REVIEW ARTICLE

THE ALKALOIDS OF CURARE

Alkaloids from Calabashes and Strychnos Barks*

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Curare is one of the many arrow poisons which have been known for centuries. Such poisons have been found in certain regions of Africa, Asia, and also Europe and South America. The African and Asiatic poisons are almost exclusively of a glucoside nature. In certain Asiatic countries the most common and most important arrow poison is made from the latex of *Antiaris toxifera*, a Moraceæ, from which a recent investigation has isolated two toxic glucosides having the formula $C_{29}H_{42}O_{11}$ and which have been given the names α- and β-antiarin. In Europe, from prehistoric times until about the fifteenth century it is likely that extracts of *Aconite*, *Veratrum* and *Helleborus* species and also snake venoms were used as arrow poisons.

Of a quite different chemical nature are the arrow poisons of South and Central America. In these, alkaloids form the toxic principles.

From their first contact with that continent, Europeans knew of the existence of the South American arrow poisons. On Magellan's first voyage around the world (1519–22) one of his sailors, whilst ashore in Patagonia, was killed by a poisoned arrow. During the invasion of the South American continent by Cortez and Pizarro the invaders were often attacked by natives using curare tipped arrows. This was for the Europeans a new and mysterious weapon and gave rise to many fantastic stories, some of which were brought back by Sir Walter Raleigh in 1596. Not until 150 years later were more reliable reports brought to Europe by more scientific workers. In 1745 La Condamine reported that curare was made mainly from a liana "Bajuco" together with other plants. The first exact and reliable description of curare production from Bajuco de Mavacure was given by Alexander von Humboldt, who also made some very good observations on the physiological and toxicological effects of the arrow poison.

In the Tupi language curare is called Urary, meaning "a liquid that can kill birds." This gives an indication of its original application.

The distribution of South American curare extends from the north of this continent to the upper La Plata river-basin; that is, it stretches from about ten degrees north to fifteen degrees south of the equator and from west to east over about twenty degrees of longitude. However, the type and the method of extraction of these arrow poisons is not the same throughout this vast area.

In Columbia the natives make an arrow poison out of a toad (*Phyllobatus melanorrhinus* Dum). According to Lewin[1], in the Orinoco region

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(Venezuela) two *Strychnos* species, *Strychnos toxifera* and *Strychnos gubleri* are said to be the sources of calabash curare. According to the same worker, in the region between the Orinoco and the Rio Negro live the most famous poison makers. Furthermore curare is produced in British Guiana, in Ecuador (probably from *Strychnos castelnaei*), in the Rio Negro region (*Strychnos castelnaei*), in the river-basin Yapura-Solimoes (tributaries of the Amazon) as well as in Peru.

The regions of curare production are obviously determined by the location of the plants necessary for poison production and therefore the natives living in such regions possess a monopoly of the curare production. The tribes of the Macusi (or Macu), Umaua, Juri, Ticua, Mesaya, Peba, Orejones and others produce high grade curare. Where those *Strychnos* species most suitable for curare production do not occur or have been exhausted, a poison of inferior quality is made out of Menispermaceæ, *Cocculus* species and other plants.

For past centuries and even up to the present day good quality curare fetches a high price and is used by the natives for bartering. In the year 1923 Lewin gave the following report. "A pot of curare which sells at four German marks on the banks of the Yagua, costs about 16 marks at the upper Içа. Twenty years ago 30 g. of the best quality curare could be bought from the Tucuna people from Marañon, near Loreto, for four marks and this same amount could be bought for 60–62 marks in Germany up to ten years ago. Many years ago in British Guiana it was possible to trade an axe, worth about eight marks, for a calabash of curare. Nowadays this curare has totally disappeared and is found only in museums. Even pot curare has already become rare. Only the so called tubocurare (or paracurare), sold in bamboo tubes, is now available."

From this it can be seen how difficult it is nowadays to get the quantities of curare necessary for chemical research, especially calabash curare, on which our work has been done and which, according to the report just cited, totally disappeared from the market fifty years ago. However after much trouble we have succeeded in finding a source, which provides a certain amount of calabash curare.

The three kinds of curare, tubocurare, pot curare and calabash curare are identified by their containers. Tubocurare is packed in bamboo tubes, pot curare in unglazed clay pots, and calabash curare in calabashes or gourds. The differences between the three types of curare are quite pronounced, they come from different plants, contain different alkaloids and show great variations in toxicity.

The calabash curare is by far the most toxic and therefore the most desirable but also difficult to obtain. In most recent times the containers are not always a guide to the type of poison in them. As an example of this, part of the calabash curare which we received, was not in gourds but in clay pots.

There are many data and numerous reports and opinions to be found in the literature concerning the plants used in curare production. However most of this information is very unreliable. Nevertheless Humboldt and various other investigators after him have pointed out the fact, that

Strychnos species are most frequently used for curare production. There were mentioned for instance: *Str. toxifera*, *Str. gubleri*, *Str. castelnaei*, *Str. rubiginosa*, *Str. hirsuta*, *Str. yapurensis*, *Str. triplinervia*, *Str. curare baillon*, etc. Most of the workers agree that besides strychnos extracts those of other plants are used, for instance *Cocculus* species (*Cocculus toxiferus*, *C. wedell*, *C. amazonum* Mart., *Menispermum cocculus* L; the latter containing the toxic bitter principle picrotoxin, which produces central nervous convulsions). Furthermore, Menispermaceæ are said to be used (for instance *Abuta rufescens* Aubl., *Abuta imene* Eichl.) as well as Aracæ (*Dieffenbachia Seguine*) and possibly Euphorbiaceæ. According to the opinion of certain investigators the addition of all these plants to the strychnos extract has little value and rather serves to make the process of poison production, which is already accompanied by much ceremonial ritual, still more mysterious.

There is already a report given by Humboldt about the technique of curare production. Another more recent one is given by the pharmacologist Freise[2,3], who reports as follows:—

"For curare production barks of various *Strychnos* species are used. In the Araraquare mountains live the Maupes indians, known to be excellent poison makers. In this region many *Strychnos* species can be found, mainly *Str. letalis*, *Str. icaja*, and *Str. lanceolaris*, some of which occur as lianas up to 200 feet long with widely spread branches. Strips of the bark, each about three to four inches wide and 20–30 inches long (about 40–60 kg. are obtained from one stem during the harvest period, that is during the dry season) are soaked in wooden troughs filled with water, the cork tissue being stripped off cautiously from the inner layers. Only the cork layer is retained. This is dried and powdered in hard mortars with wooden pestles, the process taking several days and the daily production being scarcely more than 200 g. per mortar. Each patch of bark powder produced in one mortar during one day is worked up separately. The powder is slowly extracted with warm water in wooden vessels in which the extraction temperature of 70–80 degrees Celsius has been previously produced by heated stones. Boiling is never mentioned. The amount of water for 200 g. of powder is about 2 litres. The extraction of the bark with warm water takes about four days. Then the contents of the vessel have become a red to dark brown liquid which has a penetratingly bitter taste. Straining through a bast filter separates it from the bark residues. During several days' concentration, under very cautious warming, the liquid thickens to a syrupy consistency and is poured into gourds."

Recently a different opinion is expressed by Lazzarini-Peckolt[4] on the formation of the toxic curare alkaloids. He states that these alkaloids do not occur in the plant, or if so only in small amounts, but are formed during the boiling of the various plant juices, the quaternary curare active compounds being formed by some methylation processes from the tertiary inactive bases. In his opinion three different kinds of plants are necessary to produce active curare:

1. Plants containing alkaloids capable of being alkylated.

2. Plants capable of making the mixture of the extracts alkaline during concentration.

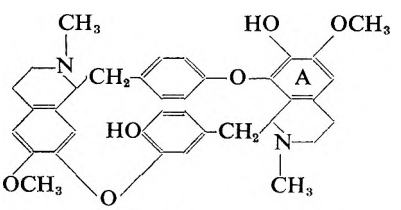
3. Plants containing methyl and other esters, which supply the necessary alkyl groups to form the active quaternary ammonium bases from the alkaloids of the first mentioned group of plants.

According to Lazzarini-Peckolt there are a great number of plants which can be used for curare production: Loganiaceæ (of the *Strychnos* species), Menispermaceæ (of the *Chondrodendron*, *Elissarrhena*, *Anomospermum*, *Sciadotenia*, *Telitoxicum*, *Abuta*, *Cocculus* and other species), Lauraceæ (of the *Nectandra* species and others), probably Solanaceæ and species of other families too. Also a great number of plants can be used to make the mixture alkaline. Those used most frequently are: *Dieffenbachia*, *Arum*, *Melothria*, *Cyclanthera*, *Clavija*, *Piper* and *Ipomoena* species. The plants used for giving the alkyl groups necessary for quaternary salt formation are mostly of the species *Aristolochia*, *Duguetia*, *Xylopia*, *Zinziber*, *Petiveria*, *Gallesia*, *Octotea*, *Fagara* and others. Therefore, according to Lazzarini-Peckolt, the composition of the curare can be changed as the producer wishes.

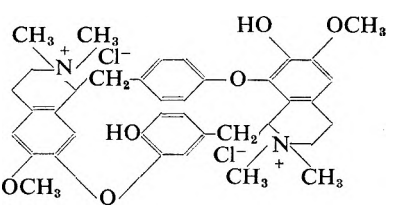
A point to note is that Wieland, who examined the calabash curare alkaloids during the years 1937–41, was not able to find the main calabash quarternary bases in the bark of *Str. toxifera*. Only very recently has more light been thrown on the origin of these toxic principles.

Chemistry

The alkaloids of tubocurare are the best known. From tubocurare Boehm and King extracted along with the tertiary base (−)-curine (I) the quaternary (+)-tubocurarine (II) as the active principle. The constitutions of both were elucidated by the work of King[5–10] and of Wintersteiner and Dutcher[11] and Dutcher[12]. Both compounds are relatively complicated di-*iso*quinoline derivatives with the following constitution:



I
(−)-Curine



II
(+)-Tubocurarine

The elucidation of the constitution of these compounds was accomplished mainly by studies of their degradation products, which are formed by zinc dust distillation, alkaline fusion and Hofmann degradation of the bases.

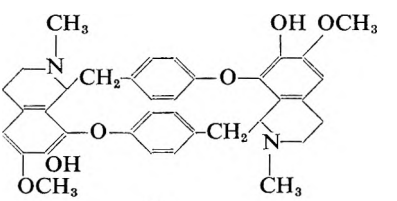
According to Wintersteiner and Dutcher, (+)-chondrocurine from *Chondrodendron tomentosum*, a tertiary base, differs from (−)-curine in

that the positions of the hydroxyl and methoxyl groups in ring A are interchanged.

Most probably tubocurare is made from Brazilian Menispermaceæ such as *Chondrodendron* species, for King and Wintersteiner succeeded in isolating the quaternary alkaloids (+)-tubocurarine and the related (+)-chondrocurarine as well as the tertiary bases (−)-curine, (+)-chondrocurine, (+)-*iso*chondrodendrine and (+)-*iso*chondrodendrine dimethyl ether from chondrodendron bark. (Once also the other tubocurarine antipode was encountered.) (+)-Tubocurarine to-day is still the only natural curare alkaloid used in medicine. Its activity and toxicity appear to be low compared with those of certain calabash alkaloids.

Pot curare, coming mainly from the Orinoco region, has relatively little toxicity and is used largely by the indians for killing small animals and birds. *Chondrodendron* species seem to be the initial material, as some substances isolated from this arrow poison, like (+)-protocuridine *i*-neoprotocuridine and (+)-*iso*chondrodendrine have also been found in *Chondrodendron* species (*Ch. tomentosum*, *Ch. platyphyllum*, *Ch. candicans*, etc.). They are tertiary bases of low activity. (+)-Protocuridine and *i*-neoprotocuridine have the formula $C_{36}H_{38}O_6N_2$. For (+)-*iso*chondrodendrine, structure III has recently been proposed:

Those quaternary alkaloids occurring in pot curare, which probably represent the main sources of activity, have not yet been isolated. Perhaps they come from a variety of *Strychnos* species.



III

(+)-*iso*Chondrodendrine $C_{36}H_{38}O_6N_2$

The most important and most active South American arrow poison is the calabash curare, the toxicity for the frog being from 0·5 to 1 mg. per kg. The first chemical work on this arrow poison dates back to the pharmacologist Boehm. However it was not until 1937–41 that Wieland and his colleagues[13–15] were able to isolate for the first time some pure alkaloids in a crystalline form. Since 1945 our Chemical Institute in Zürich has been occupied with the investigation of the components of the calabash curare[16–31]. Furthermore, King[32] in England and more recently Wieland[33,34] have worked on calabash alkaloids. Qualitative experiments on the occurrence of alkaloids in several *Strychnos* species have also been made by Bovet, Marini-Bettolo and others[35].

The calabash curare, originating from the Rio Negro region, contains a large number of different compounds. With the aid of paper chromatograms, we have been able to demonstrate the occurrence of more than thirty different alkaloids. For distinguishing the different spots on the paper chromatograms we have used the colour reactions with ceric sulphate, which with the different bases give red, blue, yellow, green, purple or yellowish green colours. Furthermore colour tests with cinnamic aldehyde, nitric acid and sulphuric acid have been made, as has also an

examination of the fluorescence shown by some of these alkaloids under ultra-violet light.

Figure 1 shows a chromatogram of the total alkaloids from calabash curare.

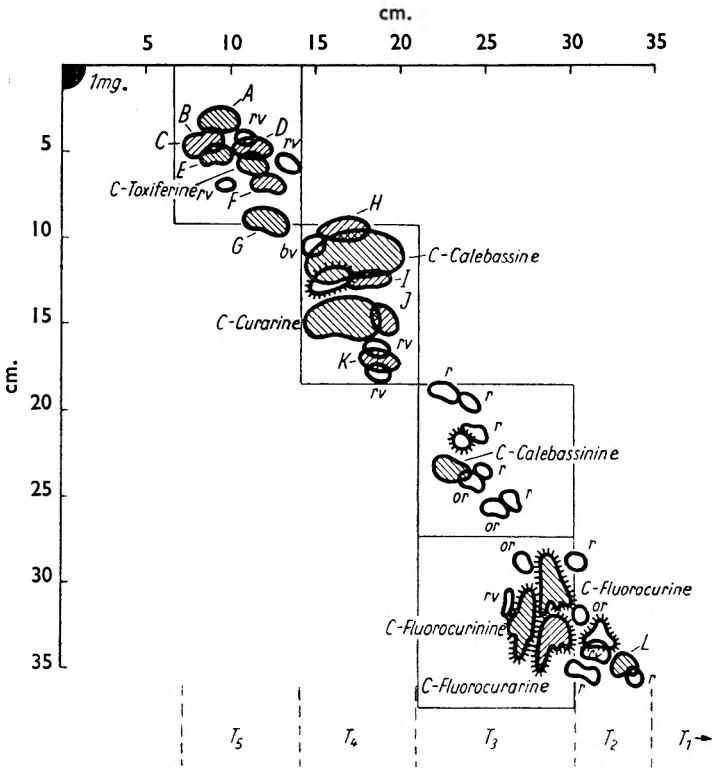


FIG. 1. Paper chromatogram of 1 mg. of purified chlorides from calabash curare No. III. The different spots were made visible by ceric sulphate or iodine solution. The hatched spots correspond to the isolated alkaloids, r = red, bv = blue-violet, rv = red-violet, or = orange spots after treatment with ceric sulphate.

The separation of the calabash alkaloids was achieved by Wieland *et al.*[13] by chromatography of the Reinecke's salts. In the beginning we also used this procedure, but lately we have obtained better results with chromatographic separations of the chlorides using a column of paper powder.

From the calabash curare at our disposal we have now been able to isolate the compounds in Table I in a chromatographically homogeneous form.

Some of the alkaloids isolated by Wieland and his colleagues from calabashes are identical with some of the members of the above mentioned groups, whilst others are different. Besides this both he and King have isolated some alkaloids from the bark of *Str. toxifera* (for example toxiferine I and II). As this was before the time of paper chromatography

TABLE I

R_c WITH SOLVENT C*

C-alkaloid A	$C_{20}H_{23}O_3N_2^+$	0·23	C-curarine	$C_{20}H_{21}N_2^+$	1·00
C-alkaloid B	$C_{20}H_{23}ON_2^+$	0·34	C-alkaloid J	$C_{19}H_{21}N_2^+$	1·04
C-alkaloid C	?	0·34	C-dihydrotoxiferine (= C-alkaloid K)	$C_{20}H_{22}N_2$	1·22
C-alkaloid D	$C_{20}H_{21}ON_2^+$	0·25	C-alkaloid UB	$C_{18}H_{22}O_3N_2$	—
C-alkaloid E	$C_{19}H_{23}ON_2^+$	0·36	C-alkaloid M		1·45
C-toxiferine I	$C_{20}H_{21}N_2^+$	0·42	C-alkaloid Y		1·59
C-alkaloid F	$C_{20}H_{25}O_4N_2^+$	0·49	C-calebassinine	$C_{19}H_{23}O_2N_2$	1·68
C-alkaloid G	$C_{20}H_{23}ON_2^+$	0·65	C-fluorocurine	$C_{20}H_{25}O_2N_2$	2·10
C-alkaloid H	?	0·71	C-fluorocurinine	$C_{21}H_{29}O_2N_2$	2·23
C-calebassine	$C_{20}H_{25}ON_2^+$	0·80	C-fluorocurarine (= C-curarine III)	$C_{20}H_{23}ON_2^+$	2·25
C-alkaloid I	$C_{19}H_{23}N_2^+$ ($C_{20}H_{23}N_2^+$)	0·89	C-alkaloid L	?	2·50
			C-mavacurine	$C_{20}H_{25}ON_2$	2·70

* $R_C = \frac{\text{distance travelled by the alkaloid}}{\text{distance travelled by the curarine}}$

Solvent C = methyl-ethyl ketone saturated with water + 1 to 3 per cent. methanol.

no suitable method was available for assessing the purity of these compounds.

We observed more than once that the composition of the poison of calabashes can show great differences. We had the opportunity to investigate two made by the indian tribe Isana, who live in the territory of the river Içа near the frontier of Columbia. These calabashes contained only one alkaloid which has been found before in other calabashes, calebassine, and even this in small quantity. But we were able to isolate from these calabashes four new alkaloids which we call C-alkaloid O and P, xanthocurine and guaianine. Xanthocurine is characterised by its intense yellow colour. Guaianine has also been found in the bark of *Strychnos guaianensis*.

Lately we have been fortunate in being able to examine the alkaloids of the bark of a *Strychnos* species, which has been identified by Prof. Frey-Wyssling as belonging to the *Str. mitscherlichii* group. In the extract of this bark we were able to identify the following alkaloids by paper chromatography and by their colour reactions[25]:

C-fluorocurinine, C-fluorocurarine, C-curarine, C-calebassine, C-alkaloid I and C-alkaloids of the B-, C-, D-group.

These alkaloids occurred in the bark in about the same proportion as in the calabashes and the main alkaloids in the bark likewise were C-curarine and C-calebassine. The two-dimensional paper-chromatogram of the alkaloids of *Str. mitscherlichii* shows very great resemblance with that of a calabash.

The identified alkaloids from the bark of *Str. mitscherlichii* are representatives of five of the eight groups or types, into which we have divided the calabash alkaloids. The representatives of the toxiferine group, occurring in *Str. toxifera*, are missing, a fact which is also found in many calabashes. Therefore it can now be stated that the natives of the middle and upper Rio Negro region use the bark of *Str. mitscherlichii* for production of calabash curare and if this contains alkaloids of the toxiferine group, it indicates that *Str. toxifera* has also been used. What further plants are used, has to be ascertained by examination of other barks.

The fact that quaternary alkaloid salts occur in the bark of *Str. mitscherlichii* shows that the assumption of Lazzarini-Peckolt, that the quaternary bases are formed by a methylation process during the concentration stage, cannot find any support in the light of our observations. But we cannot of course exclude a small measure of methylation of tertiary alkaloids during the concentration process.

The bark of a *Strychnos toxifera* species, which we received from Venezuela (the plant was grown in the district Fed. Amazonas on the mountain, Imutinava) contained partly other alkaloids. Besides mavacurine and fluorocurine and the above mentioned C-alkaloid Y we have so far isolated from this bark 10 new pure crystalline substances[30]: fedamazine (a quaternary alkaloid) caracurine I to IX (tertiary bases).

A further tertiary base which we found in this bark is of special interest. It is the nordihydrotoxiferine; the quaternary methylderivative of this is the

TABLE II

ALKALOIDS FOUND IN CALABASHES OR IN BARKS OF *Strychnos* SPECIES

	Isolated from	
	Calabashes	Barks of
C-curarine I[13,14,23,24,25]	+	*Str. mitscherlichii*
C-curarine II[25] (C-strychnotoxine Ia)[27]	+	
C-curarine III = C-fluorocurarine[15,23,24,26]	+	*Str. mitscherlichii*
C-toxiferine I = toxiferine I[16,23,24,36,40]	+	*Str. toxifera*
C-toxiferine II = C-calebassine[16,23–26,36,40] (= C-strychnotoxine I)	+	*Str. mitscherlichii*
Toxiferine II[36] (= strychnotoxine II)[27]	+	*Str. toxifera*
C-dihydrotoxiferine I = C-alkaloid K[23,24,86]	+	
C-isodihydrotoxiferine[36]	+	
C-alkaloid A[16,23–26]	+	*Str. mitscherlichii*
C-alkaloid B[16,23–26]	+	*Str. mitscherlichii*
C-alkaloid C[23–25]	+	*Str. mitscherlichii*
C-alkaloid D[23,24]	+	
C-alkaloid E[23,24]	+	
C-alkaloid F[23,24]	+	
C-alkaloid G[23,24]	+	
C-alkaloid H[23,24]	+	
C-alkaloid J[23,24]	+	
C-alkaloid I[23–26]	+	*Str. mitscherlichii*
C-alkaloid L[23,24]	+	
C-alkaloid M[28]	+	
C-alkaloid O[29]	+	
C-alkaloid P[29]	+	
C-alkaloid UB[16,23,24]	+	
C-alkaloid Y[16,28]	+	*Str. toxifera*
C-alkaloid X[16,23,24]	+	
C-calebassinine[23,24]	+	
C-fluorocurine[23,24,30]	+	*Str. toxifera*
C-fluorocurinine[23–25]	+	*Str. mitscherlichii*
C-mavacurine[27,30]	+	*Str. toxifera*
C-xanthocurine[30]	+	
C-guaianine[29]	+	
C-alkaloid 1[27]	+	
C-alkaloid 2[27]	+	
Fedamazine[14]	−	*Str. toxifera*
Caracurine I[30]	−	*Str. toxifera*
Caracurine II[30]	−	*Str. toxifera*
Caracurine III[30]	−	*Str. toxifera*
Caracurine IV[30]	−	*Str. toxifera*
Caracurine V[30]	−	*Str. toxifera*
Caracurine VI[30]	−	*Str. toxifera*
Caracurine VII[30]	−	*Str. toxifera*
Caracurine VIII[30]		*Str. toxifera*
Caracurine IX[30]		*Str. toxifera*
Nor-dihydrotoxiferine[36]		*Str. toxifera*
Melinonine A[39] (= tetrahydroalstoninechlormethylate)		*Str. melinoniana*
Melinonine B[39]		*Str. melinoniana*
"Toxiferines III to XII" of King[40]*		*Str. toxifera*
Diaboline[47,48]		*Str. diaboli*

* The homogeniousness of these compounds is doubtful and we do not know their relation to other alkaloids of calabashes and strychnos barks.

important calabash alkaloid dihydrotoxiferine. It is the first time that the tertiary base of a quaternary calabash alkaloid salt has been observed in a plant.

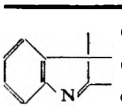
Other alkaloids occurring in this bark are still being studied in our laboratory.

We know to-day as many as 50 different alkaloids which have been found in calabashes or in barks of *Strychnos* species. Some of them are mentioned in the literature under different names (Table II).

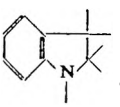
All calabash alkaloids isolated by us, contain 19 to 21, but generally about 20 C-atoms and 2 nitrogen atoms, one of which belongs to a

TABLE III

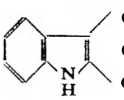
GROUPING OF CALABASH ALKALOIDS



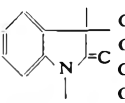
C-curarine	$C_{20}H_{21}N_2^+$
C-alkaloid G	$C_{20}H_{23}ON_2^+$
C-alkaloid E	$C_{19}H_{23}ON_2^+$(?)
C-guaianine	$C_{21}H_{25}ON_2^+$ or $C_{20}H_{25}N_2^+$



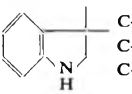
C-calebassine	$C_{20}H_{25}ON_2^+$
C-alkaloid A	$C_{20}H_{23}O_2N_2^+$
C-alkaloid F	$C_{20}H_{25}O_2N_2^+$
C-alkaloid I	$C_{20}H_{25}N_2^+$(?)
C-curarine II	$C_{20}H_{23}ON_2^+$
C-alkaloid X	



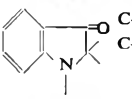
C-alkaloid J	$C_{19}H_{21}N_2^+$
C-alkaloid L	
C-mavacurine	$C_{20}H_{25}ON_2$
Toxiferine III from *Str. toxifera*	
Melinonine B from *Str. melinoniana*	



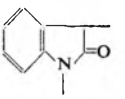
C-toxiferine I	$C_{20}H_{23}ON_2^+$
C-dihydrotoxiferine	$C_{20}H_{23}N_2^+$
C-alkaloid H	
C-alkaloid 2	



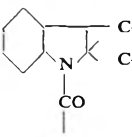
C-alkaloid B	$C_{20}H_{23}ON_2^+$
C-alkaloid D	$C_{20}H_{21}ON_2^+$
C-alkaloid C	



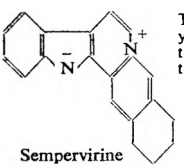
C-fluorocurine	$C_{20}H_{23}O_2N_2^+$
C-fluorocurinine	$C_{21}H_{23}O_4N_2^+$



or



C-calebassinine	$C_{19}H_{23}O_2N_2^+$
C-alkaloid UB	$C_{19}H_{23}O_3N_2^+$



Sempervirine

The spectrum of xanthocurine is very similar to those of sempervirine, *N*-methylyobyrine and flavocorynantheine, i.e., it possesses the character of the spectrum of the so-called anhydronium bases. The substance has the high specific optical rotation of more than + 800°.

quaternary ammonium salt grouping, whilst the other in most or all cases probably belongs to an indole grouping. According to the absorption spectra, colour reactions, specific rotations and degradation products most of the calabash alkaloids can be put into groups, which contain the indole ring probably variously substituted in the form of the chromophores in Table III.

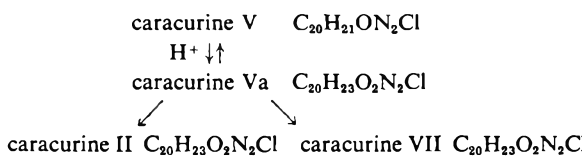
Transformation of the Alkaloids under the Influence of Acids

Several alkaloids of calabashes and of strychnos barks are very sensitive to acids and can be transformed into other compounds under the influence

of H^+. In this way Wieland and colleages[36] obtained from toxiferine-II-picrate under the influence of diluted hydrochloric acid, toxiferine IIa and the adsorption of the latter substance on aluminium-oxide lead to a new isomer, IIb. In a similar way C-toxiferine II is converted into C-toxiferine IIa by small amounts of acetic acid or hydrochloric acid[33].

Caracurine V is also very sensitive to acids. The end-products of this rearrangement are the caracurines II and VII which have been found in the same bark as caracurine V[31]. As a very unstable intermediate product of this transformation we were able to isolate a compound which possesses the characteristic ultra-violet spectrum of the C-toxiferine-alkaloids with the typical absorption maximum at 292 mμ. From this intermediate product caracurine Va the above mentioned alkaloids caracurine II and VII are formed under the influence of H^+. By heating caracurine Va caracurine V can be recovered.

The following formulæ show these various transformations:



The transformation of caracurine V in Va seems to be connected with the addition of one molecule of H_2O.

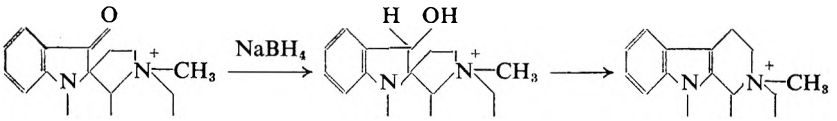
Caracurine VII shows a characteristic yellow reaction with ceric sulphate and a relatively large R_c value. Its spectrum is that of an indoline-derivative. Caracurine II however is a representative of the B,C,D-group, which migrates slowly and shows a violet ceric sulphate-reaction.

A third example of a transformation of an alkaloid of the C-toxiferine-group in a substance belonging to the B,C,D-group is the rearrangement of the C-alkaloid C-dihydrotoxiferine I into the C-alkaloid D[23] under the influence of diluted hydrochloric acid; this reaction was completed in 8 days. We observed as an intermediate compound a substance with an orange ceric sulphate reaction and a large R_c value.

So far fluorocurine is the best example we have found to illustrate the rearrangement caused by H^+. This contains the atom grouping A. By reduction it was converted into another, colourless alkaloid, which shows a characteristic indole spectrum (like the C-alkaloids J, L, I, etc.). This alkaloid has been shown to be identical with mavacurine on the basis of analyses, mixed melting point, colour reaction, ultra-violet spectrum and paper chromatograms. Mavacurine was first isolated by Wieland[37] from the South American drug "mavacurine" and from calabashes which also contained fluorocurine. We have isolated the same compound from calabashes, from the bark of *Strychnos toxifera* and *Strychnos melinoniana* and from barks of other *Strychnos* species[30], it always occurs together with fluorocurine.

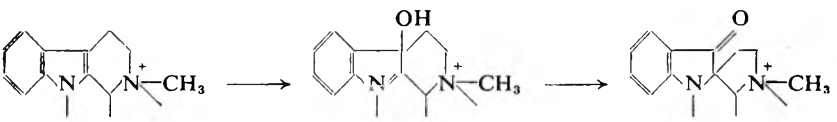
By transforming fluorocurine into mavacurine[27] we have achieved the transformation of a calabash alkaloid into another alkaloid that has been

found in calabashes. These reactions may be represented by the following formulæ:



The formation of mavacurine from hydrofluorocurine is a kind of retropinacoline rearrangement.

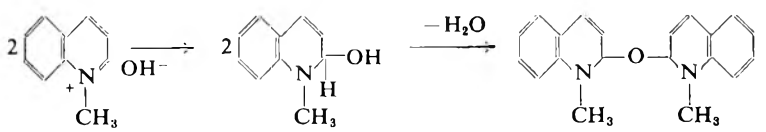
Presumably the reaction occurring in the plant is a reversal of the one observed *in vitro*, that is fluorocurine probably arises from mavacurine. From Witkop's similar observation with indole derivatives we assume that the indole compound mavacurine is first oxidised in β-position to the indole nitrogen and that the reaction product formed is immediately afterwards rearranged to give the indoxyl derivative fluorocurine:



Investigations on the Chemical Structure of the Curare Alkaloids

The elucidation of the constitution of all the calabash-alkaloids still lies in a rather distant future. Chemical examinations in this field are hindered by the difficulty of obtaining calabashes and of separating the various alkaloids; for example we chromatographed for more than a year before twenty-one alkaloids were separated. A further difficulty was due to the small quantities in which most of these compounds were obtained. Some of them we have only obtained in milligrams, whilst from others, grams have been isolated.

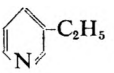
Some preliminary degradation experiments on calabash alkaloids were conducted by Wieland and his colleagues. By dehydration reactions on dehydrotoxiferine I they succeeded in obtaining β-ethylindole and *iso*quinoline whilst from C-toxiferine II some β-ethylpyridine was obtained. These observations together with the fact that C-curarine I like aromatic compounds, can be nitrated and brominated and like quaternary quinolinium bases is said to be transformed into a bimolecular ether base



caused the above mentioned authors to suspect the presence of an *iso*-quinoline or quinoline ring in C-curarine I. Or alternatively an indole ring was considered to be part of the molecule.

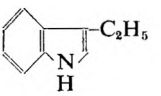
Our own investigations started with the thermal decomposition of C-curarine I chloride in high vacuum. Thereby the quaternary salt is decomposed to methyl chloride and norcurarine I. The latter compound being a tertiary base, it is not acetylatable and this indicates that the demethylated nitrogen atom must be common to two rings.

From this nor-C-curarine base the compounds IV-VII could be isolated after zinc dust distillation.



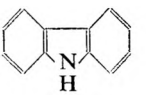
IV

β-Ethylpyridine



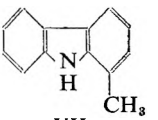
V

β-Ethylindole + scatole



VI

Carbazole



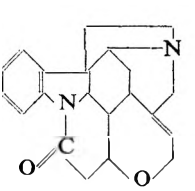
VII

1-Methylcarbazole

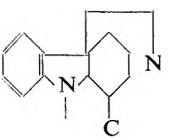
As carbazole is not changed by zinc dust distillation, it can be concluded that the ethyl group in β-ethylindole does not come from those nor-base carbon atoms, which are found in the decomposition products, carbazole and 1-methylcarbazole. Eighteen of the nineteen carbon atoms of the nor-base ($C_{19}H_{18}N_2$) are found in the above degradation-products.

The nonbasic indolo-nitrogen atom in C-curarine I is most probably substituted in a ring for these reasons: The NH-band is missing from the infra-red spectrum, the Zerewitinoff test of the base shows no active hydrogen and in the nor-base only a trace of *N*-methyl can be found.

The production of β-ethylindole after zinc dust distillation of norcurarine makes it possible that C-curarine, like many other plant bases, is derived from tryptamine. The isolation of carbazole and 1-methylcarbazole however excludes a constitution based upon the harman type (yohimbine bases). The dehydrogenation products obtained indicate a probable relationship of C-curarine with the strychnos alkaloids. Thus strychnine (VIII) for instance on vigorous degradation gave β-ethylindole β-collidine and carbazole.

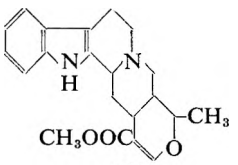


VIII



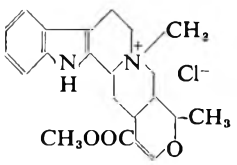
From a South American strychnos plant, *Strychnos melinoniana*, of which it is not known, whether or not the natives use it for poison production, Schlittler and Hohl[39] have isolated two alkaloids of quaternary ammonium salt character, melinonine A (X) and melinonine B. The

elucidation of the structure of melinonine A was easily achieved, whereas there is nothing yet known about the constitution of melinonine B. Melinonine A is nothing more than the methachloride of tetrahydroalstonine (IX).



IX

Tetrahydroalstonine



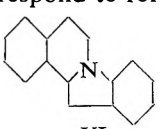
X

Melinonine A

Melinonine A has only a minimal curarising activity and has not yet been found in calabash curare.

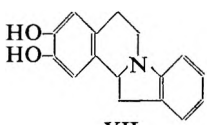
In connection with this it may be pointed out that recently a group of alkaloids has been found, which are tertiary, not quaternary bases but which nevertheless show strong curare activity. These alkaloids are found in the seeds of a member of the Leguminosæ family, *Erythrina* occurring in South America. Ramirez and Ribera[43], later Lehmann[44], Cicardo[45], Unna and Greslin[46–48] have noticed the curare activity of the extracts of these seeds and have ascertained the unusual fact that they show their activity after application by mouth, whereas tubocurare, pot curare and calabash curare only show high activity when they are directly introduced into the blood. Later Folkers[49–54] and colleagues isolated a series of pure substances from erythrina seeds, especially the so-called erythroidines $C_{16}H_{19}NO_3$. From about fifty kinds of *Erythrinæ* investigated, *E. americana*, *E. glauca*, *E. cristagalli* and *E. Eggersii* were especially rich in alkaloids.

The constitution of the erythrina bases has not yet been elucidated. Probably they are derived from the tetracyclic ring system A (XI)[56,57]; apo-erysopine (XII), a degradation product of many erythrina alkaloids, seems to correspond to formula B:



XI

(A)



XII

Apo-erysopine (B)

Some erythrina alkaloids (erysothiopine, erysothiovine) contain sulphur.

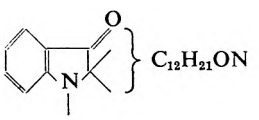
The erythrina alkaloids lower the blood pressure, have a toxic effect on the heart and, unlike the proper curare poisons, they cause central nervous system depression.

Wieland and his colleagues[34] recently studied the problem of the constitution of C-toxiferine II which is also called calebassine. This alkaloid is, according to the ultra-violet spectrum, probably an indole-derivative substituted in the α- and the β-position. This opinion is in harmony with the fact that after the dehydrogenation with sulphur β-ethylpyridine

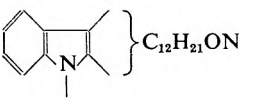
could be obtained. One of the two non-aromatic carbon double-bonds of C-toxiferine II lies, according to Wieland, in the vinyl-position to the indoline-nitrogen, because after oxidation and following saponification of the oxidised compound, formic acid was isolated. Finally the second carbon double bond of calebassine is believed to belong to an ethylidene side chain, because after oxidation with ozone, acetaldehyde was formed.

Recently we have been making experiments[57] to elucidate the constitutions of two calabash curare alkaloids, which possess a certain resemblance to the yohimbine alkaloids. The two alkaloids concerned, fluorocurine and mavacurine, always occur together and for this reason alone are presumably related in their manner of formation.

Fluorocurine exhibits the typical spectrum of an indoxyl derivative substituted at the N(1)-atom. It can therefore be assigned the partial formula XIII. Mavacurine can be recognised from its spectrum as an indole derivative and, since it can be prepared from fluorocurine, we may assign it the partial formula XIV:

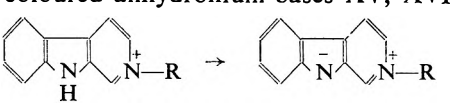


XIII
Fluorocurine



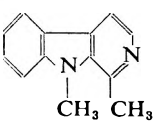
XIV
Mavacurine

Selenium dehydrogenation of nor-mavacurine yields a base which has the same spectrum as N(1)-methylharmane; since the spectrum of its chlormethylate is not altered by treatment with alkali, the indole nitrogen atom must carry a C-atom as substituent. Quaternary β-carbolinium salts, which are not alkylated at the indole-nitrogen, are transformed by alkali into deep coloured anhydronium-bases XV, XVI.

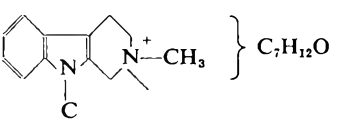


XV XVI

Hence the partial formula of mavacurine may now be expanded as follows (XVII):



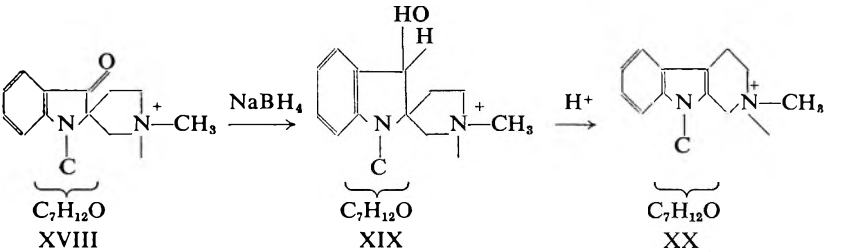
Methylharmane



XVII

Fluorocurine can be reduced with $NaBH_4$ to dihydrofluorocurine, which in turn, is readily converted to mavacurine under the influence of hydrogen ions. This conversion may now be represented by the partial formulæ XVIII, XIX, XX, which show it to be a retropinacoline rearrangement.

Mavacurine and fluorocurine each contains a C–CH_3 group and yields acetaldehyde on degradation with ozone. Consequently, their only carbon-carbon double bond must belong to an ethylidene grouping, C=$CHCH_3$.

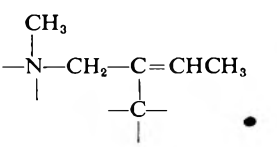


XVIII Fluorocurine — XIX Hydrofluorocurine — XX Mavacurine

The fact that the two alkaloids give one molecule of acetic acid when they are oxidised with chromic acid, according to the method of Kuhn-Roth agrees with this observation. A further proof for the ethylidene grouping could be ascertained by its selective hydrogenation and subsequent oxidation, which was effected with a new, modified carbon-methyl-determination. If during the oxidation with chromic acid the fatty acids of low molecular weight formed are continously distilled off, we are able to demonstrate in the distillate not only acetic acid but also propionic acid and further aliphatic acids with more carbon atoms, if a corresponding alkyl-group had been present in the substance which we oxidised.

Nor-hydrofluorocurine, the nor-base corresponding to the hydrofluorocurine could easily be reduced to a dihydro-derivative, which still possessed the absorption spectrum of the starting material and therefore still its chromophoric grouping. For that reason only the ethylidene group was reduced in the catalytical hydrogenation. This dihydro-nor-hydrofluorocurine gave, when oxidised with chromic acid, not only acetic acid, but also propionic acid, by which the grouping C–CH_2CH_3 was definitely proved.

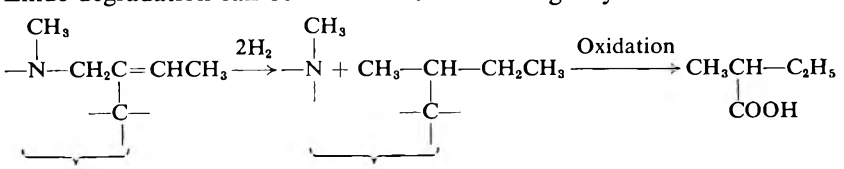
The following experiments showed that the ethylidene-grouping derives from a part of the molecule which may be represented by formula XXI.



XXI

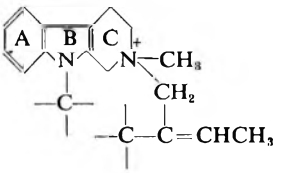
Hydrofluorocurine readily undergoes the so-called Emde degradation, i.e., when subjected to reduction with Pt and hydrogen in ethanolic solution, a C-atom attached to the quaternary nitrogen atom is split off; at the same time hydrogenation takes place at the C=C double bond.

On the degradation with chromic acid the Emde base gave as volatile acids not only acetic acid but also methylethylacetic acid, an especially important compound. Therefore the reaction which occurred in the Emde-degradation can be shown in the following way:



XXI

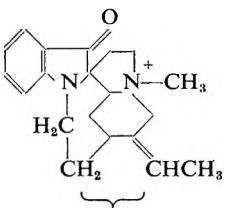
By this observation the grouping C is proved to occur in the molecule of fluorocurine. It can easily be understood that in our example the Emde degradation takes place without any difficulty, because allyl- and vinyl-amines are especially appropriate to it. We may therefore establish the partial formula XXII for mavacurine.



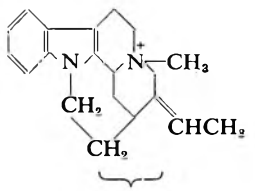
XXII

18 of the 20 carbon atoms present in the alkaloid are contained in this partial formula, in addition to the two nitrogen atoms. The two remaining C-atoms must be included in the formula in such a way that two carbon-rings more are formed, which are free of methyl-groupings.

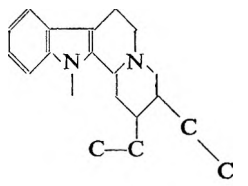
Biogenetic considerations are strongly in favour of the assumption that the missing carbon atom in the formula XXII should be so placed as to produce the carbon skeleton XXV of corynantheine, alstonine and yohimbine. If this is done, we arrive at the structural formulæ XXIII and XXIV, in which the position of the OH-group is still undetermined.



XXIII
Fluorocurine

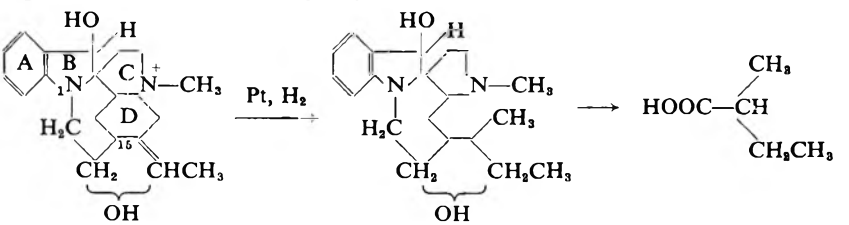


XXIV
Mavacurine



XXV

These formulæ are able to account for all the experimental findings made so far. The Emde degradation of hydrofluorocurine Emde base, and the oxidation of the latter to methylethylacetic acid, may then be represented in the following way:



XXVI
Hydrofluorocurine

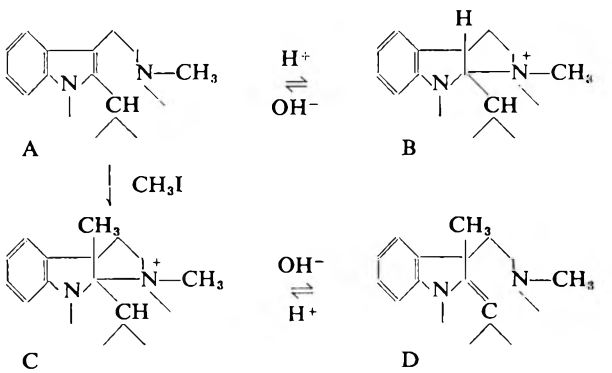
XXVIa
Hydrofluorocurine Emde base

According to this formulation, positions 1 and 15 in fluorocurine and mavacurine are connected by a carbon-carbon bridge, leading to the formation of heterocyclic 7-membered rings. Calotte models show that such structures are sterically possible.

In the same way that hydrofluorocurine XXVI is transformed by hydrogen ions into the indole derivative mavacurine, hydrofluorocurine

Emde base XXVIa undergoes rearrangement to a corresponding indole derivative under the influence of acids.

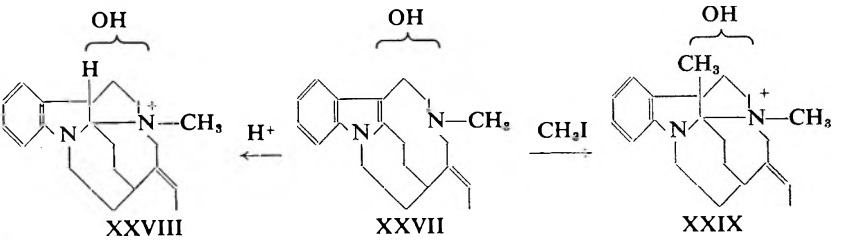
In favour of the existence of the 6-membered ring D in the two alkaloids the following further arguments can be mentioned. In the hydrogenation with platinum and hydrogen in 0·1N ethanolic potassium hydroxide mavacurine also undergoes an Emde degradation, whereby one mol. H_2 is taken up. But the reduction product contains—in contrast to the Emde base obtained from norhydrofluorocurine—only one methyl-group attached to a carbon-atom, because only one molecule of acetic acid is formed in the oxidation. The new Emde base has a typical indole spectrum, while its salts with acids possess an indoline spectrum. The same indoline spectrum was also observed in an addition product of CH_3I to the Emde base. These shifts in the spectra must be caused by a correlation of the indole chromophor and the $N_{(b)}$ of the alkaloid and they can be regarded as reversible, transannular interactions in the sense of the following formulæ:



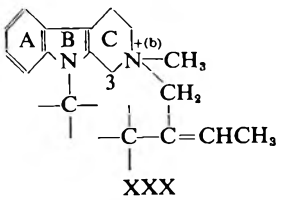
The addition product of CH_3I to the Emde-base A can be written corresponding to formula C, which like formula B, contains the indoline chromophor, while substance A, as we mentioned, possesses an indole spectrum. We could unambiguously prove that in compound C the newly entered CH_3-group is really attached to a carbon- and not to a nitrogen atom. We combined substance A with radioactive CH_3I which contained ^{14}C, and afterwards oxidised the formed addition-product with chromic acid; then the whole radioactivity was found in the acetic acid which was produced by the oxidation. Therefore the radio-active CH_3-group must have been fixed to a carbon atom.

So we come to the conclusion that during the transformation of the Emde-base XXVII into its salt XXVIII or into the methyliodide-addition product XXIX, a transannular interaction takes place which may be represented by formulæ XXVII-XXIX.

Now it is well known that such transannular interactions only occur in 8, 9 and 10 membered rings and that they consist in the formation of a new ring with 5 or 6 ring atoms. In the partial formula XXX of mava-curine, the new ring D which is to be attached to ring C can only be 5- or 6-membered. A further conclusion is, that the new ring D must be



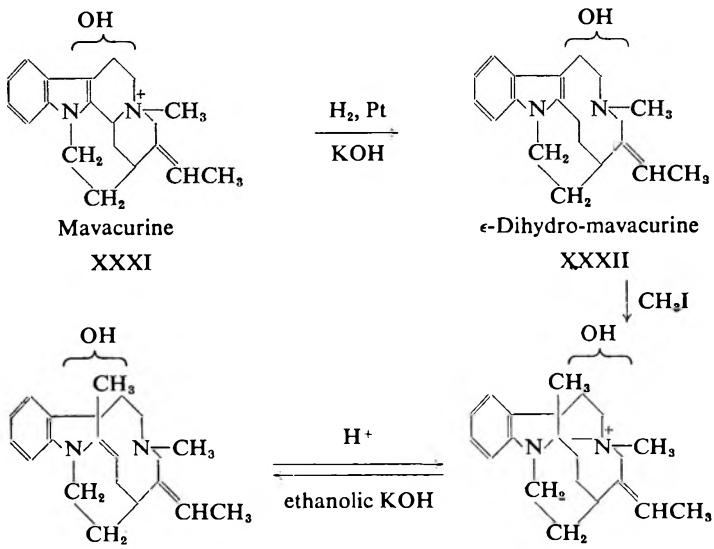
XXVIII XXVII XXIX



XXX

fixed to the carbon atom 3 of ring C. Finally we may conclude that in the Emde degradation of mavacurine the bond between carbon atom 3 and nitrogen atom b (C_3–$N_{(b)}$) is split off.

The Emde-degradation of the mavacurine can therefore be succinctly summarised by the formulæ XXXI-XXXIV:



Mavacurine — ε-Dihydro-mavacurine

XXXI XXXII

(Methylene-indoline spectrum) (Indoline-spectrum)

XXXIII XXXIV

A whole series of other transformations have been carried out with the new mavacurine and fluorocurine derivatives, but it is not possible to give details of these here. It is obvious that these substances are representatives of a new type of indole alkaloid, which is characterised by its ability to undergo a large number of rearrangements.

PHARMACOLOGY OF CURARE[47–58]

I would now like to turn briefly to the pharmacology of curare. Curare acts at the connection between the motor nerves and the muscle fibres, the so-called motor end-plates. It thereby represses or prevents

the impulse transmission from the nerve to the muscle, but does not change the conductivity of the nerve fibre itself nor the action potential of a directly stimulated nerve. The transfer of the excitation on to the muscle fibre results under normal conditions from the effect of the acetylcholine formed by the excitation of the nerve. If the muscle has been previously curarised and the nerve then excited, acetylcholine is still produced but this cannot reach the receptor cells and the muscle which therefore remains relaxed. The threshold of excitation of the muscle for the acetylcholine set free has been very much raised by the effect of curare. By using substances which repress cholinesterase and which therefore increase the duration of acetylcholine activity (for instance eserine or neostigmine) the curarisation of the muscle can be interrupted.

During the last ten years compounds with curare activity have aroused great medical interest.

TABLE IV

ACTIVITIES OF CURARE ALKALOIDS IN THE MOUSE TEST

C-Alkaloid-chlorides	Head-drop dose (HD) µg./kg.	Righting reflex abolished µg./kg.	Min. LD µg./kg.	$\frac{\text{Min. LD}}{\text{HD}}$	Time of paralysis min.
E	0·3–4·0	0·5 –5·0	0·95–8·0	3·2	18
G	0·6–5·0	0·65–7·0	0·7–12·0	1·2	7
Toxiferine	9	12	23	2·5	12
H	16	21	24	1·5	3·7
Curarine	30	35	50	1·7	4
Dihydro-toxiferine (K)	30	40	60	2·0	5·5
A	70	100	150	2·1	2
F	75	85	120	1·6	1·3
I	174	180	195	1·1	2·75
Calebassine	240	260	320	1·3	3
C	240	280	380	1·6	2·75
B	280	310	350	1·25	1·3
J	290	460	560	1·9	1·5
D	1100	1600	2000	1·8	1·3
Fluorocurarine	1800	2100	4000	2·2	2
L	1900	2200	2400	1·3	2
Fluorocurinine	2750	2900	3250	1·2	1·2
Fluorocurine	4400	5500	8000	1·8	1·5
Calebassinine	22,000	25,000	44,000	2	0·3
(+)-Tubocurarine chloride	75	100	130	1·7	1

Griffith and Johnson[59] in 1942 showed that curare compounds during anæsthesia caused complete relaxation of the skeletol muscle, thereby enabling the quantity of the anaesthetic to be reduced.

To-day it is very often applied in operations of the stomach and abdomen and in thoracic surgery, often being combined with simultaneous artificial respiration of the patient. It is useful but less important for prevention of spasms during leptazol and electro-shock. The introduction by Wintersteiner of a pure standardised curare preparation, the (+)-tubocurarine, was the beginning of its general clinical application. Good curare preparations show practically no side effects, the circulation especially remains unaffected.

For instance C-toxiferine can be given to an animal, undergoing artificial respiration, in a hundred times greater doses than the paralysing dose without it suffering any change of blood pressure or heart frequency.

The curarisation symptoms of a mouse or a rabbit begin with unsteady, trembling steps. Then the so-called head-drop symptom is seen, that is

the head cannot be held in the normal manner and slowly drops. As paralysis proceeds the animal falls in a lateral position in which it can be moved without resistance. Death then occurs as a result of paralysis of the respiratory muscles, or with small doses a recovery is made in which the various stages of paralysis are undergone in reversed order.

The calabash curare alkaloids isolated by us show very different toxicities. They lie between 0·3 μg. to 20 mg./kg. mouse corresponding to a ratio of 1 : 10^5 (Table IV).

It is of interest to observe that the highly active alkaloids have small R_c values on the paper chromatogram, that is they travel only a short distance. The far moving alkaloids like C-fluorocurine, C-fluorocurarine, C-fluorocurinine, calebassine, C-alkaloid L and mavacurine show none at all or only very small curarising effects. Within the different groups of the highly active alkaloids the biological activity decreases with increasing R_c values or increasing partition coefficient between the organic phase and water respectively. It appears therefore that the intensity of the curare activity is influenced by this partition coefficient, that is by a high water solubility (Table V).

TABLE V

CONNECTION BETWEEN ACTIVITY AND R_c VALUES OF SOME CURARE ALKALOIDS[23]

Group	C-alkaloid-chlorides	Head-drop dose μg./kg.	R_C
Curarine-group	E	0·3–4·0	0·36
	G	0·6–5·0	0·65
	curarine	30	1·0
Toxiferine-group	toxiferine	9	0·42
	H	16	0·71
	dihydro-toxiferine (K)	30	1·22
Calebassine-group	A	70	0·23
	F	75	0·49
	I	174	0·89
	calebassine	240	0·80
B,C,D-group	C	240	0·34
	B	280	0·34
	D	1100	0·35

In Figure 2 the activities of the different alkaloids are plotted taking the head-drop and lethal doses on the abscissæ and the duration of paralysis and a dose lying in between as the ordinate, both scales being logarithmic.

Each rectangle corresponds to an alkaloid, the abscissæ representing the therapeutic range and the ordinate the intensity of paralysis. Alkaloids with large rectangles, in particular C-curarine, C-toxiferine, and the C-alkaloids E, G and K, are especially fitted for curarising mice, for they show a strong paralysing activity and a large therapeutic range. Seven of the alkaloids investigated are more active than tubocurarine, for instance C-alkaloid E 250 times, C-alkaloid G 120 times. One gram of E would paralyse at least 100 tons of mice.

The importance that curare has recently gained in medicine has led numerous investigators and many pharmaceutical companies to produce synthetic curare-like substances. With the exception of the erythrina alkaloids, which have the character of tertiary bases, the naturally occurring curare alkaloids are quaternary or bi-quaternary ammonium salts.

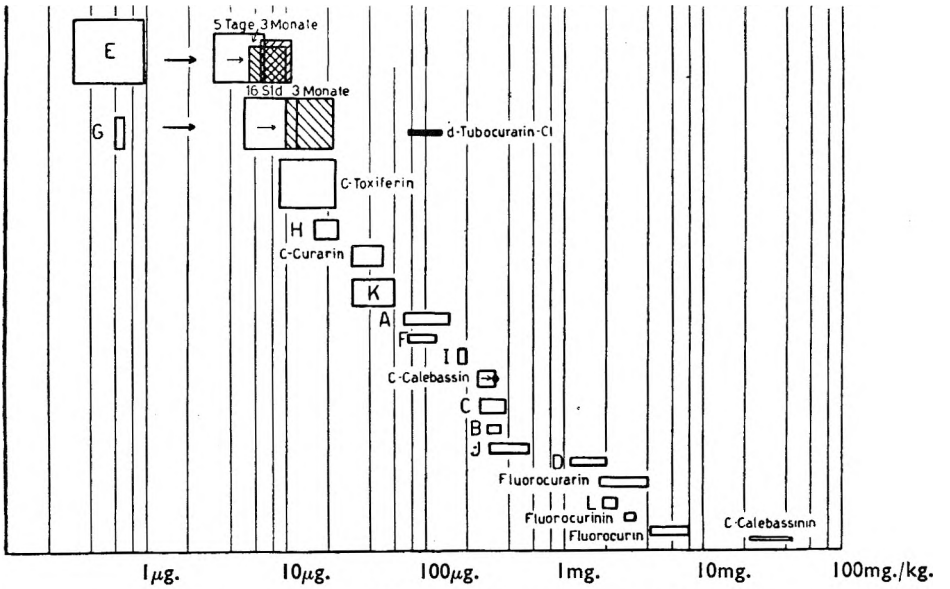


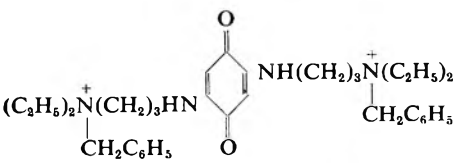
FIG. 2. Activities of the alkaloids and duration of paralysis.

This has led to attempts to synthesise various kinds of quaternary ammonium salts.

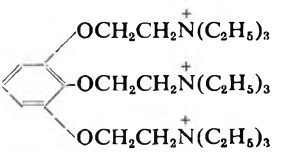
To many of these synthetic works the formula of (+)-tubocurarine served as a model, the main property of which is the two quaternary ammonium salt groups. Thus many compounds have been synthesised containing two ammonium salt groupings. Among these the following ones, because of their relatively high activity have found limited clinical application:

Decamethonium
Barlow and Ing[60]
Paton and Zaimis[61]

Succinylcholine
Ginzel, Klupp, Werner[62]



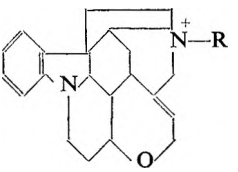
Mytolon
Cavallito, Soria, Hoppe[63]



Gallamine
Bovet[64]

In our laboratories a fairly large number of halogen alkylates of strychnidine, dihydrostrychnidine, neostrychnidine and brucidine were synthesised for pharmacological testing. These, like the calabash curare alkaloids, contain only one quaternary ammonium salt grouping. Mention has already been made that C-curarine is probably related to strychnine. Some of the synthesised and pharmacologically tested chloro- and

iodo-alkylates of strychnidine and neostrychnidine have proved to be highly curare active although they do not reach the paralysing activity and toxicity of C-curarine or even C-toxiferine I.



Strychnidine chloro-alkylates

The potency and toxicity of these compounds have been determined (Table VI).

So far little is known of the fate of these alkaloids in the organism and the form in which they are excreted. It has been known for a long time that curare when injected into the blood stream is very rapidly absorbed, whereas when administered by mouth the assimilation takes place only very slowly. The excretion in the urine takes place rapidly. In order to be able to study the excretion and distribution better, a radioactive C-curarine was made in our laboratory by reacting $^{14}CH_3I$ with norcurarine.

TABLE VI

Compound	Active doses for frogs in mg./kg.	Lethal doses for rabbits in mg./kg.
Strychnidine methylchloride	8	1·4
Strychnidine ethylchloride	10	0·6
Strychnidine propylchloride	23	?
Strychnidine butylchloride	14	1·9
Strychnidine benzylchloride	21	1·3
Dihydrostrychnidine methylchloride	35	1·7
Dihydrostrychnidine benzylchloride	28	2·3
C-curarine	0·1	0·03
C-toxiferine	0·005–0·009	0·008–0·012

High doses of this radioactive C-curarine were given to cats. In an experiment during which the normal excretion was maintained, about 25 per cent. of the injected curarine could be detected in the urine two hours after the injection and only 0·5 per cent. in the air breathed out.

TABLE VII

CAT ♂ 3·0 KG. 4·352 MG. RADIOACTIVE C-CURARINE CHLORIDE INJECTED INTRAVENOUSLY (12·2 RE. $10 \cdot 146 \cdot 10^6$ CPM.). TIME OF THE EXPERIMENT 2 HOURS

Organs	Observed activity as a percentage of the applied activity	Curarine in μg. per g. or ml. of the organ
Liver	17·54	9·51
Muscle	12·25	0·41
Gut	4·74	1·21
Kidney	2·87	8·06
Lungs	0·93	2·23
Spleen	0·33	2·09
Heart	0·10	0·34
Thymus	0·06	0·84
Thyroid	0·01	1·10
Ganglion	—	1·84
Suprarenal glands ..	0·01	0·82
Bile	11·01	159·8
Urine	1·4	10·4
Serum	5·54	1·75
Brain	traces	traces
CO_2 expired	traces	traces
	56·8	

In a second experiment the normal excretion of the curarine through the kidneys had been rendered impossible by a shunt of the kidney ducts. Now the injected alkaloid was found to be distributed over muscles, liver, intestine, kidneys and bile. Referred to the weight the muscles had taken up only a little curarine and in the air breathed out there were only traces. This is shown in Table VII.

Acknowledgement

Several pupils of mine participated in the investigations on calabash curare. I mention Prof. H. Schmid, who took part in these investigations from their beginning, Dr. P. Waser, Dr. A. Ebnöther, Dr. J. Kebrle, Dr. H. Asmis, Dr. H. Bickel, Miss E. Bächli and H. Meyer, to all of whom I offer my thanks for their assistance and help.

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RESEARCH PAPERS

PHOTOMETRIC DETERMINATION OF 2:4-DIAMINO-5-PHENYLTHIAZOLE

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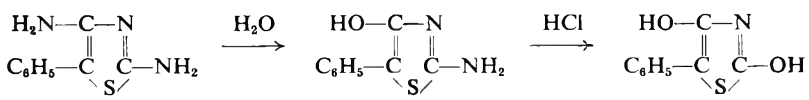
From the Apotekens kontrollaboratorium, Stockholm

Received December 11, 1955

The use of 2:4-diamino-5-phenylthiazole (amiphenazole, fenamizol, D.A.P.T.), as a morphine antagonist was reported by Shaw and Bentley in 1949[1] and 1952[2] and by Shaw and Shulman[3] in 1955. Amiphenazole has also been used by Shulman *et al.*[4] as a synergist with β-methyl-β-ethylglutarimide (4-methyl-4-ethyl-2:6-dioxopiperidine, bemegride, NP 13) in the treatment of barbiturate intoxication. In the early part of 1955 amiphenazole was studied by Canbäck, and others, in this laboratory[5–7]. During this work a rapid reliable method was required to investigate the stability of amiphenazole in pharmaceutical preparations.

From an analytical point of view, published papers offer little about amiphenazole. From a paper by Davies *et al.*[8], dealing with the synthesis and properties of 2:4-diaminothiazoles, it is known that amiphenazole is a monoacidic base [m.pt. of the picrate 189° to 191° C. (decomp.)]. Its soluble salts with halogen acids are white crystalline powders, stable to air. During the isolation of the free base, atmospheric oxidation readily produces tars. An aqueous solution of the hydrobromide or hydrochloride of amiphenazole with a slight excess of sodium hydrogen carbonate or ammonia solution deposits the base, which slowly becomes yellow and then brown. Amiphenazole is almost completely devoid of aromatic character; it neither forms Schiff's bases with aldehydes nor undergoes diazotisation, and it does not give the carbylamine reaction.[8]

In aqueous solution the halogen salts of amiphenazole slowly undergo hydrolysis, to which the 4-amino-group is extremely sensitive. By refluxing a 5 per cent. aqueous solution of the amiphenazole hydrobromide for 3 hours Davies *et al.*[8] prepared 2-amino-4-hydroxy-5-phenylthiazole. Finally, both amino-groups can be replaced by hydroxy-groups through acid hydrolysis (refluxing of the amiphenazole hydrochloride for 5 hours with diluted hydrochloric acid (1 + 2) gave 2:4-dihydroxy-5-phenylthiazole[8]).



2:4-diamino-5-phenylthiazole — 2-amino-4-hydroxy-5-phenylthiazole — 2:4-dihydroxy-5-phenylthiazole

An assay method was required for amiphenazole, specific enough to determine the parent compound in the presence of its hydrolysis products. During the introductory investigations, which will be briefly discussed

below, amiphenazole was found to give a sparingly soluble salt with Reinecke's acid. This reaction was applied to quantitative work.

Experimental

As mentioned above, amiphenazole is a monoacidic base (pKa = 7·0)[9], which can be titrated in anhydrous acetic acid with acetous perchloric acid. This method also permits a direct titration of the hydrogen halides of amiphenazole after converting the halide salts into acetates by adding an excess of mercuric acetate dissolved in acetic acid[10]. This method has, however, two drawbacks: it is not applicable to aqueous solutions of amiphenazole and it is not specific. Efforts to extract the amiphenazole with ether or chloroform after adding an excess of ammonia solution resulted in yellow-coloured solutions of the base in the organic solvent. By the titration with acetous perchloric acid after evaporating the solvent in vacuum the visual end-point was diffuse and obscure. This trouble may be attributed to the presence of 2-amino-4-hydroxy-5-phenylthiazole formed during the extraction procedure. This compound is too weakly basic to be titrated with accuracy with acetous perchloric acid using a visual end-point.

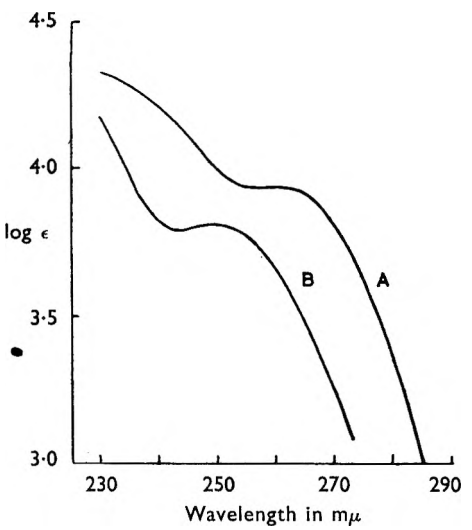


FIG. 1. Absorption curves of 2:4-diamino-5-phenylthiazole hydrochloride (A) and 2-amino-4-hydroxy-5-phenylthiazole (B) in aqueous solutions at pH 6.

The application of ultraviolet spectrophotometry to the determination of amiphenazole was also studied. The absorption curves of amiphenazole hydrochloride and 2-amino-4-hydroxy-5-phenylthiazole in aqueous solutions were recorded with a Beckman Model DU Spectrophotometer, and the effect of pH on these curves was studied. In Figure 1 the absorption curves at pH 6 are plotted. The absorption data, obtained by these measurements, did not give an ideal basis for a spectrophotometric determination of amiphenazole in presence of its hydrolysis products.

The reactions of amiphenazole with some colour forming reagents were also investigated. For instance amiphenazole may be coupled to diazotised *p*-aminoacetophenone in alkaline solution giving a yellow-coloured product. The efforts to apply this reaction to quantitative work were, however, not successful. Among tested reactions precipitation of amiphenazole by Reinecke's salt from aqueous solution was

found to be the most promising one. The precipitation of amiphenazole as reineckate was studied in detail and led to the photometric method of analysis described below.

Photometric determination of amiphenazole as reineckate

Reagents. Reinecke's salt, $NH_4[Cr(CNS)_4(NH_3)_2]\cdot H_2O$. Reinecke's salt reagent: A saturated aqueous solution, prepared by shaking 1 part of Reinecke's salt with 25 parts of distilled water. 2:4-Diamino-5-phenylthiazole hydrochloride, synthesised in this laboratory. The substance was estimated by "the acetous perchloric acid method" discussed above.

Preparation and analysis of the 2:4-diamino-5-phenylthiazole reineckate

To an aqueous solution of 2:4-diamino-5-phenylthiazole hydrochloride was added an excess of Reinecke's salt reagent. The amiphenazole reineckate, a light red, fine-grained precipitate, was collected in a G4 sintered glass crucible and washed with cold water. The precipitate was finally dried in vacuum over phosphorous pentoxide to a constant weight. Melting point: 163° to 165° C. (corr.).

The composition of the precipitate was checked by carefully charring the precipitate and then igniting the residue of chromium (III) oxide to a constant weight. 2:4-Diamino-5-phenylthiazole reineckate: $C_{13}H_{20}N_{10}S_5Cr$ Mol. wt. 510·64. The content of chromium (III) oxide found = 15·67 per cent.; calculated 14·89 per cent.

The solubility of the amiphenazole reineckate at 0° C. was also determined. Unsaturated and supersaturated aqueous solutions were shaken at a temperature of an ice-water mixture. The two methods gave similar results. Solubility: 1 part in 3000 parts of water at 0° C.

Absorption data for the solution of amiphenazole reineckate in acetone

The reineckate of amiphenazole is readily soluble in acetone, giving a red-coloured solution. The absorption curve of the acetone solution in the 470 to 580 mμ region is shown in Figure 2. The curve has a peak at 525 mμ, a molecular extinction coefficient (ϵ) = 106·3. The curve in Figure 2 is recorded with a Beckman Model DU Spectrophotometer.

The acetone solution of amiphenazole reineckate is stable for at least three hours at room temperature.

Effect of various experimental conditions on the precipitation and isolation of the amiphenazole reineckate

A quantity of a solution, containing about 30 mg. of 2:4-diamino-5-phenylthiazole hydrochloride, was measured into a small beaker and placed in an ice bath. When the solution had assumed the temperature of the bath, 5 ml. of Reinecke's salt reagent was added. The mixture was kept in the ice bath for a given time and then the precipitate collected in a G4 sintered glass crucible and washed. The reineckate was dissolved by pouring acetone on the precipitate. The solution was then slowly filtered and finally diluted to 25 ml. The extinction at 525 mμ was measured.

The following variables in the procedure were checked: (1) the time of cooling the ice bath after precipitation, (2) the washing procedure, and (3) the effect of variations in the concentration of the amiphenazole hydrochloride solution. The experiments showed, that after addition of the Reinecke's salt reagent the mixture should remain in the ice bath for at least 15 minutes. A prolongation of this time to two hours did not affect the result, while collecting the precipitate after only 5 minutes in the ice bath gave a slightly lower result.

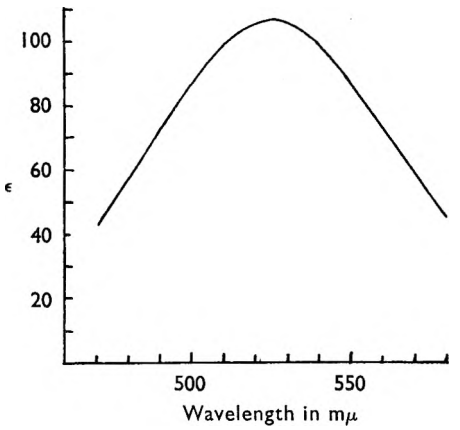


FIG. 2. Absorption curve of 2:4-diamino-5-phenylthiazole reineckate in acetone.

As shown above, the reineckate of amiphenazole is slightly soluble in water at 0° C. The effect of the solubility of the reineckate was checked by varying the washing procedure. After the precipitate had been collected in the sintered glass crucible, the beaker was rinsed with the wash liquor, which then was filtered through the crucible. The following wash liquors were used: (*a*), 2 × 2 ml. of water at room temperature (the water was however slightly cooled by rinsing the cold beaker), (*b*), 2 × 2 ml. of iced water, (*c*), 2 × 2 ml. of an iced, saturated, aqueous solution of amiphenazole reineckate, and (*d*), 2 × 5 ml. of the same wash liquor as in (*c*).

In all instances similar results were obtained. Even in variations (*a*) and (*b*) the wash water only dissolved negligible amounts of reineckate during the short time of washing. These experiments also showed that 2 × 2 ml. of wash liquor is sufficient. As the colour depends exclusively on the reineckate moiety it is essential that the precipitate is thoroughly washed.

Finally the effect of the concentration of the amiphenazole hydrochloride solution to be precipitated was checked. Solutions of about 30 mg. of the amiphenazole hydrochloride in 2, 5, 10 and 15 ml. of water were estimated. Similar results were obtained in all cases. The volume of the amiphenazole test solution taken may consequently vary between 2 and 15 ml. without any serious effects.

PROCEDURE

Measure 2 to 15 ml. of solution, containing between 15 and 30 mg. of 2:4-diamino-5-phenylthiazole hydrochloride (or hydrobromide), into a small beaker and place it in an ice bath. When the solution is cooled to the temperature of the bath add 5 ml. of Reinecke's salt reagent, mix and allow to stand in the ice bath for 15 minutes. Collect the precipitate in a G4 sintered glass crucible, wash the precipitate twice with 2 ml. of iced water, rinsing the beaker, and dry by suction.

Dissolve the precipitate in acetone by drawing the solvent slowly through the filter under negative pressure and dilute to 25 ml. with acetone. Measure E (1 cm.) at 525 mμ against water, and calculate the amount of 2:4-diamino-5-phenylthiazole hydrochloride present with the aid of a calibration curve.

The calibration curve is linear over the range 0 to at least 35 mg. of amiphenazole hydrochloride. The values in Table I have been obtained with a Beckman Model B Spectrophotometer.

TABLE I

THE RELATION BETWEEN E (1 CM.) AT 525 Mμ AND THE AMOUNT OF 2:4-DIAMINO-5-PHENYLTHIAZOLE HYDROCHLORIDE PRECIPITATED AS REINECKATE AND DISSOLVED IN 25 ML. OF ACETONE

Amiphenazole hydrochloride mg.	Extinction against water
12·4	0·240
18·1	0·360
20·9	0·415
24·1	0·475
27·8	0·550
34·8	0·678

DISCUSSION

The deterioration of aqueous solutions of 2:4-diamino-5-phenylthiazole hydrochloride, which occurs during storage, depends on the replacement of the 4-amino-group by a hydroxyl group. The 2-amino-4-hydroxy-5-phenylthiazole, which is the hydrolysis product, forms no precipitate with Reinecke's salt and consequently does not affect the determination of amiphenazole. By the estimation of a saturated aqueous solution of 2-amino-4-hydroxy-5-phenylthiazole, to which 0·5 per cent. of amiphenazole hydrochloride was added, the calculated amount of amiphenazole was found.

Moderate amounts of glycerol and ethanol (e.g., 5 per cent. of glycerol and 15 per cent. of ethanol in a 1·5 per cent. solution of amiphenazole hydrochloride) or benzyl alcohol and propylene glycol (e.g., 2 per cent. of benzyl alcohol and 60 per cent. of propylene glycol in a 1·5 per cent. solution of amiphenazole hydrochloride) do not interfere with the determination of amiphenazole. On the other hand, the test solution must not contain polyethylene glycol. As mentioned above, amiphenazole is used in the treatment of barbiturate intoxication as a synergist with β-methyl-β-ethylglutarimide. The latter compound does not interfere with the determination of amiphenazole.

An investigation of the stability of solutions of amiphenazole hydrochloride for injection is under way in this laboratory and will be published later. However, some preliminary statements of the stability of aqueous solutions will be given here. At room temperature 2 or 3 per cent. decomposition of amiphenazole in aqueous solution was found after only one week. The hydrolysis proceeded during the storage and reached the figure of about 20 per cent. after five weeks. The solution then also contained light yellow crystals of 2-amino-4-hydroxy-5-phenylthiazole. On the other hand, a refrigerated solution was stable for five weeks.

SUMMARY

1. A photometric method for the determination of 2:4-diamino-5-phenylthiazole (amiphenazole) hydrochloride (or hydrobromide) in aqueous solution has been described. It depends on the precipitation

of the compound as an insoluble reineckate. The hydrolysis products of amiphenazole have been found not to affect the determination of the compound.

2. Some other methods of determination of amiphenazole have been briefly discussed. Examples are, the titration with acetous perchloric acid and the application of ultra-violet spectrophotometry.

3. Some preliminary statements of the stability of amiphenazole in aqueous solution have been given.

My thanks are due to Dr. T. Canbäck, Head of this Laboratory, for valuable suggestions and discussions during the work.

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RELATIONS BETWEEN BACTERIOSTATIC ACTIVITY AND CHEMICAL CONSTITUTION OF CERTAIN ACRIDINE ANALOGUES OF BASIC DI- AND TRIPHENYLMETHANE DYES

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2:8-DIAMINOACRIDINE derivatives can be derived from basic di- and triphenylmethane dyes by joining positions 2 and 6′ by an imine bridge. Such derivatives can be considered as the acridine analogues of the corresponding basic di- and triphenylmethane dyes, belonging simultaneously to the acridine and the *p*-diamino-di- or triphenylmethane series. The bacteriological study of these compounds (Table I) is of interest because differences exist between some characteristic features of the acridine type bacteriostasis and the corresponding triphenylmethane effect.

One of these is the influence exerted on bacteriostatic activity by alkylating the amino groups. Methylation or ethylation increases the bacteriostatic effect in the di- and triphenylmethane series substantially[1–5], whereas no such increase has been observed in the acridine series[1]. For example, Albert *et al.*[6] found that the bacteriostatic power of acridine orange, a methylated homologue of 2:8-diaminoacridine (Table I) was not superior to that of the non-methylated compound proflavine.

A second difference is the number of benzene rings necessary for optimal bacteriostatic action. It was shown by Fischer, Garcés and López[7] that diphenylmethanes, such as Michler's hydrol and auramine, are nearly one hundred times less active than the corresponding triphenylmethanes, thus confirming the observations of Kliegler[2] on auramine, while the contrary seems to apply to the members of the acridine series. According to Albert *et al.*[6] the introduction of a phenyl radical in the position 5 of acridine yellow (2:8-diamino-3:7-dimethylacridine) causes a decrease of bacteriostatic potency, the derivative, benzoflavine (2:8-diamino-3:7-dimethyl-5-phenylacridine), an acridine analogue of the triphenylmethane dyes, being less active than acridine yellow. This circumstance is attributed by Albert *et al.* to a dystherapeutic dimensional factor.

The third difference between acridine and triphenylmethane bacteriostasis is the effect of serum on activity. Its presence increases the action of acridines, but inhibits that of diphenyl- or triphenylmethanes[1].

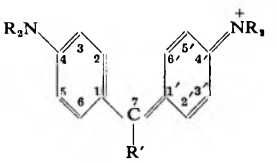
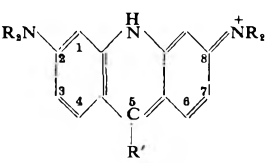
EXPERIMENTAL

In the first series of experiments the bacteriostatic effects of 2:8-diaminoacridine (proflavine), 2:8-bis(dimethylamino)acridine (acridine orange), 4:4′-diaminobenzhydrol and 4:4′-bis(dimethylamino)benzhydrol (Michler's hydrol) were compared using a culture of *Staphylococcus aureus*. The structural relation of these dyes is shown in Table I. Acridine orange possesses dimethylated amino groups, and is the higher homologue

of proflavine in the 2:8-diaminoacridine series. The same relation exists between 4:4′-diaminobenzhydrol and Michler's hydrol in the diphenylmethane series. The immonium cation forms of the two hydrols are analogues of the two acridine derivatives.

TABLE I

STRUCTURAL RELATION OF THE DI- AND TRIPHENYLMETHANE DERIVATIVES TO THEIR ACRIDINE ANALOGUES

	 4:4′-diaminodi- and triphenylmethanes (immonium cation forms)	 2:8-diaminoacridines (immonium cation forms)
R=H R′=H	Immonium cation of 4:4′-diamino-benzhydrol	Proflavine
R=CH_3 R′=H	Immonium cation of Michler's hydrol	Acridine Orange
R=CH_3 R′=C_6H_5	Malachite Green	Brilliant Acridine Orange

The diphenylmethane derivatives are prepared according to the methods given in our earlier publications[5,7]. Commercial proflavine (May and Baker) and acridine orange (Grübler) were used. The substances were dissolved in 50 per cent. aqueous ethanol (0·1 g. in 20 ml.) the necessary dilutions being obtained by mixing the stock solutions with the required amounts of broth. The technique of the bacteriological experiments was the same as in our earlier work[4,5,7].

TABLE II

GROWTH OF *Staphylococcus aureus* IN BROTH IN THE PRESENCE OF TWO TRIPHENYLMETHANE DERIVATIVES AND THEIR ACRIDINE ANALOGUES

Dilutions	Diamino-benzhydrol		Michler's hydrol		Proflavine		Acridine orange	
		+ 20 per cent. serum		+ 20 per cent. serum		+ 20 per cent. serum		+ 20 per cent. serum
1:5000 ..	±	+	−	−	−	−	−	−
1:10,000 ..	+	+	−	−	−	−	−	−
1:20,000 ..	+	+	−	±	−	−	−	−
1:40,000 ..	+	+	−	+	−	−	+	+
1:80,000 ..	+	+	+	+	+	−	+	+
1:160,000 ..	+	+	+	+	+	+	+	+

Table II shows that our earlier results[5] are confirmed, i.e. in the presence of serum the activity of the diphenylmethanes is reduced and that of the acridines is enhanced while the higher homologues show respectively an increase and decrease in activity.

In further experiments the influence of a third benzene ring on the bacteriostatic activity against *Staphylococcus aureus* was studied comparatively. The introduction of a phenyl radical into the position 7 of

diphenylmethanes transforms them into triphenylmethanes. The corresponding change in the series of 2:8-diaminoacridines is the introduction of a phenyl radical into the position 5 which produces the acridine analogues of the triphenylmethanes (2:8-diamino-5-phenylacridines). As representative members of the *p*-diamino-di- and triphenylmethane series we employed Michler's hydrol and malachite green respectively and the acridines employed were acridine orange and brilliant acridine orange, (2:8-bis-(dimethylamino)-5-phenylacridine). Commercial malachite green (Grübler) was used, brilliant acridine orange was prepared according to German Patent No. 68908 (Leonhardt).

TABLE III

GROWTH OF *Staphylococcus aureus* IN BROTH IN THE PRESENCE OF TWO TRIPHENYLMETHANE DERIVATIVES AND THEIR ACRIDINE ANALOGUES

Dilutions	Michler's hydrol		Malachite green		Acridine orange		Brilliant acridine orange	
		+ 20 per cent. serum		+ 20 per cent. serum		+ 20 per cent. serum		+ 20 per cent. serum
1:40,000 ..	−	+	−	−	+	+	−	−
1:80,000 ..	+	+	−	−	+	+	−	−
1:160,000 ..	+	+	−	−	+	+	−	−
1:320,000 ..	+	+	−	−	+	+	−	−
1:640,000 ..	+	+	−	−	+	+	−	+
1:1,280,000 ..	+	+	−	−	+	+	+	+
1:2,560,000 ..	+	+	−	−	+	+	+	+
1:5,120,000 ..	+	+	−	+	+	+	+	+
1:10,240,000 ..	+	+	−	+	+	+	+	+
1:20,480,000 ..	+	+	+	+	+	+	+	+

Table III gives the results obtained in the bacteriostatic experiments, which confirm our earlier findings[5,7] that the transformation of the diphenylmethane compound into its triphenylmethane homologue causes a spectacular (250 fold) increase of the bacteriostatic effects. A similar but quantitatively not so marked increase is observed in the acridine series, brilliant acridine orange being clearly more active than acridine orange. The bacteriostatic effect of brilliant acridine orange is diminished in the presence of 20 per cent. of serum, like that of the triphenylmethane dyes.

Our results appear to contradict those of Albert *et al.*, according to which the introduction of a phenyl radical into the position 5 of the acridine molecule constitutes a dystherapeutic dimensional factor influencing the bacteriostatic power unfavourably. But different acridine compounds, acridine yellow and benzoflavine, were used by Albert *et al.* and these were not methylated in positions 2 and 8 as were the compounds used by us. Thus it appears that the dimethylation of the amino groups changes the character of the bacteriostatic action from the "acridine-like" type toward a more "triphenylmethane-like" one. Such a supposition would be supported by the fact that the bacteriostatic action of brilliant acridine orange is not enhanced by serum, like that of the acridines with free amino groups, but is reduced, like that of the triphenylmethanes. Therefore the favourable effect of the presence of a third phenyl radical in the molecule of brilliant acridine orange could be explained by the "triphenylmethane like" character of its bacteriostatic action.

If brilliant acridine orange is now considered as a derivative of malachite green it appears that an imine bridge between the positions 2 and 6′ of the latter does not change the character of the bacteriostatic action, which remains "triphenylmethane like," but diminishes its strength to a certain degree.

Similar experiments have been made with other organisms, e.g. *Streptococcus fæcalis*, *Shigella* sp., *Eberthella* sp., *Salmonella* sp. and *Escherichia* sp. The results of these, including those with *Staphylococcus aureus*, are shown in the Table IV. The degree of activity of the assayed substances is expressed by the reciprocal of its highest inhibitory dilution, the subsequent non-inhibitory dilution being always 1 : 2.

TABLE IV

BACTERIOSTATIC ACTIVITIES EXPRESSED IN THE RECIPROCALS OF THE HIGHEST INHIBITORY DILUTIONS

Organisms	Proflavine		Acridine orange		Brilliant Acridine orange		Malachite green	
		+ 20 per cent. serum		+ 20 per cent. serum		+ 20 per cent. serum		+ 20 per cent. serum
Staphylococcus aureus	40,000	80,000	20,000	20,000	640,000	320,000	10 millions	5 millions
Streptococcus fæcalis	80,000	160,000	80,000	160,000	320,000	160,000	2·5 millions	1·25 millions
Shigella sp. ..	40,000	80,000	40,000	20,000	160,000	80,000	640,000	80,000
Eberthella sp. ..	20,000	40,000	10,000	10,000	10,000	10,000	320,000	40,000
Salmonella sp.	10,000	20,000	10,000	10,000	10,000	10,000	160,000	80,000
Escherichia sp.	10,000	20,000	10,000	10,000	10,000	10,000	10,000	10,000

The Table shows that the bacteriostatic action of proflavine is relatively uniform against the organisms employed. Its effect is more pronounced against Gram-positive (and *Shigella* strains) than against Gram-negative organisms, but these differences are not as marked as we are accustomed to see them with triphenylmethane dyes. Furthermore, it can be seen that the bacteriostatic activity of proflavine is enhanced by serum in every instance.

Malachite green is much more selective than proflavine and is always inhibited by serum. The pattern of the malachite green action is closely followed by brilliant acridine orange, whose bacteriostatic effects, though inferior, show a similar selectivity and are always inhibited by serum. The behaviour of acridine orange is not characteristic in any sense. Its activity is weak and the influence of serum on it is variable.

DISCUSSION

The 2 : 8-diamino-acridines may be considered as a special class of 4 : 4′-diaminodi- and triphenylmethane derivatives with an imine bridge between two benzene rings. The bacteriostatic activity of the simpler members of this acridine series is, however, not connected with this structure, as many other acridine derivatives not related to the 4 : 4′-diaminodi- or triphenylmethane compounds, such as 5-aminoacridine are also active[6]. Also the bacteriostatic activity of the simpler acridine derivatives shows some characteristic features which contrast with those

of the di- or triaminophenylmethanes. This "acridine like" type of activity apparently reaches its maximum with compounds without methylated amines and of reduced dimensions, whereas the corresponding di- and triphenylmethanes are almost without activity, the maximum effect being attained only with three benzene rings and two alkylated amines[5].

Albert *et al.*[6] have demonstrated in extensive experiments that the bacteriostatic activity of acridines depends on certain physicochemical properties, among which the degree of cationic dissociation seems to be the most important. According to these authors the influence of chemical configuration on the bacteriostatic strength is exerted principally through the physicochemical changes conditioned by it and modified by dimensional and other factors (cf. also ref. 8). Since the formulation of an earlier but similar theory by Stearn and Stearn[9] several attempts have been made to explain the triphenylmethane activity along the same lines. In our opinion these attempts have been unsuccessful (*cf.* ref. 10). The fact that basic triphenylmethane dyes as well as acridines show stronger action in a more basic medium than in a more acid environment was explained by Stearn and Stearn as the result of a higher ionization of anionic protein groups in relatively alkaline solutions and it was used by these authors as an argument in favour of their theory. This argument was discussed by Ingraham[11] and by Fischer and Muñoz[12]. It seems to us that the higher activity of several alkaline antibacterial agents in a more alkaline medium does not necessarily mean that the bacteriostatic activity of such compounds depends on a reaction with anionic cell constituents but can be otherwise explained, especially in view of experimental findings about the lack of parallelism between basic strength and bacteriostatic activity in the triphenylmethane series (in contrast with the acridine series[5,10]). On the basis of our previous experiments[5] we conclude that the bacteriostatic action of 4:4'-diaminodi- and triphenylmethane derivatives depended on other factors, among which a "potentially quinoid structure" seems to be the most important.

The "triphenylmethane like" character of the bacteriostatic action of brilliant acridine orange is possibly due to the fact that its chemical configuration is nearer to the structure optimal for a "triphenylmethane-like" type of activity than to that which is necessary for the "acridine-like" one.

The decreased activity of benzoflavine in the experiments of Albert *et al.* may be explained similarly, attributing it to the fact that the structure of this compound is not favourable either for an "acridine-like" type of activity because of the presence of a third phenyl radical, nor for a "triphenylmethane-like" one because of the lack of alkylation of the amino groups. This theory could also apply to acridine orange where the unfavourable factor for an "acridine-like" activity is the methylated state of the amino group and at the same time a marked "triphenylmethane-like" action is made impossible by the lack of a third phenyl radical.

Considering brilliant acridine orange from a biological point of view as a triphenylmethane derivative, it can be concluded that the presence of

an imine bridge between two benzene rings, with its inhibiting effect on the rotation of these and increasing the coplanar part of the molecule, does not impede the bacteriostatic effect or change its character, but has only a slightly unfavourable influence on it.

Summary

1. Brilliant acridine orange, a higher homologue of proflavine and an acridine analogue of malachite green, has a bacteriostatic activity, which is markedly superior to that of proflavine and somewhat inferior to that of malachite green.

2. The activity of brilliant acridine orange does not show the "acridine-like" type, but follows closely the patterns of the triphenylmethane action.

3. Acridine orange with only two phenyl radicals, but with methylated amino groups, has a structure which is neither favourable for the "acridine-like" activity, nor for the "triphenylmethane-like" type. Its action is weak and uncharacteristic.

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THE EXAMINATION OF A BACTERIOLOGICAL PEPTONE

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Peptone, by reason of its amino-acid content and other nitrogenous compounds, is an important ingredient of microbiological culture media. A knowledge of the constituent amino-acids and peptides is desirable and is a necessary preliminary to obtaining uniformity in bacteriological media.

Paper chromatography has been used previously for the analysis of both the partial and complete hydrolysates of proteins and solvents such as phenol, collidine[1], butanol-acetic acid-water[2] and butanol-formic acid-water[3] systems have been used. McFarren[4] found that buffering the paper as well as the solvent gave reproducible results. Levy and Chung[5] reported a successful two-dimensional chromatographic separation on Whatman No. 1 paper using butanol-acetic acid-water system as the first solvent, followed by spraying the dried paper with a buffer of pH 8·3 and developing in the second direction with *meta*-cresol-phenol (2 : 1) saturated with a buffer of pH 8·3.

Experimental

Analar chemicals and solvents were used throughout.

Chromatograms were formed by the ascending technique on Whatman No. 1 paper ($11\frac{1}{4}$ in. × $18\frac{1}{2}$ in.) in jars covered by glass plates. A seal was effected with Apiezon grease. The atmosphere within the jar was saturated with the solvent and water vapours by lining the jar with filter paper wetted with water saturated with the solvent[4,6]. When equilibrium was established, the solvent saturated with water or buffer solution was introduced into the dish, in which the chromatogram paper stood, down a thistle funnel passing through the lid and extending into the dish itself. The jars were lagged to reduce temperature variations.

Method

When a known mixture of twenty-two amino-acids was submitted to the separation of Levy and Chung[5] the spots showed "heading". Better results were obtained when a butanol-acetic acid-water mixture[2] was used as the first solvent. The paper was dried at 60° C. for 30 minutes[7], sprayed with a buffer of pH 6·2[4], and again dried. A two-dimensional separation was effected by using phenol saturated with a buffer of pH 6·2 as the second solvent and the positions of the amino-acids were revealed by dipping the dried chromatograms in a 0·25 per cent. solution of ninhydrin in acetone. The paper was finally dried at 60° C. for 15 minutes.

This system gave compact spots and separated methionine from leucine and *iso*leucine, but the separation of these latter amino-acids was

not complete, as shown in Figure 1. When the mixture of amino-acids was oxidised by 30 per cent. hydrogen peroxide[8], cysteic acid occupied a position below ornithine and methionine sulphone, slightly below and overlapping hydroxyproline.

Examination of a Bacteriological Peptone

Three batches A, B and C, of "Oxoid" Bacteriological Peptone (Code No. L 37) were examined qualitatively using the technique described above. Peptone gave a complex chromatogram and therefore the method of Fromageot *et al.*[9] was adopted to separate 1 ml. of a 17 per cent. w/v peptone solution into four fractions corresponding roughly to the acidic, basic, aromatic and neutral amino-acids and peptides. Each fraction was evaporated to dryness below 40° C. under vacuum and the residue dissolved in 1 ml. 10 per cent. *iso*propanol[10]. 0·003 ml. of each solution was applied to a paper buffered at pH 6·2 and developed with phenol saturated with the buffer. Five applications of the solution of the aromatic fraction of batch C were found to be necessary to obtain a sufficiently intense colour reaction. The original peptone and known amino-acid were included for comparison as shown in Figure 2. The three batches gave the same general picture except that batch C showed a more intense basic fraction and a weaker aromatic fraction. Batch B also showed a weak aromatic fraction. When these fractions were subjected to two-dimensional chromatography the pattern of the spots obtained for the basic and neutral fractions of the three batches showed a similar distribution, as illustrated in Figures 3 and 4, differing slightly in the diffuseness of the peptide spots. Free lysine and arginine and a spot corresponding to the position of ornithine were found in each basic fraction. In the neutral fractions serine, glycine, threonine, alanine, valine, methionine, proline, α-aminobutyric acid and leucine and *iso*-leucine were present. The acidic fractions were similar for each batch of peptone and showed a particularly large diffuse area, as in Figure 5. Tyrosine and phenylalanine were present in each of the aromatic fractions but the fraction from batch B did not show the four diffuse areas, numbered 2, 3, 4 and 5 in Figure 6, which were present in the batches A and C.

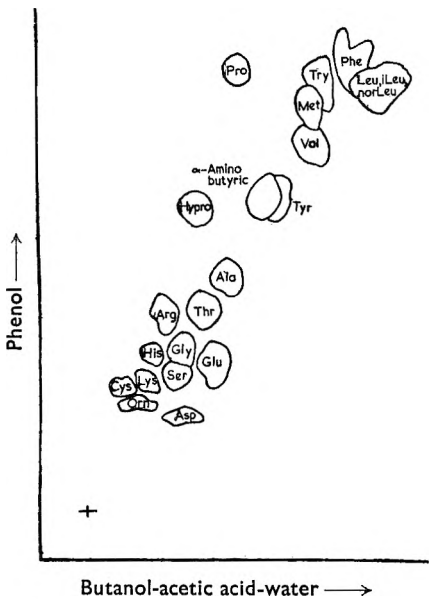

FIG. 1. Two dimensional chromatogram of a known mixture of amino-acids.

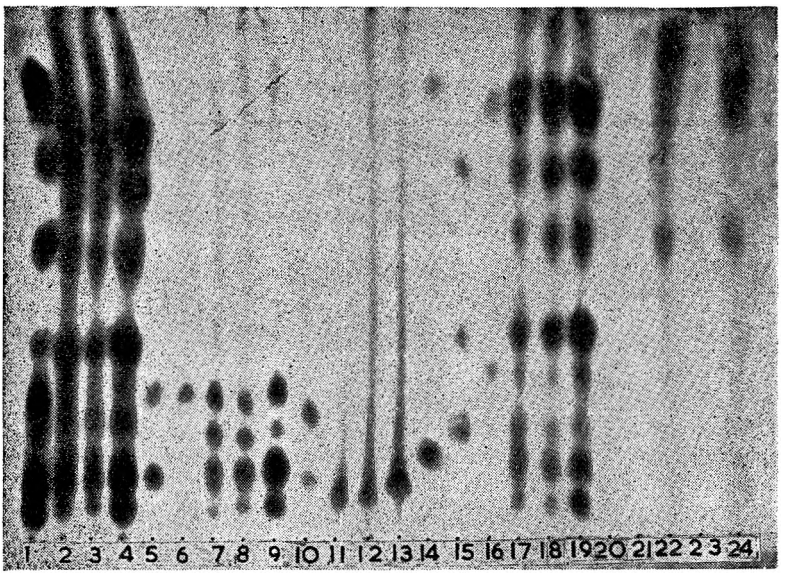

FIG. 2. One dimension chromatogram of peptone and its fractions on buffered paper (pH 6·2).

1. The mixture of amino-acids.
2. Peptone. (Batch A).
3. Peptone. (Batch B).
4. Peptone. (Batch C).
5. Lysine and Arginine.
6. Histidine.
7. Basic fraction. (Batch A).
8. Basic fraction. (Batch B).
9. Basic fraction. (Batch C).
10. Aspartic and glutamic acids.
11. Acidic fraction. (Batch A).
12. Acidic fraction. (Batch B).
13. Acidic fraction. (Batch C).
14. Serine and leucine.
15. Glycine, alanine and valine.
16. Threonine and methionine.
17. Neutral fraction. (Batch A).
18. Neutral fraction. (Batch B).
19. Neutral fraction. (Batch C).
20. Tyrosine and phenylalanine.
21. Tryptophan.
22. Aromatic fraction. (Batch A).
23. Aromatic fraction. (Batch B).
24. Aromatic fraction. (Batch C).

Hydrolysates of the Fractions

Each fraction was hydrolysed by mixing equal volumes of the *iso*-propanol solution with concentrated hydrochloric acid and heating at 100° C. for 15 hours[11]. The hydrochloric acid was removed under vacuum and the dry residue dissolved in 10 per cent. *iso*propanol. Each hydrolysate, and the hydrolysate oxidised with hydrogen peroxide, was submitted to the two-dimensional chromatography described.

The following amino-acids were found in each of the fractions from the three batches of peptone: lysine, arginine, aspartic acid, glutamic acid, serine, glycine, threonine, alanine, hydroxyproline, proline, valine, methionine, leucine, *iso*leucine, phenylalanine and tyrosine. The basic fractions yielded two spots corresponding to ornithine and histidine and the spot due to tyrosine was very faint. Each neutral fraction showed the presence of α-aminobutyric acid and an unidentified spot having an R_p similar to tyrosine in phenol, and similar to alanine in the butanol-acetic acid-water system. Batch B yielded a second unidentified spot with

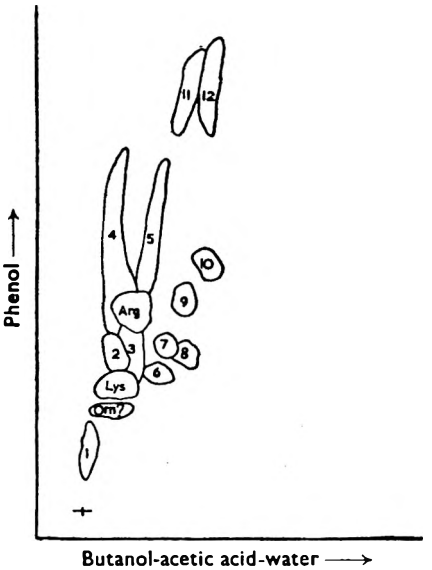

FIG. 3. Two dimensional chromatogram of the basic fraction. (Batch A).

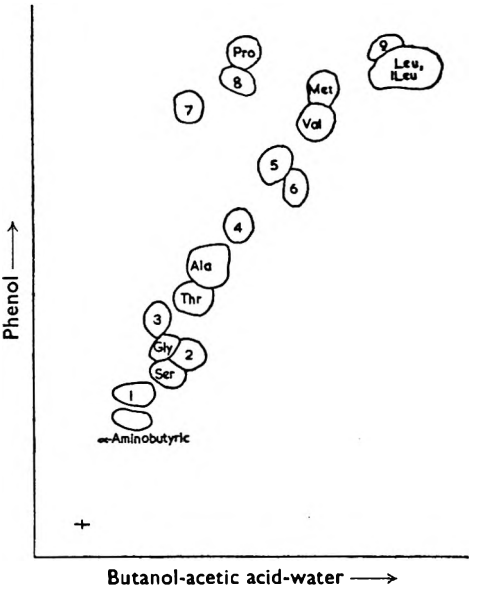

FIG. 4. Two dimensional chromatogram of the neutral fraction. (Batch A).

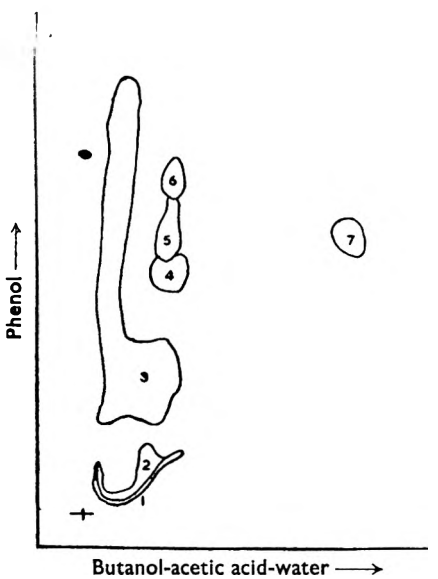

FIG. 5. Two dimensional chromatogram of the acidic fraction. (Batch A).

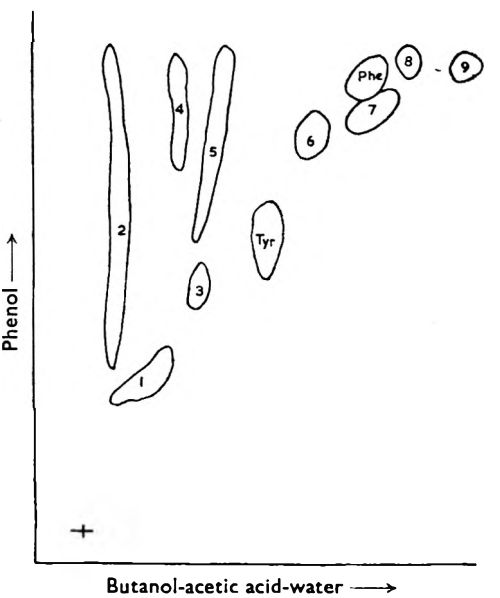

FIG. 6. Two dimensional chromatogram of the aromatic fraction. (Batch A).

an R_F value in phenol similar to valine, and slightly below α-aminobutyric acid in the butanol-acetic acid water system.

In the hydrolysate of the aromatic fraction a faint spot corresponding to ornithine was found but the spots due to tyrosine and phenylalanine were intense. The hydrolysed basic fraction of the peptone gave a more intense reaction for the basic than for the acidic amino-acids, the reverse being true for the acidic peptone fraction.

Tryptophan was found to be present in each batch of peptone when the hydrolysis was effected with barium hydroxide[5].

Discussion

The separation of the peptone into fractions by the method of Fromageot *et al.* will depend largely on the predominating amino-acids which will govern the character of the peptide, and so it is possible, for example, to obtain basic peptides containing acidic amino-acids. The adsorption on charcoal of peptides containing aromatic residues was not found to be complete; a similar result was obtained by Sanger and Tuppy[12]. Very little difference in the amino-acid content was found between the three batches of peptone which were examined qualitatively although there appears to be some variation in the peptide content. The chromatographic patterns obtained by one and two-dimensional chromatography may afford a convenient method of comparing and standardising peptones for the ninhydrin-positive substances. The variation in the spots, corresponding to peptides, may be due to variations in the manufacturing procedure.

Summary

1. The use of butanol-acetic acid-water system as the first solvent, spraying the dried paper with a buffer of pH 6·2 and developing in the second direction with phenol saturated with buffer pH 6·2 gave a well defined separation of a known mixture of twenty-two amino-acids.

2. One and two-dimensional chromatograms of the separated basic, acidic, neutral and aromatic fractions of the peptones gave a number of free amino-acids, but variations occurred in the ninhydrin-positive spots due to peptides of the three batches of bacteriological peptone examined.

3. The hydrolysed peptone yielded 20 amino-acids and one unidentified spot in all the three batches and a second spot in Batch B.

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MICROCHEMICAL IDENTIFICATION OF LOCAL ANÆSTHETIC DRUGS

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During the last fifty years the search for a substitute for cocaine has resulted in the introduction of a number of basic nitrogenous compounds that may conveniently be regarded as synthetic alkaloids. Although these substances are seldom the cause of poisoning except in circumstances where the toxic agent is beyond doubt, their identification is a matter of some importance. Like the plant alkaloids, they may be extracted from viscera by the Stas-Otto process, and yield crystalline precipitates with many of the alkaloidal reagents. Unless they can be identified with complete certainty, considerable confusion may be caused through traces of one of these substances, possibly of therapeutic origin, being mistaken for one of the more toxic vegetable bases.

The reactions of procaine, one of the first of these drugs to come into general use, have been studied in detail by Fulton[1], while tests for a number of the others have been described by Fischer[2], Offerhaus and Baert[3,4,5], Wagenaar[6], Whitmore and Wood[7], Sabon and Grignon[8], Hucknall and Turfitt[9] and Bamford[10]. Some of these tests require milligram quantities of material, while others entail special procedures. The work to be described was undertaken not only to investigate the reactions of some of the newer local anæsthetics, but also to provide a technique, based on normal alkaloidal tests, for identifying these drugs when present in μg. quantities.

Experimental Procedure

Microcrystalline tests

The hanging-microdrop technique described by Clarke and Williams[11] was used. In addition to the reagents there described, a saturated aqueous solution of trinitrobenzoic acid was employed.

The results obtained for 21 drugs are given in Table I. Owing to the importance of distinguishing between these drugs and the plant alkaloids all crystalline precipitates, not merely the most distinctive, have been recorded. Thus it is usually considered that the formation of plates with zinc chloride solution is a specific test for papaverine, whereas the Table shows that crystals very similar in shape are also formed with butacaine.

The descriptions given in the Table are intended to serve as a rough guide only. Final identification depends upon comparing the crystals formed from the test material with those formed from a known sample of the drug.

Colour tests

These are of less value than the crystal tests. Neither sulphuric acid alone nor sulphuric acid in combination with formaldehyde, ammonium

TABLE I

MICROCRYSTALLINE TESTS

Reagent	Crystals	Sensitivity in μg.
Amethocaine		
Gold bromide	Fine irregular crystals	0·025
Gold bromide hydrochloric acid	Plates and feathery needles	0·025
Lead iodide	Clusters of small plates	0·05
Mercuric chloride	Small oily crystals	1·0
Picric acid	Rosettes of fine needles	1·0
Potassium chromate	Sheaves of long needles	0·05
Potassium iodide	Needles and blades in rosettes	0·05
Trinitrobenzoic acid	Yellow rods and plates	0·25
Amydricaine		
Gold bromide	Irregular blades	0·25
Gold bromide hydrochloric acid	Bunches of irregular crystals	0·05
Gold chloride	Tufts of curved needles	0·1
Lead iodide	Serrated needles	0·05
Picric acid	Branching needles	0·05
Potassium bismuth iodide	Irregular blades and needles	0·025
Potassium chromate	Yellow plates	0·5
Potassium iodide	Small needles	0·25
Potassium permanganate	Needles and serrated plates	1·0
Potassium tri-iodide (1)	Blades and plates	0·025
Potassium tri-iodide (3)	Serrated yellow blades	1·0
Amylocaine		
Gold bromide	Long thin needles	0·05
Gold bromide hydrochloric acid	Long thin needles	0·05
Gold chloride	Serrated plates	0·05
Lead iodide	Rosettes of rods	0·1
Picric acid	Bunches of thin rods	0·05
Benzamine		
Picric acid	Clusters of plates and rods	0·05
Potassium iodide	Rosettes of rods	0·5
Trinitrobenzoic acid	Hedgehogs	0·25
Benzocaine		
Gold bromide	Dendrites and rosettes (ON)	0·05
Gold bromide hydrochloric acid	Dendrites and rosettes (ON)	0·05
Lead iodide	Short rods	0·1
Mercuric chloride	Long rods	0·1
Picric acid	Dendrites	0·05
Platinum chloride	Needles from edge	0·5
Platinum iodide	Branching rods	0·25
Potassium bismuth iodide	Rosettes of branching rods	0·25
Potassium chromate	Small rods	0·25
Potassium tri-iodide (1)	Yellow rhomboids	0·5
Sodium carbonate	Short rods	0·25
Sodium phosphate	Rosettes of rods	0·25
Butacaine		
Gold bromide hydrochloric acid	Very small needles and plates	0·025
Picric acid	Branching rods	1·0
Potassium chromate	Serrated rods and needles	0·1
Potassium iodide	Small rods in bunches	0·05
Zinc chloride	Plates	1·0
Butyl Aminobenzoate		
Gold bromide	Small blades in stars	0·1
Lead iodide	Long plates	0·1
Picric acid	Tufts of needles	0·25
Platinum chloride	Needles	0·1
Platinum iodide	Dark needles	0·1
Potassium tri-iodide (1)	Dark rods	0·5
Potassium tri-iodide (3)	Long red plates	0·5
Sodium carbonate	Long plates	0·05
Sodium phosphate	Long plates	0·5
Trinitrobenzoic acid	Long needles	1·0
Cinchocaine		
Gold bromide	Bunches of small rods (2 days)	0·25
Gold bromide hydrochloric acid	Irregular plates and needles	0·025
Gold chloride	Dense rosettes	1·0
Lead iodide	Dense rosettes	0·1
Mercuric chloride	Dense rosettes	0·1
Platinum chloride	Stout needles	1·0
Potassium bismuth iodide	Needles	0·25

TABLE I—*continued.*

Reagent	Crystals	Sensitivity in μg.
Cinchocaine (*contd.*)		
Potassium tri-iodide (1)	Branching needles	0·5
Potassium tri-iodide (3)	Branching needles	1·0
Sodium carbonate	Bunches of rods	0·25
Trinitrobenzoic acid	Small rosettes of needles	0·1
iso*Butyl Aminobenzoate*		
Gold bromide	Yellow blades	1·0
Platinum chloride	Tufts of fine needles	0·5
Platinum iodide	Rosettes	0·1
Potassium chromate	Plates	0·5
Sodium carbonate	Segmented rods	0·25
Sodium phosphate	Segmented rods	0·25
Trinitrobenzoic acid	Long needles	1·0
Larocaine		
Gold bromide	Bunches of serrated plates	0·025
Gold bromide hydrochloric acid	Bunches of plates	0·025
Lead iodide	Rosettes	0·25
Platinum chloride	Shell-like rosettes (2 days)	1·0
Platinum iodide	Rosettes of branching needles	0·1
Potassium mercuric iodide	Serrated rods	0·25
Lignocaine		
Gold bromide	Serrated needles	0·05
Gold bromide hydrochloric acid	Serrated needles	0·25
Gold chloride	Yellow plates	0·5
Lead iodide	Long branching needles	0·25
Picric acid	Yellow plates and rods	0·1
Platinum iodide	Small plates	0·1
Potassium cadmium iodide	Serrated blades	0·1
Potassium mercuric iodide	Bunches of irregular rods	0·1
Potassium permanganate	Mass of needles	0·05
Potassium tri-iodide (1)	Yellow hexagonal crystals	0·05
Monocaine		
Gold bromide	Needles and serrated plates	0·025
Gold bromide hydrochloric acid	Long needles	0·1
Picric acid	Rosettes of plates	0·1
Potassium iodide	Prisms	0·5
Potassium tri-iodide (3)	Prisms	0·5
Orthocaine		
Gold bromide	Small plates	
Gold bromide hydrochloric acid	Small plates	0·5
Mercuric chloride	Rosettes of rods	0·1
Picric acid	Curved needles	0·05
Trinitrobenzoic acid	Long prisms	0·25
		1·0
Panthesine		
Gold bromide	Serrated plates (2 days)	0·5
Gold bromide hydrochloric acid	Dense rosettes (2 days)	0·1
Sodium carbonate	Branching needles	0·25
Trinitrobenzoic acid	Hedgehogs	0·5
Piperocaine		
Gold chloride	Irregular plates	0·1
Lead iodide	Small irregular needles	0·05
Picric acid	Rosettes of blades	0·025
Trinitrobenzoic acid	Rosettes of needles	0·25
Procainamide		
Gold bromide hydrochloric acid	Rosettes of plates	0·5
Platinum chloride	Rosettes of pointed plates	0·5
Potassium bismuth iodide	Rhomboidal plates	0·025
Trinitrobenzoic acid	Fans of plates	1·0
Procaine		
Gold bromide	Plates and needles	0·05
Gold bromide hydrochloric acid	Needles and plates	0·25
Mercuric chloride	Overlapping prisms	0·5
Platinum chloride	Plates or rosettes	0·25
Platinum iodide	Bunches of prisms	0·05
Potassium tri-iodide (1)	Rosettes of rods	0·25
Ravocaine		
Gold bromide hydrochloric acid	Bunches of small serrated plates	0·1
Platinum chloride	Bunches of blades, some dense rosettes	0·25

TABLE I—*continued*

Reagent	Crystals	Sensitivity in μg.
Tropacocaine		
Gold bromide	Bunches of serrated needles	0·025
Gold bromide hydrochloric acid	Bunches of serrated needles	0·025
Gold chloride	Bunches of serrated needles	0·025
Lead iodide	Plates and rods	0·025
Mercuric chloride	Segmented rods and plates	0·05
Picric acid	Feathery rosettes	0·025
Platinum chloride	Serrated needles	0·025
Platinum iodide	Curved needles	0·5
Potassium bismuth iodide	Branching needles	0·025
Potassium cadmium iodide	Rosettes of feathery needles	0·025
Potassium chromate	Serrated plates	0·5
Potassium iodide	Long plates	0·05
Potassium permanganate	Irregular plates	0·025
Potassium mercury iodide	Plates and rods	0·025
Potassium tri-iodide (1)	Plates	0·025
Potassium tri-iodide (3)	Plates	0·5
Trinitrobenzoic acid	Rosette of needles	0·25
Tutocaine		
Gold bromide	Rosettes	1·0
Gold bromide hydrochloric acid	Dendrites and irregular rods	0·25
Unacaine		
Gold bromide	Dense rosettes	0·5
Platinum iodide	Rosettes of branching needles	0·1

ON (overnight) indicates that the crystals do not usually form until the following day.

vanadate, ammonium molybdate or selenium dioxide gives any colours that are of use for the identification of μg. quantities of these drugs. Three tests, however, while not specific, are of some value. These are: (*a*) The diazo reaction. Many of these drugs contain a primary arylamino group, and may be diazotised and coupled with β-naphthol to give red dyes[12]. On the microscale, this test is carried out as follows: A microdrop (0·1 μl.) of the test solution is placed on a piece of opal glass.

TABLE II

VITALI'S TEST

Substance	Colour with nitric acid	Colour of residue after heating	Colour with potassium hydroxide
Amethocaine	Yellow	Yellow	Pink–purple
Benzocaine	—	"	Orange
Butacaine	—	"	"
Butyl aminobenzoate	—	Yellow brown	Yellow
*iso*Butyl aminobenzoate	—	"	"
Larocaine	—	Yellow	"
Monocaine	—	"	"
Orthocaine	Black	Red brown	Dark brown
Panthesine	—	Yellow	Orange
Procainamide	—	Light brown	Yellow brown
Procaine	—	Yellow	Orange
Ravocaine	Red orange	Brown	Brown
Tutocaine	—	Light brown	Yellow
Unacaine	—	Yellow	"

Microdrops of N hydrochloric acid, of 1 per cent. sodium nitrite solution and of a 4 per cent. solution of β-naphthol in 2 N sodium hydroxide solution are added in that order. Bright red colours are given by benzocaine, butacaine, butyl aminobenzoate, *iso*butyl aminobenzoate, larocaine, monocaine, orthocaine, panthesine, procaine, procainamide, ravocaine, tutocaine and unacaine. With orthocaine the drop slowly becomes plum coloured. The limit of the reaction is about 0·1 μg. (*b*) Paradimethylaminobenzaldehyde. The reagent is made by adding

20 drops of concentrated sulphuric acid to a solution of 1 g. of the substance in 100 ml. of ethanol[10]. A microdrop of the test solution is evaporated to dryness on opal glass, and a microdrop of reagent added. A bright yellow colour is given by benzocaine, butacaine, butyl aminobenzoate, *iso*butyl aminobenzoate, larocaine, monocaine, orthocaine, panthesine, procaine, procainamide, ravocaine, tutocaine and unacaine, the limit of the reaction being about 0·025 μg. Amethocaine and amylocaine give a pale yellow colour, but with these substances the sensitivity of the test is considerably less, being near 0·5 μg. (*c*) Vitali's test. This is carried out by the micromethod described by Clarke and Williams[11]. The results obtained are given in Table II. The sensitivity is about 0·1 μg.

Discussion

Whereas each of the substances tested yields a number of microcystalline derivatives, several of them give no colour reactions at all. It follows that identification must be established by means of the former test, the latter being of value for confirmatory purposes. As most of the microcrystalline tests are very delicate no difficulty should be experienced in identifying a few μg. of one of these substances. This high sensitivity is desirable owing to the fact that many of these drugs are rapidly hydrolysed and excreted by the body, so that the quantity of unchanged substance likely to be isolated from cadaveric material is extremely small.

The results described in this paper were obtained using pure substances. A technique for extracting and purifying μg. quantities of these and other basic nitrogenous drugs will be described in a subsequent paper.

Summary

Crystal and colour tests are described for the identification of μg. quantities of 21 local anæsthetic drugs.

I wish to express my gratitude to Professor E. C. Amoroso for the interest he has taken in this work, and to acknowledge most gratefully gifts of drugs from the Alwitt Trading Company Ltd.; Messrs. Bayer Products Ltd.; Messrs. May and Baker Ltd.; The Pharmaceutical Manufacturing Company; Messrs. Roche Products Ltd.; Messrs. P. Samuelson and Company; Messrs. Sandoz Products Ltd., and the S. S. White Company Ltd. I am also much indebted to Miss A. Stanley for technical assistance.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Aneurine, Microbiological Assay of. J. R. Villanueva. (*Nature, Lond.*, 1955, **176**, 465.) Aneurine, biotin, riboflavine, pyridoxine, nicotinic acid, inositol and pantothenic acid (this latter in the form of its calcium salt) were tested as growth-promoting factors for *Tuberculina persicina*. The vitamins were added aseptically to a synthetic basal medium containing potassium phosphate, magnesium sulphate, potassium nitrate, dextrose and agar, the final pH being adjusted to 5·2. After incubation for 20 days at 20° C., the plates to which aneurine had been added showed good growth of the fungus colony and displayed a pigmentation of the medium; those lacking this factor remained colourless. Further experiments, devised to ascertain the optimum levels of aneurine concentration for pigment production, were made with liquid cultures which were incubated for thirty days, at the end of which time striking differences in the colour of the liquids were observed. Measured spectrophotometrically, these differences were related to the amount of aneurine present in the original culture medium. Negligible pigment formation occurred in the liquids of the control flasks, or in those in which the aneurine concentration was lower than 5×10^{-3} μg./l. Above 5×10^{2} μg./l. of aneurine there was a marked decrease in pigment formation. Further experiments are being made with *T. persicina*.

R. E. S.

Barbiturates, Interference to the Ultra-violet Spectrophotometry of. A. S. Curry. (*Nature, Lond.*, 1955, **176**, 877.) The presence of a phenolic substance, isolated in four cases along with barbiturates during the pathological examination of four livers from various cases of sudden death, is reported. The substance exhibits ultra-violet absorption characteristics in both 0·5N ammonia and in acid solution, which are very similar to those of barbiturates. Interference by this substance in the spectrophotometric estimation of barbiturates isolated from such material is therefore possible, the quantity of the substance present being such as to suggest barbiturate concentrations of 1 to 4 mg./100 ml. The interfering substance is readily separated from barbiturates by chromatography on paper using *n*-butanol/5N ammonia (R_F 0·95). The spots fluoresce in ultra-violet light, give no reaction with the mercuric sulphate/diphenylcarbazone reagent for barbiturates, but give a blue colour with ferric chloride/ferricyanide. Another compound having similar ultra-violet absorption characteristics has also been isolated during the course of examination of the contents of a stomach. The liver of the same person yielded a similar substance in the alkali-soluble and ether-soluble fraction.

J. B. S.

Corticotrophin, Chromatographic Studies on. H. B. F. Dixon and M. P. Stack-Dunne. (*Biochem. J.*, 1955, **61**, 483.) Corticotrophin concentrates have been chromatographed in sodium phosphate buffers (pH 6·7) on ion exchange resins. The resin used was of the weak carboxylic acid type specially prepared by grinding and elutriation and of such particle size as to give a flow rate of 5 ml./sq. cm./hour under gravity when packed in a column 30 to 40 cm.

high. The active components have been resolved into a number of fractions, of which the main one has been designated corticotrophin A_1. This is probably identical with the β-corticotrophin of Bell. The chromatographic results suggest that this fraction is essentially homogeneous. The resolved component fractions were separated from salts by adsorption at pH 3 on a carboxylic acid resin or by extraction of the material into phenol. The other components, corticotrophins A_2 and A_3 moved more rapidly than A_1 on the resin columns. A_2 is formed from A_1 by alkaline treatment, which is thought to cause some loss of amide ammonia. The formation of A_3 and the fact that A_2, unlike A_1, shows two spots on paper ionophoresis suggests that other changes are involved, such as the unmasking of acid groups or the masking of amino groups by acyl migration. J. B. S.

Galenical Preparations, Chromatographic Examination of. P. Lundgren. (*Svensk farm Tidskr.*, 1955, **16**, 389.) A method which has been applied to the assay of a number of galenical preparations is based on distribution chromatography on silica with an aqueous phase of suitable pH. Examples are given of the application of the method to the extraction of nitroglycerin, bromural, phenacetin, etc., from tablets, of alkaloids from their salts and to the analysis of a number of tablets containing alkaloids with synthetic chemicals. The method, as applied to a tablet containing barbitone, phenacetin and papaverine hydrochloride, is as follows: 1½ tablets are rubbed down with 2 ml. of 2M hydrochloric acid and then mixed with silica (Hyflo Super Cel) to form an almost dry powder which is then packed into a chromatograph column (column 1). Another column (column 2) is prepared from 2 g. of silica and 1 ml. of 5M sodium hydroxide suspended in ether. Column 1 is washed through with 150 ml. of ether at 1 to 2 ml. per minute, which is allowed to drip on to column 2 which itself is dripping at the same rate. Removal of the solvent from the eluate gives the phenacetin. To column 2 is added a solution of 0·5 ml. of acetic acid in 10 ml. of ether and, when this has been taken up by the column, 50 ml. of ether is added. The eluate is evaporated to dryness and the residue is disolved in 20 ml. of sodium carbonate solution (10 per cent.) washed twice with two 10 ml. portions of sodium carbonate solution, and filtered. The filtrate is titrated with 0·1N silver nitrate solution to a permanent turbidity. A blank test is carried out with 40 ml. of the sodium carbonate solution. One ml. of the silver nitrate corresponds to 0·01842 g. of barbitone. Column 1 is eluted with 50 ml. of chloroform and the eluate is evaporated to dryness. The residue is dissolved in 25 ml. of 2M hydrochloric acid and made up to 250 ml. The papaverine is then determined by the spectrophotometer at 309 mμ, using 0·2N hydrochloric acid as blank. The value for E (1 per cent. 1 cm.) for papaverine hydrochloride is 219·7. G. M.

Lignocaine, Titrimetric Assay with Reinecke's Salt. J. M. Hanquin and C. Lapiere. (*J. pharm. Belg.*, 1955, **10**, 246.) Lignocaine and other local anæsthetics may be assayed by precipitation from an acid solution with a standard solution of ammonium reineckate. The precipitate is removed by filtration and the excess of reagent in an aliquot quantity of the filtrate is determined by hydrolysing in alkaline solution, and titrating with silver nitrate in the presence of nitric acid. If the substance under examination is in the form of its hydrochloride, allowance must be made for the quantity of chloride ion which reacts with the silver nitrate. This method is applicable to lignocaine, procaine and tutocaine, using samples of about 20 mg. An alternative method in which the precipitated reineckate is washed, dissolved in acetone and titrated with silver

nitrate after hydrolysis with alkali is satisfactory for lignocaine, but losses occur in washing the precipitates obtained from procaine and tutocaine. The method has not proved to be successful with other local anæsthetic agents. G. B.

Morphine, Determination of, by Exchange Resins. E. Brochmann-Hanssen. (*Medd. Norsk farm. Selsk.*, 1955, **17**, 76.) The apparatus used consists of a stoppered extraction tube which has a stop-cock below and is kept warm by means of heating tape. A plug of glass wool is placed in the bottom. For the assay of opium, 0·1 g. of the drug is mixed in the tube with 1 g. of Dowex 50-X_2 (H^+) and 25 ml. of hot water. The mixture is shaken mechanically for 15 minutes, being kept at 70° to 80° C. The liquid is drained off and the tube washed with water and placed on top of a column containing Dowex 1-X_1, previously washed with 50 ml. of 4 N ammonia in methanol (70 per cent. by volume). The alkaloids are eluted from the extraction tube with 50 ml. of methanolic ammonia onto the column which is washed with methanol and then with water to remove the ammonia. The amphoteric alkaloids are finally removed from the column by 0·1 hydrochloric acid, collecting the eluate in a 50-ml. volumetric flask. The morphine is determined by the method of Pride and Stern (*J. Pharm. Pharmacol.*, 1954, **6**, 390) using iodic acid and nickel chloride. In a modification the final assay is done by spectrophotometry, with an allowance for the slight yellow colour of the solution. The results obtained are about 25 per cent. higher than those by the lime method of the United States Pharmacopeia XIV (which does not use a correction factor).

G. M.

Organic Phosphate Insecticides, Paper Chromatography of. J. W. Cook. (*J. Assoc. off. agric. Chem. Wash.*, 1955, **38**, 826.) An investigation of the conversion of organic phosphates to *in vitro* cholinesterase inhibitors by *N*-bromosuccinimide and ultra-violet light has been made. Chromatograms were produced by depositing at the origin point, from anhydrous ether solution, either 2 or 20 mg. of the organic phosphate on strips of paper followed by spraying with a 4 per cent. solution of mineral oil in anhydrous ether. After chromatography with water as the mobile phase, they were dried at room temperature and sprayed with a fresh solution of *N*-bromosuccinimide in methyl chloroform. Other strips were spotted with the organic phosphate but were sprayed with *N*-bromosuccinimide at the origin before being treated with oil and chromatographed. Further strips were spotted by the same procedure as before but the papers were exposed to a strong ultra-violet light for 45 minutes before they were treated with oil and chromatographed. All strips were tested for *in vitro* cholinesterase inhibitors as described previously (*J. Assoc. off. agric. Chem. Wash.*, 1955, **38**, 150). In general, it was found that systox, parathion, diazinon, sulphatepp, TEPP and triethylphosphate were converted to more potent cholinesterase inhibitors by treatment with *N*-bromosuccinimide and ultra-violet light; the resulting compounds were more soluble in water than in oil.

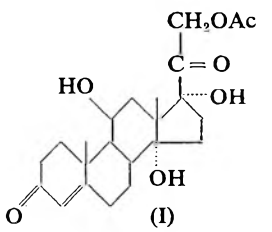
R. E. S.

***Rhamus frangula*, Determination of Anthranols in.** H. Mühlemann. (*Pharm. Acta Helvet.*, 1955, **30**, 350.) Absorption curves of chrysazin-9-anthranol triacetate, 10-glucosylchrysazin-9-anthranol hepta-acetate and emodin-9-anthranol tetra-acetate all show the same form with three peaks, of which the central one is the higher. In the case of the first two compounds, the difference between this maximum and the minimum of the curves was found to run parallel with the molecular weights, and it is reasonable to assume that the same

relation holds for emodin-9-anthranol tetra-acetate and reduced glucofrangulin acetate. On this basis the difference between E (1 per cent. 1 cm.) maximum (395 mμ) and minimum (320 mμ) for the latter compound was calculated as 65·7. This method was used for observing the concentration of frangula extracts in the course of attempts at purification. Actually it was found necessary to take the minimal extinction between 320 and 350 mμ rather than a definite wavelength. By using this method a fraction was finally obtained which should represent 100 per cent. of the glucofrangulinanthranol acetate. This compound could not be obtained in a crystalline form, but the figures obtained by elementary analysis and for acetyl content agreed with the theoretical values. It gave a value for E (max.–min.) of 64, which agreed well with that previously calculated. In these glucosides, an increase in the hydroxyl groups causes a displacement of the maximum towards longer wavelengths, while the entry of sugar into the molecule flattens the maximum. G. M.

ORGANIC CHEMISTRY

Corticoids, Biologically Active, A New Class of. E. J. Agnello, B. L. Bloom and G. D. Laubach. (*J. Amer. chem. Soc.*, 1955, **77**, 4684.) A new class of corticosteroids which display varying degrees of glucocorticoid activity are reported. The compounds are derivatives of hydrocortisone and cortisone functionally substituted in ring D. The introduction of the various nuclear substituents was readily effected by chemical transformations of a dihydroxylated product obtained from the microbiological oxygenation of 11-desoxy-17α-hydroxycorticosterone. The dihydroxylated product was shown to be I; it is more active than hydrocortisone acetate in the thymus involution assay and has been found to be an active anti-inflammatory agent in rheumatoid arthritis. A. H. B.



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BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Diethyltin Dichloride and Triethyltin Sulphate, Biochemistry of. W. N. Aldridge and J. E. Cremer. (*Biochem. J.*, 1955, **61**, 406.) It has been shown that diethyltin dichloride and triethyltin sulphate influence the biochemical mechanisms of rat-brain brei and rat-liver mitochondria in different ways. The response of diethyltin dichloride is similar to that of phenlyarsenious acid, but the biochemical symptoms of triethyltin sulphate and diphenylchloroarsine are not identical. Evidence is given that diethyltin dichloride inhibits α-keto acid oxides with citrate as substrate, as does phenylarsenious acid. There is also some evidence that diethyltin dichloride has some other actions at the concentrations which inhibit α-oxoglutaric oxidase. Unlike diphenylchloroarsine, triethyltin sulphate possesses little or no affinity for sulphydryl groups, but with rat-brain brei or rat-liver mitochondria it causes a consistent lowering of α-keto acid levels. The various steps of the respiratory chain are unaffected by approximately 100 times the concentration effective against oxidation. Triethyltin sulphate is a highly specific inhibitor of phosphorylation processes associated with oxidation. The hypothesis that the triethyltin ion replaces an essential ion in the phosphorylation mechanism is considered, and rejected. It appears to interfere with oxidation as a consequence of its inhibition

of phosphorylation. There is some evidence that the inhibition of phosphorylation is responsible for the generalised muscular weakness, which is a clinical symptom of poisoning by triethyltin sulphate. J. B. S.

BIOCHEMICAL ANALYSIS

Esterified Fatty Acids, Assay of, and its Application to Blood Serum. M. Jarrier and J. Polonovski (*Bull. Soc. Chim. biol., Paris*, 1955, **37**, 495.) The method depends on the conversion of the esters into alcohols and hydroxamic acids according to the equation: $RCOOR' + NH_2OH = R'OH + RCONHOH$. The hydroxamic acids yield a purple-red ferric complex while free fatty acids do not react. The method is especially useful for the determination of small quantities of esters (of the order of 1 mg.) mixed with free fatty acids. Quantities of ethereal solution representing 1 to 6μ equiv. of ester are placed in tubes and the contents dried *in vacuo*. The residue is dissolved in 0·3 ml. of 2·5 per cent. ethanolic hydroxylamine hydrochloride solution and 0·3 ml. of 2·5 per cent. ethanolic sodium hydroxide solution is added. After 1 hour, 10 ml. of ferric perchlorate solution is added and the tubes are kept at 25° C. for 20 minutes, after which the extinction is measured at 250 mμ. The quantity of ester is determined by means of a reference curve prepared by plotting extinctions obtained in a similar experiment with a pure sample of ester, for example ethyl palmitate. For the determination of fatty acid esters in blood serum, a sample of about 0·25 ml. of serum or 2 mg. of lipids is required, a preliminary treatment being necessary, either precipitation with a 3 : 1 mixture of ether and ethanol or extraction with methylal/methanol mixture. G. B.

Fibrin, Determination of, in Plasma. K. Christensen (*Scand. J. clin. Lab. Invest.*, 1955, **7**, 246.) A new and accurate method is described for the estimation of fibrin in plasma, based on the digestion of fibrin by trypsin. Blood is withdrawn into a 2 ml. citrated centrifuge tube, centrifuged and 0·5 ml. of plasma transferred to a siliconed test tube. The plasma is recalcified by addition of 0·5 ml. 1/40M calcium chloride. The mixture is stirred with a roughened 5 mm. glass rod and the rod is left in the tube for 30 minutes. Serum is squeezed from the clot attached to the rod and it is washed by immersion in physiological saline. The rod is transferred to a 5 ml. graduated test tube containing 1 ml. of a 0·1N ammonium chloride-ammonia buffer of pH 9. To this is added 0·125 ml. of a solution containing 50 mg. of trypsin in 20 ml. of 0·0025N hydrochloric acid. After incubation for 1 hour at room temperature, when the clot is digested, the rod is removed, rinsed with buffer and the volume made up to 5 ml. The extinction of the solution at 280 mμ is measured, buffer containing the same amount of trypsin being used as a blank. The result is obtained from a standard calibration curve. G. F. S.

Mercury in Biological Materials, Determination of. D. Polley and V. L. Miller. (*Analyt. Chem.*, 1955, **27**, 1162.) A sample of biological material or soil was prepared by digestion in concentrated sulphuric acid with dropwise additions of 50 per cent. hydrogen peroxide. The analysis of the solution for mercury was based on the addition of an excess of an alcoholic solution of a diorganic mercurial which reacted with a weakly acid solution of mercury to form two molecules of the corresponding organic mercury compound; the resulting organic mercurial was determined by the colour formed from reaction with dithizone. Four dimercurials di-*p*-tolylmercury, diphenylmercury, dinitrophenyl-mercury, and bis-*m*-($\alpha\alpha\alpha$-trifluorotolyl) mercury, were found to react quantitatively but required different reaction conditions; the ditolyl compound

was chosen as it was more stable towards acid and had the most favourable solubility ratio. The spectrophotometric curve for the resulting dithizonate had a minimum at 480 mμ. Fuming sulphuric acid was tried in place of the concentrated acid in the digestions but it offered no advantages and caused more violent digestions. Much of the success of the digestion was believed to be due to a gentle oxidation, avoiding a sudden vigorous reaction when mercury was lost. In the absence of chloride there was an increasing loss of mercury due to adsorption on glass with increasing acidity, reaching 18 per cent. at 1·8N; there was no adsorption of mercury in 24 hours from a solution 0·3N in chloride. 1 mg. of iron, cobalt, nickel, zinc, cadmium, lead, iron, copper, manganese, or bismuth did not interfere, but silver required an additional extraction of the chloroform phase. Using a spectrophotometer amounts down to 0·5 μg. could be determined; the precision of the procedure as applied to mercury (0 to 100 μg.) in soil samples was 5 per cent. R. E. S.

Mercury in Urine, Determination of. A. C. Rolfe, F. R. Russell and N. T. Wilkinson. (*Analyst*, 1955, **80**, 523.) A trial of published methods gave low recoveries of mercury when it was added to fresh urine as mercuric sulphate; if the urine was allowed to stand after the addition of the mercuric sulphate the results obtained by each method were even lower. In the method developed, 50 ml. urine was oxidised by heating with nitric acid and potassium permanganate in a 350 ml. glass pressure bottle; excess permanganate was removed with ammoniacal hydroxylamine solution. An aliquot of this solution containing up to 100 μg. of mercury was extracted with dithizone in toluene followed by re-extraction into 5N hydrochloric acid. Ammoniacal hydroxylamine was then added with a further dithizone extraction, excess being removed with sodium hydroxide. After dilution with toluene the extinction of the solution was measured with a Spekker photoelectric absorptiometer, using Calorex H 503 heat filters and Ilford No. 602 blue filters in conjunction with a tungsten-filament lamp. Toluene was used in the comparison cell and it was necessary for a blank determination to be carried out on all reagents used. The amount of mercury was obtained from calibration curves prepared using known amounts of standard mercuric sulphate solution. The method could be used in the presence of copper. Mercury was determined over a range of 0 to 100 μg. with an accuracy of ± 1 μg. below 50 μg. and ± 3 μg. for amounts between 50 and 100 μg. R. E. S.

CHEMOTHERAPY

Bis-*iso*quinolinium and Bis-quinolinium Salts, Antifungal Activities of. H. O. J. Collier, M. D. Potter and E. P. Taylor. (*Brit. J. Pharmacol.*, 1955, **10**, 343.) The antifungal activities of polymethylene bis-quinolinium and bis-*iso*-quinolinium salts with 10 to 20 methylene groups were determined against a human strain of *Trichophyton mentagrophytes*. In both series activity increased with chain length up to the tetradecamethylene member; activity showed a marked drop with the eicosane member. The tetradecamethylene and hexadecamethylene members of both series inhibited growth in Sabourand's broth of 11 strains of 6 species of pathogenic fungi, at concentrations between 0·3 and 10 μg./ml. Human serum and hair slightly decreased the antifungal power of the tetra- and hexa-decamethylene bis-*iso*quinolinium salts; bovine bile markedly antagonised the action of the hexadecamethylene member. Spores of *T. mentagrophytes* in saline suspension were still viable in the presence of hexamethylene bis-(*iso*quinolinium methosulphate) after 24 hours at 20° C. However, incubation of the spores in Sabourand's broth containing 1·25 μg./ml. of the compound, caused 100 per cent. mortality after 7 days at 27° C. G. P.

PHARMACY

NOTES AND FORMULÆ

Enteric Coated Tablets, Disintegration of, in Simulated Digestive Juices. R. Crisafio, J. Taylor and L. G. Chatten. (*Drug Standards*, 1955, **23**, 1.) Disintegration tests were carried out with an apparatus similar to that described in the U.S.P. XIV, modified to provide a regulated rubbing action on each tablet, and using a larger disintegration chamber. Commercial enteric-coated tablets were tested using simulated gastric juice prepared according to the formula of Toplis (pH 1·6) and the one proposed for the U.S.P. XV (pH 1·2). Of 64 products examined, 31 failed to resist the action of the gastric media for a period of 1 hour under the conditions of the test. Tablets which did not disintegrate in the acid media were tested in simulated intestinal juice, Toplis (pH 8·0) and U.S.P. XV (pH 7·5) and the disintegration times recorded. No significant difference was found to exist between the results with the two gastric media, but significant differences were observed between the alkaline media. With some products the two alkaline media gave similar results, whereas others showed considerable differences, possibly as a result of the difference in pH between the media. The application of this method of testing is discussed in the light of previous investigations and of the relationship between *in vitro* and *in vivo* tests. G. B.

Enteric Coated Tablets, The Disintegration of. N. E. Brindamour and H. G. DeKay. (*Drug Standards*, 1955, **23**, 10.) Tablets coated with cellulose acetate hydrogen phthalate, which had been shown to be 93 per cent. efficient when tested radiographically, were compared with commercial enteric-coated tablets, using a series of artificial gastric and intestinal juices and rat gastric juice, *in vitro*. The apparatus described in the U.S.P. XIV was used, tablets being weighed before the test, and after periods of 1, 2 and 3 hours in the gastric juices. Only unbroken tablets were used in estimating the loss in weight due to the corrosive action of the gastric juices. Disintegration times were measured in the alkaline juices, using the same apparatus. The tablets were also tested in the rat stomach and intestine, *in vivo*. As a result of these experiments, the authors recommend the following solutions for testing the tablets: gastric solution, sodium chloride 2 g., pepsin 3·2 g., hydrochloric acid 2·5 ml., water to 1000 ml.; intestinal solution, pancreatin 10 g., calcium chloride 10 per cent. solution 10 ml., 0·2M dipotassium phosphate 250 ml., 0·2M sodium hydroxide 118 ml., ox bile extract 4 g., water to 1000 ml. G. B.

Neomycin, Release of, from Selected Ointment Bases. W. T. Hill, Jr., J. F. Bester and O. H. Miller. (*Drug Standards*, 1955, **23**, 80). Steel cylinders filled with ointments containing neomycin in various bases were placed on agar plates seeded with a standardised suspension of the spores of *Bacillus subtilis*, and incubated. The degree to which neomycin was released from the bases was assessed from measurements of the zones of inhibition produced. Neomycin was most readily released by washable emulsion bases such as hydrophilic ointment U.S.P., carbowax bases and jelly bases such as those containing methylcellulose and carboxymethylcellulose, and from a base containing Spans 40 and 45. Oily and absorption bases released neomycin to a lesser extent. Bentonite base was not suitable since the antibiotic, being cationic, becomes firmly bound to the bentonite. Neomycin cracks emulsions formed with sodium lauryl sulphate and precipitates certain gums from jelly bases. The ointments were re-examined after 30 and 60 days' storage at room temperature

and a slight decrease in potency was observed in aqueous ointments over 60 days. It is recommended that these ointments should be freshly prepared and the packages dated. G. B.

Polyvinylpyrrolidone as an Adjunct to Antibacterials. H. Sheinaus and G. J. Sperandio. (*Drug Standards*, 1955, **23**, 96.) Solutions of many antibacterial agents were tested by the cylinder-plate method against *Micrococcus pyogenes* var. *aureus*. Tests were repeated with the addition of varying amounts of polyvinylpyrrolidone, which had previously been shown to have a negligible antibacterial action. The zones of inhibition remained unchanged for most of the preparations tested, including antibiotics, mercury compounds, quaternary ammonium compounds and dyes. Polyvinylpyrrolidone decreased the effect of chloroxylenol, zinc sulphate and iodine, while in the presence of polyvinylpyrrolidone the effect of sodium hypochlorite was substantially increased. In the case of hexachlorophene, an optimum concentration was observed, beyond which the antibacterial effect decreased. The sodium hypochlorite/polyvinylpyrrolidone mixtures were assayed for available chlorine, and an inverse relationship was found to exist between size of zone of inhibition and volume of titration solution required. It is suggested that either sodium hypochlorite combines with polyvinylpyrrolidone, or iodine which is released in the assay process (U.S.N.F.IX) may be held as a complex with it. G. B.

Quaternary Ammonium Salts, The Relationship of Charge Density, Antibacterial Activity and Micelle Formation of. J. A. Cella, L. A. Harriman, D. N. Eggenberger and H. J. Harwood. (*J. Amer. chem. Soc.*, 1955, **77**, 4264.) A series of four phenyl-containing quaternary ammonium salts $[R(CH_3)_2C_{12}H_{25}N]Cl$ was compared with the series of *cyclo*hexyl analogues. In both series the cyclic structure is separated from the nitrogen atom by from 0 to 3 methylene groups. The antibacterial results do not substantiate an earlier hypothesis that the antibacterial activity of quaternary ammonium salts is influenced by the charge density on the nitrogen atom. In the series studied, an inverse relationship between antibacterial activity and critical micelle concentration was shown. It is suggested that simple steric effects are a major factor in determining the tendency towards micelle formation and the biological activity of cationic surface active agents. These phenomena may be a function of the ability of the molecules to undergo close packing which in turn influences the size of the micelle or the extent of interaction with the bacterial surface. A. H. B.

Tablet Lubricants, Water-soluble. M. Smilek, F. P. Cosgrove and E. P. Guth. (*Drug Standards*, 1955, **23**, 87.) Tablets of sodium bicarbonate, ascorbic acid, calcium lactate and nicotinic acid were prepared from granules lubricated with polyoxyethylene monostearates (Myrj 51 and 53) and polyoxyethylene lauryl alcohol (Bryj 35). The lubricating agents were dissolved in acetone and added to the granules which were dried in air. Lubricating properties were assessed by measuring the force required to eject a tablet from a hand machine, and by determining the minimum quantity of lubricant necessary to prevent granules sticking to the punches of a rotary machine. All polyoxyethylene compounds tested had about the same efficiency as lubricants, approximately one third that of magnesium stearate. The tablets disintegrated rather more rapidly than those made with magnesium stearate. They did not swell, but gradually dissolved, leaving thin wafer-like films which finally also dissolved. It is suggested that these substances would be useful as lubricants for the preparation of lozenges and buccal tablets. G. B.

PHARMACOLOGY AND THERAPEUTICS

Alphaprodine Hydrochloride and Levallorphan Tartrate; Effects on Respiration. M. Swerdlow, F. F. Foldes and E. S. Siker. (*Amer. J. med. Sci.*, 1955, **230**, 237.) Alphaprodine hydrochloride (1 : 3-dimethyl-4-phenyl-4-propionoxypiperidine hydrochloride) is a short-acting analgesic which can be used with advantage to supplement nitrous oxide-oxygen thiopentone sodium anæsthesia. Levallorphan tartrate ((−)-3-hydroxy-*N*-allylmorphinan tartrate) is a narcotic antagonist. A study of the effect of these two drugs on respiration was carried out on 210 conscious patients who underwent surgery under low spinal or epidural anæsthesia. The changes of respiratory rates were measured in 120 patients, in three groups of 40, breathing room air, who were given 1 mg./kg. of alphaprodine. The drug was given to one group before, to the second together with, and to the third after levallorphan tartrate in doses of either 0·01 or 0·02 mg./kg. It was shown that alphaprodine caused a marked depression of the respiratory rate; that levallorphan tartrate given after, together with or before, corrected or prevented this fall to a large degree; that there was no marked difference between the two dose levels of levallorphan; and that there were no significant effects on pulse rate or blood pressure. In the second part of the study kymographic tracings were made of the respiration of 90 patients, in three groups of 30, breathing oxygen in a closed circuit. One mg./kg. of alphaprodine was given to all patients, and levallorphan was given to the first group before, to the second together with, and to the third after the analgesic in doses of 0·01, 0·02 and 0·04 mg./kg. It was shown that alphaprodine affected alveolar ventilation rate, minute volume, and respiratory rate to a greater extent than depth of respiration. Levallorphan given after, together with or before alphaprodine corrected or prevented the respiratory depression, but was more effective in counteracting the decrease in depth of depression than the rate. The best effects were obtained with a dose of 0·04 mg./kg. of levallorphan. Good results were also obtained, especially with the higher dosage of levallorphan, against the alphaprodine-induced decrease of tidal volume, depression of minute volume and depression of alveolar ventilation. The authors conclude that levallorphan tartrate offers considerable protection against the respiratory depression induced by alphaprodine hydrochloride. S. L. W.

Bradykinin and Substance P, Comparative Study. B. Pernow and M. Rocha e Silva. (*Acta physiol. scand.*, 1955, **34**, 59.) Both these substances are known to be polypeptides having a slow contracting effect upon several smooth muscle structures but causing a fall in blood pressure. Such effects are not influenced by atropine, antihistamines or ganglion-blocking drugs. Both substances are rapidly destroyed by chymotrypsin. However, bradykinin is less strongly adsorbed on aluminium oxide than substance P. Bradykinin has a higher R_F value than substance P, using butanol/acetic acid/water solvent. Furthermore, these two substances give separate peaks of activity when subjected to paper electrophoresis. On pharmacological test preparations, the effect of bradykinin on smooth muscle structures is slower than that of substance P. The hen cæcum is very sensitive to substance P but rather insensitive to bradykinin. The depressor action of substance P is more pronounced than that of bradykinin. M. M.

Chlorpromazine, Reversibility of Induced Psychosis with. B. E. Schwarz, R. G. Bickford and H. P. Rome. (*Proc. Mayo Clin.*, 1955, **30**, 407.) Psychic disturbances induced by 50 μg. of lysergic acid diethylamide (LSD-25) or by

400 mg. of mescaline, given orally to normal human subjects, had similar symptomatic patterns. The most striking effects common to both drugs were affective reactions, such as mania, anxiety, uncontrollable laughing and crying, withdrawal and suspicion. Visual hallucinations, synæsthesia and disturbances in the body image were particularly prominent. Thought disturbances consisted of loosening of association, blocking, increased communicability and loquaciousness. Catatonia was present to some degree in most subjects. In addition, mescaline frequently, and LSD-25 occasionally, caused nausea, vomiting, mydriasis and blood pressure variations. Chlorpromazine (25 mg. intramuscularly) administered at the height of the psychosis (two hours after LSD-25 administration or three hours after mescaline) returned mental conditions to normal. EEG changes induced by the hallucinogenic drugs also reverted to normal patterns, in most cases, after chlorpromazine. The implication of neurohumoural agents in the actions of these drugs is discussed. G. P.

2:4-Dichloro-6-phenylphenoxyethyl Diethylamide, a Potentiating Agent, Inhibition of Drug Metabolic Pathways by. J. R. Fouts and B. B. Brodie, (*J. Pharmacol.*, 1955, **115**, 68,) Blockade of drug metabolism has been described for diphenylpropylacetic acid and its β-diethylaminoethyl ester (SKF-525-A). Similar activity has now been demonstrated for 2:4-dichloro-6-phenylphenoxyethyl diethylamine HBr (Lilly 18947). This compound prolonged duration of sleep induced in mice by hexobarbitone, by inhibiting the metabolism of the barbiturate. *In vitro*, the enzyme systems present in the supernatant fractions of centrifuged liver homogenates which effected the oxidation of the side-chain of hexobarbitone, dealkylation of aminopyrine, deamination of amphetamine, ether cleavage in codeine, hydroxylation of acetanilide and conjugation of morphine were inactivated by the potentiating agent. The inhibitory activity of Lilly 18947 for most of these reactions was comparable with that of SKF-525-A (on the codeine ether-cleavage and hydroxylation of acetanilide, however, Lilly 18947 was considerably the more potent) and it is likely that the two substances have similar modes of action. Preliminary evidence suggests that Marsilid, (1-*iso*propyl-2-*iso*nicotinyl hydrazide), also acts on the same metabolic pathways. G. P.

Diethylaminoethoxyethylphenyl-1-*cyclo*pentane Carboxylate, Antitussive Activity of. S. Levis, S. Preat and F. Moyersoons. (*Arch. int. Pharmacodyn.*, 1955, **103**, 200.) This compound, one of a series of phenyl*cyclo*alkanecarboxylic acids studied for antitussive activity, is more active than codeine phosphate and has a low toxicity. Antitussive activity was determined in anæsthetised cats in which the superior laryngeal nerve was stimulated from a constant current square wave stimulator. Spasms of coughing were recorded from a thread attached to the skin of the abdomen. The percentage of inhibition and duration were determined for each compound. This compound has only a weak vasodepressor action and no effect on respiration. The compound also has antispasmodic, local anæsthetic and weak mydriatic actions. G. F. S.

Glycyrrhetinic Acid, Effects of, on Salt and Water Metabolism. E. E. Galal. (*Brit. J. Pharmacol.*, 1955, **10**, 305.) DOCA-like activity on sodium and potassium metabolism in man has been reported for liquorice extracts and glycyrrhetinic acid. Beneficial effects were also obtained with these drugs in patients with Addison's disease. In this study, however, they had no effect on sodium or potassium balance in adrenalectomised rats. Even with prolonged treatment with glycyrrhetinic acid there was no significant improvement in the

level of serum electrolytes, body weight or survival time. The acid had an anti-diuretic action in normal water-loaded rats, conscious or anæsthetised, and on a conscious dog. This action was still present in neurohypophysectomised rats. Increased tubular reabsorption was suggested as the mode of action where the acid was given parenterally; delay in water absorption from the alimentary tract was sufficient to account for the water-retaining effect of the drug when given orally. It was concluded that the actions of the drug are different from those of DOCA. G. P.

5-Hydroxytryptamine in Mental Diseases and its Antagonism to Lysergic Acid Derivatives. A. Cerletti and E. Rothlin. (*Nature, Lond.*, 1955, **176**, 785.) 2-Brom-(+)-lysergic acid diethylamide (BOL-148), like lysergic acid diethylamide (LSD-25), antagonised peripheral actions of 5-hydroxytryptamine (5-HT). The antagonism of 5-HT on the isolated uterus and kidney of the rat, and on the blood pressure and bronchi in cats, by BOL-148 was equal to or slightly greater than that of LSD-25. The potentiation of barbiturates by 5-HT was also antagonised to the same degree by both agents. Specificity for the antagonism was high. There was no anti-adrenaline, antihistamine or anti-acetylcholine activity with the doses used. However, BOL-148 had none of the central excitatory actions of LSD-25; on the contrary it induced sedation in normal and waltzing mice. Also, LSD-25 in small doses caused bradycardia and a fall in blood pressure in cats, whereas doses up to 1 mg./kg. of BOL-148 were inactive in this respect. In man BOL-148 had none of the hallucinogenic actions shown by LSD-25; the only effects experienced were sedation fatigue and sometimes nausea. It was concluded that the pharmacological antagonism of 5-hydroxytryptamine by LSD-25 can no longer be held to explain the hallucinogenic activity of LSD-25 on the brain. G. P.

Iron-Dextran Intramuscular Hæmatinic, Pharmacology of. L. E. Martin, C. M. Bates, C. R. Beresford, J. D. Donaldson, F. F. McDonald, D. Dunlop, P. Sheard, E. London and G. D. Twigg. (*Brit. J. Pharmacol.*, 1955, **10**, 375.) Iron combined with low-molecular-weight dextran (iron-dextran or "Imferon") was compared with saccharated iron oxide for hæmatinic activity and general toxicity. Uptake of iron-dextran from the blood by the cells of the reticulo-endothelial system was slower, and *in vitro* anticoagulant activity less, with iron-dextran than with saccharated iron oxide. The urinary and fæcal iron excretion was of the same order for the two agents. Acute toxicity in mice of iron-dextran was about one-third of that of saccharated iron oxide. Heavy iron precipitation and hæmorrhage in the lungs of rabbits was observed after doses of saccharated iron oxide equivalent to 150 mg. Fe/kg. Iron-dextran at a dose-equivalent of 500 mg. Fe/kg. caused only slight deposition and no hæmorrhage. Domestic piglets, which suffer from a naturally-occurring hypochromic anæmia during the first weeks of neonatal life were given intramuscularly a dose of iron-dextran corresponding to 26 mg. Fe/kg. During the first 14 days after injection they utilised 93 per cent. of the dose of iron administered. G. P.

6-Mercaptopurine in Acute Leukæmia. F. G. J. Hayhoe. (*Lancet*, 1955, **269**, 903.) This report is based on a study of 15 adults with acute leukæmia treated with 6-mercaptopurine during the last two years. Four of the cases were lymphoblastic, 6 myeloblastic, and 5 monocytic. The 6-mercaptopurine was given usually in a single daily oral dose at an initial rate of 2·5 mg./kg. Most patients therefore received 120 to 200 mg. daily during the early stages of treatment. Dosage was maintained at this level until suppression of leukæmic

proliferation became manifest by a fall in the peripheral leucocyte-count and by a reduction in the proportion of primitive cells in the blood and bone-marrow or, in unresponsive patients, until an adequate therapeutic trial had been continued for 3 weeks. When evidence of response was obtained intermittent therapy or maintained treatment at a lower dosage was administered until definite leucopenia was produced. When a complete remission was obtained by this means the drug was discontinued and not resumed until relapse threatened. Where remission was only partial frequent intermittent courses of treatment were given in an attempt to prevent recrudescence. Seven of the patients experienced remission, which was partial in 5 and complete in 2. The complete remissions occurred in patients with myeloblastic and lymphoblastic leukæmia and lasted respectively 3 months and more than 8 months. The partial remissions were obtained on 1 patient with myeloblastic, 1 with lymphoblastic and 3 with monoblastic leukæmia. Gastro-intestinal toxic effects were not observed in most of the patients, but in 2 cases anorexia, nausea and vomiting were unpleasantly severe. The author concludes that 6-mercaptopurine is at present probably the agent of choice in myeloblastic and monoblastic leukæmia of adults. S. L. W.

Nitrogen Mustard and Tretamine (Triethylene Melamine), Hæmopoietic Depression from. R. G. Mrazek and T. J. Wachowski. (*J. Amer. med. Ass.*, 1955, **159**, 160.) Nitrogen mustard and tretamine have been proved to be effective drugs in the treatment of the malignant lymphomas but they may cause severe and uncontrollable depression of the hæmopoietic system. In the present study 100 patients with malignant lymphoma were treated with either one or other of the drugs, a total of 189 courses being given. Hæmopoietic depression occurred in 54 of the 100 patients; 8 had alarming bone marrow depressions and 5 of these died. The incidence of complications did not appear to be related to the specific disease process. Previous treatment with irradiation or chemicals was the factor most directly related to the occurrence of peripheral blood depression. A second factor of importance was the general condition of the patient; the bone marrows of those patients who were debilitated or had widespread disease were affected most by the medication. The patients with early disease tolerated initial courses of either drug without appreciable drops in blood cell count. It is imperative that the hæmatological status of all patients be known before treatment is instituted and that frequent checks be made as treatment progresses. Hæmopoietic depression may be observed 10 to 51 days after the beginning of treatment and careful observation should continue for at least this period. Once a serious leucopenia or thrombocytopenia has occurred the only effective treatment is repeated transfusion. Eighty-one per cent. of the patients in this series improved under treatment, but residual malignant lymphoma was present in all the patients who came to autopsy. S. L. W.

Noradrenaline and Adrenaline Content of Cat Organs, Effect of Amine Oxidase Inhibitors on. U. S. von Euler and S. Hellner-Björkman. (*Acta physiol. scand.*, 1955, **33**, Suppl. 118, 21.) A study is made of the effect of various amine oxidase inhibitors (propamidine, methylamphetamine, choline-*p*-tolyl-ether, ephedrine and cocaine) on the adrenaline and noradrenaline content of the cat heart, spleen and liver. In no instance were highly significant changes observed, though increased noradrenaline figures in the heart were observed after propamidine in several cases. Increased adrenergic nerve activity caused by carotid occlusion, carotid sinus denervation or electrical stimulation of the splenic nerves had no consistent effect on the catechol amine content of the organs in the presence of amine oxidase inhibitors. M. M.

Noradrenaline and Adrenaline, Urinary Excretion during Recumbency and Standing. U. S. von Euler, R. Luft and T. Sundin. (*Acta physiol. scand.*, 1955, **34**, 169.) The free catechol amines in the urine of 15 healthy adults were assayed, subsequent to extraction, on the blood pressure of the cat and the hen rectal cæcum. It was found that when the person was placed on a tilting table at an angle of 75 degrees for 3 or 4 hours, there was a considerably increased urinary output of noradrenaline in comparison with the corresponding excretion in the recumbent position. This increased production of noradrenaline is interpreted as the result of reflex activation of the vasomotor system induced by the orthostatic fall of the systolic blood pressure. The excretion of adrenaline in urine during the tilting test showed a slight to moderate increase, probably depending on various stress factors during standing. M. M.

Noradrenaline in the Treatment of Severe Shock. I. E. W. Gilmour. (*Brit. med. J.*, 1955, **2**, 1248.) (−)-Noradrenaline bitartrate was used in the treatment of 4 cases of shock caused respectively by hæmorrhage and post-operative collapse (two cases), post-anæsthetic inhalation of gastric contents combined with anoxia, and toxæmia. One of the hæmorrhage cases died from pulmonary embolism but in the other three cases almost immediate improvement in the blood pressure followed its administration. The noradrenaline was given by intravenous drip in the form of 4 ml. of Levophed in 1000 ml. of glucose-saline, the rate of administration varying from 18 to 80 drops per minute but in one case where 80 drops per minute was insufficient the concentration was doubled. The blood pressure must be taken every minute initially until it is stabilised, the rate of drip being varied accordingly. It is suggested that noradrenaline should only be used in severe shock when other "orthodox" methods have failed. H. T. B.

Pennyroyal Poisoning. W. B. Vallance. (*Lancet*, 1955, **269**, 850.) This is a report of a fatal case of poisoning after the use of pennyroyal as an abortifacient. The drug was taken by a healthy married woman aged 24, in the third month of pregnancy, and produced abortion, vaginal bleeding, hæmolytic anæmia, and acute tubular necrosis of the kidney, with death from uræmia on the 14th day. Before admission to hospital, and subsequent to the abortion which was followed by heavy bleeding, she developed a widespread rash and pyrexia; she also complained of nausea, vomiting, abdominal pain, and diarrhœa. It was not possible to ascertain how much pennyroyal was taken, but in any case the effect of the drug on any particular person is unpredictable. One teaspoonful of the oil has been known to produce convulsions, and there is a case on record where a patient recovered from coma following ingestion of 15 ml. of essence of pennyroyal. S. L. W.

***Rauwolfia serpentina* Benth., Anti-acetylcholine and Antihistamine Actions of the Total Alkaloids.** M. L. Chatterjee and H. F. Hausler. (*Nature, Lond.*, 1955, **176**, 701. Experiments were carried out on isolated strips of guinea-pig ileum in Dale's bath, containing oxygenated Ringer-Locke's solution at 35° C., and the modifications caused by varying concentrations (10^{-6} to 10^{-4} g./ml.) of extracts of *Rauwolfia serpentina* (total alkaloids extracted in 2 per cent. hydrochloric acid) on the action of acetylcholine (10^{-8} g./ml.) or of histamine (10^{-8} to 10^{-7} g./ml.) were studied. A concentration of the extract of 5×10^{-5} g./ml. and stronger, completely annulled the effect of 10^{-8} g./ml. of acetylcholine. Extracts of rauwolfia in concentrations of 5×10^{-5} g./ml. and stronger, applied 30 to 50 sec. before the addition of histamine, inhibited the action of 10^{-8} or 5×10^{-8} g./ml. of the latter. A stage of slight potentiation of the action of

(ABSTRACTS *continued on p.* 224).

PHARMACOPŒIAS AND FORMULARIES

INTERNATIONAL PHARMACOPOEIA
FIRST EDITION VOLUME II*

REVIEWED BY K. R. CAPPER

In spite of a promise in the preface that the second volume would contain monographs on injections, tablets, certain tinctures and "certain newer drugs, for instance, antibiotics . . ." the first volume of the *International Pharmacopoeia* allowed an assessment of the quality of the book but not its scope. The 217 monographs in Volume II now completes the first edition and gives a pharmacopœia containing 435 monographs, over 300 less than in the British Pharmacopœia. It would be quite wrong to deduce from these figures that the International Pharmacopoeia is inadequate as a book of standards. The number of monographs on inorganic chemicals and on organised and unorganised drugs of vegetable origin has been rapidly decreasing in recent editions of most of the national pharmacopœias. It is understandable that a new pharmacopœia should be short of these types of drugs. However, the major cause of the relatively small number of monographs is the omission of all except a few types of formulated products, i.e., injections (including sterile powders for preparing these), tablets, standardised vegetable powders, solutions, tinctures and a few diluted products such as dilute hydrochloric acid, liquefied phenol, diluted ethanol and diluted glycerol. The B.P. on the other hand still contains 34 different types of preparations. Although it is probable that products such as capsules and implants will find their way into later editions of the Ph.I. the decision to omit preparations such as ointments, lotions and syrups is wise. These vary much more from country to country than do the drugs from which they are made or the simple dosage forms such as injections and tablets. Any country adopting the International Pharmacopoeia as its national book of standards will presumably supplement it with a formulary for products likely to be dispensed and it may be that this is the most satisfactory arrangement even in countries with national pharmacopœias.

The promised antibiotics include all those in the B.P., mostly under names which in spite of being in a sadly un-English style of Latin are recognisable; a possible exception is aureomycin hydrochloride which bears the latinised version of the U.S.P. title, chlortetracycline hydrochloride. The other "newer drugs" include many which have been added to the B.P. and B.P.C. in the last five years, for example, amodiaquine hydrochloride, cortisone acetate, cyanocobalamin, ethinylœstradiol, dimercaprol, gallamine triethiodide, methadone hydrochloride, sodium aminosalicylate and a number of antihistamines. It would be an easy matter to criticise this selection. It is a pity for instance that a place could not be found for a monograph on sulphadimidine which is at the least the equal of any sulphonamide in the International Pharmacopoeia and that all the principal antimalarial drugs are now included

*Pp. xx + 350. World Health Organisation, Geneva, 1955. 35s. Available from The Pharmaceutical Press, 17, Bloomsbury Square, London, W.C.1.

except pyrimethamine and primaquine. The narcotic drugs hydrocodone hydromorphone, metapon and oxycodone have not found much favour in this country nor have acetylcholine chloride and aminoacetic acid. Nevertheless this sort of criticism can be and is levelled at all pharmacopœias and it is admirable that so reasonable a list of contents could be selected by international agreement. It is more regrettable that agreement cannot be achieved on names. Those who have mastered the approved names issued by the British Pharmacopœia Commission may well be excused if they do not recognise benzhexol as trihexyphenydyl, noradrenaline as levarterenol and ethopropazine as profenamine.

Approximately half the monographs in Volume II are on injections and tablets. A general monograph is included in each case. That for Injections closely resembles Appendix XX in the B.P. in that it gives details of methods of sterilisation including that of "heating with a bactericide," describes containers, deals with the addition of bacteriostatics, the treatment of rubber caps, and excess volumes in ampoules. Two points of difference are the requirements that all aqueous vehicles must comply with the test for pyrogens and that fixed oils used as vehicles must conform with specifications for acid, iodine and saponification values and with a test for the absence of mineral oil. The first difference is largely one of style as Water for Injection B.P. must comply with the test for pyrogens and now the 1955 Addendum has extended this requirement to Injection of Dextrose and Injection of Sodium Chloride. A few monographs have been included for sterile powders for the preparation of injections of mepacrine methanesulphonate, phenobarbitone sodium, procaine benzylpenicillin, tetracaine hydrochloride and tryparsamide.

Tablets have been given the title "Compressi" a title which from the definition can apply to moulded as well as to compressed tablets. The disintegration test which applies only to uncoated tablets is a simple one requiring no special apparatus. Each of six tablets is immersed separately in a flask containing water at 37° C. and rotated every half minute without touching the sides of the flask. After 30 minutes the tablets must either disintegrate or else soften so that they disintegrate at a slight touch. In spite of its simplicity, the test is tedious and likely to give results difficult to interpret. There is no statement on colouring.

The standards for the drugs and preparations are similar in style and stringency to those in the B.P. and U.S.P. There are exceptions to this, for example, folic acid with a minimum of 85·0 per cent. determined on the dried substance compared with 94·0 per cent. in the B.P., glycerol with a lead limit five times the B.P. figure, while chlorophenothanum technicum is a material of significantly lower grade than B.P. dicophane. On the other hand there are some specifications which are more stringent than the corresponding B.P. requirements and assays have been included for several drugs not assayed in the B.P.

Those Appendices in Volume II which are not supplementary to those in Volume I include two which give detailed descriptions of the determination of methoxyl and of water by the Karl Fischer method. Two

other Appendices give descriptions of fluorometry and of spectrophotometry and related subjects, these however are so general that they could have been omitted from a book of standards published in 1955. Biological assays are described for the gonadotrophins, insulin and protamine zinc insulin, tubocurarine chloride and certain of the antibiotics. The test for pyrogens is arranged somewhat differently from that in the 1955 B.P. Addendum; the test is in two stages, the first with three rabbits and if this is not passed a second with five rabbits. It seems likely that the Ph.I. test is more severe than the four stage B.P. test.

The doses are not given at the foot of the monographs but, as in Volume I, in an Appendix as a table of usual and maximal metric doses. There is also a table giving the usual daily doses for children of many drugs, one column giving these for children of up to 30 months and another for older children, doses for toxic substances are usually on a weight basis. This table is likely to be useful to many who might not otherwise consult the International Pharmacopoeia. It is unfortunate that the milligram is not used in giving doses as, in spite of the increasing application of the metric system to dosage, many prescribers and pharmacists dislike long strings of noughts after a decimal point.

The Expert Committee on the International Pharmacopoeia are to be congratulated on the results of their efforts. It is however generally recognised that the major source of inspiration of the International Pharmacopoeia and its detailed planning was the late Dr. Hampshire, and the two volumes of the first edition are his monument. The Chief of the Pharmaceutical Section of the WHO, Mr. P. Blanc, who acted as secretary, and his assistant, Mr. G. R. Brown, are also entitled to our congratulations.

BOOK REVIEWS

OFFICIAL METHODS OF ANALYSIS, A.O.A.C., 1955, Eighth Edition. xvi + 1008 (including Index). Published by the Association of Official Agricultural Chemists, Washington, D.C., U.S.A. (U.S.A. $12.00; elsewhere $12.50).

The eighth edition of this work presents the Official Methods of the Association as revised during the five years since the publication of the seventh edition in 1950. "Official Methods" is well known in most analytical laboratories and it is chiefly of interest, therefore, to note the additions and alterations which have been made since 1950.

Changes have, in fact, been considerable with an expansion of about 100 pages chiefly in the sections devoted to pesticides, flavourings, drugs, extraneous materials, microchemical methods and nutritional adjuncts; chapters on spectroscopic methods and hormone drugs have also been added. Agricultural commodities are divided into six main parts: (1) soils and related materials; (2) miscellaneous materials other than foods and drugs; (3) foods; (4) drugs and cosmetics; (5) general methods; and (6) reference tables.

A substantial advance has been made towards the more rigid standardisation of methods. No longer is copper permitted as a catalyst in the Kjeldahl determination of total nitrogen; mercury or mercuric oxide alone are now allowed and other experimental details are prescribed following an extensive collaborative study.

The chapter on colouring matters has undergone fundamental revision and

the classical methods of isolation of coal-tar colours by dyeing on and stripping from wool, and identification of the colours by spot reactions have been replaced by determinations using chromatography and spectrophotometry. The micro-chemical chapter which in the seventh edition included only methods for micro Kjeldahl and alkoxy group determinations has been expanded by the addition of standardised methods for the elemental analysis of bromine, chlorine, carbon, hydrogen and sulphur.

The vitamin chapter has been renamed "Nutritional Adjuncts," the title being designed to recognise the potentialities of nutritionally significant factors such as amino-acids and antibiotics. The fermentation method for thiamine has no longer been included in this chapter but microbiological methods for pantothenic acid and vitamin B_{12} have been added. As a minor criticism it is a little surprising that the standard for vitamin B_{12} should consist of a weighed quantity of standard reference material; a quantitative standardisation by spectrophotometry would be more precise.

In view of the fact that collaborative trials precede the adoption of many of the Association's methods it would be most helpful if the limits of error to be expected could be given, particularly for biological and microbiological assays.

In conclusion it can be stated that the eighth edition of this work represents a considerable advance over the previous editions. "Official Methods" is an essential volume for any large analytical laboratory and it is a credit to all concerned that the book is so widely used for its methods in fields of chemistry outside those for which it is written. R. E. STUCKEY.

STATISTICS OF THERAPEUTIC TRIALS, by G. Herdan. Pp. xvi + 367 (including Index). Cleaver-Hume Press, Ltd., London, 1955. 50*s*.

This elegantly produced book is written to help people who are concerned with therapeutic trials and who find mathematics distasteful. Explanations of mathematical processes are avoided and emphasis is placed on a clinical approach. Most of the chapters deal with trials in specific diseases. The acute diseases which are particularly considered are the pneumonias, poliomyelitis, scarlet fever, diphtheria, typhoid fever and pneumococcal meningitis, and the chronic ones are cancer, tuberculosis of the lung, rheumatoid arthritis, congestive heart failure, hypertension, diabetes and nephritis. It is a little difficult to see why this list was selected. It omits some very widespread disorders, such as peptic ulceration, schizophrenia and the common cold, in which properly designed therapeutic trials are urgently needed to separate effective treatments from useless ones. On the other hand, the diseases included are sometimes rather similar to each other and sometimes extraordinarily indefinite (e.g., cancer), and unlikely always to be amenable to the same kind of therapeutic trial. This sort of difficulty seems to arise from trying to avoid general treatment of the logic and mathematics of therapeutic trials, and distinctly limits the usefulness of this book.

Once general principles are discarded in favour of a series of examples, there is no end to the material which must be brought in, and there is always uncertainty whether a new situation may not arise which will not be filled by any of the available examples. It may also be questioned whether anyone who does not understand the principles which underlie therapeutic trials can safely decide which example it is appropriate to follow in a given instance. Even as a set of recipes this book therefore has limitations: as a reasoned guide to procedure it is quite bewildering.

It must also be admitted that the style of writing is rather ponderous and

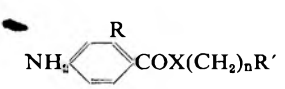
sentences such as this are common. "The therapeutic comparison between the disease behaviour in the control and the trial group is necessarily restricted to that of one or the other nosographic criterion which has been chosen for observation by the investigator because he considered it representative of the disease." The author is also inconsistent in his use of abbreviations (e.g., the tangent of an angle is referred to as tg. on p. 169 and tang on p. 355, and it is difficult to see why the orthodox symbol tan is avoided) and not always accurate in his references (e.g., to Burns, for Burn, on p. 255 and p. 349). The non-mathematical should be warned that in spite of the clinical approach, they will not be spared much algebra and an occasional and perhaps unnecessary flavour of trigonometry. MILES WEATHERALL.

(ABSTRACTS *continued from p.* 219).

both acetylcholine and histamine was noted in a few cases during the phase of recovery of the action of acetylcholine and histamine on repeated changing of the bath fluid after an earlier observation of the action in the presence of rauwolfia extract. A. H. B.

Riboflavine Excretion Technique, Reliability in Determining Availability of Coated Tablets. D. G. Chapman and J. A. Campbell. (*Canad. J. Biochem. Physiol.*, 1955, **33**, 753.) Determination of the urinary excretion of riboflavine in human volunteers is a valid and reliable procedure for determining the physiological availability of coated tablets. Eight volunteers receiving a normal diet were given 1, 3 and 5 mg. amounts of riboflavine. Excretion was the same whether these amounts were given as single or divided doses. With doses of 1, 3, 5, 7·5 and 10 mg. there was a linear relationship between the excretion and the dose, but the response line did not pass through the origin. In calculating the availability of riboflavine from a tablet, a curve for each subject should be referred to and there is a suggestion of a slope difference between subjects. G. F. S.

Thiocaine and Related Compounds, Alkoxy Analogues of, Corneal Anaesthetic Activity and Toxicity. F. P. Luduena and J. O. Hoppe. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 393.) Procaine and a series of its derivatives of the general formula (I) below were tested for anæsthetic activity on the rabbit cornea. The concentration required to produce anæsthesia lasting for 5 minutes was calculated and compared with the concentration of cocaine required to produce the same effect. The LD50 of each substance was determined in mice and compared with that of cocaine. Replacement of the oxygen atom (X) in procaine by S resulted in a sixfold increase in activity and toxicity. In the sulphur analogue (thiocaine), introduction of a 2-propoxy group at R produced a 130-fold increase in activity with a 13-fold increase in toxicity. In this series (where X = S) activity increased with the length of the 2-alkoxy side chain, the 2-hexyloxy derivative being particularly active. Toxicity increased with the length of side-chain, but to a lesser extent. In the procaine series (X = 0), activity and toxicity also increased with the length of the 2-alkoxy group. A moderate increase in activity was obtained by substituting a methylpiperidyl group for the diethylamino group in the thiocaine series. The ratio of activity to toxicity (taking cocaine as 1) varied from 0·16 for thiocaine to 8·3 for its 2-hexyloxy derivative. All the compounds except procaine, thiocaine and 2-ethoxyprocaine were more active than cocaine, and all the 2-alkoxy derivatives of thiocaine were more active than cinchocaine. G. B.



(I)

X=O or S