REVIEW ARTICLE

THE PHARMACOLOGY OF TROPANE COMPOUNDS IN RELATION TO THEIR STERIC STRUCTURE*

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ATROPINE, hyoscine and cocaine are the best know naturally occurring tropane alkaloids. The first two are active inhibitors of structures innervated by postganglionic cholinergic nerves: cocaine, in suitable concentrations, prevents conduction in all nerves. Because of this specific biological activity, their pharmacological properties and those of several other tropane alkaloids, and also their degradation and synthetic products, were investigated.

Research over the last thirty years in the field of atropine-like structures has led to the discovery of many synthetic parasympathetic blocking agents. With the knowledge of the structure of cocaine originated the wide search for new local anaesthetic compounds.

The naturally occurring tropane compounds are tropine esters which act both centrally and peripherally. Atropine stimulates some areas in the brain and blocks the action of muscarine-like parasympathomimetic drugs and most postganglionic cholinergic nerve end-organs. Hyoscine is unlike atropine in its central actions because it is usually depressant, and in its more widespread central action, for example on cortical and subcortical areas. But it is like atropine in its peripheral actions. Cocaine causes intense central nervous stimulation and a blockade of conduction in nerve, at synapses and at neuromuscular junctions.

The aim of this work is to survey the compounds of tropane and related structures, collecting and collating the available data relating chemical structure and pharmacological action. Much valuable information has been derived from the study of the stereostructure of the tropane alkaloids.

CHEMISTRY AND NOMENCLATURE OF TROPANE COMPOUNDS

The basic skeleton of the tropane alkaloids is the ring system tropane (I) which is an *N*-methylated 8-aza-bicyclo-3:2:1-octane. Pharmacologically the oxyderivatives of tropane have most significance: these are tropine and ψ-tropine, 6-oxytropine, 6:7-dioxytropine or teloidine, 6:7-epoxytropine or scopine and ecgonine.

Stereostructure of Tropine and ψ-Tropine

Tropine and ψ-tropine are chemically 3-oxytropanes. The two compounds are isomers[1]. They differ in the position of the OH group in relation to the nitrogen, tropine being the *trans* form (II) and ψ-tropine the *cis* form (III).

* Dedicated to Professor B. Issekutz on his 70th birthday.

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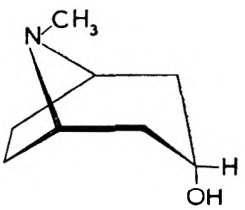
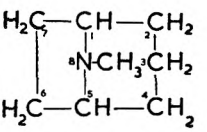
The steric position of the OH group was first determined by Fodor and Nador[2] and independently by Nickon and Fieser[3]. Fodor and Nador found that in the case of *N*-acetyl- or *N*-benzoyl-nor ψ-tropine the N → O or O → N directed acyl migration can be effected by H^+ and OH^- ions respectively. On the other hand this reaction does not succeed with *N*-acetyl or *N*-benzoyl-nor-tropine. From this it follows that in ψ-tropine



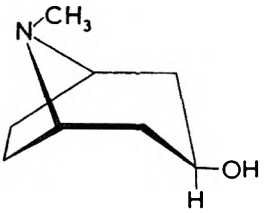
(II)

Tropine
(*trans*)



(I)

Tropane



(III)

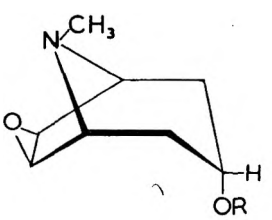
ψ-Tropine
(*cis*)

the OH group and the nitrogen are in spatial proximity, that is, in the *cis* position; with tropine the OH is distal to the nitrogen and *trans*. For these reasons the authors assigned formula II to tropine and III to ψ-tropine. A proof of the validity of these proposed formulae was given by Hardegger and Ott[4] who prepared a ring oxamine ψ-tropine derivative. The structures of tropine and ψ-tropine have since been established by the physical and chemical measurements of others[5,6]. By analogy with the nomenclature of steroids, tropine is 3α-tropanol or 3α-oxytropane, and ψ-tropine is 3β-tropanol or 3β-oxytropane. Here, α assigns a *trans* configuration of the grouping to the nitrogen and β a *cis* configuration.

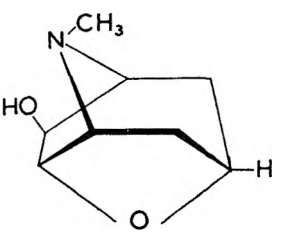
It follows that in atropine, hyoscyamine, convolvamine, poroidine and *iso*poroidine, all of which on hydrolysis yield tropine, and also in convolvine which yields nor-tropine, the esterified OH group of the tropane ring is *trans* to the nitrogen. In tropacocaine and tigloidine, which on hydrolysis yield ψ-tropine, the esterified OH group occupies the *cis*-position in relation to the nitrogen.

Stereostructure of Hyoscine (*Scopolamine*)

Hyoscine is the (−)-tropic acid ester of scopine, the latter being a 3-oxytropane which contains an epoxy ring (IV). The observation that scopine can easily be transformed into the ring structure scopoline (V)



(IV)



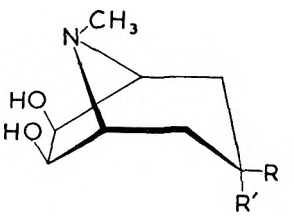
(V)

(R = H = scopine: R = COCH·(CH$_2$OH)C$_6$H$_5$ = hyoscine)

proves the *trans*-position of the 3-oxy group to the nitrogen. This reaction can take place with the epimeric ψ-scopine only with difficulty. On the basis of this and further investigations made by Fodor and colleagues[7–10], which showed the epoxy group to be in the β-position, hyoscine is 6:7β-epoxy-3α-tropyloxytropane and its stereostructure is represented by (IV).

Teloidine (3:6:7-*trioxytropane*) *and its* 3-*Tiglic Acid Ester Meteloidine*

The stereochemistry of teloidine may be elucidated using the following arguments. Teloidine can be prepared according to the classic synthesis of Robinson starting from *meso* tartaric acid-dialdehyde. This synthesis proves that the two OH groups at C(6) and C(7) are in *cis*-position to each other. This was first demonstrated by Schöpf[11], but the position of these OH groups to the nitrogen was still questionable, as there were four possible steric structures of teloidine (VI*a* and *b*; VII*a* and *b*). Fodor[9] determined the steric position of the 6:7-dihydroxy groups to the nitrogen, and Heusner[12] and Sheehan and Bissel[13] ascertained the configuration of the OH group on C(3) to the nitrogen. Correlation of this data showed the correct stereostructure of teloidine to be represented by formula VI*a*, where the 6:7-OH groups are in *cis*-position to each other and in the *cis*-position to the nitrogen. Therefore teloidine is 3α:6β:7β-trioxytropane.



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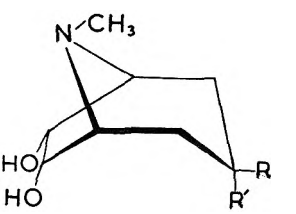
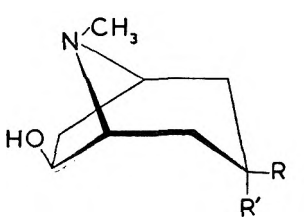


(VII)

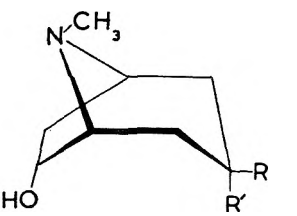
(*a*, R = H, R′ = OH: *b*, R = OH, R′ = H)

6-*Oxytropine and* 6-*Oxy-*ψ-*tropine*

Naturally occurring valeroidine is the *iso*valerianic acid ester of the 6-oxytropine. Its stereostructure was ascertained by Stoll and colleagues[14]. Stereochemically four different structures may be supposed (VIII*a* and *b*; IX*a* and *b*). Two of the formulae are sterically similar to



(VIII)



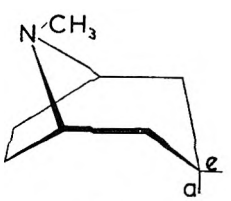
(IX)

(*a* R = H, R′ = OH: *b*, R = OH, R′ = H)

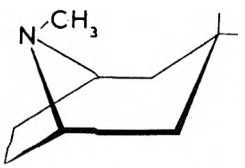
tropine and two to ψ-tropine in the relation of OH groups on C(3), while the position of the OH group at C(6) may be *cis* or *trans* to the nitrogen. The steric position of both of these OH groups was determined by Martin, Mitchell and Tranter[15,16]. According to these authors and latterly Fodor and colleagues[9], who by the results of the catalytic hydrogenation of scopolamine added a further proof to the 6-oxytropine stereostructure, the position of the OH on C(6) is *cis* and that of the OH on C(3) is *trans* to the nitrogen. Therefore the configuration (VIII*a*) was assigned to 6-oxytropine. Thus valeroidine is 3α-valeroyloxy-6β-oxytropane.

The Conformation of the Piperidine Ring in the Tropane Compounds

In the preceding sterostructural formulae the piperidine ring is represented by a "chair" form (X*a*). However, acyl migration experiments which established the steric position of the C(3)–OH group in tropine and ψ-tropine on the one hand, and the development of a cyclic oxazine structure according to Hardegger and Ott[4] in ψ-tropine on the other, can be explained only by the "boat" form (X*b*). The reduction of tropinone to ψ-tropine can be explained on the basis of an assumption of the chair-form, because a reduction with complex metal hydrides, for example lithium aluminium hydride, gives an equatorial OH group relative to the axis of the ring system which has a *cis*-position relative to the nitrogen. Reduction with catalytic agents, however, produces an axial OH group in



(X*a*)
Chair-form

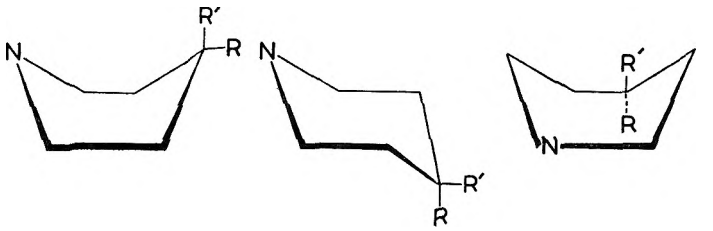


(X*b*)
Boat-form

the *trans*-position. The differences between the rate of saponification of the ester linkages in tropine and ψ-tropine compounds can be explained by a chair-form structure. This contradiction is explicable in that tropane can be thought of as either a piperidine ring strengthened by an ethylene chain, or as a *cyclo*heptane ring which is fixed by an *N*-CH_3 bridge. In this hypothesis the chair-form of the piperidine structure is also simultaneously the boat-form of the *cyclo*heptane ring. Work to solve the problem is still in progress. The present opinion is that, considerations of dynamic equilibria suggest the chair conformation of the piperidine ring the more frequent and the more probable.

Many difficulties have arisen in solving conformational arrangements in the piperidinium compounds investigated by us[17]. Some experimental data allow the hypothesis that 4-oxypiperidine and its compounds can be characterised by one of the chair-forms or by the *trans* boat-form among the possible six varieties of conformation. (XI*a* and *b*; XII*a* and *b*;

XIII*a* and *b*.) It is important to know the correct steric relations of piperidine compounds to the naturally occurring tropane derivatives. Only with this knowledge is it possible to establish the relation between chemical structure and pharmacological action.



(XI) (XII) (XIII)

(*a*, R = H, R′ = OH: *b*, R = OH, R′ = H)

Steric Configuration about the Tropane Nitrogen

Fodor[9,18] and Findlay[19] independently described quaternary tropine compounds which were pseudoasymmetric about the nitrogen. Findlay did not prove the steric position of the substituents on the nitrogen. According to Fodor[9,18], the *N*-carbethoxymethylscopolaminium iodide prepared from hyoscine with iodoacetic ester, hydrolysed by acid gives the same lactone of *N*-carbethoxymethyl scopoline as that obtained from (±)-scopoline. This indicates that the carbethoxymethyl group is bound to the nitrogen of these compounds from the direction of the endoethylene bridge. The experimental results indicate that in these compounds the N–CH_3 group is orientated towards the piperidine ring. Fodor and colleagues[18] stated further that ψ-tropine, with ethyl bromoacetate, yields only a single quaternary ammonium salt. When the sequence of the quaternisation is reversed, by preparing the *N*-carbethoxymethyl-nor-ψ-tropine first and then quaternising with methylbromide, a different *N*-epimeric tropinium salt is obtained. Each of these compounds proved to be uniform but not the same by crystallographic and X-ray studies. The situation is the same with the two quaternary carbethoxymethyl tropinium compounds obtained by two different ways.

The results concerning the N–CH_3 group show that the ring system in tropane compounds leads to a strong configurational stability of the methyl group attached to the tertiary nitrogen, and it is orientated towards the piperidine ring. The consequence of this is that in quaternising the tropeines, the second substituent, that is, the quaternary one, is given a determined steric orientation towards the pyrrolidine ring, and whether the two substituents—tertiary and quaternary—can change their position by transvibration and under what circumstances has yet to be elucidated.

Stereochemistry of Cocaine and Related Compounds

The stereostructure of cocaine is more complicated than that of the tropane alkaloids, because cocaine epimers must be considered to belong to one of two different systems. It is known that the acid hydrolysis of

cocaine yields ecgonine. Neglecting stereochemistry, this latter is 3-oxytropane-2-carboxylic acid. Wilstätter[20] stated that oxidation of ecgonine gives tropinone through the β-ketonic acid. Einhorn and Marquardt[21] observed that as a result of treatment with alkali, ecgonine is transformed into (+)-ψ-ecgonine, which proved to be the diastereoisomeric form of the ecgonine. Based on these results Willstätter[22] denoted (+)-ecgonine as (+)-ψ-ecgonine without having proved C(3) epimerism. Recent investigations in organic chemistry have almost completely elucidated the stereochemistry of ecgonine and related compounds. There were two important questions to be solved: (i) the relative steric position of C(3)–OH group to the nitrogen, and (ii) the establishment of the relative position of the functional groups on C(2) and (3) in cocaine.

The configuration of the C(3)–OH group relative to the nitrogen was investigated in ecgonine using methods similar to those for tropine and ψ-tropine derivatives. These are acyl migration, and oxazine formation by *p*-nitrobenzaldehyde. This research led to the conclusion that in both ecgonine and ψ-ecgonine the C(3)–OH group has a *cis*-position to the nitrogen[23–27]. The investigations also dealt with 2-methyl-3-tropanol epimers, prepared from the ecgonines, and showed that ecgonine and ψ-ecgonine are C(2) epimers.

Findlay[25] showed that ecgonine methyl ester reacts with methyl iodide to give a methiodide identical with that obtained from the ψ-ecgonine methyl ester (the positive charge on the nitrogen atom facilitating the epimerisation). It is known that the α-hydrogen of esters of this type is more labile than that of the corresponding acid under alkaline conditions. Ecgonine can be converted to the ψ-isomer in 2 per cent yield with 10 per cent alcoholic potash, but in 39 to 54 per cent yield from the methyl ester (or cocaine) under the same conditions. These results also indicate that ecgonine and ψ-ecgonine are C(2) epimers.

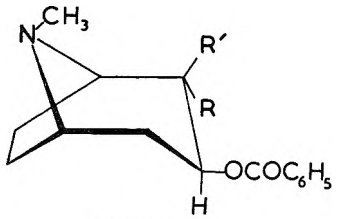
The determination of the relative steric position of the substituents of C(2) and C(3) atoms required much research. Fodor[9,23] showed that acyl migration does not succeed with the *N*-benzoyl derivative of 2-amino-3-tropanol which was prepared from ψ-ecgonine by the Curtius reaction. But 2-benzamido-3-tropanol obtained from ecgonine showed a quantitative and reversible N → O acyl migration.

When studying the chemistry of nor-ecgonine, in preparing *O*-benzoyl-nor-ecgonine Findlay[25] found that inadvertent use of insufficient acid yielded an isomer not previously reported. He confirmed that the new compound was the result of rearrangement to give the *N*-benzoyl nor-ecgonine, proving a *cis* arrangement of the C(3) OH group to the nitrogen.

On the basis of this and other work[28] it can be stated that in ecgonine the C(2) and C(3) groups are in the *cis*-position to each other, while in ψ-ecgonine they are *trans*. Also cocaine and ψ-cocaine differ only in the configuration of the C(2) grouping.

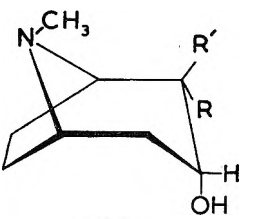
Thus cocaine is 2-β-carbomethoxy-3β-benzoyloxypropane (XIV*a*) and ψ-cocaine is 2α-carbomethoxy-3β-benzoyloxytropane (XIV*b*). In the cocaine group four racemic pairs may exist, two racemates being well

known for a long time, one of them being used clinically as the local anaesthetic psicaine. The third racemate of ecgonine was prepared in a small quantity by Willstätter[29] by the large-scale reduction of 2-carbomethoxytropinone. Its configuration is represented by XV*a*[25]. The fourth racemate was prepared recently by Fodor[30] and has the structure (XV*b*).



(XIV)

(*a*, R = H, R′ = $COOCH_3$:
b, R = $COOCH_3$, R′ = H)



(XV)

(*a*, R = COOH, R′ = H:
b, R = H, R′ = COOH)

Thus most of the structural problems of tropane and related compounds have been successfully resolved in recent years, and the up-to-date nomenclature of tropane compounds expresses these stereochemical features. This nomenclature should be introduced into pharmacology without delay, because of the great importance of the relation of steric structure to activity.

Atropine-like Tropane Compounds

The tropane alkaloids are widely distributed in nature especially in plants of the Solanaceae. Of the alkaloids, atropine [(±)-tropyl-tropine] has been the most studied pharmacologically. Atropine is a racemic mixture of hyoscyamine, the (−)-isomer of which is considerably more active than the (+)-isomer in its peripheral anti-acetylcholine effects; on the central nervous system the isomers are equiactive[31]. (−)-Hyoscine [(−)-tropyl-α-scopine], is much more active than (+)-hyoscine. Its peripheral actions are very like those of atropine but central effects differ in that it has a direct depressant effect on the central nervous system in reducing excitement and fear. The (+)-isomer has a much weaker atropine-like effect but its central depressant activity is similar to that of the (−)-isomer[32,33].

This review will endeavour to describe only the correlation of the most characteristic pharmacological effects and chemical structure, and therefore it will not cover many of the actions which these alkaloids possess. There are several reviews which deal with this aspect[31,34,35].

Groups

The Role of the Acid Ester Groups in the Pharmacological Activity of Tropine Esters (Tropeines)

The high activity of atropine (an ester) and the relative inactivity of its parent amino alcohol, tropine, led to the preparation and investigation of many tropeines in which tropine was esterified by acids other than tropic

acid. The role of the acid ester group in the α-tropine compounds has been summarised by von Oettingen[31]. In general it can be stated that in blocking post-ganglionic parasympathetic effects, tropine esters of oxy-aromatic acids are the most active. But, in view of the diversity of methods used by the authors quoted by von Oettingen in his review, no absolute quantitative comparison of their results can be made.

Tropeines can be divided into three groups of differing atropine-like potency shown in Table I. It seems that the requirement for high activity is an asymmetric carbon atom in the aromatic acids esterifying tropine. The activity can be enhanced by an OH group[31]. Substitution of the oxyaromatic tropic acid in hyoscine by other acids, i.e., acetic, cinnamic, benzoic, greatly diminishes the atropine-like activity[36].

TABLE I

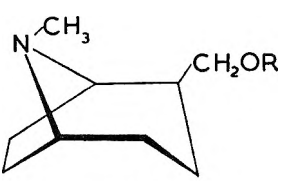
ATROPINE-LIKE ACTIVITY OF TROPEINES RELATED TO ESTER GROUPING

Tropeines without activity	Tropeines with mild activity	Tropeines with a high activity
Glycolyl tropine	Acetyl tropine	(±)-Tropyl tropine
Tartaryl tropine	Lactyl tropine	(−)-Tropyl tropine
Fumaryl tropine	Succinyl tropine	Acetyltropyl tropine
Phthaloyl tropine	Methylparaconyl tropine	(±)-Mandelyl tropine
	α-Phenyl-β-chlorpropionyl tropine	(−)-Mandelyl tropine
	α-Phenyl-β-brompropionyl tropine	*o*-Methylmandelyl tropine
	Atrolactyl tropine	*m*-Methylmandelyl tropine
	Atroglyceryl tropine	*p*-Methylmandelyl tropine
	Atropyl tropine	Fluorenyl tropine[64]
	Hydratropyl tropine	Oxyfluorenyl tropine[64]
	Phenylacetyl tropine	Xanthen 9-carboxyl tropine[66]
	Phenylaminoacetyl tropine	Phenyl-thienyl tropine[63]
	Cinnamyl tropine	Diphenylacetyl tropine[63]
	*iso*Cumarin-carboxyl tropine	
	α-Phenyl-β-hydroxypropionyl tropine	
	α-2-Pyridyl-β-hydroxypropionyl tropine	
	Hippuryl tropine	
	Phenylcarbamyl tropine	
	Dibenzylacetyl tropine	
	Benzylyl-β-tropine	

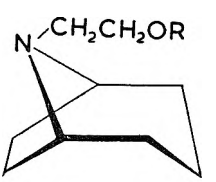
Note: The data without references were collected from the review of von Oettingen.[31].

The Position of the Ester Group on the Tropane Ring System and Activity

von Braun and Müller[37] synthetised various homatropanol esters with general formula XVI (where R = (±)-atropyl, (±)-mandelyl or benzoyl). Wichura[38] found these esters weaker and not identical in their action with 3 α-tropanol esters. Of the compounds of von Braun and Räth[39] (the



(XVI)



(XVII)

esters of *N*-alkyl nor-tropane with general formula XVII where R = acyl), only the tropic acid ester of *N*-oxyethyl-nor-tropane has atropine-like effect[26]. By the application of the principle of heterotopy to these compounds a wide range of investigations developed with compounds of the

general formula R_2N alkyl-COO-aryl, where R_2N is usually a piperidine ring or dialkylamino groups. From these series of substances which do not contain the tropane ring system have come many of the so-called neurotropic spasmolytics.

The Steric Position of Ester Groups attached to the C(3) of Tropane and activity

The pseudoderivative of (−)-hyoscyamine has been mentioned as a mydriatic[40], but the (±)-tropyl and (±)-mandelyl β-tropines[41] do not show any mydriatic activity. Benzoyl β-tropine is a much weaker atropine-like agent than benzoyl α-tropine, and this applies to their alkyl quaternary derivatives also[42,43].

Although the relation of stereostructure of the esterifying group to activity has not yet been investigated systematically, it seems that an aromatic acid ester group on the C(3) of tropane directed in the α-position to the nitrogen is necessary for activity. According to Pfeiffer's theory[44] acetylcholine acts at cholinergic neuro-effector junctions by three functional groups, a "cationic head" ^{+}N–CH_3 group and an ether and a ketone O atom displaced from the ^{+}N–CH_3 group by 5 and 7 Å respectively. Most of the specific and typical antagonists of acetylcholine at the autonomic post-ganglionic receptor site contain both the ^{+}N–CH_3 group and the ether and ketone O atoms again at a distance of 5 and 7 Å. But these antagonists have the active groups protected sterically to give a large umbrella-like molecule by aromatic groups as with tropane and some piperidine compounds, or surrounded by simple aliphatic groups. A ring system around the nitrogen is not essential for atropine-like effect, as can be seen from the commercially available compounds which have alkyl groups attached to the nitrogen and no saturated ring system, for example in methantheline, oxyphenonium or lachesine. With these compounds the steric flexibility of the carbon chain between the functional groups can assure the correct fit on the receptor surfaces, but with tropane compounds, the rigid ring system 3-tropanol and the directed localisation of the ester groups attached to its C(3)–OH group determine the steric position of the functional groups. The difference between the α- and β-position of C(3)–OH group appears to be of importance for atropine-like activity.

The Effect of an Ester Group on Atropine-like Activity

The hydrolysis products (tropine and ψ-tropine) of the naturally occurring tropeines, were mentioned as almost devoid of atropine-like activity[45,46]; in addition, both 3α- and 3β-tropanol have only slight activity at ganglia[17,47]. Thus it may be deduced that this activity also is connected with an ester function.

The Nitrogen of Tropine Esters in Atropine-like Activity

Secondary amine derivatives of tropeines. Demethylation of the nitrogen in atropine and (−)-hyoscyamine to give the secondary amines noratropine and nor-(−)-hyoscyamine reduces the activity about eight times. The latter compound occurs in a natural form in *Scopolia japonica*[31].

Quaternary ammonium derivatives of tropeines. Our recent experience shows that the most pronounced alterations in the pharmacological effects of tropane compounds can be obtained by varying the groupings on the quaternised nitrogen.

Methyl quaternary derivatives of atropine were first investigated as early as 1869 by Crum Brown and Fraser[48]. In 1906 Hildebrand[49] described the curare-like effect of the methyl- and benzyl-quaternaries of atropine. Issekutz[50] dealing with the methyl quaternary derivatives of atropine and homatropine pointed out that, especially in the case of homatropine, quaternisation with a methyl group enhances the vagus-blocking and curare-like capacity and diminishes the central stimulant activity. In 1948 Kimura, Unna and Pfeiffer[51,52] described various bis-quaternary derivatives of atropine. Among these the decamethylene bis-atropinium diiodide was most active, having very pronounced curare-like activity besides a strong atropine-like effect. In 1950 Issekutz and Nador[53] found that the 1:4-xylilene quaternary derivative of atropine, (±)-mandelyl- and benzoyl-tropines are very active curare-like compounds. These have only relatively moderate atropine-like activity[54]. In 1951 Gyermek and Sztanyik drew attention to the ganglion-blocking activity of quaternary tropeines of the atropine group[55]. This was confirmed later by others[56,57]. The following year we prepared several new tropane compounds, with aralkyl quaternary groups, which were the most active ganglion-blocking agents then known. At the same time, Wick[58] independently dealt with the quaternary derivatives of hyoscine and made some new observations on their mode of action. These investigations led to the derivative butyl scopolammonium bromide (buscopan) being used in therapy. Between 1952–54, parallel with the development of sterochemistry of tropane compounds, we made systematic investigations for anticholinergic nerve actions with tropane and related compounds. In 1954, Rothlin and his colleagues[59] made new observations with the 6-alkoxytropine compounds and their quaternary salts prepared by Stoll and Jucker[60]. Recently Archer, Cavallito and Gray[61] and Lape, Fort and Hoppe[62] described asymmetric bis-quaternary compounds of tropane structure. The latter group especially seems to merit attention.

The changes in the pharmacological activity of quaternary tropane derivatives effected by alterations of chemical structure will now be described.

The Methyl Quaternary Group in the Tropine Esters

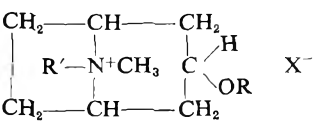
Aliphatic acid esters of tropine such as acetyl and dimethylcarbaminoyl tropines quaternised with methyl halides yield compounds with parasympathomimetic activity. They cause a blood pressure fall and spasm of isolated organs which can be antagonised by atropine. Their effects are 100 to 500 times weaker than those of acetylcholine. These tropine compounds are similar in action to the acetyl ester of 1-methyl piperidine[63]. Thus it is not essential for the parasympathomimetic activity of acetyl and dimethylcarbaminoyl esters of basic alcohols to have CH_3 groups on the nitrogen, which itself can be in a ring system. The "umbrella"-like

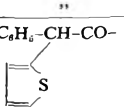
structure of the tropine compounds only decreases and does not abolish the parasympathomimetic activity. For atropine-like activity it is unnecessary for the nitrogen-containing basic esters to have a ring structure. Neither parasympathomimetic nor atropine-like activity in the basic ester type of compounds appears to be dependent upon a rigid saturated ring system around the terminal nitrogen.

A summary of the changes which can be observed with the methyl quaternisation of aromatic acid tropine esters can be seen in Table II.

TABLE II

THE EFFECT OF METHYL QUATERNARY GROUPS ON THE ESTERS WITH ATROPINE-LIKE PROPERTIES



R	R′	X	Atropine-like activity Atropine = 1*	Ganglion-blocking activity Tetraethyl-ammonium bromide = 1†	Curare-like activity Tubocurarine = 1‡
C_6H_5–CO–	H	HCl	0·006	0·1	—
,,	CH_3	Br	0·04	2·0	0·10
C_6H_5–CH(OH)–CO–	H	HCl	0·08	0·35	—
,,	CH_3	Br	0·3	4·5	0·07
C_6H_5–CH(CH_2OH)–CO–	H	H_2SO_4	1·0	0·35	—
,,	CH_3	Br	1·0	4·0	0·15
$(C_6H_5)_2$–CH–CO–	H	HCl	0·06	—	—
,,	CH_3	I	0·08	5·3	0·05
	H	HCl	0·2[83]	—	—
,,	CH_3	Br	0·5	—	—

* Isolated rabbit and rat gut; † cat nictitating membrane; ‡ frogs.

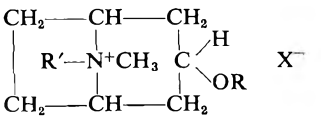
The methyl quaternisation, besides introducing curare-like activity, enhances atropine-like and especially ganglion-blocking activity. This alteration in atropine-like activity is general, not only with the compounds having a tropane ring but also with 6-methoxytropine compounds[59] and with hyoscine[35]. An increase in this activity can be induced by the methyl quaternisation of compounds containing a piperidine ring, or *N*-aliphatic groups[64]. Quaternisation of tropine esters by aralkyl groups gives compounds with very different actions: atropine-like activity diminishes, ganglion-blocking activity increases, and the curare-like effect alters (Table III).

Quaternisation with the groups of the general formula X_1–Ar–CH_2–X (where X_1 = a halogen or phenyl group, Ar = aryl group and X =

halogen group) containing the halogen or phenyl group in the *para* position, yields active ganglion-blocking compounds. Among the quaternary compounds containing condensed rings the most active were those that were coaxially condensed.

TABLE III

THE PHARMACOLOGICAL PROPERTIES OF SOME ARALKYL QUATERNARY TROPEINES



R	R'	X	Atropine-like activity Atropine = 1*	Ganglion-blocking activity Tetraethyl-ammonium bromide = 1†	Curare-like activity Tubocurarine = 1‡
C_6H_5–CO–	C_6H_5–CH_2–	Br	0·004	3·0	0·10
,,	*p*BrC_6H_5–CH_2–	Br	0·003	8·2	0·17
p$NH_2C_6H_5$–CO–	C_6H_5–CH_2–	Br	0·005	8·0	0·20
,,	*p*ClC_6H_5–CH_2–	Br	0·0025	22·0	0·40
C_6H_5–CH(OH)–	C_6H_5–CH_2–	Br	0·005	3·0	0·10
,,	*o*BrC_6H_5–CH_2–	Br	0·015	4·1	0·17
,,	*m*BrC_6H_5–CH_2–	Br	0·025	6·8	0·20
,,	*p*BrC_6H_5–CH_2–	Br	0·015	19·0	0·12
,,	C_6H_5–C_6H_5–CH_2–	Br	0·015	40·0	0·12
C_6H_5–CH(CH_2OH)–	C_6H_5–CH_2–	Br	0·10	2·1	0·18
,,	*p*BrC_6H_5–CH_2–	Br	0·07	8·5	0·17
,,	C_6H_5–C_6H_5–CH_2–	Br	0·15	28·0	0·10

* Isolated rabbit and rat gut; † cat nictitating membrane; ‡ frogs.

Between 1951 and 1955 we determined the relation between the structure and effect of more than 50 mono-quaternary tropine ester compounds[17,65], and found that the various anti-acetylcholine activities changed quite independently of each other and of the type of quaternisation. We observed that several parasympathetic blocking tropine esters could be changed into selectively acting ganglion-blocking or curare-like compounds.

Some examples of ganglion-blocking selectivity can be seen in Table IV.

p-*Diphenylmethyl Quaternary Groups*

All the compounds investigated containing benzyl quaternary groups with or without halogen and alkyl substituents show only blocking activity on autonomic ganglia. Some *p*-diphenylmethyl quaternary derivatives of benzoyl and *p*-aminobenzoyl-tropine and ψ-tropine,

however, have ganglion-stimulating effects too. The diphenylmethyl quaternary derivative of *p*-aminobenzoyl α-tropinium bromide is so effective that its stimulant activity exceeds that of nicotine by about 50 times. It has the highest ganglion-stimulating activity hitherto known. The tropane skeleton is only partly responsible for this activity. The *p*-amino group on the benzoic acid ester seems to be of importance[66].

TABLE IV

GANGLION-BLOCKING SELECTIVITY

Compounds	Ratio of Ganglionic blocking activity† to	
	Curare-like action‡	Atropine-like action*
p-Chlorbenzyl(*p*-aminobenzoyl) α-tropinium bromide ..	55	8,800
p-Brombenzyl[(±)-mandelyl]-α-tropinium bromide ..	160	1,250
p-Diphenylmethyl[(±)-mandelyl] α-tropinium bromide ..	235	2,700
p-Diphenylmethyl[(±)-tropyl] α-tropinium bromide ..	168	280
p-Brombenzyl[(±)-tropyl] α-tropinium bromide	50	57
Tubocurarine	3	—
Atropine	—	0·3
Tetraethylammonium bromide	15	—

* Isolated rabbit gut; † cat nictitating membrane; ‡ frogs.

The changes effected in the structure of the mono-quaternary tropeines have produced the most active ganglion-blocking and stimulating compounds known until 1954, and the results suggest the use of these compounds as starting materials for further research.

Quaternary Compounds of Hyoscine

With atropine and homatropine, the higher alkyl quaternary derivatives proved to be weaker atropine-like and ganglion-blocking agents than the methyl quaternary derivatives[65]. Contrary to this, the butyl quaternary compound of hyoscine is a highly active ganglion-blocking agent[58]. Its atropine-like side effects, mydriasis and tachycardia, are weak, and it is used clinically. The successful experiments with *p*-diphenylmethyl quaternary derivatives of atropine[67] and hyoscine[68] show that these tropeines are more potent than butyl scopolammonium bromide and they advantageously unite important and therapeutically useful pharmacological effects. The atropine derivative was clinically tested and proved to be therapeutically active[69].

In our opinion, the specific quaternary group attached to the tropane nitrogen of hyoscine is more important for activity than the epoxy group[68,70]. We have observed several instances in which the epoxy group decreases ganglion-blocking activity. This effect is shown in Table V.

Rothlin and his colleagues[59] reporting the activity of the compounds of 6-alkoxytropine esters described by Stoll and Jucker[60] stated that some of these have favourable pharmacological properties. Therapeutically useful hyoscine and butyl scopolammonium bromide-like compounds were sought. Although some of the 6-methoxytropine compounds have a marked atropine-like activity they do not show the central actions characteristic of hyoscine. Apparently the (−)-tropic acid ester of

6-methoxytropine was not prepared. This compound would have given valuable information had it shown hyoscine-like central depressant effects.

TABLE V

ATROPINE-LIKE AND GANGLION-BLOCKING ACTIVITY OF SOME ATROPINE AND HYOSCINE QUATERNARY DERIVATIVES*

Compound	Atropine-like activity*	Ganglion-blocking activity†
Atropine sulphate	1·0	0·35
Methyl atropinium bromide	1·5	4·0
Butyl atropinium bromide	0·02	—
p-Diphenylmethyl atropinium bromide	0·1	23·0
Hyoscine hydrobromide	2·0	0·1
Methyl scopolammonium bromide	4·0	0·4
Butyl scopolammonium bromide	0·025	3·0
p-Diphenylmethyl scopolammonium bromide	0·2	10·0
Tetraethylammonium bromide	—	1·0

* Rabbit and rat gut; † cat nictitating membrane.

Bis-quaternary Tropeines

Results of the investigations into the structure of naturally occurring curare alkaloids led to a search for new curare-like derivatives. Many bis-quaternary ammonium compounds were prepared and tested. Because of the competitive nature of tubocurarine for acetylcholine receptors it seemed interesting to produce and investigate bis-quaternary atropine compounds. Kimura, Unna and Pfeiffer[51,52] described the first of such compounds. Two atropine molecules were connected by the OH groups of their tropic acid moieties or by their nitrogen atoms by quaternisation. The most active compound was the 1 : 10-decamethylene-bis-atropinium diiodide, which showed about the same activity as tubocurarine but also had a very pronounced atropine-like effect. The aim of the investigations of Issekutz and Nador with bis-quaternary tropeines[71,72] was to find compounds with practical value. The bis-atropinium, mandelyl, and benzoyl tropinium compounds prepared with 1 : 4-xylylene dibromide showed a high curare-like activity. It was noteworthy, however, that these compounds had only relatively mild atropine-like activity compared with their methyl quaternary compounds[54]. The *cis*-position of the benzoyl ester group and the lack of other ester groups decreases the curare-like effect[71]. However, of the α- and β-forms of the hexamethylene-1 : 6-bis-benzoyltropinium halides, the β-form proved to be the more effective[73].

Many authors consider the chain length between the two nitrogen atoms, the so-called interquaternary distance, as the most important structural requirement for the curare-like activity of the bis-quaternary ammonium compounds; we have found with several bis-quaternary tropeines that the character of the quaternary group is at least as important. In the case of bis-quaternary tropeines prepared by quaternisation with alkyl or aralkyl dihalides, the reaction predetermines the structure of the quaternary ammonium group. Therefore it was decided to prepare bis-quaternary tropeines in which, besides an optimal quaternary

distance, a choice of quaternary groups could be made. The investigations with some succinyl and phthaloyl tropeines resulted in compounds with curare-like effect which were equal to, or more active than, tubocurarine[74]. Also, recently some bis-quaternary belladonnine compounds of Hotovy and colleagues[75] were mentioned as being very active.

There are not yet enough of the bis-quaternary tropane compounds to decide which kind has the optimal characteristic for curare-like action. Because some of them have proved to be very potent curare-like compounds, a comparison of a few derivatives is made in Table VI.

TABLE VI

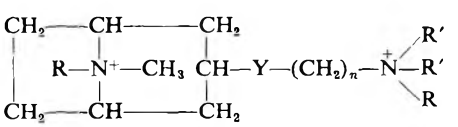
BIS-QUATERNARY TROPANE DERIVATIVES

Compound	Curare-like effect Tubocurarine = 1		Atropine-like activity Atropine = 1*	Ganglion-blocking activity Tetraethyl-ammonium bromide = 1†
	Frogs	Rabbit head drop		
Methyl atropinium bromide	0·05	0·015	1·0	4·0
1 : 10-Decamethylen-bis-atropinium diiodide ..	0·25	2·5	2·0	—
1 : 4-Xylylene bis-atropinium dibromide ..	1·5	1·0	0·2	0·4
1 : 4-Xylylene bis-benzoyl α-tropinium dibromide	1·0	1·5	0·01	0·4
m-Brombenzyl-succinyl α-tropinium dibromide	2·0	2·5	0·02	3·0
1 : 6-Hexamethylen-bis-benzoyl β-tropinium dibromide	2·0	3·0	0·002	<0·5

* Rabbit and rat gut; † cat nictitating membrane.

Asymmetric Bis-quaternary Ammonium Compounds with the Tropane Ring System

Two series of these compounds are known. The first one can be characterised by the general formula: $(CH_3)_3{-}^+N{-}(CH_2)_3{-}^+N{-}R$ where $^+N{-}R$ = tropane, tropine, tropinone or atropine[61]. The preparation of these compounds was suggested by the observation that various asymmetric bis-quaternary compounds containing $-(CH_2)_3-$chain and hydrated *iso*quinoline and carboline rings proved to be strong and long-lasting vasodepressants. The other group of compounds[62] has the general formula XVIII. Some members of this series show very strong and



(XVIII)

(R′ = Alkyl or aralkyl; R = Alkyl; Y = NH, *N*-alkyl or 0; n = 2 or 3)

long-lasting blocking activity on autonomic ganglia and on vasopressor reflexes. It is interesting to mention that the intensity and duration of action were the greatest with those compounds having an aralkyl group, with halogen substituent (*m*–Cl–benzyl group), on the chain nitrogen. In this respect the compounds are similar to the active mono-quaternary tropane compounds described previously by us[17]. None of these asymmetric compounds with $(CH_2)_n$, where $n = 2$ or 3, have significant curare-like activity in spite of their bis-quaternary character.

The Tropane Ring System and its Stereostructure in Relation to Pharmacological Activity

Changes in structure of tropane derivatives and synthesis of new compounds did not show more effective atropine-like activity than that produced by atropine, hyoscyamine and hyoscine. The many active alkylamine compounds not possessing a tropane ring system on the one hand, and the relative inefficacy of the tropane compounds without ester groups on the other, showed that the tropane ring system is not responsible for the atropine-like activity of the tropane alkaloids.

The role of the tropane skeleton in the synaptic (ganglion-blocking and curare-like) effects remained unsolved even after the preparation of the highly active tropine ester compounds. Knowledge of the stereostructure of the tropane ring system appeared necessary to solve this problem.

In 1951 on the basis of observations on a few quaternary derivatives of tropine we supposed that, in the ganglion-blocking actions, the C(3)–OH group of the tropine compounds depressed activity[53]. The striking difference in the ganglion-blocking activity between some alkyl tropinium salts and the corresponding quaternaries of 2-6-dimethylpiperidines led to a search for correlation of stereostructure and anti-acetylcholine effects in tropane and some piperidine compounds.

The Steric Position of the C(3)–OH Group of the Tropane Skeleton and Activity

As previously mentioned, the C(3)–OH group in tropine may be in a *trans*- (α) or *cis*- (β) position to the ring nitrogen, its configuration determining the steric position of ester groups connected to it.

To study the pharmacological effect of the configuration of the C(3)–OH group, benzoyl-α-tropine (benzoyltropine) and benzoyl-β-tropine (tropacocaine) seemed to be most suitable. Preparation of several quaternary derivatives of these was undertaken, and their atropine-like, and ganglion-blocking, and local anaesthetic effects were assessed.

As the distance of the ketone and ether O atoms in the ester linkage from the nitrogen seems to be an important factor for atropine-like activity[44], it appeared from experiments that the optimum distance is present only in α-tropine and not in the β-tropine esters. Further, the effectiveness of a group of compounds is already determined by the configuration of tropine, and no quaternary group is capable of influencing this in β-tropine compounds, in which the steric structure probably does not permit the O atoms to be at the required distances from the ring nitrogen[42,43]. The configuration of the C(3) grouping of the tropine derivatives does not affect ganglion-blocking activity as the members of the benzoyl β-tropinium series are no less active in this respect than those of the α-tropinium series. Curare-like effect appears to run parallel with ganglion-blocking activity. Because the steric position of the aromatic ester groups on C(3) has no significant influence on these latter two actions it was assumed and later confirmed that the presence of these groups was unnecessary for activity.

The Effect of the C(3)–OH Group of Tropine on Activity

This was investigated using the quaternary derivatives of 3-tropanol (tropine) and tropane series. Parallelism on ganglion-blocking activity is found between the members of both series and the corresponding quaternary derivatives of aromatic acid tropine esters. The correlations obtained can be seen in Table VII, which also shows the depressant effect of C(3)–OH groups on ganglion-blocking activity. The aromatic ester group is not responsible for this effect as the tropane compounds are similar in activity to the esterified compounds. For ganglion-blocking activity in tropane compounds, the nature of the quaternary group appears to be the most important factor.

TABLE VII

THE GANGLION-BLOCKING ACTIVITY OF TROPINE, TROPANE AND TROPINE ESTER COMPOUNDS. CAT NICTITATING MEMBRANE

Tetraethylammonium bromide = 1

Quaternary group	Tropine	Tropane	Mandelyl-α-tropine	Tropyl-α-tropine
Tertiary	0·5	2·0	0·2	0·35
Methyl	1·0	2·4	4·5	4·0
Benzyl	0·8	4·0	3·0	2·1
p-Brombenzyl	2·5	17·0	19·0	8·5
p-Diphenylmethyl ..	6·0	28·0	40·0	28·0

2:6-DIMETHYLPIPERIDINIUM COMPOUNDS

The "opening" of the endoethylene bridge in the tropane ring by substituting two methyl groups not only breaks the chain character but it may also affect the conformation of the ring system. Therefore the 2:6-dimethylpiperidine compounds may serve as only imperfect paper-plane models of the tropane compounds.

Among the 2:6-dimethylpiperidine compounds three kinds of derivatives are known: (i) 2:6-dimethyl-4-acyloxypiperidinium compounds, (ii) 2:6-dimethylpiperidinium compounds[76], (iii) 2:6-dimethylpiperidino-1-(ethan-2-ol) esters[77].

The first compounds were made for comparison with α- and β-tropine esters, and were found to have great similarity in atropine-like activity to the β-tropine esters[17]. From this it may be deduced that an ester grouping on C(4) has a β- rather than an α-configuration assuming the existance of the unproved chair conformation of the piperidine ring. The ganglion-blocking and curare-like effects of the compounds were not comparable to those of the α- and β-tropine esters. This fact shows that the 2:6-dimethylpiperidine ring, which stereochemically is not a rigid ring system as is tropane, under certain circumstances alters its steric form; for example, besides the alternations of the chair and boat conformations the steric orientation of the 2:6-dimethyl groups can change in addition. This view is confirmed by the results with 2:6-(*cis*- and *trans*-)dimethylpiperidinium compounds.

The members of this series were used to answer the following questions: (i) can one replace the endoethylene bridge of tropane compounds by methyl groups in the 2 and 6 position of the piperidine ring?; (ii) has the

possible *cis* and *trans* position of methyl groups in C(2) and C(6) of piperidine a significant role on the synaptic-blocking effects?; (iii) can the change in the order of the substituents (primary and secondary) on the nitrogen alter the pharmacological activity?

Some results of experiments made with 2:6-dimethylpiperidine quaternary derivatives are shown in Table VIII when it can be seen that the

TABLE VIII

GANGLION-BLOCKING ACTIVITY OF 2:6-DIMETHYL(*cis*)*N*-METHYL-PIPERIDINIUM AND TROPINIUM COMPOUNDS. CAT NICTITATING MEMBRANE

Tetraethylammonium bromide = 1

Compound	2:6-Dimethylpiperidine group	Tropane group
Tertiary	2·0	2·0
Methyl quaternary	2·5	2·4
Benzyl quaternary	3·4	4·0
p-Diphenylmethyl quaternary ..	8·0	28·0

cis members of the series have an activity similar to the corresponding members of the tropane series except for the *p*-diphenylmethyl quaternary compound which is weaker than the similar tropane compound. The difference between the two diphenylmethyl quaternary compounds can be explained by the postulation that this large group, with a greater steric requirement, deforms the "original" *cis* configuration of the methyl groups on C(2) and C(6) which in normal circumstances seems to be sterically similar to the endoethylene bridge. Evidence for this supposition is the observation that compounds with *trans* methyl groups are weaker than those with *cis* methyl groups, only the latter being similar in ganglion-blocking activity to tropane compounds.

These observations show that for ganglion-blocking action the stereostructure of the rigid ring system of tropane with the endoethylene bridge is a very favourable basic chemical structure. Therefore building up various quaternary groups on this skeleton may give promising results.

Similarly Winbury[76] stated that for ganglion-blocking activity in aliphatic quaternary ammonium compounds an α-branched aliphatic chain attached to the nitrogen is necessary. In our opinion this statement is valid only if the stereostructure of such a branched aliphatic carbon chain is known. Very probably this steric orientation is similar to that of the α-branched tropane ring system.

The 2:6-dimethylpiperidinium ring, in contrast to the tropane ring, is not a rigid ring system. Therefore in piperidinium compounds which differ in the position of the quaternary groups about the nitrogen, the steric position of these groups and their relation to the elastic ring system is not the same as in the rigid quaternary tropane compounds. The results obtained with 2:6-dimethylpiperidinium derivatives showed indeed that in one instance the potency of the R′–$^{+}$N–R and the R–$^{+}$N–R′ epimeric form, was exchanged[17].

From data on the 2:6-dimethylpiperidinium compounds, some having ester groups on C(4) and other attached to a carbon chain on the nitrogen,

one can state that generally they do not have such a high ganglion-blocking activity as tropane compounds[17,77,78]. This also applies to their curare-like activity[17].

N-Alkyl and Aralkyl-nor-tropine Compounds

The problem of the role of the absolute steric position of the substituents on the nitrogen in relation to pharmacological activity can be investigated only in nor-tropine or tropane compounds which have the desired substituents in a different order. The investigation of these compounds can answer the question whether with the tropine and tropane compounds, the presence of, and the steric position of, the N–CH_3 group is optimal for pharmacological activity or not.

Pharmacological data for this kind of tropine compound have been limited until recently. Stoll and Jucker[60] mention the observation of Rothlin and colleagues[59] with the butyl-quaternary derivative of benzylyl-6-methoxytropine and its *N*-epimeric methyl-quaternary *N*-butyl-benzylyl-6-methoxy-nor-tropine. They did not find significant differences in the spasmolytic action of these compounds. Our experiments with some aralkyl tropinium compounds quaternised in a reversed order resulted in compounds exhibiting marked differences in atropine- and curare-like and ganglion-blocking effects[17,79].

Piperidinium Compounds

Experiments made with compounds containing simple piperidine (piperidinium) rings show that their structure does not fulfil the requirements necessary for synaptic-blocking activity compared with the tropane compounds[17].

The Tropane Compounds and Local Anaesthetic Activity

Local anaesthetic tropane compounds can be divided into three groups: compounds with (*a*) ecgonine, (*b*) tropine and (*c*) 6-alkoxytropine ring systems.

The best known and one of the most active among these is cocaine (2β-carbomethoxy-3β-benzoyloxytropane)[80]. As psicaine (2α-carbomethoxy-3β-benzoyloxytropane) has about the same local anaesthetic activity as cocaine[81], the configuration of the carbomethoxy group in C(2) is not important for this activity. In fact tropacocaine, which has no carbomethoxy group, is a potent local anaesthetic. However, in all of these substances the benzoyl ester group is found in the 3β-position. Compounds with ester groups attached to the OH group in the 3α-position have weaker activity. It seems that for the blockade of (sensory) nerve conduction, ester groups in the 3β-position are important. The benzoyl ester prepared from the third racemate of ecgonine has no local anaesthetic properties according to Wick[82]. Ecgonine proved to be ineffective too.

Considering the role of nitrogen in compounds with the ecgonine ring, the secondary amine derivative corresponding to cocaine is more effective than cocaine itself, but the methyl-quaternary cocaine derivative shows little activity[83]. von Braun and Müller[37] investigated some nor-ecgonidine derivatives (compounds not having a substituent on C(3), their benzoyl

ester group being on an aliphatic chain attached to the nitrogen). One of these compounds, Ekkain [*N*-(3-benzoyloxy-*n*-propyl)2-carbethoxy-nortropidine], proved to be more effective and to possess a better therapeutic index than cocaine[84].

In the group of local anaesthetic compounds with the tropine ring system, tropacocaine, benzoyltropine and *p*-aminobenzoyl-α-tropine are known. Methyl quaternisation of these compounds, as with cocaine, decreases their activity, but higher alkyl and aralkyl quaternary groups retain or even increase the local anaesthetic activity in comparison with the tertiary compounds[42,43]. Recently it has been pointed out[85] that some higher alkyl and aralkyl quaternaries of atropine were absorbed from the gastrointestinal tract more efficiently than the methyl derivative. Presumably in other tissues the rate of penetration depends significantly on the nature of the quaternary groups.

Of the 6-methoxytropine derivatives, the 3α-benzoyloxy-6-methoxy tropane and the 3α-diphenylglycolyl-6-methoxy-tropane show a definite local anaesthetic activity[59]. Their methyl and butyl quaternary derivative were, however, inactive. The activity of the former compounds proves that the methoxy group at C(6) does not affect local anaesthetic activity.

From a practical point of view, the stereochemical features of tropane and ecgonine compounds with local anaesthetic activity do not seem to be of as great an importance as those of ganglion-blocking and curare-like substances. The large number of synthetic local anaesthetics of high activity and their wide application shows that for local anaesthetic effect, unlike atropine-like activity, some simple alkamines without a complicated ring system can exert optimal effects. However, in the search for new local anaesthetics, without undesirable side effects, the derivatives of tropane and ecgonine, with their elucidated stereostructure, still merit further attention.

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RESEARCH PAPERS

THE WEIGHT AVERAGE MOLECULAR WEIGHT OF POLYVINYLPYRROLIDONE PREPARATIONS AS DETERMINED BY LIGHT SCATTERING

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The commonly accepted method for the characterisation of polyvinylpyrrolidone solutions used as plasma substitute is the measurement of the viscosity. The method is also applied to fractions separated from them by fractional precipitation, usually by the addition of acetone. From these measurements, reported in terms of intrinsic viscosity or K (Fikentscher)[1] values, figures, reputed to represent molecular weights, have been derived. Levy and Frank[2] have discussed these derivations and have listed a number of equations proposed by various investigators to relate intrinsic viscosity to molecular weight. The graphical representations of several such equations are presented in Figure 1. From these the interpretation may be made that an intrinsic viscosity of 0·2 dl./g. corresponds to a molecular weight between 15,000 and 43,000 while an intrinsic viscosity of 0·4 dl./g. corresponds to a molecular weight between 50,000 and 150,000. Obviously these interpretations must be treated with some reservation.

Since many of the *in vivo* characteristics of a particular plasma substitute depend on its molecular size it would seem desirable to characterise these materials by a procedure which measures molecular weight directly rather than by the interpretation of viscosity data. Light scattering photometry, which measures directly the weight average molecular weight of a polymer, has been used for this purpose on polyvinylpyrrolidone by several investigators[2,5,7]; but a detailed method has not been published. Since polyvinylpyrrolidone presents some problems in light scattering photometry, it appeared of interest to report the results of studies conducted in these laboratories.

Experimental

Seven commercial plasma substitutes from three sources, four dried polyvinylpyrrolidone fractions suitable for clinical use, and five special samples of molecular weight above and below the clinical ranges were studied. It was at once evident that the usual light scattering procedure, such as that applied to dextran plasma substitutes[8], could not be applied directly to polyvinylpyrrolidone solutions, which exhibited fluorescence when exposed to blue light of 436 mμ, the wavelength commonly used. The procedure adopted was as follows.

Aliquots of an approximately 1·0 to 3·5 per cent (w/v) solution of polyvinylpyrrolidone in water were pipetted into 50-ml. volumetric flasks

to produce, after dilution to the mark with optically-clear glass-distilled water, dilutions of approximately 1:10, 2:10, 3:10, 4:10, and 5:10. The higher concentration starting solutions were used with the lower molecular weight material. The carefully mixed dilutions were filtered under positive pressure through an ultra-fine sintered glass filter (nominal maximum pore size 1 to 2 μ) into clean dissymmetry cells. Clear, glass-distilled, filtered water was used as the blank. Readings were taken in the Brice-Phoenix Light Scattering Photometer, series 1000, using blue light (λ 436 mμ). The procedure described by Brice, Nutting and Halwer[9]

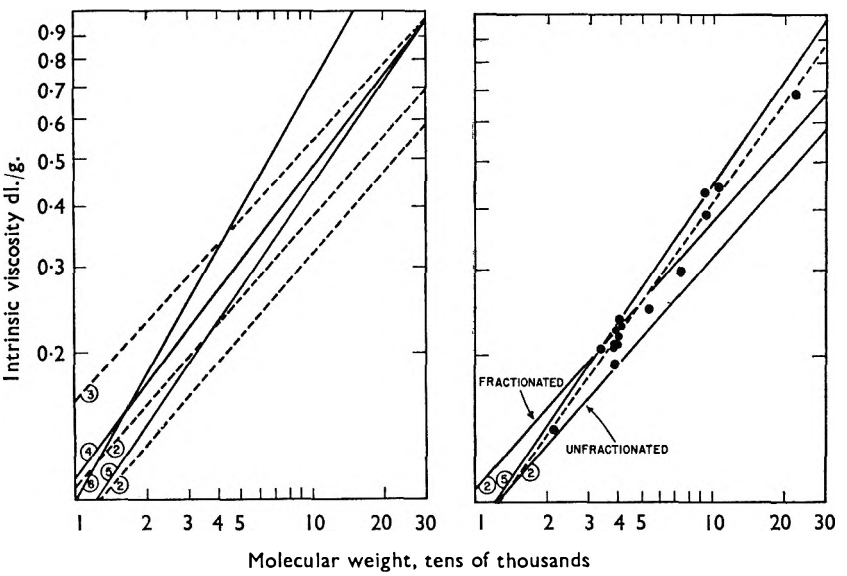

FIG. 1. Lines representing published relationships found between molecular weight and intrinsic viscosity of polyvinylpyrrolidone. The numbers refer to bibliographic references.

FIG. 2. Lines representing the relationship between molecular weight and intrinsic viscosity of polyvinylpyrrolidone found by Levy and Frank[2] and by Hengstenberg and Schuch[5]. The dashed line represents the line of best fit for the plotted data obtained in the present investigation.

was used to correct for fluorescence. In this procedure the unpolarised incident light is passed through the test solution, and the intensity of the polarised light scattered at 90° is compared with the intensity of the transmitted light for both horizontal and vertical vibrations. The depolarisation of the fluorescent light is measured by inserting a yellow filter (which transmits only fluorescent light) in the 90°-scattered light path and determining the proportion of horizontally to vertically polarised fluorescent light. This value is used to correct the true scattering ratio.

The intensity of the light scattered at 45° and 135° is determined to assess the dissymmetry of scattering. These values are corrected also for fluorescence. Under the conditions of these investigations, properly

clarified solutions of polyvinylpyrrolidone in the molecular weight range 20,000 to 100,000 regularly exhibited corrected dissymmetry ratios of less than 1·15 and usually less than 1·10.

The turbidity data obtained from the photometer readings were used to calculate the relation Hc/τ.* These values were plotted against the concentration c and the line of best fit was determined by eye and extrapolated to zero concentration. The line thus obtained usually showed some upward curvature as c increased. The intercept with the Y axis is the reciprocal of the weight average molecular weight. From the data shown in Table I, the coefficient of variation of the method was found to be less than $\pm$4·3 per cent. The 95 per cent confidence limits of the mean amounted to $\pm$3·1 per cent or less.

TABLE I

REPRODUCIBILITY OF LIGHT SCATTERING MOLECULAR WEIGHT DETERMINATIONS

	Sample			
	G1	S37	G2	IBH
	Weight average molecular weight			
	22,900	33,300	56,500	77,000
	21,300	33,800	55,000	80,000
	21,400	33,400	53,500	79,000
	22,400	33,000	58,600	80,000
	22,700	32,000	55,000	74,000
	22,200	31,300	54,000	71,500
	22,000	33,000	55,100	80,000
		33,000	55,100	80,000
		31,700	58,800	80,000
		32,000	58,800	74,200
Mean	22,130	32,650	55,660	76,990
Coefficient of variation	2·75	2·55	4·27	4·00
Standard error of the mean as per cent	1·04	0·81	1·35	1·27
95 per cent confidence limits of the mean as per cent	2·45	1·84	3·06	2·88

As a check on the calibration of the photometer the Rayleigh ratio for freshly distilled benzene was measured at 436 mμ and found to be 48·25 $\times$ 10^{-6}. This is 99·8 per cent of the value reported by Brice, Halwer and Speiser[11] and 102·3 per cent of the value reported by Oster[12].

Rather than use blue incident light which causes fluorescence, green light 546 mμ may be used. At this wavelength fluorescence is low in intensity and the corrections are small. At the same time the intensity of the scattered light is much reduced and the precision was found to be about the same as when blue incident light was used.

Some results obtained at the two wavelengths are shown in Table II. It will be noted that higher results were obtained using the longer wavelength. When the molecular weight found with green light was plotted against the molecular weight found with blue light, for the range of molecular sizes examined, the linear regression coefficient for the data

* For the calculation of the value for H, the refractive index increment dn/dc was found to be 0·185 for aqueous solutions. This is larger than the 0·175 reported by Hengstenberg and Schuch[5] but agrees with the 0·185 found by Levy and Frank[2]. Where the solvent was methanol the value 0·197 was used for dn/dc. Solutions were analysed for polyvinylpyrrolidone content by the colorimetric iodine method of Thrower and Campbell[10] as modified for use in this laboratory along the lines suggested to us by Drs. Levy and Fergus.

was 1·247. This was significantly different from 1 ($P = 0{\cdot}01$). This finding confirms similar results reported by Hengstenberg and Schuch[5].

Experiments also were conducted to compare the results obtained by light scattering photometry using methanol as solvent for the polyvinylpyrrolidone with those obtained using water. Where dry polymer samples

TABLE II

EFFECT OF INCIDENT LIGHT WAVELENGTH ON THE MOLECULAR WEIGHT DETERMINED BY LIGHT SCATTERING

Wavelength	Sample				
	G1	S37	G2	IBH	G4
	Weight average molecular weight				
436 mμ	22,000	33,800	55,000	74,000	235,000
546 mμ	25,200	40,000	63,500	85,000	290,000
Ratio	0·87	0·85	0·87	0·87	0·81

were used as the starting materials the results, in most instances, agreed fairly well regardless of the solvent. With the commercial solutions where the polyvinylpyrrolidone was precipitated by the addition of excess acetone, dried *in vacuo*, and redissolved in anhydrous methanol the results invariably were high compared with those obtained with water as

TABLE III

EFFECT OF SOLVENT ON THE MOLECULAR WEIGHT DETERMINED BY LIGHT SCATTERING

A. Dry powders

Solvent	Sample			
	G1	S37	G2	G4
	Weight average molecular weight			
Water	21,400	33,800	55,000	235,000
Methanol	22,700	34,800	58,700	232,000

B. Clinical solutions

Solvent	Sample			
	M4	M9	S86	S88
	Weight average molecular weight			
Water	40,000	39,200	41,000	38,500
Precipitated by acetone, redissolved in methanol	85,000	80,000	83,000	71,000
Precipitated by acetone, redissolved in water ..	41,500	—	—	39,600

the solvent. If the samples, after acetone precipitation and drying, were redissolved in water, the results agreed with those found with the original aqueous solution for direct analysis. Apparently some material, precipitated by acetone, failed to dissolve completely in methanol although it was readily soluble in water. This precipitate was in such a finely divided state that it passed through the ultra fine filter and caused high

turbidities. Illustrative results are presented in Table III. Intrinsic viscosities of the methanol solutions, unlike the light scattering measurements, were not affected by the treatment but were in the range to be expected from the relations reported by Levy and Frank[2]. This indicates that the treatment had not caused aggregation of the molecules.

It has been reported by Frank and Levy[7] that the weight average molecular weight of polyvinylpyrrolidone, measured in methanol, is not

TABLE IV

EFFECT OF SALTS ON THE DETERMINATION OF THE MOLECULAR WEIGHT OF POLYVINYLPYRROLIDONE BY LIGHT SCATTERING

Solvent	Sample		
	GI	S37	IBH
	Weight average molecular weight		
Water	21,400	33,800	74,000
0·9 per cent NaCl	21,600	33,300	74,000
1·8 per cent NaCl	—	33,000	71,000
3·6 per cent NaCl	—	32,000	—
Mixed salts*	—	33,000	80,000
Double strength mixed salts*	—	33,000	74,200
Mean	21,500	33,000	74,700

* For composition see text.

identical with that measured in water. It has been noted too that polyvinylpyrrolidone in methanol fluoresces more strongly than does an aqueous solution of the same concentration. In view of these findings it has been found desirable to make the light scattering measurement on aqueous solutions.

Investigation was made of the possible effect of the presence of sodium chloride, and of other salts used in physiological solutions, in the test solutions of polyvinylpyrrolidone. Equal weights of the same dry polyvinylpyrrolidone sample were dissolved in water, in 0·9, 1·8, or 3·6 per cent (w/v) aqueous sodium chloride solutions, or in a physiological salts* solution of usual or twice usual concentration. These starting solutions containing 1 to 3 per cent (w/v) polyvinylpyrrolidone were used to prepare the 5 dilutions in optically clear, glass-distilled water and these were subjected to filtration and light scattering measurements in the usual manner. The results of these analyses, presented in Table IV, indicate that the presence of these salts in the amounts indicated had no significant effect on the molecular weight determination by light scattering.

Frank and Levy[7] reported that 1M sodium chloride solution had little effect on the intrinsic viscosity of polyvinylpyrrolidone solutions. On the other hand, Jergensons[13] found the intrinsic viscosity to be reduced by the presence of 1·0M KCl and 0·25M $MgCl_2$ and to be slightly increased by

* Usual concentration physiological salts solution.

NaCl	0·8 g.	$MgCl_2 \cdot 6H_2O$	0·0005 g.
K Cl	0·042 g.	Na HCO_3	0·168 g.
$CaCl_2 \cdot 6H_2O$	0·05 g.	N HCl	1·171 ml.
		Water to make	100 ml.

1·0M $MgCl_2$. In Table V are presented data on the intrinsic viscosity† of the same solutions used to assess the effect of salts on light scattering measurements. Viscosity determinations were made as described previously for dextran[8]. It may be seen that there appeared to be no significant effect of salts on intrinsic viscosity measurements. On the basis of these experimental results it was considered unnecessary to free the commercial solutions of salts before performing viscosity and light scattering measurements.

TABLE V

EFFECT OF SALTS ON THE INTRINSIC VISCOSITY OF POLYVINYLPYRROLIDONE

Solvent	Sample		
	G1	S37	IBH
	Intrinsic viscosity dl./g.		
Water	0·142	0·210	0·284
0·9 per cent NaCl	0·142	0·209	0·282
1·8 per cent NaCl	—	0·208	0·286
3·6 per cent NaCl	—	0·216	—
Physiological salts solution*	—	0·207	0·284
Double strength physiological salts solution*	—	0·211	—
Mean	0·142	0·210	0·284

* For composition see text

As pointed out earlier, attempts to establish a reliable correlation of weight average molecular weight and intrinsic viscosity have not been too successful. It would appear that the viscosity of a particular sample of polyvinylpyrrolidone may bear a different relation to its weight average molecular weight than may be the case for another sample. Levy and Frank[2] have presented evidence which suggests that this is due to variations in homogeneity of the samples. In the lower ranges, application of the Levy and Frank formulae[2] for fractionated and unfractionated polyvinylpyrrolidone samples to the viscosity data assembled here yielded molecular weight values which bracketed most of the actual values obtained by light scattering. In the range above molecular weight 75,000 the data obtained here showed considerable divergence from the Levy and Frank relationships. The equation for the line of best fit over the entire range considered here, determined by least squares and converted to the usual form, was $[\eta] = 1{\cdot}575 \times 10^{-4} M^{0{\cdot}68}$. This line is shown in Figure 2 together with the lines representing the relationships found by Levy and Frank[2] for "fractionated" and "unfractionated" material and by Hengstenberg and Schuch[5].

SUMMARY

1. A method for the determination of the weight average molecular weight of polyvinylpyrrolidone is described. Corrections for the effects of fluorescence are applied.

† "Intrinsic viscosity" here is in some instances more properly termed "apparent intrinsic viscosity" since water alone was used to calibrate the viscosity pipettes as would be the case if the analyst were unaware of the presence of salts.

2. Measurements are carried out in aqueous solution in preference to methanol solution.

3. The presence of physiological concentrations of salts such as sodium chloride in the solutions analysed did not significantly alter light scattering or intrinsic viscosity measurements.

4. A relation between intrinsic viscosity and weight average molecular weight is derived, but any interpretations of intrinsic viscosity in terms of molecular weight must be regarded as approximations.

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THE STRUCTURE OF THE ROOT AND STEM OF *RAUWOLFIA CAFFRA* SOND.

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During the past twenty years the root of *Rauwolfia serpentina* Benth. has been the subject of extensive chemical and pharmacological investigation[1,2]. The isolation of the alkaloid, reserpine and its use in psychiatric and hypertensive conditions has stimulated the study of other plants of the same genus. Of the African *Rauwolfia* species, reserpine has been isolated from *R. vomitoria* Afz[3]., *R. caffra* Sond[4]. and *R. cumminsii* Stapf.[5] and extracts of *R. mombasiana* Stapf. have been shown to have hypotensive properties[6]. *R. vomitoria* is used commercially and has now largely replaced *R. serpentina* as a source of reserpine; its histological structure has been investigated[7,8] and a number of non-African species have been similarly studied[9,10,11]. As *R. caffra* is a potential African commercial source of reserpine and is of similar geographical distribution to *R. vomitoria*, it was thought desirable to undertake a histological study of the root and stem; the results are recorded below.

R. caffra, one of the largest species of the genus, is a tree 15 to 25 m. high[12] with a trunk up to about 120 cm. in diameter. It is of widespread, but not abundant, occurrence in the secondary fringe forest of the high rainfall areas of Central, East and South Africa[13,14]. The plant has many vernacular names [13,15,16] and native tribes have employed it for a wide range of ailments varying from skin rashes to intestinal disorders[13,15].

A number of species of *Rauwolfia*, originally considered to be distinct[17], are now generally regarded as synonymous with *R. caffra*. These include *R. natalensis* Sond.[18], *R. ochrosioides* K. Schum.[12], *R. inebrians* K. Schum., *R. obliquinervis* Stapf., *R. goetzei* Stapf.[19], and *R. welwitschii* Stapf.[20] Pichon in his classification[21] of the genus, has grouped them under Section Afrovolfia.

In 1901 Juritz[22] demonstrated the presence of a bitter crystalline alkaloid in the bark of *R. natalensis*. Rindl and Groenewoud[23] (1932) could not confirm this finding but obtained amorphous alkaloids which they did not characterise and Koepfli[14] (1932) isolated a crystalline alkaloid rauwolfine, which had hypotensive activity. Recently Schüler and Warren[4] have isolated reserpine and ajmaline from the root bark and a small quantity of intractable alkaloidal material from the stem bark.

Plant Material

The following material was used in this investigation:

1. *R. caffra* roots, stem wood and stem bark collected by D. B. Fanshawe, Esq., on the banks of the Ndola River, Northern Rhodesia.

2. *R. caffra* root bark from large roots, obtained by D. B. Fanshawe, Esq.

3. *R. caffra* roots supplied by John Ronaldson Ltd., London.
4. *R. caffra* roots supplied by B. O. G. Schüler, Esq., Natal University.

Macroscopy

The roots occur as cylindrical or flattened, occasionally branched pieces of varying lengths and up to about 10 cm. in diameter. The bark of larger roots separates from the wood and when dry, occurs as strips about 2 to 5 mm. thick. Externally, the irregularly furrowed, buff or light brown cork is frequently rubbed away revealing the buff cortical tissue which on careful examination shows glistening points of calcium oxalate. Often pieces of bark have broken away to reveal the reddish-brown longitudinally striated outer layer of the wood. Small roots may possess rootlet scars or stumps and rootlets with a marked longitudinal furrowing of the cork. The inner surface of the yellowish-brown root bark with its reddish-brown and greyish-brown patches, is marked by irregular longitudinal ridges. The surface exhibits glistening points of calcium oxalate.

Smoothed transverse surfaces of the roots show a narrow granular bark usually 0·5 to 3 mm. thick, but swelling considerably on soaking in water, and an inner buff, or yellowish finely-radiate, porous wood possessing a few distinct growth rings.

The dried root is almost odourless; the outer corky layer and the wood almost tasteless, and the outer cortex and phloem very bitter. The fracture of the bark is short and that of the wood splintery; small roots are brittle but larger roots are tough (Fig. 1 : A).

The dark brown stem bark occurs as irregular pieces up to about 15 mm. in thickness. Externally it shows buff patches where cork has rubbed off, deep fissures being apparent between the patches. The smoothed transverse surface is characterised by a narrow buff cork layer external to a wide granular phloem (Fig. 6).

Microscopy

In the following description the symbols R, T and L refer to measurements made in the radial, tangential and longitudinal directions respectively of material mounted usually in Berlese mountant. The ranges of measurements have been obtained from as wide a variety of specimens as possible, but the limits may not prove to be absolute.

Root

The appearance of the transverse section of the root shows considerable variation due, mainly, to the degree of development of stone cell layers within the secondary phloem. This development varies from a few isolated groups of stone cells in some small roots to about six interrupted concentric bands in some of the larger roots (Fig. 1 : B-E).

The radially arranged cork cells are lignified and suberised showing no alternation of lignified and non-lignified zones. For the cork cells, R = 16 to **40** to **80** to 140 μ, T = 32 to **72** to **120** to 180 μ and L = 24 to **40** to **60** to 108 μ. As the soft cork cells rub off easily, most specimens

show relatively little cork, although samples with up to 26 radial rows have been encountered. In surface view, the cork cells appear polygonal (Fig. 2: A, C).

The phellogen, consists of 3 to 4 rows of regularly arranged, radially flattened cells with thin cellulosic walls and the phelloderm is a zone of

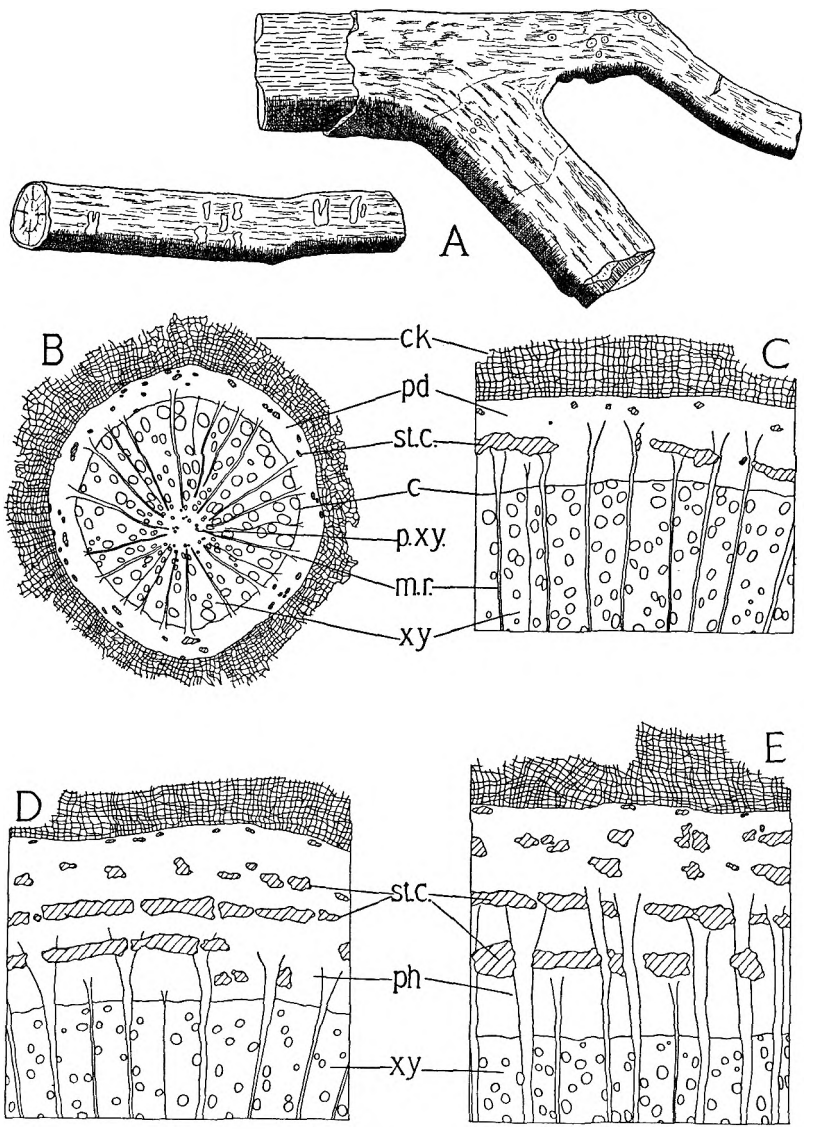

FIG. 1. *Rauwolfia caffra* Sond. Root. *A*, root segments × 1, *B-E*, general diagrams of transverse sections of roots, all × 15. *B*, 2·5 mm. diameter; *C*, 14 mm. diameter; *D*, 5 cm. diameter; *E*, 11 cm. diameter. *c*, cambium; *ck*, cork; *m.r.*, medullary ray; *pd*, phelloderm; *ph*, phloem; *p.xy.*, protoxylem; *st.c.*, stone cell group; *xy*, xylem.

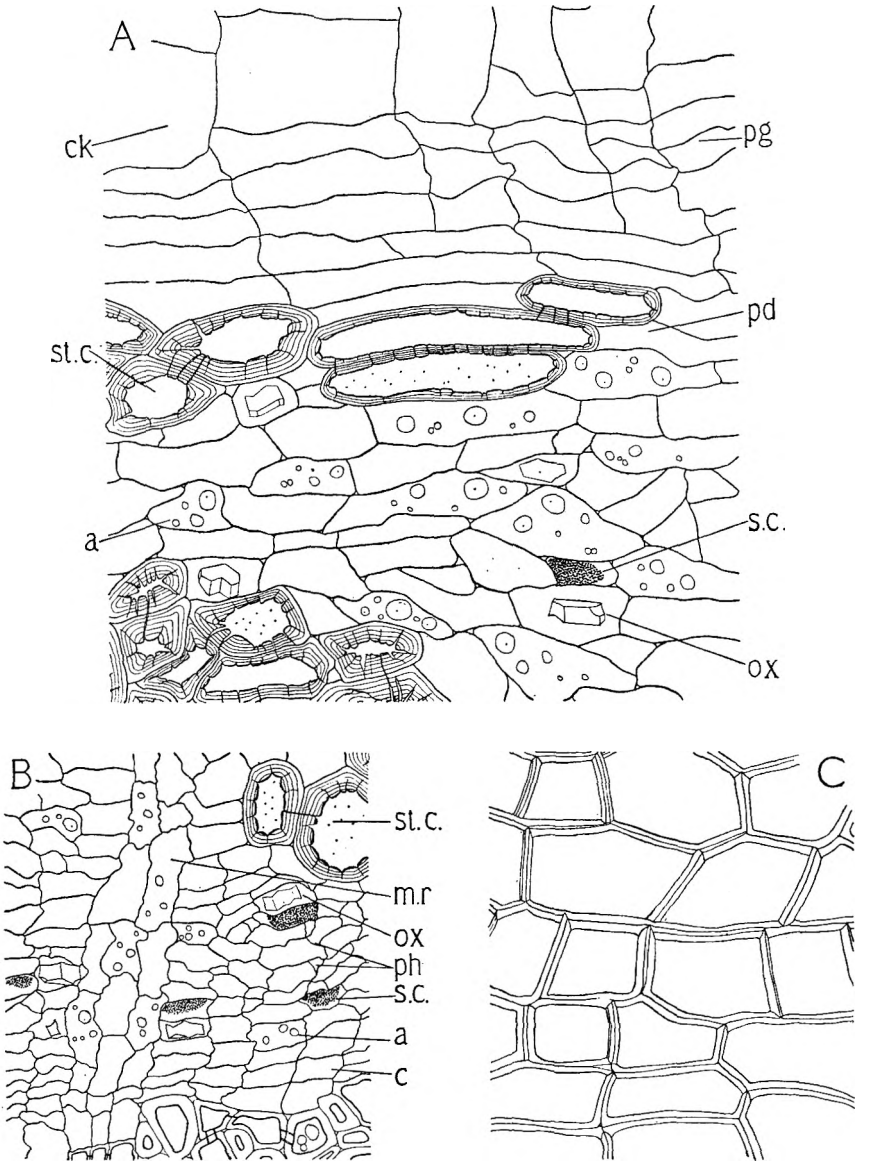

FIG. 2. *Rauwolfia caffra* Sond. Root. *A*, transverse section of the outer tissues, root diameter 33 mm. *B*, transverse section of inner phloem. *C*, cork cells in surface view. All × 200. *a*, starch grain; *c*, cambium; *ck*, cork; *m.r*, medullary ray; *ox*, calcium oxalate crystal; *pd*, phelloderm; *pg*, phellogen; *ph*, phloem elements (companion cell and sieve tube); *s.c.*, secretion cell; *st.c.*, stone cell.

about 5 to 25 rows of cells dependent on the size of the root. The cells adjacent to the phellogen are usually regularly arranged in radial rows whilst the inner ones are more oval in shape with intercellular spaces and showing evidence of sliding growth. R = 16 to **24** to **40** to 72 μ,

T = 40 to **64** to **100** to 184 μ and L = 16 to **40** to **56** to 80 μ. The walls of most of the phelloderm cells are thickened with cellulose, but, with the exception of small roots, some are lignified, forming either isolated or groups of stone cells. Individual stone cells are frequently tangentially elongated and most are relatively thin walled with marked pitting and striation of the walls. R = 20 to **32** to **52** to 108 μ, T = 20 to **48** to **92** to 268 μ and L = 20 to **40** to **64** to 108 μ (Fig. 2: A; 3: A). Starch and scattered twinned prisms of calcium oxalate occur in the phelloderm. The starch consists chiefly of single, rounded grains 1 to **3** to **6** to 27 μ in diameter, with occasional 2 to 4 compound grains which may split into individual plano-convex or angular grains. The hilum is usually apparent as a central point or star-shaped cleft and many grains, with the exception of the larger partially gelatinised ones, show a Maltese cross effect when examined in polarised light (Fig. 3: E).

Internal to the phelloderm is the wide zone of secondary phloem, which, in the larger roots consists of an inner functional zone and an outer non-functioning secondary phloem characterised by up to about 5 interrupted bands of stone cells. The phloem consists of sieve tubes, companion cells, phloem parenchyma, secretion cells, medullary ray cells and groups of stone cells (Fig. 2: B). The heterogeneous rays consist of groups of small cells often with wavy walls, 3 to 5 cells wide and up to 28 cells in height with upper and lower uniseriate extensions consisting of 1 to 4 larger cells (Fig. 3: C). For the smaller cells R = 16 to **28** to **48** to 104 μ, T = 12 to **24** to **48** to 96 μ and L = 12 to **24** to **40** to 64 μ, and for the larger cells R = 12 to **20** to **40** to 64 μ, T = 24 to **36** to **56** to 132 μ and L = 36 to **48** to **68** to 104 μ. Occasional ray cells adjacent to the stone cell groups may be lignified to form radially elongated sclereids (Fig. 3: B).

The irregular stone cell groups in the outer phloem are up to about 8 cells in radial thickness and 30 cells in depth. Individual cells vary greatly from isodiametric to irregularly elongated fibre-like structures, R = 16 to **32** to **64** to 108 μ, T = 20 to **48** to **80** to 140 μ and L = 20 to **40** to **80** to 456 μ. Stone cells isolated by maceration using chromic-nitric acid reagent measured 44 to **60** to **108** to 576 μ in length and 20 to **36** to **52** to 108 μ in breadth. All the stone cells have funnel-shaped and occasionally branched pits and stratified walls. Calcium oxalate crystals completely fill the lumina of some cells (Fig. 3: B; 5: A, B).

In radial and tangential longitudinal sections of the secondary phloem long rows of calcium oxalate crystals are evident (Fig. 3: C). These crystals consist of monoclinic prisms often twinned on one of the hemi-pyramid faces and exhibit, in polarised light, a bicolouration effect. Length of prisms, 18 to **24** to **32** to 50 μ; breadth, 8 to **10** to **16** to 28 μ. The occasional angular masses of calcium oxalate which can be seen probably arise from fracture of the prisms during sectioning (Fig. 3: F).

Starch grains occur freely in the outer non-functional phloem, but are less frequent in the inner functional phloem. In size and shape they resemble those of the phelloderm.

Secretory cells occur occasionally in the phelloderm and scattered

throughout the outer and inner phloem. The amorphous contents of these cells stain with iodine solution, Sudan III and Tincture of Alkanna. No latex vessels have been observed although occasional short vertical

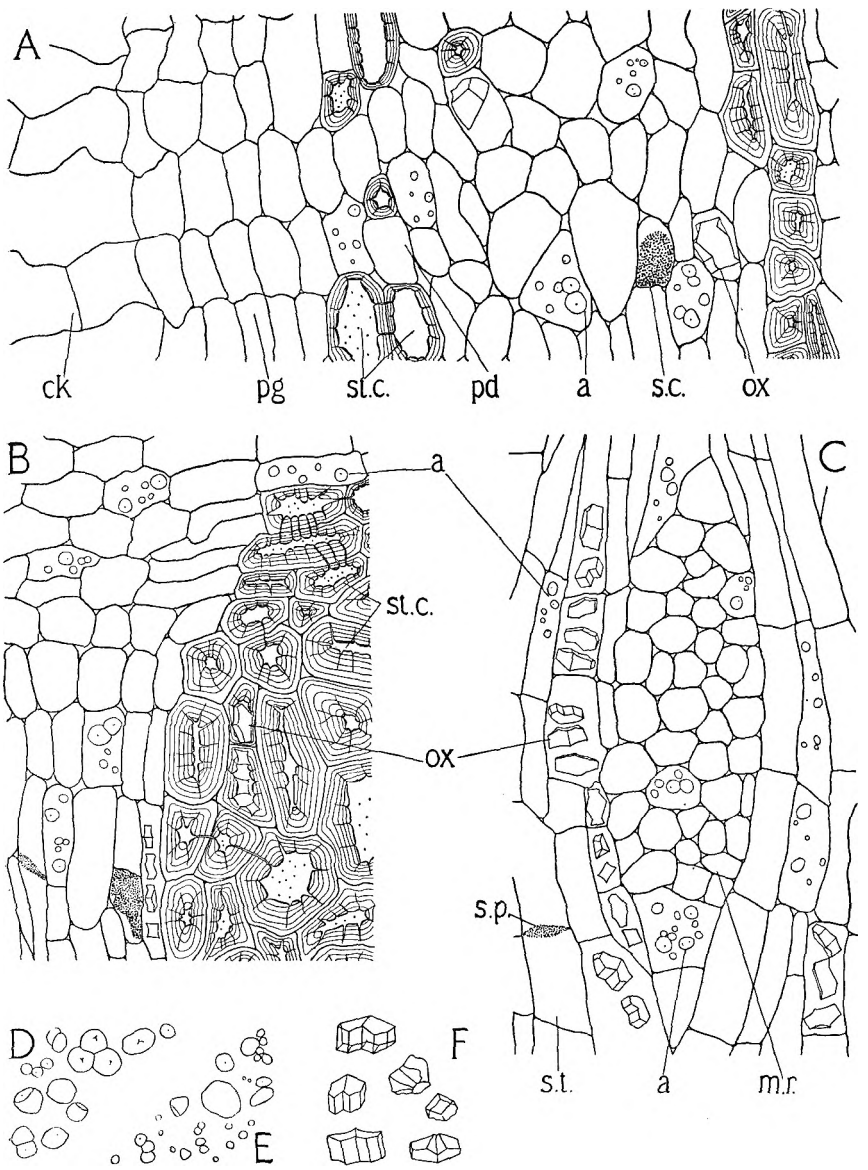

FIG. 3. *Rauwolfia caffra* Sond. Root. *A-C*, longitudinal sections of the bark, root diameter 33 mm. *A*, radial section of outer tissues. *B*, radial section of inner phloem. *C*, tangential section of inner phloem. *D*, starch grains from the wood. *E*, starch grains from the bark. *F*, calcium oxalate crystals from the root bark. All × 200. *a*, starch; *ck*, cork; *m.r.*, medullary ray; *ox*, calcium oxalate crystal; *pd*, phelloderm; *pg*, phellogen; *s.c.*, secretion cell; *s.t.*, sieve tube; *s.p.*, sieve plate; *st.c.*, stone cell.

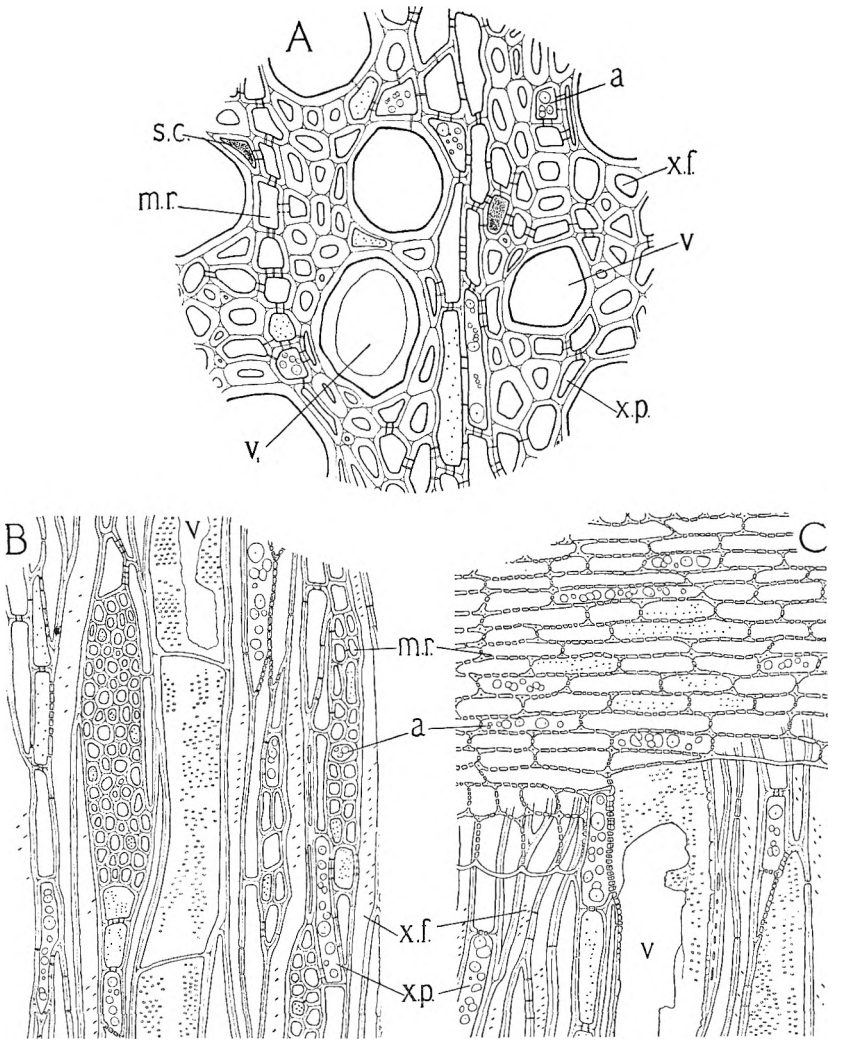

FIG. 4. *Rauwolfia caffra* Sond. Root. Secondary Wood. *A*, transverse section × 200. *B*, tangential longitudinal section, × 100. *C*, radial longitudinal section, × 100. *a*, starch; *m.r.*, medullary ray; *s.c.*, secretion cell; *v*, vessel; v_1, vessel showing portion of end plate; *x.f.*, xylem fibre; *x.p.*, xylem parenchyma.

rows of secretion cells occur. Individual cells can be isolated from alkali macerations (Fig. 5: D).

The primary xylem is indicated by four to six small groups of vessels near the centre of the root and the completely lignified secondary xylem is composed of medullary rays, vessels, fibres and wood parenchyma (Fig. 4: A, B, C). In transverse sections the rounded or somewhat radially elongated vessels occur singly or occasionally in pairs. The relatively thin vessel walls bear numerous alternately arranged bordered

pits, and isolated vessel segments show transverse and oblique perforation plates. Occasional vessels are occluded by brown amorphous material. R = 44 to **99** to **151** to 195 μ, T = 20 to **79** to **127** to 167 μ and for isolated segments, length = 137 to **412** to **715** to 962 μ, breadth = 68 to **104** to **152** to 224 μ. (Fig. 4; 5: G).

The numerous xylem fibres which support the vessels possess thick lignified walls with spirally arranged slit-like pits (Fig. 5: H). The length of the fibres is about 850 to **1290** to **1950** to 2340 μ, and breadth 20 to **28** to **36** to 56 μ. The ends of the fibres are frequently contorted, occasionally bifurcated and may show scalloping corresponding to the positions of adjacent medullary ray cells.

The apotracheal wood parenchyma occurs in short uniseriate rows connecting the vessels and medullary rays. In longitudinal sections the cells are arranged in vertical rows. The walls bear simple or half bordered pits, dependent on the nature of the adjacent cell structure. R = 10 to **28** to **40** to 88 μ, T = 12 to **28** to **40** to 64 μ and L = 56 to **100** to **140** to 240 μ.

The heterogeneous medullary rays resemble those of the bark being 3 to 5 cells wide, up to 20 small cells in height with uniseriate upper and lower extensions of about 1 to 4 large cells. For the small cells R = 28 to **72** to **120** to 248 μ, T = 10 to **12** to **20** to 40 μ and L = 10 to **18** to **28** to 56 μ, and for the larger cells R = 12 to **20** to **40** to 64 μ, T = 24 to **36** to **56** to 132 μ and L = 36 to **48** to **68** to 104 μ. In tangential sections, the small cells have a rounded appearance and intercellular spaces are apparent (Fig. 4: B).

Starch grains, 2 to **6** to **15** to 33 μ in diameter, similar to those in the bark, fill the wood parenchyma and medullary ray cells (Fig. 3: D). Occasional cells contain material staining with iodine and Sudan III. Some medullary ray cells of a few root specimens contained calcium oxalate prisms.

Stem

The general tissue distribution and cell dimensions of the stem bark resemble those of the root bark (Fig. 6: C). The soft cork layer is generally more extensive than that of the root, stem bark with up to 65 radial rows of cork cells having been examined. The phelloderm and cortex consist of about 30 radial rows of cells resembling the corresponding tissues in the root bark. The pericycle is not a well defined zone, but is indicated by scattered highly refractive non-lignified fibres 8 to **12** to **24** to 40 μ in diameter occurring either isolated or in association with groups of stone cells. After maceration in alkali, the isolated fibres, particularly of older, thicker bark show marked swellings 28 to 92 μ in diameter (Fig. 7: E).

The cortex and secondary phloem are characterised by up to about 16 interrupted bands of stone cells, individual stone cell groups being up to 12 cells wide and 19 cells deep.

Occasional latex vessels, containing granular matter which stains with iodine, occur in the outer non-functional secondary phloem and

cortex. In transverse sections they appear circular or ellipsoidal and in macerated material occasional branching is evident. R = 44 to 140 μ, T = 76 to 200 μ and length up to 0·5 mm. (Fig. 7: B, F).

The medullary rays are usually 4 to 6 small cells in width and 9 to 23 small cells high. Many medullary rays in older, thicker bark are com-

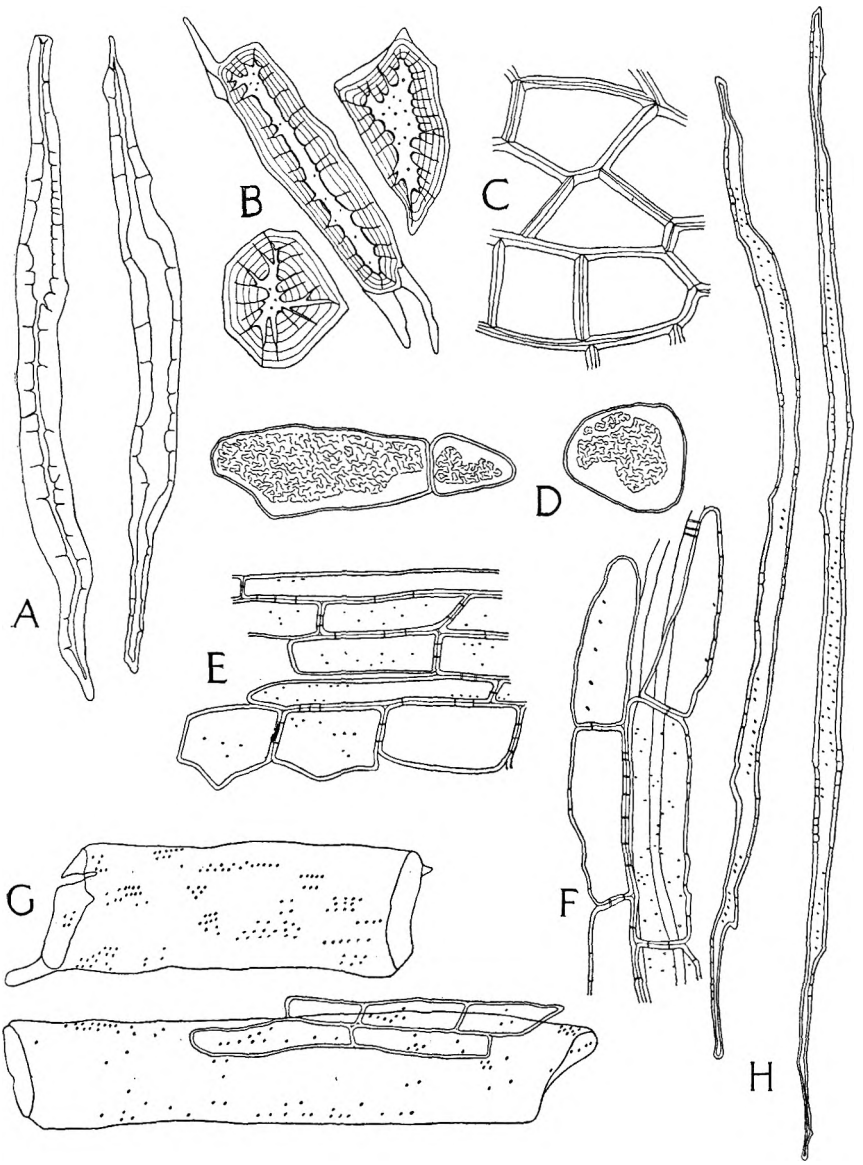

FIG. 5. *Rauwolfia caffra* Sond. Isolated elements of the root. *A*, elongated stone cells, × 100. *B*, stone cells, × 200. *C*, cork cells, × 200. *D*, secretion cells, × 200. *E*, lignified medullary ray cells, × 200. *F*, xylem parenchyma, × 200. *G*, portions of xylem vessels, × 100. *H*, xylem fibres, × 100.

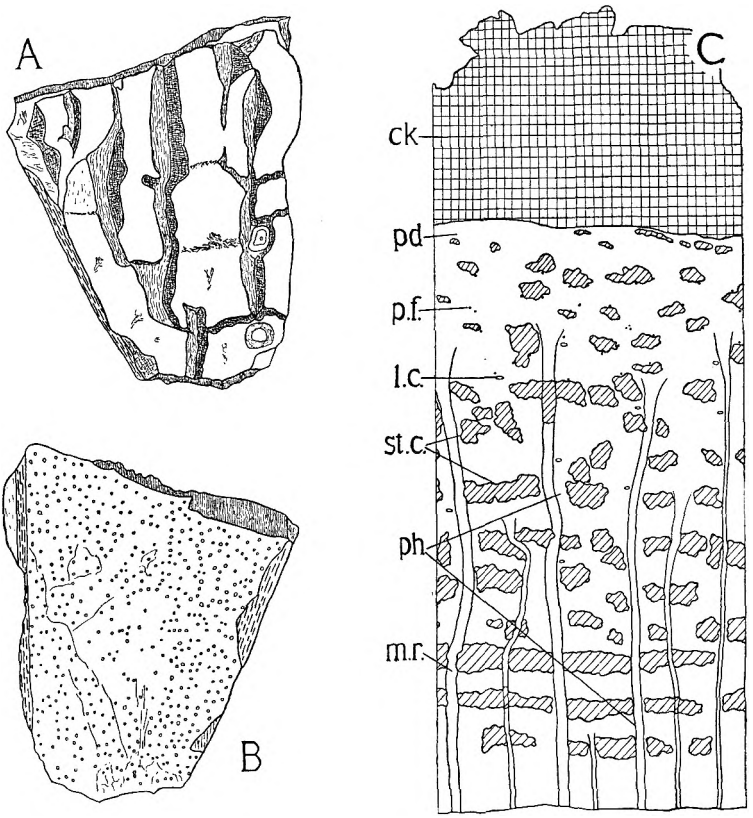

FIG. 6. *Rauwolfia caffra* Sond. Stem bark. *A*, outer surface. *B*, inner surface. Both × 1. *C*, transverse section, × 10. *ck*, cork; *l.c.*, latex canal; *m.r.*, medullary ray; *pd*, phelloderm; *p.f.*, pericyclic fibre; *ph*, phloem; *st.c.*, stone cell group.

pletely lignified forming groups of radially elongated sclereids. R = 40 to **52** to **88** to 136 μ; T = 24 to **32** to **40** to 68 μ and L = 20 to **24** to **32** to 48 μ.

The innermost layer of the phloem is the functional zone consisting of sieve tubes, companion cells, phloem parenchyma and medullary ray cells. Many cells, which often appear yellowish in chloral hydrate mounts, contain granular material staining with iodine solution and Sudan III. (Fig. 7: C, D). Calcium oxalate and starch resemble that present in the root bark.

The stem wood resembles the root wood although the fibres appear to be somewhat larger, length 627 to **1710** to **2310** to 2772 μ and breadth 20 to **28** to **40** to 56 μ. (Fig. 8).

Calcium oxalate which is only occasionally found in the root wood occurs more freely in the ray cells and xylem parenchyma of the stem wood and consists of the usual monoclinic and twinned prisms.

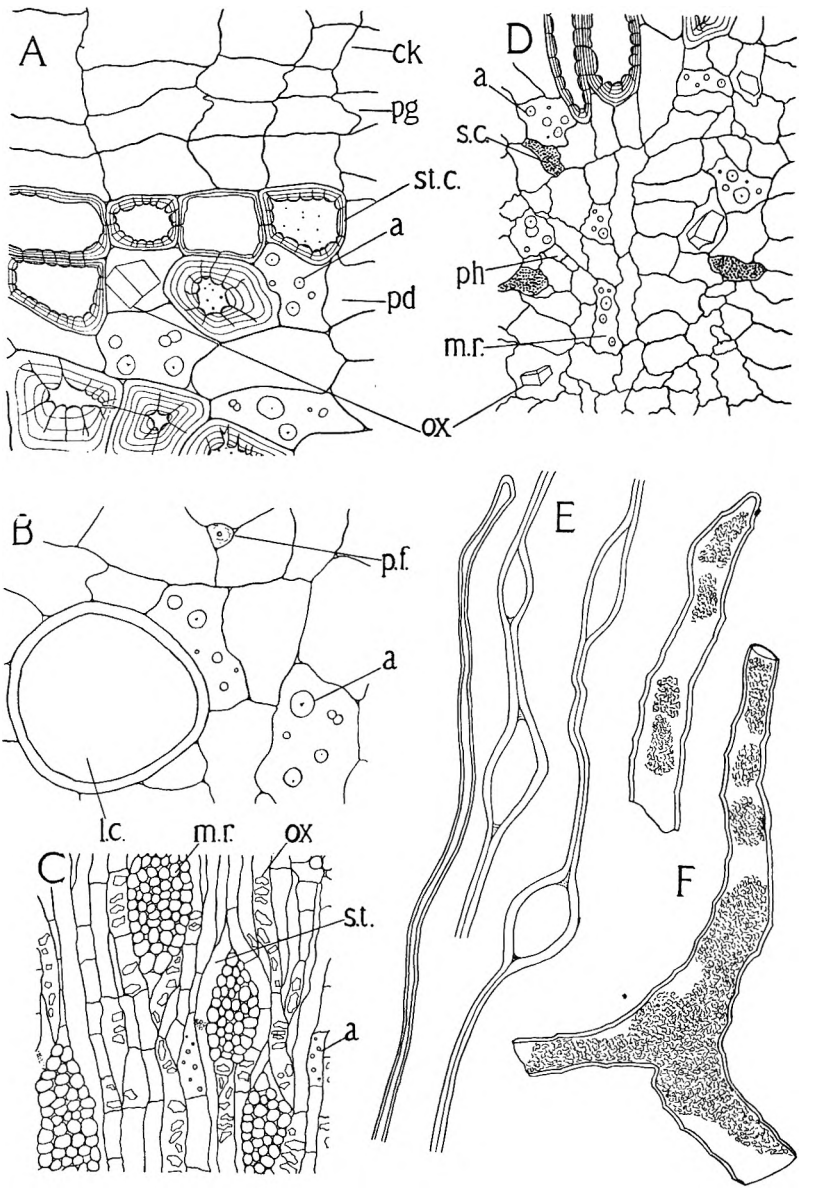

FIG. 7. *Rauwolfia caffra* Sond. Stem bark. *A*, transverse section of outer tissues, × 200. *B*, transverse section in region of pericycle, × 200. *C*, tangential longitudinal section of inner phloem, × 75. *D*, transverse section of inner phloem, × 200. *E*, isolated pericyclic fibres, × 100. *F*, portions of isolated latex canals, × 100. *a*, starch; *ck*, cork; *l.c.*, latex canal; *m.r.*, medullary ray; *ox*, calcium oxalate crystal; *pd*, phelloderm; *p.f.*, pericyclic fibre; *pg*, phellogen; *ph*, phloem elements; *s.c.*, secretion cell; *s.t.*, sieve tube; *st.c.*, stone cell.

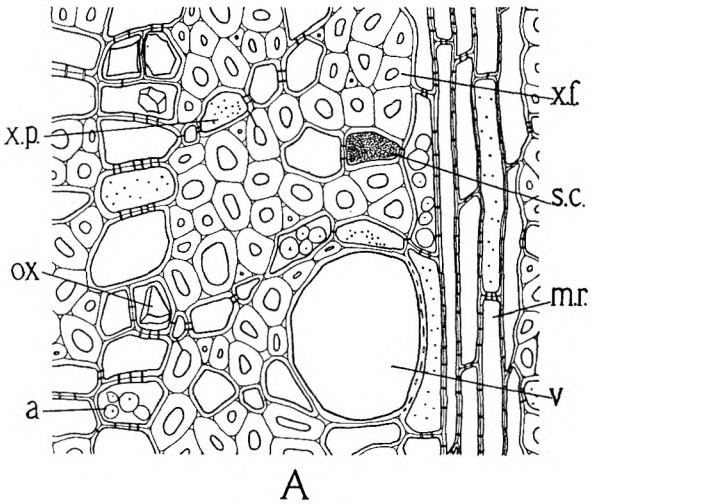

FIG. 8. *Rauwolfia caffra* Sond. Stem wood. *A*, transverse section, × 200. *B*, tangential longitudinal section, × 100. *C*, radial longitudinal section, × 100. *a*, starch; *m.r.*, medullary ray; *ox*, calcium oxalate crystal; *s.c.*, secretion cell; *v*, vessel; *x.f.*, xylem fibre; *x.p.*, xylem parenchyma.

THE POWDERED ROOT

The principal features of the powdered root are:

1. Thin-walled yellow, lignified cork cells appearing polygonal in surface view.

2. Thin-walled cellulosic elements of the phelloderm and phloem containing starch grains, calcium oxalate crystals and resinous material.

3. Rounded, ovoid, plano-convex and concavo-convex starch grains about 1 to 3 to 15 to 33 μ in diameter; occasional 2 to 4 compound grains.

4. Single or twinned monoclinic prisms and irregular crystalline masses of calcium oxalate.

5. Isodiametric, elongated or irregularly shaped lignified stone cells either singly or in groups. Occasional stone cells contain calcium oxalate crystals.

6. Large amounts of lignified xylem elements comprising xylem fibres, thin-walled vessels with alternately arranged bordered pits and elongated xylem parenchyma and medullary ray cells usually containing starch grains.

Discussion

The structure of *R. caffra* root and stem is typical of the family Apocynaceae and of other members of the genus. The dried roots are readily distinguishable from *R. serpentina* in both the whole and powdered conditions due to the complete absence of stone cells in the latter. Its structure however closely resembles that of *R. vomitoria* root, the principal distinguishing feature being the absence of alternating lignified and unlignified cork cells in the transverse sections of *R. caffra*. As a consequence the cork of the whole root does not tend to flake off in small scales as with *R. vomitoria*. A comparison of the microscopical measurements of *R. caffra* with those recorded for *R. vomitoria*[7] indicates that the ranges of measurements are so similar in the two species to be useless for differentiation purposes. Further work will therefore be necessary to obtain a more suitable method for the differentiation of the powdered roots. It is hoped to attempt this after other African species have been studied in detail.

Summary

1. The gross morphology and histology of the stem and root of *R. caffra* Sond. have been described and illustrated.

2. The dimensions of the principal cell structures and contents are recorded.

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THE SYNTHESIS OF D-1 : 2-BISDECANOYLGLYCEROL AND D-1 : 2-BISDODECANOYLGLYCEROL*

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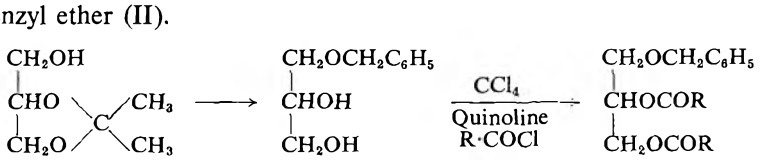
IN the course of investigations reported elsewhere[1] it became necessary to prepare 1 : 2-diacylglycerols of D configuration and containing normal fatty acid chains with approximately 10 carbon atoms. The synthesis of 1 : 2-bisdecanoyl and 1 : 2-bisdodecanoylglycerol reported below follows the elegant procedure of Sowden and Fischer[2], each step of which has been shown to proceed with full retention of configuration.

Thus D-1 : 2-*iso*propylidineglycerol (I) was obtained from D-1 : 2 : 5 : 6-di-*iso*propylidinemannitol by oxidative cleavage with lead tetra-acetate followed by catalytic hydrogenation of the intermediate glyceraldehyde. Benzylation of (I) followed by mild acid hydrolysis furnished D-glyceryl 3-benzyl ether (II).



(I) (II) (III)

Acylation with the appropriate acid chloride in carbon tetrachloride/quinoline gave the requisite diacylglyceryl benzyl ethers (III) (R = C_9H_{19} and $C_{11}H_{23}$) as undistillable liquids. Although purification of (III; R = C_9H_{19}) was achieved by crystallisation from ether at −70°, chromatography on silica gel proved simpler and applicable to either case (Fig. 1), a graded elution technique with continuously increasing concentrations of acetone in light petroleum (b.p. 60 to 80°) being used. This method was also used to effect separation of D-1 : 2-bisdecanoylglycerol from its benzyl ether (see Figure 2 for a typical separation).

Catalytic hydrogenolysis of the D-1 : 2-diacylglyceryl 3-benzyl ethers furnished the required D-diglycerides as solids of low melting point. Their optical rotations were too small to permit of accurate determination, and only the values for the 3-benzyl ethers are recorded.

EXPERIMENTAL

Melting and boiling points are uncorrected. Microanalyses were performed by Mr. G. S. Crouch of this school.

D-*Glyceryl 3-benzyl ether.* D-1 : 2-*iso*Propylidineglyceryl 3-benzyl ether

* This paper forms part of a thesis submitted by one of us (D.W.R.) in part fulfilment of the requirements for the Degree of Doctor of Philosophy in the University of London.

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was prepared from D-1 : 2-*iso*propylidineglycerol (35 g.) by the method of Howe and Malkin[3]. The product was distilled once *in vacuo* and without *further purification was hydrolysed with dilute acetic acid to give* D-glyceryl 3-benzyl ether. After redistillation, 21·2 g. (56 per cent) of the required product was obtained, b.p. 138 to 139°/0·2 mm., $n_D^{16°} = 1·5342$, $[\alpha]_D^{20°} = +5·7°$.

D-1 : 2-*Bisdecanoylglyceryl 3-benzyl ether.* To a solution of D-glyceryl 3-benzyl ether (8·4 g.) in dry carbon tetrachloride (25 ml.) was added dry

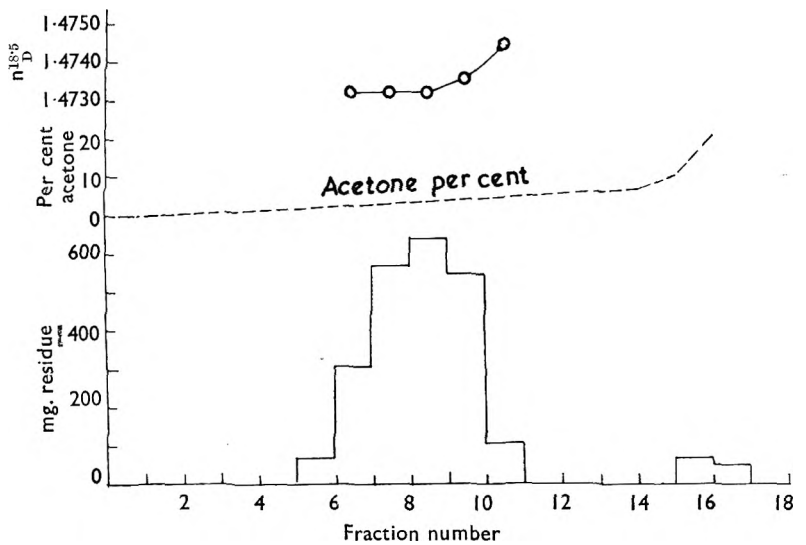

FIG. 1. Chromatographic purification of D-1 : 2-bisdecanoylglyceryl 3-benzyl ether.

quinoline (17·0 ml.). The flask containing the solution was immersed in a cooling bath and a solution of decanoyl chloride (17·4 g.) in the same solvent (25 ml.) added dropwise over a period of five minutes with gentle agitation. The reaction mixture was allowed to stand, with occasional shaking, for 20 hours, then extracted with ether (200 ml.). The filtered ethereal extract, washed successively with N hydrochloric acid, water, 1 *per cent sodium hydrogen carbonate and water*, was dried over anhydrous sodium sulphate. Evaporation of the solvent furnished 21·4 g. (96 per cent) of crude product, $n_D^{20°} = 1·4733$.

Purification was effected by chromatography on silica gel[4] (Hopkin *and Williams Ltd.). The adsorbent (20 g.) in light petroleum (b.p.* 60 to 80°) was packed in a tube of internal diameter 2·0 cm. The above 1 : 2-diglyceride 3-benzyl ether (2·4 g.) in light petroleum (b.p. 60 to 80°) (30 ml.) was placed on the column and elution carried out with graded concentrations of acetone (0 to 2·5 per cent) in light petroleum (b.p. 60 to 80°). Fractions of 30 ml. eluate were collected and the progress of the elution was followed by determining the refractive index of the residue obtained on evaporating the solvent from each fraction (Fig. 1). For analysis the central, isorefractive fractions were again chromatographed.

Found: C, 73·1; H, 10·08. $C_{30}H_{50}O_5$ requires C, 73·4; H, 10·27 per cent; $n_D^{20°}$ 1·4744; $[\alpha]_D^{22°}$ +11·3°; $d_4^{22°}$ 0·966; m.p. 6 to 7°.

A further portion of the crude product was purified by crystallisation from ether at −70°. Three crystallisations gave a product of m.p. 5 to 6°; $n_D^{20°}$ 1·4740; $[\alpha]_D^{20°}$ +10·3°.

D-1 : 2-*Bisdodecanoylglyceryl 3-benzyl ether* was similarly prepared in 78 per cent crude yield, and purified chromatographically. Found: C, 74·9; H, 10·54. $C_{34}H_{58}O_5$ requires C, 74·7; H, 10·69 per cent; $n_D^{15°}$ 1·4672; $n_D^{20°}$ 1·4747; $n_D^{24°}$ 1·4731; $[\alpha]_D^{24°}$ +9·8°; $d_4^{22°}$ 0·957.

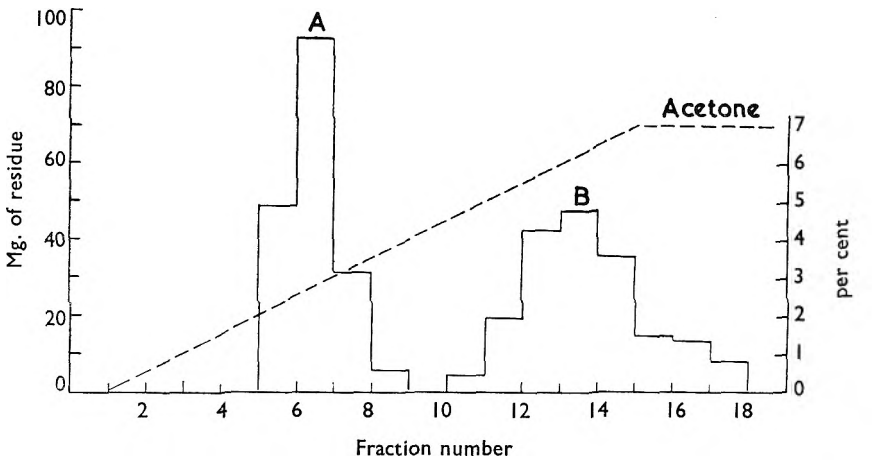

FIG. 2. Chromatographic separation of D-1 : 2-bis-decanoylglycerol from its 3-benzyl ether.

A. Diglyceride benzyl ether. B. Diglyceride.

The substance exhibited dimorphism, a phenomenon of common occurrence among the glycerides[4,5]. The modification stable at room temperature melted at 20·5 — 21·5°.

D-1 : 2-*Bisdecanoylglycerol.* D-1 : 2-Bisdecanoylglyceryl 3-benzyl ether (4·9 g.), in *n*-hexane (40 ml.) was shaken at atmospheric pressure with palladium black (Johnson Matthey and Co.)* (0·5 g.) in an atmosphere of hydrogen. When the theoretical volume of gas had been absorbed (*c.* 4½ hours) the solution was filtered, the catalyst washed with a little solvent, and the combined filtrate and washings concentrated to dryness *in vacuo.* The residue (3·6 g., 89 per cent, m.p. 28 to 29°) consisted of almost pure D-1 : 2-bisdecanoylglycerol. For analysis it was twice chromatographed on silica gel as already described (acetone concentrations of 0 to 10 per cent in light petroleum b.p. 60 to 80° being used). Found: C, 69·0; H, 10·86. $C_{23}H_{44}O_5$ requires C, 69·0; H, 11·07 per cent, m.p. 28·5 to 29°.

* Of a number of palladium black preparations tried in this laboratory this catalyst was the only one which proved to be sufficiently active and reproducible in catalytic debenzylation.

D-1 : 2-*Bisdodecanoylglycerol* was similarly prepared in 95 per cent yield; m.p. 44·5 to 45°, not raised by further chromatography. Found: *C*, 71·2; *H*, 11·39. $C_{27}H_{52}O_5$ requires *C*, 71·0; *H*, 11·46 per cent.

Chromatographic separation of D-1 : 2-*bisdecanoylglycerol from its* 3-*benzyl ether* (Fig. 2)

A sample of 0·2 g. of each of the above substances in *n*-hexane (30 ml.) was applied to a column of silica gel (8 g. column diameter 2·2 cm.) made up in the same solvent. Elution was carried out with light petroleum (b.p. 60 to 80°) in which the acetone concentration continuously rose from 0 to 7 per cent as shown: 30 ml. fractions of eluate were collected. The elution curve (Fig. 2) showed satisfactory separation of the two substances.

Summary

The synthesis of two new D-1 : 2-diacylglycerols is reported, and a chromatographic method for the purification of their 3-benzyl ethers is described.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Atidine, a New Diterpene Alkaloid from *Aconitum heterophyllum*. S. W. Pelletier. (*Chem. Ind.*, 1956, 1016.) The strongly basic alkaloid fraction from the roots of *Aconitum heterophyllum* was chromatographed on alumina and after the removal of atisine from the column with benzene, benzene-methanol (50:1) was used as the eluant and yielded heavy prisms m.p. 176 to 181°. An analytical sample ($C_{22}H_{33}NO_3$) showed m.p. 182·5 to 183·5° $[\alpha]_D^{29}$ − 47° (*c*, 1·7 in $CHCl_3$) pKa 7·53; the hydrochloride crystallised from acetone as prisms, m.p. 204 to 215°. The sample (atidine) had an infra-red spectrum (in KBr) which indicated the presence of –OH, >C=O, >C=CH_2 and C–Me. It formed an amorphous diacetate, and a crystalline diacetate hydrochloride m.p. 182 to 190°. Treatment of atidine with hydroxylamine acetate gave an amorphous oxime. Reduction of atidine with sodium borohydride in 80 per cent methanol afforded an amorphous dihydroderivative which gave an infra-red spectrum devoid of carbonyl absorption. Catalytic reduction gave an amorphous tetrahydroderivative, pKa 8·47, which could be oxidised with lead tetra-acetate to form glyoxal, demonstrating the presence of an ethanol-amine system. The data indicate a pentacyclic, tertiary base of the dihydroatisine type containing a carbonyl group in a six-membered ring. A. H. B.

ANALYTICAL

Barbituric Acid Derivatives, Microscopic and X-ray Diffraction Methods for the Identification of. W. G. Penprase and J. A. Biles. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 585.) For the identification of barbiturates when only small specimens were available, a sample was dissolved in water with the aid of sodium hydroxide and the solution filtered and acidified with dilute sulphuric acid. After the addition of sodium bicarbonate (to prevent extraction of salicylates) the solution was extracted with ether, the ether evaporated and the residue recrystallised from dilute ethanol. Crystals were obtained by recrystallisation from water or strong solution of ammonia, or by sublimation on a microscope slide, and examined under the microscope; photographs illustrating the crystalline habits of the various barbiturates are provided for comparison. The melting point was determined by the Kofler melting point apparatus. Crystallisation from various solvents, fusion and sublimation provide a basis for the rapid identification of barbituric acid derivatives, except for quinalbarbitone, which does not crystallise readily under the usual conditions. Purified samples of barbiturates were finely ground and submitted to X-ray diffraction analysis. The d-distances are reported, and may be used as further evidence for the identification of these compounds. G. B.

Digitalis, Chemical Assay of. H. Knöchel. (*Pharmazie*, 1956, **11**, 378.) The author considers digitoxin the most pharmacologically active component of digitalis. Since gitoxin and digitoxin both give the same extinction when assayed by the Soos method, whereas there is considerable difference in the

extinction when determined according to the method of Howland, it is possible by combining these two methods to determine the digitoxin, and details of the method are given. This includes a hot extraction procedure in which the extracting water is passed through a steam-heated condenser before reaching the drug, so that extraction occurs at about 80 to 90°. The method may also be used for determination of the active glycosides of *D. lanata*. G. M.

Morphine in Individual Poppy Capsules, Determination of. S. Pfeifer. (*Pharmazie*, 1956, **11**, 387.) About 0·2 g. of the finely powdered dried material is rubbed down with 1 ml. of water and, after 15 minutes, treated with 5 g. of "acid" aluminium oxide. The resulting powder is eluted with water, about 20 ml. being collected. This is treated with 1 ml. of ammonia (10 per cent NH_3) and 0·2 g. of ammonium chloride, and extracted twice with 20 ml. portions of chloroform—*iso*propyl alcohol (3 + 1), then once with 10 ml. The chloroformic solution is evaporated to dryness and the residue is taken up in 5 ml. of chloroform, then extracted with 15 ml. of 0·1N hydrochloric acid: 5 ml. of this solution is mixed with 2 ml. of 1 per cent solution of sodium nitrite and shaken for 15 seconds. After exactly 15 minutes 3 ml. of ammonia (10 per cent) is added and then 2·5 ml. of water. After a further 5 minutes the colour is measured (Filter S47). A further 5 ml. of the acid solution is heated for 5 minutes on the water bath with 4·5 ml. of water and 3 ml. of ammonia: this used as a comparison solution and compensation for narcotoline. G. M.

Reserpine, Determination of. C. R. Szalkowski and W. J. Mader. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 613.) A rapid method for the determination of reserpine depends on the formation of a greenish-yellow colour when an ethanolic solution of reserpine is treated with dilute sulphuric acid and sodium nitrite. A sample containing 0·05 to 0·1 mg. of reserpine dissolved in 5 ml. of methanol is mixed with 3 ml. of 0·5N sulphuric acid and 2 ml. of a 0·3 per cent solution of sodium nitrite. After the solution has been allowed to stand for 1 hour, the light absorption at 390 mμ is measured against a compensating blank, consisting of 5 ml. of methanol, 3 ml. of 0·5N sulphuric acid and 2 ml. of water. At the same time, experiments are carried out using known quantities of a standard preparation of reserpine, and the reserpine content of the sample is calculated by comparison. The green colour is given by reserpic acid, methyl reserpate and rescinnamine in addition to reserpine, but alstonine, rauwolscine, sarpagine, raunescine, deserpidine and yohimbine do not interfere. For the assay of reserpine tablets, citric acid solution is added to the powdered tablets. The material is extracted with chloroform, and, after washing with sodium bicarbonate solution, the chloroform solution is evaporated and the residue assayed as above. The extraction process is satisfactory in the presence of acetylsalicylic acid, amphetamine and caffeine, but hydrallazine, mannitol hexanitrate, phenacetin and theophylline inhibit the formation of the colour. In the assay of reserpine tablets, good agreement was obtained between this method and assays by phase solubility analysis and ultra-violet absorption methods. G. B.

Sodium Tetraphenylboron in the Identification and Isolation of Alkaloids. W. E. Scott, H. M. Doukas and P. S. Schaffer. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 568.) Sodium tetraphenylboron derivatives were prepared from 21 alkaloids and in all cases the reagent was at least as effective as Mayer's reagent for detecting the alkaloids. In the concentrations used in the test, Mayer's

reagent failed to detect ephedrine, solanine, tomatidine, tomatine, *N*-acetyltomatine and betaine, all of which gave a turbidity with sodium tetraphenylboron. When using sodium tetraphenylboron as a reagent for alkaloids, potassium and ammonium ions must be absent. In the recommended method, 20 mg. of alkaloid is dissolved in 15 ml. of water and 6 drops of glacial acetic acid. The solution is heated to 70° and 0·1M sodium tetraphenylboron is added until no further precipitation occurs. The alkaloids may be regenerated by adding a 5 per cent solution of sodium hydroxide to the acetone or ethanolic solution, heating on a water bath for 15 minutes, removing the solvent and extracting with chloroform. The melting points of the sodium tetraphenylboron derivatives of 21 alkaloids are reported. G. B.

BIOCHEMISTRY

BIOCHEMICAL ANALYSIS

Carbutamide, Determination of. A. Häussler. (*Arzneimitt.-Forsch.*, 1956, **6**, 393.) For determination in urine, 1 ml. of the sample (containing 10 to 100 g.) is mixed with 2 ml. of water and 2 ml. of N hydrochloric acid, and diazotised in the cold with 1 ml. of 0·1 per cent solution of sodium nitrite. After 3 minutes 1 ml. of 0·1 per cent sulphamic acid is added and, after 3 minutes, 3 ml. of a solution containing 2 per cent of potassium guaiacol sulphonate and 10 per cent of sodium carbonate. The absorption at 470 mμ is compared with a standard curve. For determination in serum, 3 ml. is treated with 3 ml. of a mixture of 9 parts of 13 per cent trichloroacetic acid and 1 part of N hydrochloric acid. After standing 10 minutes and centrifuging, 3 ml. of the clear liquid is mixed with 1 ml. of water and 1 ml. of N hydrochloric acid, and the analysis is completed as above. The standards are prepared with serum to which is added known quantities of carbutamide. For faeces, 3 g. is mixed with 3 ml. of water and 3 ml. of the trichloroacetic acid solution. After centrifuging, the liquid is filtered twice and to 3 ml. of the filtrate is treated as above. These determinations show only the non-acetylated compound: the total may be determined after hydrolysis by the usual methods. To distinguish between other compounds which also give a colour in this reaction, a chloroform extract is taken up in hydrochloric acid and the absorption curve determined. Carbutamide shows a maximum at 255 mμ. G. M.

5-Hydroxytryptamine in Brain, Identification and Assay. D. F. Bogdanski, A. Pletscher, B. B. Brodie and S. Udenfriend. (*J. Pharmacol.*, 1956, **117**, 82.) A fluorimetric method for the determination of 5-hydroxytryptamine (5-HT) in brain is described. A spectrophotofluorimeter capable of activating compounds and measuring their fluorescence over the range 250 to 650 mμ was used. The 5-HT content of rat's and rabbit's brain was identified and determined by this method and by countercurrent distribution and subsequent fluorimetric assay. Estimates of brain 5-HT content by these methods correlated well with bioassay on the clam heart. G. P.

Lysergic Acid Diethylamide, Metabolism of. J. Axelrod, R. O. Brady, B. Witkop and E. V. Evarts. (*Nature, Lond.*, 1956, **178**, 143.) The development of a sensitive method for estimation of the hallucinogenic agent, lysergic acid diethylamide (LSD), in biological materials has enabled the study of its distribution and metabolism in the body. The LSD was extracted from a salt-saturated suspension of biological material into heptane containing 2 per

cent *iso*amyl alcohol. It was then returned to dilute hydrochloric acid and after activating at 325 mμ, the fluorescence was measured at 445 mμ in a spectrophotometer. The method was sensitive to as little as 0·001 μg. of LSD. After administration of LSD to a cat the drug was recovered from all tissues, in order of decreasing concentration: bile, plasma, lung, liver, kidney, brain, cerebrospinal fluid, spleen, intestine, muscle and fat. It was calculated from the figures obtained that LSD in man exerts its action at a concentration of 0·0003 μg./g. of brain tissue. The drug was almost completely metabolized in the body, only traces appearing in the urine and faeces. Transformation was by liver microsomal enzymes, mainly to 2-oxylysergic acid. This metabolite was shown to have no effect on transmission through the lateral geniculate nucleus at a dose level ten times that at which LSD produced 80 per cent block. Similarly 300 μg. of 2-oxylysergic acid diethylamide given orally had no psychological effects in human subjects who responded to 30 μg. of LSD.

G. P.

Trichloroethylene, Trichloroacetic Acid and Trichloroethanol in Urine, Determination of. T. A. Seto and M. O. Schultze. (*Analyt. Chem.*, 1956, **28**, 1625.) Advantage is taken of the Fujiwara pyridine-alkali reaction for the determination of these trichloro compounds. This depends upon the formation of a crimson colour formed by heating traces of these compounds with pyridine in strong alkaline solution. Although the reaction is not generally considered to be very specific, methods are given for the direct determination of the above three chloro compounds in urine in the presence of each other, special attention being directed to the need for careful adjustment of alkali concentration and temperature and time of the reaction in each case. For trichloroethanol, preliminary oxidation with chromic oxide was necessary. 0·07 to 0·50 μg. of trichloroethylene could be determined and quantities of 10 to 50 μg. of the other compounds to within about ±10 per cent accuracy.

D. B. C.

PHARMACY

Aminosalicylic Acid, Decomposition of Solutions of. R. F. Rekker and W. T. Nauta. (*Pharm. Weekbl.*, 1956, **91**, 693.) The decomposition of solutions of *p*-aminosalicylic acid at various pH values was followed by observing the changes in absorption spectrum. Below pH 6·3 the decomposition results from decarboxylation, which reaches a maximum at pH 2·7. In addition, long-term storage of strongly acid solutions leads to the development of discoloration. At pH above 6·3 the stability decreases slowly, but without formation of *m*-aminophenol: the brownish discoloration observed in alkaline solution is not identical with that developing in acid solution. It is concluded that solutions of aminosalicylic acid should not be kept at a pH below 6·3, and that the storage of the acid is undesirable unless moisture can be satisfactorily excluded.

G. M.

Senegin, Stability of. P. Finholt. (*Dansk tidsskr. farm.*, Suppl. II, 1956, 92.) A previous determination of the stability of senegin (Schou and Toft Madsen, *Dansk tidsskr. farm.*, 1937, **11**, 153) was based on the haemolytic activity, and indicated that the stability was at a maximum at a pH of 3. Since this investigation did not take into account the sensitivity of the dilute solutions used to diffused daylight, which may lead to a loss of activity of 50 per cent in 1 hour, a new investigation was made. The removal of sugars from senegin was followed iodometrically, and changes in the aglycone by spectrophotometry.

The stability, as determined from the haemolytic activity, is at a maximum at pH 3 to 5, and is very low at pH 10 to 13. In acid media (pH 1) and to some extent in strongly alkaline ones, sugar is split off, while changes in the aglycone occur most rapidly when alkaline. This process also proceeds at the lowest rate at pH 3 to 5. It is the changes in the aglycone in particular which affect the haemolytic activity. G. M.

PHARMACOLOGY AND THERAPEUTICS

Alkyl Sulphonates, Tumour Growth-inhibitory. A. Haddow and W. C. J. Ross. (*Nature, London*, 1956, **177**, 995.) 2-Chloroethyl methanesulphonate inhibited growth in the transplanted Walker rat carcinoma, both in its solid and ascites form. The drug was effective both by a single intraperitoneal injection of 100 mg./kg. in arachis oil or by daily feeding of an aqueous solution (10 mg./rat/day) for the duration of the experiment (13 days). With these doses there appeared to be no significant depression of the bone marrow. The compound had a mutagenic action on *Drosophila melanogaster*, but the mode of action appears to differ from that of other biological alkylating agents such as the sulphur and nitrogen mustards, dimethylsulphonates, diepoxides and polyethyleneimines. The fluorine analogue had similar cytotoxic activity. The hydrogen analogue, ethyl methylsulphonate was noteworthy in that daily oral administration was much more effective than daily injections of the same aqueous solution (20 mg./rat/day). G. P.

Azacyclonol Hydrochloride, An Ataractive Agent, Pharmacological Activity of. B. B. Brown, D. L. Braun and R. G. Feldman. (*J. Pharmacol.*, 1956, **118**, 153.) Azacyclonol hydrochloride (Frenquel) is the 4-isomer of pipradol. In mice it has a low toxicity. Small doses cause a mild depression characterised by decreased spontaneous activity. Lethal doses cause convulsions. Hexobarbitone sleeping time is increased in mice and hypnosis can be re-induced by azacyclonol after the mice are fully awake. In rats low doses produce a peculiar paralysis of the hind legs, depressed spontaneous activity and increased responsiveness to auditory stimuli. Lethal doses cause tremors and death without convulsions. In dogs and cats doses of 25 and 50 mg./kg. cause increased irritability, tremors and inability to rest. Doses of 100 mg./kg. cause the dogs to be in constant motion. In monkeys single oral doses as high as 160 mg./kg. cause no observable changes in behaviour and no signs of toxicity. Azacyclonol antagonises increased coordination activity induced in mice by pipradol, amphetamine, morphine and cocaine and it antagonises morphine induced stimulation in cats. In man intravenous doses promptly relieve LSD-induced psychoses. G. F. S.

Bemegride and Amiphenazole in Allyl*iso*propylbarbituric Acid Poisoning. J. Pedersen. (*Lancet*, 1956, **2**, 965.) In twenty-two cases of poisoning with a barbituric acid derivative, allyl*iso*propylbarbituric acid, bemegride with and without amiphenazole had a good arousing effect. Patients usually reacted within thirty minutes to strong stimuli, reflexes reappeared and in many cases became hyperactive. However, in most cases patients lapsed into coma again after the first injection and a further dose had to be given. Compared with seventy-four control cases bemegride did not curtail the period of unconsciousness or restore consciousness at higher blood levels of barbituric acid.

G. F. S.

Bemegride and Amiphenazole in Respiratory Paresis. C. Clemmesen. (*Lancet*, 1956, **2**, 966.) Treatment of seventy cases of severe barbiturate poisoning with bemegride and amiphenazole are reported. In seven of the cases there was respiratory paralysis with total apnoea. In each of these cases the paresis was abolished and respiration permanently restored to normal shortly after the administration of bemegride and amiphenazole. If only because of their effect in respiratory paralysis which has hitherto been regarded as an extremely serious complication, bemegride and amiphenazole are valuable adjuvants in the treatment of acute barbituric acid poisoning. G. F. S.

Bemegride, Delirious Psychosis and Convulsions due to. J. Kjaer-Larsen. (*Lancet*, 1956, **2**, 967.) During the treatment of fifty acute cases of barbituric acid poisoning with bemegride, return of consciousness was followed by psychosis in fifteen cases. Their onset was from the first to the fourth day after waking. Visual experiences predominated in the form of "black specks", smoke, or fire and in several cases coloured patterns. Auditory hallucinations were less outstanding. There was an impairment of consciousness, deficient orientation and an inability to sleep. The psychotic states persisted for two to six days. The frequency of psychoses was higher after large doses of bemegride. The psychoses resembled intoxication due to mescaline and lysergic acid. Nine of the cases of psychoses occurred among twelve barbiturate addicts, while among thirty-eight non-addicts there were only six cases of psychosis. These psychoses are apparently exogenous reactions provoked by bemegride in barbituric acid addicts. They resemble spontaneous withdrawal psychoses, but there are certain differences. During treatment with bemegride sixteen of the fifty patients developed convulsions, four had from one to eight severe, typical grand-mal seizures and twelve had petit mal. G. F. S.

Bemegride in Barbituric Acid Poisoning. A. Louw and L. M. Sonne. (*Lancet*, 1956, **2**, 961.) In severe cases of barbituric-acid poisoning bemegride (Megimide), stimulated respiration and restored reflex activity, reduced coma and helped to bring about a safe state. It did not shorten the period of coma, hasten elimination of barbituric acid, or cause patients to recover consciousness at a higher blood-level of barbituric acid than normal. Bemegride induced electroencephalographic changes before any clinical effect was seen. The results did not support the hypothesis that bemegride is a true antagonist of barbituric acid, like nalorphine is in morphine poisoning. They suggest that bemegride counteracts barbituric-acid by a central stimulant action. G. F. S.

Brom-lysergic Acid Diethylamide, a Highly Potent 5-Hydroxytryptamine Antagonist. L. Sollero, I. H. Page and G. C. Salmoiraghi. (*J. Pharmacol.*, 1956, **117**, 10.) 2-Brom-(+)-lysergic acid diethylamide (BOL) more effectively antagonised the action of 5-hydroxytryptamine (5-HT) on the isolated uteri of rats and guinea pigs than did (+)-lysergic acid diethylamide (LSD). LSD did not alter the action of 5-HT on guinea pig's ileum and rabbit's duodenum, whereas BOL was a potent antagonist of the spasmogen in these tissues. The actions of the antagonists were relatively specific in that the actions of acetylcholine, histamine, adrenaline and noradrenaline were not affected. These results suggest that the postulation of two types of tryptamine receptors, one type (rat's uterus and rabbit's ear) where LSD is a highly active antagonist of 5-HT and another where LSD is without effect (guinea pig's ileum), is unnecessary; BOL effectively antagonises the action of 5-HT at both receptor sites. G. P.

Bufotenine, Intravenous Injection in Man. H. D. Fabing and J. R. Hawkins. (*Science*, 1956, **123**, 886.) Bufotenine, in doses up to 16 mg., was injected intravenously into four normal healthy male subjects, the injections being made slowly over a three-minute period. Subjective effects similar to those of the hallucinogenic drugs LSD and mescaline were experienced, but the onset of activity was more rapid and the duration of action shorter with the bufotenine. The presence of nystagmus and mydriasis after the administration of bufotenine indicates that part of its action is located in the brainstem. Cardiovascular effects were slight; blood pressure changes were never more than 15 mm. Hg nor pulse rate variation more than 12 beats per minute. The subjects became cyanosed, presumably by a bronchoconstrictor action akin to that of 5-hydroxytryptamine (bufotenine is the *NN*-dimethyl derivative of this amine). 5-Hydroxytryptamine does not, however, induce model psychoses of the type seen with bufotenine. G. P.

Carbutamide, Effect of Different Doses of. G. Mohnike and U. Hagemann. (*Arzneimitt.-Forsch.*, 1956, **6**, 389.) High doses (1 g. or 0·75 g./kg.) of carbutamide administered intravenously to rabbits produce hyperglycaemia, which may be observed 30 minutes after administration. Hyperglycaemic doses are highly toxic, many animals dying with convulsions which were not the result of glycopenia. The increase in blood sugar must be due to mobilisation of liver glycogen, and a decrease in the latter was observed histochemically. Together with the convulsions there were observed brain changes. The toxicity was shown especially by necrotic processes in the tubules of the kidney. The most favourable hypoglycaemic action was observed with small doses (0·25 g./kg. i.v.), and only with these was there any positive relation between dose and blood sugar level. The experimental results do not indicate an insulin-like action, stimulation of the β cells, or inhibition of a facultative antagonist of islet cells. G. M.

Carbutamide, Effect of, on Blood Sugar Level. G. Mohnike. (*Arzneimitt.-Forsch.*, 1956, **6**, 388.) When administered at a level of 100 mg./kg. to rabbits with meta-alloxan diabetes, carbutamide caused a distinct drop in blood sugar when the sulphonamide content of the blood exceeded 10 mg. per cent. If this dose was given shortly before the administration of insulin, there was a greater drop in blood sugar level than when the insulin alone was given. This effect was marked in the period when the insulin action was especially distinct and when the sulphonamide level of the blood showed its highest value. These observations suggest that carbutamide and related substances increase the effect of the nsulin administered on blood sugar, and it may be assumed that the same applies to the insulin of the body. The actual mechanism is not clear. G. M.

Chlorhexidine in Urology. H. Beeuwkes and H. R. de Vries. (*Lancet*, 1956, **2**, 913.) Chlorhexidine (Hibitane) is of great value as a urological disinfectant. Its antibacterial spectrum covers all the organisms likely to be encountered, its oral and local toxicity is extremely low and in the concentrations used it is non irritant to the mucosa. Chlorhexidine is used in the following way for cystoscopy. The rinsing fluid for the irrigator is a 1 : 10,000 solution in tap water. For bladder irrigation and as the cystoscopy medium the concentration is 1 : 5,000 and for disinfecting the external genitalia 1 : 1,000. The top of the irrigator tube is stored in a 1 per cent solution. The hands of the staff are rubbed with a cream containing 0·5 per cent chlorhexidine hydrochloride. Before the introduction of chlorhexidrine 70 per cent of the urine samples became infected (56 per cent *Ps. pyocyanea*, 10 per cent proteus and 4 per cent *Staph. albus*). After the introduction of chlorhexidine only 8 per cent became infected, all with *Staph. albus.* G. F. S.

Contaminants in Stored Blood. M. G. McEntegart. (*Lancet*, 1956, **2**, 909.) Two fatal transfusion reactions are reported because blood was contaminated with an organism (resembling but not identical with *Klebsiella cloacae*) which grew at 4°. Both bottles of blood were in the third week of storage. The source of the infection was not found, but when the blood-taking equipment was checked bacteria were found in the fluid used to preserve the plastic caps. These did not grow at 4°. Thus the caps are a possible source of contamination and must be sterilised by autoclaving before use. A study of the growth rate of the organism showed that infected blood is unlikely to be dangerous until the third week of storage. The risk of severe reactions of the "infection" type could be very greatly reduced if, before any blood in its third week of storage is issued, a stained film is examined to exclude the presence of bacteria. In cases of severe reactions stained films of the blood should be examined at once. If the blood is infected the patient should be treated at once. Treatment should correct the acute peripheral circulatory failure which develops and the administration of noradrenaline and plasma to maintain the blood pressure should be considered. In the two fatal cases reported the infecting organism was unable to grow at body temperature. It is therefore unlikely that multiplication of bacteria in the body plays any important part in these reactions. G. F. S.

Cortisone Acetate in Status Asthmaticus, Controlled Trial of Effects of. Report to the Medical Research Council by the Subcommittee on Clinical Trials in Asthma. (*Lancet*, 1956, **2**, 803.) A comparison was made at 13 centres of the effectiveness of cortisone with that of antispasmodic drugs in the treatment of status asthmaticus. The trial was made on adult patients admitted to hospital in status asthmaticus who had suffered at least one previous severe attack of asthma and had not previously received cortisone therapy. For the first 24 hours they were given the treatment normally adopted by the physician in charge. This might include adrenaline subcutaneously, aminophylline intravenously, isoprenaline inhalation, oxygen, antibiotics or sedatives. During the first 12 hours endeavour was made to establish the dose of antispasmodic required and this dose was continued during the second 12 hours. Patients still in status asthmaticus at the end of 24 hours were admitted to the trial. In one group the treatment was continued and cortisone acetate was given in addition; in the second group also the treatment was continued but placebo tablets were given in addition. All the patients in the treated group received the same dosage of cortisone. Starting with 350 mg. in divided doses on the first day and ending with 25 mg. in two doses on the 9th day, the total amount administered was 1·25 g. Clinical assessment of each patient was made twice daily. By day 4, 10/15 patients in the treated group no longer had disabling bronchial obstruction whereas only 4/17 in the control group were relieved. This difference was maintained to the end of the 14-day treatment period. 6 patients whose condition caused concern were withdrawn from the trial. 5 who were successfully treated with cortisone or corticotrophin were found to belong to the control group; the sixth patient, who was receiving cortisone, had mitral stenosis and developed signs of cardiac failure on day 6. One patient in the cortisone group died; he had considerable bronchial infection and bronchopneumonia. The smallness of the number of patients admitted to the trial was due to the success of the standard treatment during the first 24 hours and it seems desirable to use antispasmodic drugs except for extremely exhausted and dehydrated patients. Observation of the patients for 3 months after the trial showed that they reverted to their usual asthmatic condition; status asthmaticus recurred in 9/11 in the cortisone group and 7/14 in the control group. H. T. B.

Diphenhydramine, 2-Methyl Derivative of (Mephenamine), Pharmacology of. U. G. Bijlsma, A. F. Harms, A. B. H. Funcke, H. M. Tersteege and W. T. Nauta. (*Arch. int. Pharmacodyn.*, 1956, **106**, 332.) In contrast to the 4-methyl derivative of diphenhydramine, which has greater antihistamine activity than the parent compound, the 2-methyl derivative has much weaker antihistamine effects, but increased anti-acetylcholine activity. The 2-methyl derivative (mephenamine, Disipal, BS 5930) had low acute and semi-chronic toxicity in rats and mice. Clonic spasms were obtained after subcutaneous injection of 40 mg./kg. mephenamine into a normal cat; this action was absent in the thalamic cat. An effect on the diencephalon was observed in thalamic cats (in contrast to decerebrate or spinal cats) after injection of mephenamine into the vertebral artery; an inhibition of the ipsilateral flexor reflex similar to that seen with scopolamine was obtained. The gait of thalamic cats was also altered by both mephenamine and scopolamine, scopolamine being 20 times the more active. A vasodilator action on perfused frog blood vessels and coronary vessels of the isolated mammalian heart was demonstrated. The force of contraction of isolated frog or mammalian hearts was reduced. Atropine-like activity was 1/25th that of atropine on the guinea pig's ileum, 1/350th in inhibiting salivary secretion in rabbits and 1/250th in mydriatic activity in mice. A ganglion-blocking action was not observed. In the nicotine tremor test in rabbits, mephenamine resembled atropine and scopolamine. Surface anaesthesia with the drug was several times greater than that of procaine and conduction anaesthesia about half that of procaine. Mephenamine had no protective effect in an anti-emetic test in pigeons. The drug prolonged barbiturate sleeping-time, but was less potent than diphenhydramine. Clinical reports of the use of the drug in Parkinsonism have been favourable. G. P.

Diphenhydramine, Enhancement of the Central Nervous System Effects of Strychnine and Pentobarbitone by. J. F. Sherman. (*Science*, 1956, **123**, 1170.) Diphenhydramine given subcutaneously to mice in a dose of 20 mg./kg. increased both the sleeping time with pentobarbitone sodium and the convulsive activity of strychnine. Such a dose of diphenhydramine had no gross effects of its own. Two modes of action were considered. The first entails an inhibition of the metabolic transformation of pentobarbitone and strychnine, similar to an action already described for β-diethylaminoethyl diphenylpropyl acetate hydrochloride (SKF 525-A), to which diphenhydramine is related structurally. An alternative to this is a more direct effect on elements of the central nervous system, the result of which would be an alteration in the levels of neuronal activity; this would then affect the degree of response to either strychnine or the barbiturate. G. P.

5-Hydroxytryptamine-releasing Activity Limited to Rauwolfia Alkaloids with Tranquillizing Action. B. B. Brodie, P. A. Shore and A. Pletscher. (*Science*, 1956, **123**, 992.) Of a number of drugs exerting a tranquillising action, only rauwolfia alkaloids reduced brain 5-hydroxytryptamine (5-HT) levels. Rabbits received the drugs intravenously and were killed four hours later. Their brains were removed as rapidly as possible and 5-HT determined fluorimetrically. In the rauwolfia alkaloid series, only reserpine, rescinnamine and deserpidine induced sedation and caused a significant alteration in brain 5-HT levels. A variety of hypnotics, narcotic analgesics and central nervous stimulants had no effect on brain 5-HT. Chlorpromazine, which resembles reserpine in its potentiation of barbiturate sleeping times, also failed to release 5-HT from the

brain, indicating a more direct action on receptor sites in the brain. Azacyclonol, a new drug currently under investigation in the treatment of psychiatric disorders, also failed to release 5-HT. Lysergic acid diethylamide, which antagonises the central action of reserpine, had no effect on the release of 5-HT from the brain tissues; this supports the idea that LSD inhibits the action of reserpine by blocking the released 5-HT. G. P.

Isoniazid in the Treatment of Lupus Vulgaris. B. Russell and N. A. Thorne. (*Lancet*, 1956, **2**, 808.) 111 patients, ranging in age from 8 to 82 years, suffering from lupus vulgaris for periods of from 6 to 74 years were treated with isoniazid. 8 were treated by mouth and by injection, 1 by injection only and the remaining 108 received the drug by mouth only. 104 patients completed treatment. In nearly all cases the daily dose was either 300 mg. or 400 mg. in divided doses of 100 mg. 6 to 12 months treatment was necessary to clear the lesions, and isoniazid was continued for 14 weeks after apparent clinical clearance. There was complete clinical clearance in 99 patients in an average of 29 weeks, the extremes being 8 weeks and 79 weeks. There were 3 deaths, 2 of them from carcinoma superimposed on long-standing neglected lupus. The skin of the affected areas becomes either normal in colour or slightly pigmented with a reticular pattern and slight atrophy. No serious side-effects were noted. 12 patients had mild side-effects at the commencement of treatment; they included nausea, dyspepsia, indigestion, giddiness and, in one case, anosmia. Treatment had to be abandoned after 23 weeks in the case of a pregnant woman because of nausea; she gave birth to a stillborn anencephalic monster at 39 weeks. The only evidence of the development of resistant strains of tubercle bacilli was in 4 cases who improved at first but failed to clear after prolonged treatment. Relapses have occurred in 11 cases between 8 and 130 weeks after completing treatment. In 4 of these a further course of isoniazid is effecting improvement; in the remainder a cure has been achieved by other treatment. Isoniazid was also effective in a case of tuberculosis verrucosa cutis, in 3 cases of scrofuloderma and in 2 cases of erythema induratum. It was ineffective in a case of lupus miliaris disseminatus faciei. H. T. B.

Mecamylamine, Hypotensive Action of. A. E. Doyle, E. A. Murphy and G. H. Neilson. (*Brit. med. J.*, 1956, **2**, 1209.) Mecamylamine (Inversine, 3-methylamino*iso*camphane) was given to 25 hypertensive patients for 6 to 10 months and to a further 15 patients for 3 to 6 months, the effects being compared with those obtained with subcutaneous or oral pentolinium. The fall in blood pressure was more gradual and more prolonged than that produced by pentolinium whether given by injection or by mouth. A dose sufficient to reduce the systolic blood pressure to 140 to 120 mm. Hg often gave a substantial reduction even 12 hours after the dose was given. Minor changes in dosage were occasionally necessary but no tolerance was observed during up to 10 months' treatment. The action is not usually exerted until one hour after administration and reaches a maximum in 3 to 5 hours; even after intravenous administration the action may not occur for ½ to 1 hour. The effective oral dose of mecamylamine is 1 to 2 times the subcutaneous dose of pentolinium required to produce the same effect. Side effects are mainly those due to parasympathetic blockade with gastrointestinal symptoms predominating; they tend to be less severe but more protracted than those due to pentolinium although constipation is more severe. Persistent nausea occurred in 6 patients and in 2 of these persistant vomiting made it necessary to change to pentolinium. Control of the blood pressure was good in 24/45 patients and side effects were mild, while

in 12/45 good control of blood pressure was accompanied by severe side effects. In 9/45 the side effects made it difficult to establish good control and in 5 of these patients treatment with mecamylamine had to be abandoned. Because of the prolonged effect caution is necessary when there is doubt about the safety of reducing blood pressure, as in coronary ischaemia, cerebral vascular disease and impaired renal function, and parenteral hexamethonium is preferable until the reduction is known to be safe. In general an initial dose of 2·5 mg. twice daily is suggested, increments of 2·5 mg. being added at 3-day intervals until an effective hypotensive dose is obtained; the final dose is usually between 10 mg. and 25 mg. twice daily. Prophylactic routine administration of laxatives is an advantage. H. T. B.

Meprobamate, Controlled Trial of. E. D. West and A. Fernandes da Fonseca. (*Brit. med. J.*, 1956, **2**, 1206.) The value of meprobamate was investigated in a straight trial in psychiatric out-patients and in two other trials in which the compound was compared with an inert tablet and with sodium amylobarbitone respectively. In the straight trial, 151 patients were given doses ranging from 400 mg. twice daily to 800 mg. three times daily with a fourth dose at night. Treatment was continued for up to 4½ months. Meprobamate was noticeably most effective in a group of 62 patients classified as anxious and tense, of whom 36 (58 per cent) improved. Only about 25 per cent of patients with tension headache were relieved. Somatic manifestations of anxiety such as tremor, sweating and tachycardia, and cases of muscle spasm such as spasmodic torticollis and writer's cramp, showed very little change. Patients with difficulty in getting off to sleep and those with undue irritability were particularly helped. Severe anxiety states did not respond so well as mild ones and often side effects such as drowsiness occurred. Some benefit occurred in other groups, for example in reactive depression. No marked effect was noted in patients with endogenous depression or when the compound was used as a premedication for electroconvulsion therapy. Minor improvement occurred in 3 patients with globus hystericus. A slight improvement occurred in some patients with obsessive-compulsive neurosis but motor forms such as compulsive handwashing were unaffected. There were no serious side effects but tiredness occurred and 5 patients had transient urticarial rashes. In the comparison with the inert tablets 13/26 patients were given the placebo and half the meprobamate, the tablets being identical in appearance. At each attendance the alternative substances was substituted. The results showed a statistically significant superiority of meprobamate. In a similar comparison with sodium amylobarbitone, mainly in anxiety and tension states, no marked differences in effectiveness were found but meprobamate often seemed more useful where there was marked irritability. H. T. B.

Meprobamate, Toxic Reactions to. H. T. Friedman and W. L. Marmelzat. (*J. Amer. med. Ass.*, 1956, **162**, 628.) Detailed reports are given of 7 cases of severe toxic reactions following administration of meprobamate in therapeutic doses. Five of these were allergic in character, consisting of cutaneous reactions, chiefly purpuric and accompanied by intense itching; in one case one 400-mg. tablet was sufficient to produce the skin lesions without previous exposure. In another case diplopia accompanied by nausea developed following administration of a dose of 800 mg., and in yet another severe diarrhoea, with cramps, gas and watery stools resulted from two doses of 400 mg. In addition to these 7 cases, 3 cases of paradoxical reaction to meprobamate of extreme excitement rather than tranquillisation are recorded. S. L. W.

Morphine Antagonists: Distribution and Excretion of Morphine ^{14}C in the Presence of Nalorphine and 5-Aminoacridine. L. B. Achor and E. M. K. Geiling. (*J. Pharmacol.*, 1956, **117**, 16.) Previous observations have indicated strongly that antagonism of morphine by nalorphine involves changes in the tissue concentrations of morphine. These concentrations were investigated in male white mice using ^{14}C-labelled morphine, 10 mg., before and after the administration of nalorphine 25 mg./kg. or 5-aminoacridine 2·5 mg./kg. (also a morphine-antagonist). The antagonists affected tissue morphine concentration to some degree in all tissues examined, but changes in the liver, kidney, small intestine and urine appeared to be the most significant. Although the control and nalorphine-treated series presented qualitatively similar morphine distribution patterns, the liver concentration in the nalorphine-treated mice decreased more rapidly with time, indicating (*a*) a diversion of significantly larger amounts of morphine from the liver to the urinary system in the unconjugated form and (*b*) facilitation of more rapid conjugation of morphine by the liver. Bladder urine accumulated radioactivity at the same rate in both series, but in the nalorphine-treated mice the urine voided at the end of two hours had approximately the same radioactive content as that of the controls. 5-Aminoacridine was even more active in reversing this antidiuretic action of morphine; other differences in morphine distribution patterns after 5-aminoacridine indicate a mode of action differing from that of nalorphine. The distribution data suggest that both antagonists alter the free/bound morphine ratio in the urine.

G. P.

Neodymium 3-Sulpho*iso*nicotinate and Blood Coagulation. R. B. Hunter and W. Walker. (*Brit. med. J.*, 1956, **2**, 1214.) Although salts of lanthanum, cerium, praseodymium and neodymium are known to prolong the clotting time of blood, most of them are toxic. Neodymium 3-sulpho*iso*nicotinate is a water-soluble salt which prolongs the clotting time in animals without producing toxic effects and its mode of action was investigated. Intravenous administration to dogs, rabbits or mice in a dose of 50 mg./kg. greatly prolongs the clotting time of whole blood. The effect is not reversed by intravenous injection of protamine nor by addition of thrombin, showing that there is no interference with the thrombin-fibrinogen reaction. The one-stage prothrombin time (Quick test) was only moderately prolonged and the two-stage test was normal. The most striking abnormality was gross impairment of the generation of intrinsic blood prothrombin, both the plasma factor and the serum factor concerned being affected. In man the relatively much smaller dose of 250 to 375 mg. intravenously did not prolong the clotting time of whole blood, showing a normal thrombin-fibrinogen reaction. The clotting time of oxalated plasma in the Quick test showed a variable prolongation of 0 to 14 seconds which was corrected by adding aged normal serum, indicating a deficiency of factor VII. Two-stage prothrombin measurements were normal. The impairment of intrinsic blood thromboplastin generation was again striking but the abnormality was found in the patient's serum only. On giving a correspondingly small dose to rabbits, the effects were found to be identical with those in man. The effects in man were not prevented by oral premedication with 50 mg. of vitamin K_1. The effect on thromboplastin generation was investigated in a series of tests using normal serum, Christmas disease serum, and serum from patients treated with phenindione or neodymium. Thromboplastin formation was normal in mixtures of neodymium serum with Christmas serum, or with phenindione serum, but only if the proportions of neodymium serum were respectively below 10 and 2·5 per cent. The addition of normal serum to neodymium serum

was more effective than either of the other additions. Addition of equal parts of phenindione and Christmas sera to neodymium serum was as effective as an addition of normal serum. It appears therefore that neodymium serum contains an inhibitor, weakened by dilution, both of Christmas factor and of factor X. Addition of neodymium to normal serum *in vitro* had this inhibiting effect so that it does not need to be present during clotting to produce its action on those factors. It is possible that neodymium acts by displacing calcium from combination with one or more proteins involved in the reaction but a more probable explanation is its selective combination with plasma proteins.

H. T. B.

New Cholinesterase Inhibitor, Studies on. R. G. Herrmann and R. H. Tust. (*J. Pharmacol.*, 1956, **117**, 75.) The anticholinesterase activity of 4:4′-oxy-bis-(phenacyl-pyridinium) chloride was compared with that of neostigmine *in vitro* on cholinesterase preparations from rat's brain and serum and *in vivo* on brain, serum and salivary gland cholinesterase of the rat. *In vitro* the new drug was one tenth as potent as neostigmine in inhibiting rat brain cholinesterase. Results *in vivo* were similar, but the enzyme-inhibitor complex formed by the new inhibitor was much more readily dissociated than that of neostigmine. In the dog the two anticholinesterases were similar pharmacologically in respect of changes in gut motility, blood pressure and respiration, toxic symptoms and duration of action. Again neostigmine was ten times the more potent. On the rat gastrocnemius muscle preparation the new drug was less effective as an antagonist of tubocurarine, than was neostigmine, but its onset of activity was more rapid. In high dosage a neuromuscular blocking action was seen, which was not seen with neostigmine under similar experimental conditions. G. P.

Phthalylglutamic Imide, a New Sedative. W. Kunz, H. Keller and H. Mückter. (*Arzneimitt.-Forsch.*, 1956, **6**, 426.) *N*-Phthalylimidoglutaric imide (Contergan) has pharmacological properties which suggest that it would be a valuable sedative. The compound melts at 271° (uncorr.), and is only slightly soluble in the usual solvents, but readily soluble in dioxan, dimethylformamide or pyridine. The sedative effect was determined by recording the motility of mice in special cages, and a comparison was made with other sedatives including barbiturates and pentynol. Parenteral and peroral administration were employed, the compound being suspended in water. The onset of the sedative effect was rapid and of long duration, comparable with long term hypnotics. There was no initial excitement or disturbance of co-ordination. Owing to the low solubility it was not possible to determine the toxicity; no side effects were observed with mice, rats, guinea pigs or rabbits. Clinical trials are reported below. G. M.

Phthalylglutamic Imide, Clinical Experience with. H. Jung. (*Arzneimitt.-Forsch.*, 1956, **6**, 430.) This new sedative has been tried on more than 300 patients. It may be given to patients with serious liver damage; there was no effect on the blood picture and blood sugar. Side effects resulted from over dosage (drowsiness, vertigo, tremor, constipation) but not with normal doses. Good results were obtained in all types of vegetative dystonia, and in less marked conditions of hyperthyreosis and thyreotoxicosis; and thyreostatic drugs could be discontinued or reduced. Effects in nervous gastric troubles and labile hypertension and bronchial asthma were favourable. G. M.

Pilocereine, a Cactus Alkaloid. C. E. Powell and K. K. Chen. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 559.) Pilocereine, an alkaloid of molecular formula $C_{30}H_{42}O_4N_2$, isolated from *Lophocereus schottii* and *Pachycereus marginatus* was administered intravenously to a series of anaesthetised animals. Pilocereine caused a decrease in the blood pressure of dogs and roosters, and an increase in the case of rats. Small doses administered to cats produced a decrease, followed by an increase in blood pressure. Pilocereine was shown to relieve pituitary-induced spasm in the isolated guinea pig uterus, and adrenaline-induced spasm in the rabbit uterus. Similarly, it relieved methacholine- or histamine-stimulated spasm of the rabbit or guinea pig small intestine. In canaries infected with *Plasmodium relictum*, pilocereine showed an antimalarial action approximately equal to that of quinine. It was shown to be toxic to mice when given intravenously, the LD50 being approximately 52 mg./kg.

G. B.

***n*-Propyl Nitrate, Pharmacological Effects of.** E. F. Murtha, D. E. Stabile and J. H. Wills. (*J. Pharmacol.*, 1956, **118**, 77.) A study of *n*-propyl nitrate, a by-product of the chemical industry, has shown it to be not very toxic, the LD50 in rabbits being 225 mg./kg. Doses of 200 mg./kg. i.v. were fatal to anaesthetised cats or dogs. In anaesthetised dogs intravenous doses of 50 to 250 mg./kg. caused immediate hypotension and arrest of gut activity. The higher doses caused a precipitous fall in blood pressure and respiratory paralysis. Non-lethal doses caused a transient apnoea followed by a persistent hypernoea accompanied by cyanosis. In cats, doses of 100 to 250 mg./kg. i.v. caused death in one minute, but the methaemoglobin values showed only 0 to 4 per cent of the total haemoglobin oxidised to methaemoglobin. In dogs, ECG changes were observed after 30 mg./kg.—including bradycardia, arrhythmia, inverted or widened QRS complex and inverted T-wave and terminal ventricular fibrillation. Contractile studies in the dog heart showed that the contractile force fell to 10 to 15 per cent of normal with a simultaneous fall in systolic pressure. The results suggest that a direct cardio-toxic effect is the cause of the hypotension which may be lethal. Respiratory depression and a direct action on vascular smooth muscle may also play a part. G. F. S.

Rauwolscine, Pharmacological Action of. J. D. Kohli and N. N. De. (*Nature, London*, 1956, **177**, 1182.) Like yohimbine, rauwolscine (α-yohimbine) has local anaesthetic and adrenaline-blocking activity; it lowers the convulsant threshold level to leptazol and has aphrodisiac activity. In guinea pigs a 2 per cent solution of rauwolscine causes complete surface anaesthesia of the cornea which starts 5 to 7 minutes after installation of the drug into the eye and lasts for up to 15 to 20 minutes. By the guinea pig weal method a 2 per cent solution induced local anaesthesia for up to 2 hours, but there was local tissue damage. 10 to 20 mg./kg. injected intraperitoneally into guinea pigs gave signs of psychic excitement and erection in the males. 25 mg./kg. given intraperitoneally to rats lowered the convulsant threshold for leptazol. Adrenaline-blocking activity of rauwolscine was comparable with that of tolazoline on the isolated seminal vesicle of the guinea pig. 12 to 15 mg./kg. injected intravenously in rabbits induced sudden clonic convulsions lasting 2 to 3 minutes, accompanied by increased respiratory rate and followed by sexual excitement; intraperitoneal injection of 20 mg./kg., on the other hand, had no convulsant action, but the other effects appeared in 5 to 10 minutes. G. P.

Reserpine, Antagonists of the Action of, on Smooth Muscle. C. N. Gillis and J. J. Lewis. (*Nature, Lond.*, 1956, **178**, 859.) Since reserpine antagonises non-selectively the contractions of the guinea pig ileum caused by acetylcholine, histamine, serotonin or barium, it is possible that it is affecting the underlying mechanisms connected with the production and utilisation of the energy necessary for the contraction. These mechanisms probably involve the breakdown and resynthesis of carbohydrate through the glycolytic and tricarboxylic acid cycles. Thus an investigation was carried out on the influence of adding known intermediates of carbohydrate metabolism to the isolated ileum of the guinea pig immediately before the addition of the reserpine. The ileum was stimulated by either acetylcholine, histamine, serotonin or barium. All metabolites were tested over a wide dose range. Glucose-1-phosphate, fructose-1 : 6-diphosphate, fructose-6-phosphate, pyruvate, fumarate, succinate and adenosine triphosphate were ineffective as reserpine antagonists. Citrate, *cis*-aconitate, α-keto-glutarate and maleic acid were highly active. Malate and oxalo-acetate had intermediate activity. Removal of calcium from the bath by disodium versenate did not cause antagonism to reserpine. The possibility of release of histamine or acetylcholine by the metabolites was excluded. Since the most active reserpine antagonists are intermediates of the tricarboxylic acid cycle, it seems possible that this is a site of action of the alkaloid. M. M.

Staphylococcal Pneumonia in Adults. W. Hausmann and A. J. Karlish. (*Brit. med. J.*, 1956, **2**, 845.) Eighteen cases of staphylococcal pneumonia in adults are reported to have occurred in a series of 122 consecutive cases of pneumonia admitted to one medical unit in 1952–4. There was no epidemic influenza in the area during that period. The outstanding feature in these cases was the severity of the clinical course and the number of suppurative complications. All of the 18 cases recovered, but only 6 responded to penicillin. Seven responded to oxytetracycline or chlortetracycline, 2 to chloramphenicol, and 2 others to streptomycin. In one man, erythromycin effected a dramatic improvement. There is good evidence that the incidence of staphylococcal pneumonia is growing. A review of the literature in the last 5 years suggests that these cases now constitute from 3 to 10 per cent of all cases of pneumonia admitted to hospital. The average age of the patients is over 50 years and a history of previous chest disease is obtained in at least 40 per cent of cases. The authors conclude that in future staphylococcal infections are likely to present a major problem in the treatment of pneumonia. S. L. W.

***Staphylococcus aureus*, Transmission of.** R. Hare and C. G. A. Thomas. (*Brit. med. J.*, 1956, **2**, 840.) Experiments carried out with nasal carriers of *Staphylococcus aureus* suggest that this organism is not transported from person to person by droplets or droplet nuclei but by an indirect route. The first step is the transport of the staphylococci by hand, handkerchiefs or any object coming into contact with the noses to the skin, clothing, bedding, and other objects within the immediate vicinity of the carrier. The second step is the release of the organism into the atmosphere, which may result from friction and dislodgment of dried particles from the skin or hair, from the spattering of water droplets while washing, or from shaking of the clothing during activity. The third step involves the transport of these infected particles by air currents to other individuals. In this way the organisms may reach the anterior nares of normal persons to produce the carrier state in them, or they may reach highly susceptible tissues such as the conjunctiva of the newborn infant, or open wounds in operating theatres, to produce in due course post-operative infection. There

is evidence that some carriers can contaminate the atmosphere in their neighbourhood with much larger numbers of *Staph. aureus* than the majority of carriers. Such persons wearing a sterile gown over their ordinary underclothing may cause potentially dangerous degrees of air contamination during activity; even when wearing a complete set of sterile operating clothes large numbers of *Staph. aureus* may still be detectable in the surrounding atmosphere. Such carriers are probably more dangerous in the hospital environment than members of the staff suffering from a minor degree of skin sepsis. S. L. W.

Substance P, Neurotropic Effects of. U. S. von Euler and B. Pernow. (*Acta physiol. scand.*, 1956, **36**, 265.) Intracisternal or intraventricular (IIIrd ventricle) injection of substance P, a biologically active polypeptide occurring in extracts of brain and intestine, caused increased rate and depth of respiration in anaesthetised cats and rabbits; there was a variable effect on blood pressure, usually a transient moderate fall, followed by a small rise. Diuresis was little affected by the intraventricular injections, while intravenous injection had a slight antidiuretic effect. In the cat, intravenous injection had no consistent effect on serial carotid occlusion tests; nor was transmission through the superior cervical ganglion altered. Large intravenous doses depressed the amplitude of respiration. Introduction of the polypeptide into one of the lateral ventricles of the cat, through a permanent cannula, caused an increase in depth and rate of respiration. Behaviour was also affected, the animals showing lack of spontaneity of movement, except for occasional vigorous tail movements. Aggressive tendencies were induced in one normally docile animal. These central neurotropic actions bear some resemblance to those of acetylcholine and nicotine, although the drugs differ in several other respects. G. P.

Sulphonamide Hypoglycaemic Agents, *In Vitro* Studies of. M. Vaughan. (*Science*, 1956, **123**, 885.) It has been suggested that a possible mode of action of the sulphonamide hypoglycaemic agents, *N*-*p*-aminobenzenesulphonyl-*N'*-*n*-butyl urea (carbutamide, BZ55), and *N*-toluenesulphonyl-*N'*-*n*-butyl urea (tolbutamide), is through some direct effect on the reactions involved in the conversion of liver glycogen to blood sugar. In this connection, neither carbutamide nor tolbutamide had any inhibitory activity against rat liver insulinase, ruling out an indirect effect of the drugs through hyperinsulism of this type. Nor did tolbutamide interfere with the conversion of liver glycogen to blood sugar at the level of glucose-6-phosphatase. However, the increased release of glucose from rat and rabbit liver slices incubated with adrenaline or Glucagon was markedly diminished by the addition of tolbutamide. If, as has been suggested, adrenaline and Glucagon act by promoting phosphorylase reactivation by phosphokinase, then tolbutamide may decrease this by inhibiting phosphokinase. G. P.

Tetracycline and Chlortetracycline in Pneumonia. Report from the City General Hospital, Sheffield. (*Brit. med. J.*, 1956, **2**, 1146.) Thirty-two patients were treated with tetracycline and 24 with chlortetracycline in a controlled trial of the two antibiotics in clinical pneumonia. The dosage was 0·5 g. orally six-hourly for 3 days, followed by 0·25 g. six-hourly in either case. Three desperately ill patients received initial intravenous administration (0·5 g.) of one or other of the drugs. The patients given tetracycline were slightly older and more of them had lobar consolidation and a positive blood culture than those given chlortetracycline; otherwise the two groups were comparable. In most cases

(ABSTRACT *continued on page* 272.)

BOOK REVIEWS

THE CONDENSED CHEMICAL DICTIONARY. Fifth Edition by A. and E. Rose. Pp. xix + 1200 (double-column). Chapman & Hall, London, 1956. 100s. Reinhold Publishing Corporation, New York. $12.50.

There can be few who do not regularly refer to glossaries, dictionaries and reference books to enlighten their regularly exposed ignorance. But even multi-volume dictionaries do not solve the problem of providing easy access to up-to-date information on names and technical terms. A single-volume chemical dictionary, because it can be revised more frequently, therefore attracts attention even though it is necessarily either restricted in its scope or in the extent of detail.

The fifth edition of this dictionary is stated to contain over 30,000 revised up-to-date entries. Data has been supplied by 355 named manufacturers, all except three having North American addresses. It is claimed that the material presented is specifically "tailored" to the needs of the busy chemist while at the same time providing ready answers to the more commonplace questions of the non-scientist. Its usefulness to the non-scientist appears likely to be considerable. Its usefulness to the scientist appears to the reviewer to be in those fields of chemistry with which the reader is less or not at all familiar. Thus the pharmacist or pharmacologist looking through the entries on names and terms about which he is knowledgeable will often be disappointed by what seems inadequate or over-simplified information. As merely one example in this connection the entry on insulin commences with the formula $C_{45}H_{69}O_{14}N_{11}S.3H_2O$ and continues "The pancreatic hormone which greatly increases the combustion of sugar and leads to a reduction of the amount of glucose in the blood, a systemic deficiency of which is the cause of diabetes". Its properties are given as "white powder, amorphous or crystalline; soluble in water" and yet the "Grade" is stated as "U.S.P.XV, a sterile solution in water", "containers" as "Glass bottles; ampoules; vials" and "Uses: medicine". There is no indication that it is used other than as a watery solution, no reference to complexes such as protamine zinc insulin, globin zinc insulin, isophane insulin or the insulin zinc suspensions. Very many similar examples could have been chosen to illustrate the limited nature of the information. And yet that very limitation can be of considerable help to the reader who merely wants a little information on a large number of items. Among the subjects stated to be covered are adhesives, agricultural chemicals, antibiotics, biologicals, brand names (all American though some are also used in the United Kingdom and elsewhere), catalysts, cellulose derivatives, ceramics and glass, colloids, cosmetics and perfumes, detergents, dyes and pigments, engineering materials, fats, oils and waxes, fertilizers, food and nutrition, isotopes, lubricants, medicines, metals and alloys, minerals, nuclear terminology, organic coatings, pesticides, petrochemicals, petroleum products, pharmaceuticals, plasticizers, plastics and rubber, rare earths, reagents, refractories, solvents, synthetic organics, textiles, trade names. Extensive sampling during review provided much new information and enlightenment; all of it clearly had an American flavour, U.S.A. shipping and transport regulations and available U.S.A. grades being indicated. Some entries were surprising, such as "Zwitterion" which is merely defined as "Trade name for N-coco-beta-amino-butyric acid; a 50-55% aqueous gel". American spelling is used throughout, some of it unexpected such as "heaver" for "heavier" at page 134. However, thumb-indexed, easy to handle, and of admirable format, this dictionary cannot fail to provide much help in concise form to all outside America who care to meet its cost.

FRANK HARTLEY.

Gathercoal and Wirth's PHARMACOGNOSY. Third Edition by Edward P. Claus. Pp. 731 (including 307 illustrations and Index). Henry Kimpton, London, 1956. 93s. 6d.

The presentation of the subject matter of this book reflects a change in emphasis which is being accepted in the United States of America and to some extent in this country, stress being laid on the chemistry of crude drugs rather than on their morphology or taxonomy. Monographs on crude drugs are found in chapters devoted to Carbohydrates, Glycosides, Tannins, Fixed Oils, Fats and Waxes, Volatile Oils, Resins, Alkaloids, Endocrine Products, Vitamins, Enzymes, and Proteins. The taxonomical classification of previous editions is relegated to a short but adequate appendix. Macroscopical and microscopical descriptions of crude drugs are inadequate, the reader being referred throughout to the United States Pharmacopeia and the American National Formulary. For some drugs no descriptive matter is included, for others macroscopical characters only are given, while for yet others the microscopical features of the powdered drug only are described. However, the book is freely illustrated with drawings, photographs and photomicrographs, many of them excellent, though in most magnification is not stated. The key for the identification of powders suffers the defect of inflexibility and in some instances gives misleading information, such as the absence of sclereids from cardamom seed and the presence of lignified tissue in rhubarb. The introduction gives a clear outline of the scope of Pharmacognosy, but the quantitative microscopy of powdered drugs merits more prominence, and the section on chromatography would have benefited by the inclusion of examples. There is a good general account of the cultivation of drug plants, and of the collection and preparation for the market, although details for individual drugs are lacking. The chemical constituents of drugs and the characters of many of the isolated pure principles receive adequate treatment. Fuller accounts could have been given of digitalis, rauwolfia and senna leaf in the light of recent work. While specific references to original literature are few, general reading references are quoted for the chemistry of crude drugs, and for the new and useful chapters on Antibiotics, Immunizing Biological Products, Allergens and Pesticides. Another new feature of this edition is that proprietary products, some available in Britain, are named under the crude drugs from which they are derived. The nomenclature would benefit by the use of a uniform system and adherence to convention. The text is almost free from spelling mistakes and is reasonably well indexed. Presentation has been improved by standardising the size of print, and the quality of paper and the binding are excellent. This is a very comprehensive work with few omissions, and both student and drug analyst will find it a useful reference book.

FRANCIS FISH.

(ABSTRACT *continued from page* 270.)

the pneumonia was pneumococcal and about half the patients in each group had chronic chest disease. The results show that both antibiotics were equally effective in the treatment of bacterial pneumonia. Deaths were few (3 with tetracycline; 2 with chlortetracycline) and were confined to elderly patients with serious complicating diseases. The incidence of side-effects, mostly mild, was about 30 per cent with each antibiotic. This does not confirm the reported lower toxicity of tetracycline. The only serious complication followed tetracycline therapy, namely, one case each of staphylococcus enteritis, pulmonary moniliasis, and aspergillosis; in the last case the patient died. S. L. W.