cent. Tables showing the concentrations of acetic acid under physiological conditions, in human blood and in the blood, liver, kidney and muscle of the guinea-pig and rat are given.

R. E. S.

Glucose in the Presence of Maltose, Estimation of. D. Ramon. (*Bull. Soc. Chim. biol. Paris*, 1951, **33**, 1756.) A mixture of 27 ml. of sugar solution with 3 ml. of phosphate buffer at *p*H 7 is warmed and mixed with 0·3 g. of baker's yeast at 37° C. The mixture is allowed to ferment at 37° C. for 30 minutes and centrifuged. Ethanol is removed from the supernatant liquid by distillation and estimated by treatment with nitric acid-potassium dichromate solution and titration of the excess of reagent iodimetrically. A duplicate experiment is performed using distilled water in place of sugar solution and any necessary allowance made. The result is calculated from the datum that for solutions containing 0·025 to 0·040 per cent. w/v, the yield is 54 per cent. of the theoretical 2 molecules of ethanol per molecule of glucose. Maltose does not yield ethanol under these conditions and the accuracy of the method is fairly high for a reaction of such complexity. During 30 minutes' fermentation the whole of the glucose is destroyed but in spite of the low yield of ethanol other possible products such as acetone or acetaldehyde do not appear in the distillate. When fermentation continues for more than 45 minutes the yield of ethanol from glucose is not increased but maltose undergoes slight fermentation. Toluene should be avoided as it interferes with the hydrolysis of the glucose, but sugar solutions may be preserved with chloroform, which is removed by heating before making an estimation of the glucose content. G. B.

Vitamin B_{12}, Selective Assay of by Chromatography. F. Patte. (*Ann. pharm. franç.*, 1951, **9**, 660.) The following method is suitable for the determination of vitamin B_{12} (cyanocobalamin) in the presence of desoxyribonucleosides and desoxyribonucleotides which interfere in the ordinary microbiological assay. Solutions of the vitamin B_{12} standard containing 0·8, 1·6, 3·2 and 6·4 g. per ml. are prepared in distilled water, and suitable solutions of the preparation under test are also prepared. Using a special pipette delivering 0·0025 ml. drops, a row of drops of these solutions is placed on the filter paper and developed by ascending chromatography until the solvent (butanol saturated with water) has risen 7 cm. This takes about 45 to 60 minutes. The chromatogram is allowed to dry and placed on a seeded agar plate for incubation at 37° C. for 16 hours. The maximum diameter of the circles of growth stimulation due to vitamin B_{12} is displaced about 2 mm. above the origin of the chromatogram. Diameters of the circles are plotted on log-linear graph paper and should yield straight lines of equal slope from which the potency is calculated. Areas of growth stimulation due to the desoxyribonucleic matter are generally separated completely from the areas due to cyanocobalamin and its analogues. To obtain truly circular areas for measurement, the samples have to be diluted with water and not buffer solution as excessive amounts of salts disturb the chromatography. In samples containing much desoxyribonucleic material, flattening of the part of the growth-stimulation zone which is in contact with the desoxyribonucleic derivatives may occur. Some samples contain growth-stimulating factors which are not separated from the vitamin B_{12} by this method. The method was shown to be accurate with prepared mixtures of vitamin B_{12} and desoxyribonucleosides. This method is also useful for the qualitative detection of desoxyribonucleosides in samples of vitamin B_{12}. G. B.

CHEMOTHERAPY

Aminoalkyl Morphine Ethers. J. Cheymol, R. Giudicelli, P. Chabrier and K. Kristensson. (*Thérapie*, 1952, **7**, 21.) Diethylaminoethylmorphine was prepared by warming morphine in sodium hydroxide solution with the hydrochloride of diethylaminoethyl chloride. Piperidylethylmorphine was prepared in a similar manner. When injected intravenously into mice, diethylaminoethylmorphine and piperidylethylmorphine were more toxic than codeine while morpholinoethylmorphine was less toxic. The compounds exhibited a respiratory depressant action similar to codeine, but had a smaller convulsive effect. Piperidylethylmorphine and codeine showed about the same analgesic effect, which was absent in the case of the morpholinoethyl and diethylaminoethyl compounds. The dibromomethylates, prepared by reaction with methyl bromide in ethanol, exhibited a curare-like action, the ratio of curarising dose (rabbit head-drop method) to LD50 in mice being greater than for D-tubocurarine chloride. G. B.

Dithiocarbamic Esters, Antibacterial Activity of *N*-disubstituted. A. Quevauviller, P. Chabrier, G. Nachmias and G. Maillard. (*Ann. pharm. franç.*, 1951, **9**, 638.) Derivatives of dithiocarbamic acid dissolved in acetone were mixed with melted agar medium, which was poured into Petri dishes and allowed to set. The proportion of acetone was kept below 5 per cent. v/v to prevent inhibition of growth by the solvent. The plates were seeded in layers with 24-hour broth cultures of *Staphylococcus pyogenes aureus*, *Escherichia coli*, *Bacillus mesentericus* and *Pseudomonas æruginosa*, and incubated for 24 hours at 37° C. before comparison with similarly prepared agar plates to which the same quantity of solvent had been added. Derivatives of dithiocarbamic acid were more active against Gram-positive than against Gram-negative organisms. The sodium salts of the *N*-substituted derivatives, dimethylamino, morpholino and piperidino carbodithioic acids were of high antibacterial activity, the piperidino derivative being the most active and the morpholino derivative the least active. Esters of these substances having alcohol, nitrile or carboxylic acid groups were practically without antibacterial action, but the ketonic esters had a certain amount of activity against *B. mesentericus*. β-Dimethylaminoethyl *N*-dimethyldithiocarbamate had an activity similar to that of the sodium salt of the parent acid, but the corresponding quaternary ammonium salt was inactive. G. B.

Salicylamide, Analgesic Properties of Certain Derivatives of. M. Carron, J. Tabart and Mme. Jullien. (*Thérapie*, 1952, **7**, 27.) The analgesic effect of 42 substances administered orally to mice was examined by the application of a pain stimulus in the form of radiant heat applied to the tails of the mice. Analgesia was assessed by absence of movement of, or delay in moving, the tail when the radiation was applied to the base of it. All compounds were submitted to a screening test, a fixed dose of the drug being administered and the animal examined for analgesia after 30, 60, 90, 120 and 150 minutes. For the more active compounds, the minimal active dose and the toxicity on oral administration were determined in mice, and the therapeutic index calculated. *N*,*N*-diethylsalicylamide had the highest therapeutic index. Substitution in the phenolic group:—the introduction of hydrophilic functions, $-CH_2{\cdot}CO_2H$, $-CH_2NH_2$, $-CH_2{\cdot}CH_2OH$, $-CH{\cdot}CH(OH){\cdot}CH_2OH$ decreased analgesic potency compared with salicylamide. Methylation increased the activity while ethylation decreased it. Substitution in the amido group:—*N*-dimethyl

and *N*-butyl derivatives were inactive, while *N*-diethyl and *N-iso*propyl derivatives were active. When two salicylamide molecules were joined at the amido groups by a 3-carbon atom chain the compound was active; corresponding compounds joined by 1 or 2 carbon atoms were inactive. 3 derivatives of gentisamide were inactive, but *o*-cresotinamide was more active than salicylamide. The therapeutic indices, $\left(\frac{LD50}{MAD50}\right)$, of some compounds were found to be as follows:—*o*-ethoxylbenzamide, 3·5; *N-iso*propylsalicylamide, 2·6; *N*-methylolsalicylamide, 3·54; trimethylene *bis*-salicylamide, 3·65; *N*-diethylsalicylamide, 5·66; *O*-acetylsalicylureide, 1·5; cresotinamide, 1·97; salicylamide, 2; amidopyrine, 2·8; acetylsalicylic acid, 2·07 G. B.

PHARMACY

DISPENSING

Neostigmine Methylsulphate, Decomposition of Solutions of. A. W. M. Indemans and J. A. C. Pinxteren. (*Pharm. Weekbl*, 1951, **86**, 773.) Hydrolysis of solutions of neostigmine may be detected from the dimethylamine formed. About 0·1 ml. of the solution (= 50 μg.) is distilled with buffer solution of *p*H 8 in a microdistillation apparatus, the dimethylamine being collected in a drop of 0·1 N hydrochloric acid. The acid solution is evaporated to dryness, the residue taken up in 0·02 ml. of water and transferred to a micro tube. After making alkaline with sodium hydroxide, the mixture is distilled. The dimethylamine is detected by a piece of filter paper in the vapour, soaked in a solution of 1 g. of sodium nitroprusside in 10 ml. of freshly distilled and neutralised acetaldehyde. It is possible to detect 0·5 μg. of dimethylamine. G. M.

GALENICAL PHARMACY

Disintegrating Agents, A Comparative Study of. H. M. Gross and C. H. Becker. (*J. Amer. pharm. Ass. Sci. Ed.*, 1952, **41**, 157.) Tablets were prepared by mixing lactose with a suitable quantity (generally 17 per cent.) of disintegrating agent and granulating with the aid of a solution containing 5 per cent. of zein, in a mixture of syrup, ethanol and water. For disintegrating agents which react with water, 5 per cent. of zein in *iso*propanol was used. Granules were lubricated with leucine (2 per cent.), and in an alternative procedure, some of the disintegrating agent was added with the lubricant. Tablets were tested for hardness with a Monsanto Hardness Tester, and for disintegration by the U.S.P. XIV method, results being extrapolated to a standard hardness of 7 kg. A variety of disintegrating agents, such as aveeno, pectin, corn starch, locust bean gum, methyl cellulose, magnesium peroxide, sodium carbonate-peroxide, citric acid-calcium carbonate and pectin-calcium carbonate, gave successful results, in many cases comparing favourably with commercial tablets prepared by precompression. Some gums such as algin, sodium alginate, veegum, tragacanth and karaya gave tablets which did not disintegrate within 10 minutes and often remained covered with a water-impervious gum layer. Tablets which disintegrated readily were prepared with dried citrus pulp and powdered dried sponge, but the former gave grainy, and the latter, tan-coloured tablets. Storage at room temperature for 500 hours did not increase disintegration times, except for tablets prepared with pectin. Similarly storage at 4° C. had little effect, but deodorised some tablets. Exposures to 45° C. for 500 hours had a deleterious effect in most cases. G. B.

PHARMACOGNOSY

Belladonna Leaves, Phosphatase of. J. E. Courtois, C. Anagnostopoulos and M. Khorsand. (*Bull. Soc. Chim. biol., Paris*, 1951, **33**, 1813.) The phosphatase occurs in two fractions, the greater part soluble in water, and the remainder insoluble and firmly bound to the cell tissues. The following three preparations were tested:—(1) the soluble enzyme prepared by extraction of the powdered leaves with water followed by precipitation with acetone, (2) the insoluble material after removal of the water-soluble material, washed with acetone and dried and (3) (2) after treatment with sodium cyanide to remove metallic ions. There was little difference in action between the different preparations, when incubated at 37° C. for 48 hours in contact with 0·04M glycerophosphate solution and a suitable buffer solution. The phosphatases present belonged to types II and III, and hydrolysed a number of phosphorus compounds, but not phytic acid. A distinctly greater proportion of the type II phosphatase occurred in the samples purified by a process of fractionation with ammonium sulphate, and the phosphatase was not significantly different from others of the same type. The type III phosphatase was markedly different from those obtained from sources other than leaves. It was strongly activated by the bivalent ions Mg^{++}, Sn^{++}, Ni^{++} and Co^{++}. G. B.

***Datura stramonium*, Location of Alkaloids in.** R. Hegnauer. (*Pharm. Weekbl.*, 1951, **86**, 935.) *Datura stramonium* was grafted on roots of tomato, previously tested for the absence of mydriatic alkaloids. It was possible to demonstrate a translocation of alkaloids in the plant in all directions and towards all organs, including the tomato root. When the grafted tops were cut off and immersed in alkaloidal solutions, the latter accumulated in greater concentration (calculated on the dry matter) in the midribs of the leaves than in the laminae of the leaves. Neither the demonstration of the presence of alkaloids in all organs, not the typical manner of accumulation of the alkaloids in the leaf, can be considered as a definite indication of alkaloid synthesis taking place in the underground organs. G. M.

Digitalis, Relation of Biological Activity to Time of Collection of. F. Neuwald. (*Arch. Pharm., Berl.*, 1951, **284**, 382.) It is generally assumed that digitalis leaves should be harvested in the afternoon, owing to the glycosides being synthesised in sunlight and broken down in the dark. Actually the differences in values found in the original experiments which were supposed to prove this fact were not significant. The author has re-investigated the problem, taking samples of leaves at 5 a.m. and 5 p.m. respectively, and testing the activity both biologically on cats, guinea-pigs and frogs, and chemically. The results show that there is no significant difference either in biological activity or in content of heart-active glycosides within a period of 24 hours. Similar results were also obtained with *D. lutea* and *D. ambigua*. There is therefore no reason to specify that the leaves should be collected in the afternoon. G. M.

***Scilla maritima*, Vegetative Reproduction.** I. Z. Devetak. (*Acta pharm. Jug.*, 1951, **1**, 83.) Pieces of bulb consisting of 2 or 3 scales joined to a small piece of the base were either set aside on the laboratory shelf or buried in sand. At the same time a number of scales were completely separated from the bulb and set aside either whole or cut into 2, 3, or 4 portions. After 3 months all the specimens had produced small bulbs weighing 5 to 15 g. When planted

in March, the bulbs began to grow normally in October. By this means one parent bulb weighing 3 kg. can be made to yield about 100 new plants in one season. G. R. K.

PHARMACOLOGY AND THERAPEUTICS

Aconite, Biological Assay of. R. Paris and J. Vavasseur. (*Ann. pharm. franç.*, 1951, **9**, 718.) A biological test is necessary because of wide variations in toxicity of aconite powders which cannot be distinguished microscopically. The method of the French Pharmcopœia 1949, using a guinea-pig is open to criticism because (1) only one animal is used and (2) only a minimum potency requirement is given, and consequently highly toxic aconites are not excluded. Upper and lower limits of potency are recommended, as in the U.S.P.X. A simpler and more precise method is based upon subcutaneous injection into mice, and it can be made more exact by making the determination of LD50 in comparison with a standard aconitine. The LD50 for pure aconitine varies between 8 and 9 μg./kg. according to the strain of mouse used. In a series of 10 commercial tinctures containing about 0·05 per cent. of total alkaloids and tested in mice, wide variations in potency were observed. In 3 of the tinctures, potency was similar to that of a control prepared from genuine *Aconitum napellus*, and 3 other tinctures had lower potencies, presumably having been prepared from old aconite roots. One specimen was highly toxic, resembling a control tincture prepared from *A. deinorrhizum*. G. B.

Adrenaline and Noradrenaline; Release from the Suprarenal Gland. A. Lund. (*Acta Pharmacol. Toxicol.*, 1951, **7**, 309.) This investigation was carried out to show the course of the release of adrenaline and noradrenaline from the glands in circumstances, e.g. blood-letting and electrical stimulation, known by experience to give rise to a pronounced release of adrenaline. Venous blood from the left suprarenal gland of dogs was collected continuously from the suprarenal vein, while at the same time a corresponding amount of donor blood was infused through the jugular vein. In other words, the suprarenal gland was perfused *in situ*. The concentrations of adrenaline and noradrenaline in the blood samples were estimated fluorimetrically. Under gentle anæsthesia no adrenaline or noradrenaline is released to the blood. As blood pressure falls, from electric stimulation of the splanchnic nerve and at the time of death, a mixture of adrenaline and noradrenaline is released, of which from 25 to 50 per cent. is noradrenaline. In each experiment the concentration of noradrenaline to adrenaline remained constant, irrespective of the duration of the experiment or the nature of the stimulant. The suprarenal glands from non-anæsthetised animals contained on an average 1·56 mg. of adrenaline and 0·48 mg. of noradrenaline per g. The glands from anæsthetised animals contained only about half these amounts. S. L. W.

Digitoxoside and Gitoxoside, Some Comparative Pharmacological Data. R. Jequier, M. Peterfalvi and C. Plotka. (*Ann. pharm. franç.*, 1951, **9**, 730.) Pigeons were anæsthetised with ether and fitted with a cannula in the alar vein. Test solutions of the glycosides, purified by chromatography, were injected in 1·5 ml. quantities every 5 minutes until death of the animal by cardiac arrest occurred. The concentration of glycoside was adjusted to cause death in 65 to 95 minutes as described in the U.S.P. XIV. The average lethal dose showed a wide difference between the glycosides, being 0·40 mg./kg. for digitoxoside and 0·80 mg./kg. for gitoxoside, whereas in cats gitoxoside is only

slightly less toxic than digitoxoside. The dose necessary to produce cardiac irregularities, determined by the electrocardiograph in pigeons is 0·20 mg./kg. for digitoxoside and 0·25 mg./kg. for gitoxoside. It is pointed out that this variation in the ratio of toxicities of the glycosides according to the animal used and the method of testing may be the reason for the discrepancies in the results obtained by different biological methods of assay of digitalis preparations. In experiments in the isolated rabbit or guinea-pig heart, digitoxoside was found generally to increase the coronary output, and gitoxoside to diminish it. This effect was confirmed in experiments with a rat heart connected to the circulatory system of a living rat, this method having the advantage of using blood as perfusion fluid, a pulsating pressure instead of the constant pressure in the isolated organ method, and an animal of low sensitivity to the toxic action of digitalis on the heart. The opposing action of the two glycosides may serve to explain conflicting results previously reported in experiments on the coronary effect of digitalis preparations. G. B.

Hetrazan in the Treatment of Human Ascariasis. E. H. Loughlin, I. Rappaport, A. A. Joseph and W. G. Mullin. (*Lancet*, 1951, **261**, 1197.) A comparison was made on three groups of patients with ascariasis, whose ages ranged from 3 to 70 years, of the efficacy of different dosages of a syrup of hetrazan, containing 30 mg. of the dihydrogen citrate per ml., with that of the 6 mg./kg. given in tablet form. It was shown that the tablets given in this dosage three times daily for 5 days expelled numbers of ascaris comparable to those removed by the syrup given in single doses of 13 mg./kg. on the first day and 20 mg./kg. on the second and third days, or in single doses of 13 mg./kg. for 4 days; the syrup, however, possesses the advantage that it obviates multiple daily doses and is easier to administer to small children. The authors conclude that syrup of hetrazan, because of its excellent anthelmintic efficiency (91 to 94 per cent. of ascaris removed), its almost complete lack of toxicity in doses not exceeding 20 mg. daily, and its ease of administration, is the anthelmintic of choice for the treatment of ascariasis and would be useful for mass treatment. S. L. W.

Hexamethonium Bromide in Hypertension. E. A. Murphy. (*Lancet*, 1951, **256**, 899.) Encouraging results were obtained in 43 cases of hypertension of different degrees and in patients of different ages treated with hexamethonium bromide either orally or subcutaneously, some of whom had previously been treated without success by sympathectomy or with potassium thiocyanate. All cases with clinical or electrocardiographic evidence of coronary disease or evidence of cerebral thrombosis were excluded and the sensitivity of the patients to hexamethonium was established by the Freis test. The initial dose was 0·25 g. by mouth 3 times daily, gradually increased to a satisfactory level, a total dose of 1 g. 4 times daily being regarded as a maximum. If this failed, hypodermic injections were given, beginning with 25 mg. twice daily, and increasing gradually to a maximum of 200 mg. 3 times daily. The minimum effective dose was 0·5 g. twice daily by mouth or 25 mg. twice daily by injection. The most common side-effects, in the order of their frequency, were constipation, light-headedness, drowsiness, dry mouth, visual disturbances, nausea, diarrhœa and vomiting. All these side-effects responded to symptomatic treatment. The risk of bromism is small. The most dramatic improvements were in patients with severe retinopathy and placid temperament. The results in this series of patients seem to indicate that it is possible by this treatment to maintain a lowered blood pressure for a considerable period. As there is no correlation

between the serum-bromide level and the fall in blood pressure the theory that the long-term improvement is due solely to the bromide ion is ruled out; moreover, other salts of hexamethonium (especially the bitartrate) are also effective.

S. L. W.

Isotonic Saline Solution, Diuretic Effect of, Compared with that of Water. G. Blomhert, J. Gerbrandy, J. A. Molhuysen, L. A. de Vries and J. G. G. Borst. (*Lancet*, 1951, **261**, 1011.) The diuretic effects were observed in normal men, big meals and excessive intake of fluids being avoided for at least 7 hours before the experiments, which were preceded by a control period. During the experiments the subjects lay with the body slightly elevated. Following the ingestion of 2 l. of water at night, only 70 per cent. was excreted within 4 hours. The administration of 2 l. of isotonic saline solution during the day, either by mouth or by intravenous injection during 1 hour was followed by a diphasic diuresis. The first phase was similar to that after drinking plain water during the day, about 750 ml. of urine of low chloride content being excreted in the first 2 hours. A protracted mild saline diuresis followed. At night only a brief water diuresis averaging 630 ml., followed the drinking of 2 l. of isotonic saline solution, and when the solution was given by intravenous injection it was retained in the body. The intake of isotonic saline solution was followed by a temporary increase in the volume of circulating blood plasma and in the central venous pressure. It is suggested that the secretion of the antidiuretic hormone depends on both the osmotic pressure of the body fluids and on the circulation.

G. B.

Phenobarbitone Narcosis, Effect of Iodides on. J. C. Krantz, Jr. and M. J. Fassel. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 511.) Organidin, a substance obtained by the interaction of glycerin and iodine, significantly increased the duration of narcosis of phenobarbitone sodium when both were administered intraperitoneally in rats. Similar effects were produced by the administration of an equivalent amount of iodine as sodium iodide and also by glycerin, sucrose and sorbitol, although in each instance the increase was smaller than with organidin. A mixture of glycerin and sodium iodide of similar composition to organidin, however, produced an equivalent increase. Oral administration of organidin produced only a slight increase. The intraperitoneal administration of organidin and an otherwise non-narcotic dose of phenobarbitone sodium produced narcosis in all of nine rats, indicating that the threshold for narcosis was diminished by the presence of organic iodide; oral administration of organidin, sodium bromide and sodium iodide produced a similar effect. That the presence of iodide in the tissues appears to lower the threshold of narcosis was shown by adding 0·5 per cent. of sodium iodide to the drinking water for seven days and then giving a dose of 9 mg./100 g. of body weight of phenobarbitone sodium; narcosis lasting for an average of 75 minutes was produced in 6 out of 10 rats whereas without the iodide the same dose of phenobarbitone sodium had failed to produce narcosis in any of the rats. The potentiating action of the substances with high osmotic pressure when given intraperitoneally may be due to osmotic pressure changes in the animal since fluid will be drawn from the extracellular spaces, tissues, and blood, thereby increasing the concentration of the narcotic in the blood. G. R. K.

Polymer-fume Fever. D. K. Harris. (*Lancet*, 1951, **261**, 1008.) Polytetrafluoroethylene ("Teflon," "Fluon") gives rise to toxic fumes when heated above 300° C. Exposure to the fumes produces toxic symptoms which may start with discomfort in the chest followed by an irritating cough. Within a few hours

there is a gradual increase in temperature, pulse-rate and possibly respiration rate, followed by shivering and sweating. Administration of oxygen is recommended. It appears that the fume consists of a fine sublimate with adsorbed hydrofluoric acid. Although the symptoms are similar to those of metal-fume fever, only traces of metal are present in the sublimate. G. B.

Sodium γ-Resorcylate in Rheumatic Fever. J. Reid, R. D. Watson, J. B. Cochran and D. H. Sproull. (*Brit. med. J.*, 1951, **2**, 321.) The chelate structure of salicylic acid is considered as a possible explanation of its activity in rheumatic fever, the *m*- and *p*-hydroxybenzoic acids being inactive. The pharmacological effects of increasing the chelate structure have been studied by investigating the antirheumatic action of the mono-sodium salt of γ-resorcylic acid. Oral administration of smaller doses was found to be as effective as salicylate in the 7 cases under treatment, and resulted in relief from acute arthritis in 1 to 4 days and a fall in temperature and pulse rate. The side effects accompanying the drug and the changes in fluid, nitrogen and chloride balances were similar to those reported with salicylate therapy, with the exception that profuse sweating, tinnitus and deafness were absent. The effect of the drug on the erythrocyte sedimentation rate is about the same as that of salicylate. Attention is drawn to the similarity in clinical and side-effects of salicylate, γ-resorcylate, adrenocorticotrophic hormone and cortisone. J. R. F.

Strophanthin, Heart Activity of, When Administered Gastro-enterically. G. Zöllner and K. Foth. (*Arch. Pharm., Berl.*, 1951, **284**, 253.) Experiments were carried out on 137 cats in order to determine the activity of strophanthin when administered by the gastro-enteric route. The materials used included a proprietary preparation "Strophoral," stated to be a derivative of strophanthin. The results were as follows. On the administration in the stomach of a simple intravenous toxic dose of g- or k-strophanthin or of strophoral, there was no absorption, while a similar test with digitoxin gave an absorption of 60 per cent. The upper parts of the intestine, and especially the ileum, are important in the action of strophoral. It was not proved whether the lower part took any share in the absorption. In the upper parts of the gastro-intestinal canal (stomach and duodenum), a reduction in the activity of strophanthins and of strophoral occurred. No proof could be obtained of any absorption from an isolated section of intestine, apparently because the length of the section was too small. Digestion of strophanthins or of strophoral in human gastric juice, followed by intravenous injection into cats, produced a reduction in heart activity of about 50 per cent. None of the experiments indicated any significant difference between g- and k-strophanthins and strophoral. G. M.

Veratrum Alkaloids, Depressor Action of. G. S. Dawes, J. C. Mott and J. G. Widdicombe. (*Brit. J. Pharmacol.*, 1951, **6**, 675.) From the results of comparative animal experiments using veratridine and veriloid, a mixture of veratrum alkaloids used clinically for producing a fall of blood pressure, it is concluded that the latter does not differ materially in its mode of action from the pure alkaloids. It elicits the Bezold reflex and sensitises or excites the pulmonary stretch receptors. The fall of blood pressure and heart rate caused by a slow infusion of veratridine in cats is abolished by cooling the vagi, and a similar result is obtained in some dogs under chloralose anæsthesia. In view of this apparent species difference it would be unwise to draw any conclusions about the precise mode of action of these drugs in man. There may be both a peripheral Bezold reflex and a central nervous action. S. L. W.

BOOK REVIEW

CLARK'S APPLIED PHARMACOLOGY. Eighth Edition, revised by Andrew Wilson and H. O. Schild. Pp. x + 670 (including 120 illustrations) and Index. J. and A. Churchill, Ltd., London. 1952. 37s. 6d.

There can be few pharmacologists who have not read and profited from the writings of the late Professor Clark. His book on the "Mode of Action of Drugs on Cells," published in 1933, was hailed at the time "as the most important contribution made to pharmacology in recent years." This book on "Applied Pharmacology" was first published in 1923, and 7 editions had appeared by 1940 shortly before Professor Clark's death. In preparing this new edition Professor Andrew Wilson and Dr. H. O. Schild have carried out a most commendable task. This could not have been easy, bearing in mind the rapid advances in pharmacology since 1942. They have painstakingly maintained the original broad foundations of the earlier editions, and yet have adequately included the new advances and new discoveries. This book was originally written in order to bridge the gap between the laboratory science of pharmacology and the clinical practice of therapeutics, so as to demonstrate as clearly as possible the connection between the two subjects. It consists of 31 chapters, giving a full and comprehensive account of pharmacology and chemotherapy based on a solid foundation of physiology. This is how pharmacology should be taught. There is a logical and systematic application of the principles of pharmacology in the treatment of disease, which gives the book a valuable place in therapeutics. Throughout, the book deals with the pharmacology of the various organs and physiological processes, rather than with the individual drugs themselves, the properties of which must be sought in several chapters. Inevitably, in a book of this size, space limits an adequate description of all the drugs and one might say "the book does not go far enough." However, the revisers have recognised this and included at the end of each chapter a list of general literature for supplementary reading. Here, too, there is selected list of official preparations, together with their dosage given entirely in the metric system. The chapter on the pharmacology of the autonomic nervous system seems all too short for such an important branch of pharmacology, and should be extended in subsequent editions. The chapter on the pharmacology of the heart is rather difficult to understand. There is certainly a need for a clear, concise and accurate account of the actions of the cardiac glycosides. This book offers to medical students, practitioners and pharmacists, a readable account of the principles and practice of pharmacology in relation to human disease. G. F. SOMERS.

ABSTRACTS (*Continued from page* 599.)

Veriloid (*Veratrum viride*), Treatment of Arterial Hypertension with. R. Kauntze and J. Trounce. (*Lancet*, 1951, **261**, 1002.) Veriloid is a mixture of ester alkaloids of *Veratrum viride*, standardised for hypotensive activity in dogs. It acts on the afferent vagus nerve and central nervous system direct. Administered by mouth or intravenous infusion, veriloid lowers the blood pressure in about 66 per cent. of hypertensive patients. Owing to its toxicity, the drug is useful in only 20 to 30 per cent. of cases, and dosage needs continuous supervision. Renal insufficiency is not adversely affected. Toxic symptoms which include nausea, recurrent vomiting and collapse are best treated with phenobarbitone. The same side reactions are observed with the pure alkaloids, germitrine and germidine. G. B.