'DEGRADATIVE AND SYNTHETIC STUDIES ON TUBOCURARINE AND SOME RELATED COMPOUNDS'

THESIS

submitted to

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by

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SUMMARY

The history of curare, its production, the botanical sources and biologically-active constituents of the various curares are reviewed. The derivation of the structure of curine and tubocurarine is discussed together with the total synthesis of (±)-tubocurarine by Tolkachev, Voronin and Preobrazhenskii.

The pharmacological role of acetylcholine related to nerve impulse transmission at the neuromuscular junction, and the action of depolarizing neuromuscular blocking agents are briefly reviewed. The use of neuromuscular blocking agents in surgery and the compounds employed in practice are discussed. Structure-action relationships between tubocurarine, its isomers and certain of their derivatives are examined and the marked differences in potency noted.

An experimental approach to the determination of the absolute configuration of (+)-tubocurarine has been examined. The method, based on the measurement of optical rotations of benzyltetrahydroisoquinoline compounds in solvents of increasing polarity, required the cleavage of the bisbenzyltetrahydroisoquinoline molecule of (+)-tubocurarine into its constituent benzyltetrahydroisoquinoline moieties. Attempts to convert (+)-O,O-dimethyltubocurarine iodide, required for this purpose, to (+)-O,O-dimethyltubocurine with lithium aluminium hydride were unsuccessful. N-Dealkylation of the quaternary iodide with ethanolamine, however, yielded the required product in 58% yield. N-Dealkylation of (+)-tubocurarine chloride with ethanolamine similarly yielded the tertiary base (+)-tubocurine in high yield. Successful conversion of the resulting (+)-tubocurine to (+)-O,O-dimethyltubocurine by treatment with excess diazomethane and subsequent methylation to the corresponding tubocurarine derivatives provided evidence that dealkylation proceeds without racemisation.

Examination of methods for the cleavage of (+)-O,O-dimethyltubocurine with sodium and liquid ammonia, which in the closely related (-)-O,O-dimethyl-curine is reported to yield (-)-O-methylarmepavine and (-)-N-methylcoclauri in benzene-toluene and (-)-O-methylarmepavine,(-)-N-methylcoclaurine and (-)-laudanidine in dioxan, led to the isolation of two non-phenolic and three phenolic compounds. Separation of the reaction products on alumina columns resulted in the identification of starting material from the non-phenolic fraction, and (-)-laudanidine and (+)-N-methylcoclaurine from the phenolic fraction, implying that the course of the reaction is not influenced by solvent, but merely facilitated by the presence of methoxyl substituents ortho to the phenolic ether undergoing cleavage, in accordance with the observations of Tomita. Sodium and liquid ammonia fission of phaeanthine revealed evidence of fission on both sides of the phenolic ether linkage in further support of these conclusions. Optical rotation measurements on (+)-N-methylcoclaurine and (-)-laudanidine in different solvents confirmed the hypothesis of Tomita who in 1962 deduced absolute configuration at both optical centres of the tubocurarine molecule.

(-)-O,O-Dimethylcurine was similarly produced by the action of diazometh on curine. The four tertiary bases, (+)-tubocurine, (+)-O,O-dimethyltubocuri (-)-curine and (-)-O,O-dimethylcurine were treated with a series of alkyl halides to produce their respective series of quaternary halides.

ACKNOWLEDGEMENTS

The author wishes to take this opportunity to gratefully acknowledge the guidance and understanding shown by Professor J.B. Stenlake who kindly suggested the problem and also to thank Mr. G.A. Smail for his stimulating interest and valuable advice during the course of this study. The author would like also to record his thanks to Mr. R. Nugent and his staff and Mrs. J.A.R. Thomson for technical assistance and to the D.S.I.R. and the University of Strathclyde for financial support.

CONTENTS

H I S T O R I C A L

INTRODUCTION

Curare, the so-called flying death, is a dart or arrow poison prepared by the South American Indians in the primeval forests of the upper Amazon and upper Orinoco basins. The preparation of curare was the monopoly of only a few tribes since the raw materials were found in only limited areas of the Amazonian hileia. In consequence curare represented a source of wealth to the manufacturi tribe and its processes of manufacture were shrouded in ritual and superstition.

The final product is a dark brown, gummy decoction; the actual constituents including the toxic principles vary considerably from area to area of the region from which the material is collected depending upon the plant source used in the preparation. On the one hand trees of the genus Strychnos supply most of the toxic material for some curares whereas Menispermaceous plants are mainly involved in the others.

The primary use of curare was in the hunting of small mammals (monkeys etc.) which die by respiratory paralysis leaving the meat untainted. The dart points are treated with curare and fired through a blow-pipe called a sarabatane. The effective distance and accuracy of the blow-pipe was dependent on its length, the longer the pipe the more effective it was. The dart could be fired with accuracy up to a distance of 45 metres. Although seldom used for war purposes arrows and spears were occasionally treated in the same way as the darts.

Despite the obvious armigerous potential of curare its

infrequent use as an instrument of war springs from the superstitions of the people of the region who believed that curare had been given to them by their spirits for hunting and if used in warfare the curare would lose its potency. If the potency was lost they could not hunt and starvation would ensue. At present curare and the sarabatana are fast disappearing with the advance of modern civilisation which has infiltrated into tribal hunting areas. Traditional patterns of hunting and warfare have been abandoned and the weapons which made use of curare rendered obsolete.

Originally three main varieties of curare were recognised in commerce. The containers in which they were exported were at one time deemed diagnostic of the type, now recognised as no longer being a satisfactory method of classification. These varieties were:

1. Calabash or Gourd Curare;
2. Pot Curare;
3. Tube Curare.

1. Calabash Curare.

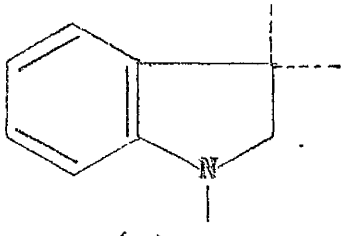
The main sources of calabash curares are different species of Strychnos trees, a wide variety of which are used to produce this curare but notably S. toxifera, S. melinoniana, S. mitscherlichii and S. guianensis.[1]

Systematic investigations of the alkaloids of C*-curare were initiated in 1937 by H. Wieland[2,3,4,5] and his co-workers who showed that alkaloids similar to those isolated from C-curare were present in

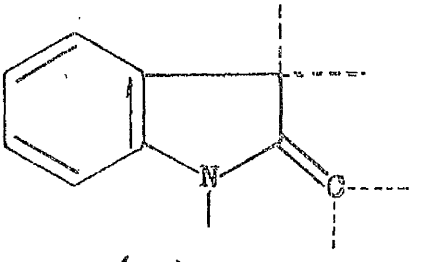
* C=Calabash

S. toxifera. A later investigation of S. toxifera in 1949 by King corroborated the results.[6]

About seventy alkaloids have so far been isolated from C-curares and Strychnos species but the structures of only account thirty of these have been elucidated. The majority are indole derivatives which may be correlated by their ultraviolet absorption spectra with one of several related chromophores including indoline (I), methylene indolene (II), indole(III), oxindole (IV), N-acylindole (V), ψ - indoxyl (VI), and the β-carbolinium ion (VII).



(I)



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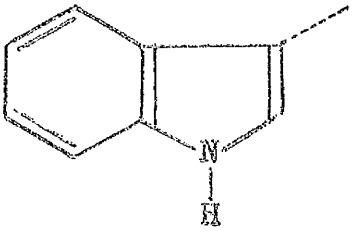

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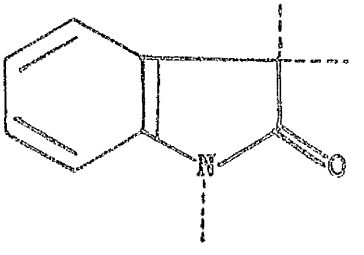

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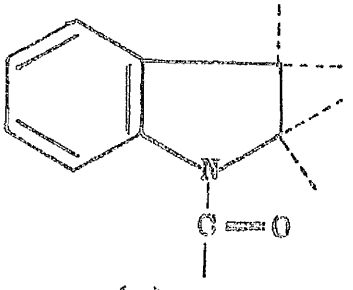

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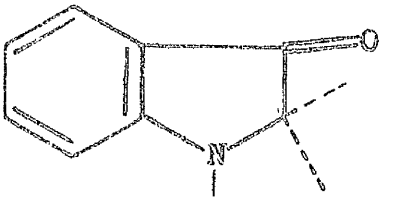

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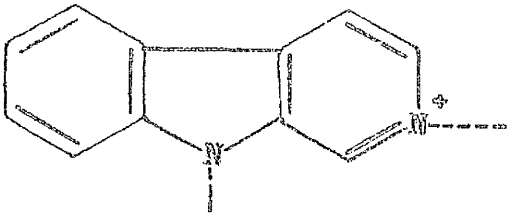
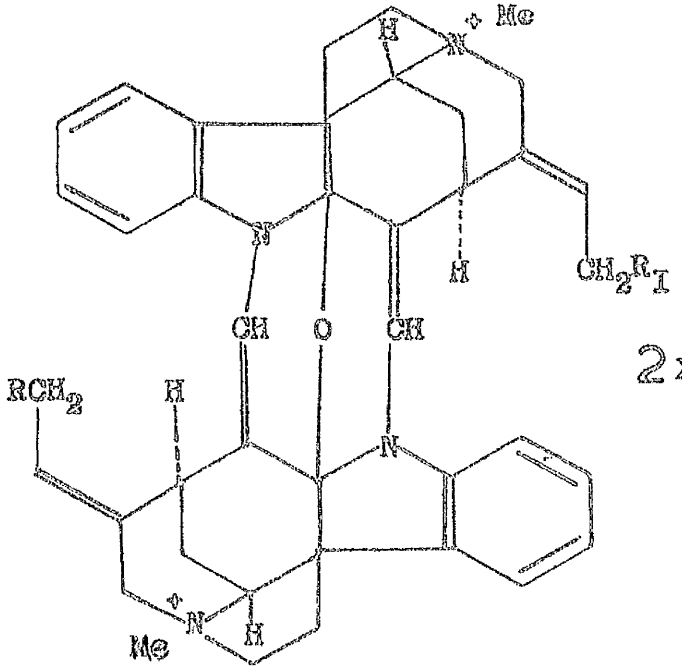


(VII)

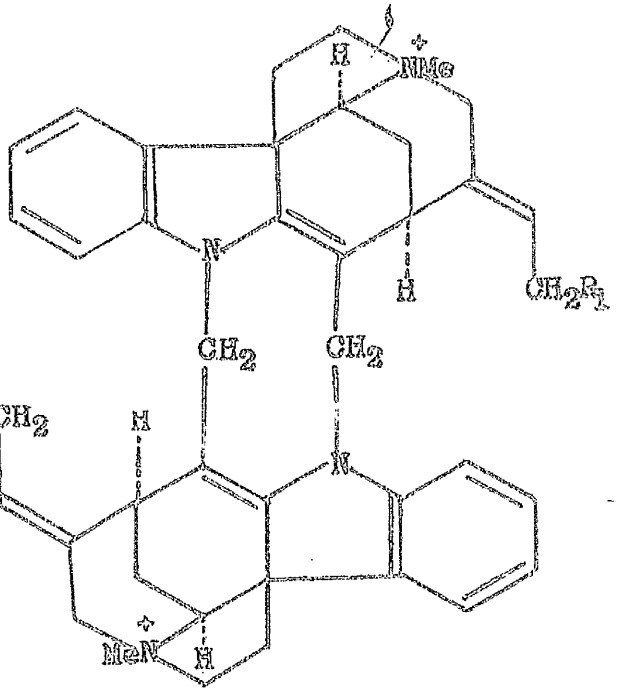
Separation of the complex mixture of alkaloids from samples of calabash curare or from S. toxifera plants was effected by intensive fractionation involving repeated chromatography on cellulose or alumina. The complexity of the problem of isolation can be gauged from the demonstration by Schmid, Kebrle and Karrer[7] of 41 alkaloids in a single sample of calabash curare from the Amazon basin. A single plant S. toxifera was shown by Battersby, Binks, Hodson and Yeowell[8] to contain at least 30 quaternary alkaloids.

Karrer, Schmid, Asmis, Bächli, Giesbrecht, Kebrle, Meyer and Waser from paper chromatographic and other evidence deduced that the alkaloids fell into two distinct groups - mono-quaternary and bis-quaternary alkaloids. The former had a high R_F value, relatively low toxicity and little pharmacological activity whereas the latter had low R_F values and much greater activity.

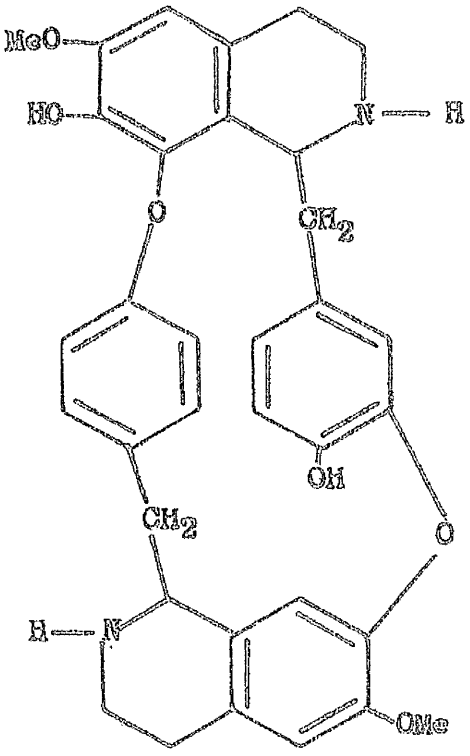
Originally all the alkaloids were considered to contain 19-21 carbon atoms but Karrer et al,[9] deduced from investigations involving the partial quaternisation of norcurine (VIII) that in some cases the molecular weights were much higher than that predicted by Wieland[2,3,4,5]. The bis- quaternary group which includes the important alkaloids C-curarine I (IX, $R=R_1=H$) C-dihydrotoxiferine (IXA, $R=R_1=H$) toxiferine I (IXA, $R=R_1=OH$) thus contained 38-42 carbon atoms and four nitrogen atoms and the mono-quaternary group 19-21 carbon atoms and two nitrogen atoms.


Me
H
N
N
H
CH_2R_1
CH
O
CH
RCH_2
H
N
N
Me
H
$2X^-$

(IX)


H
NMe
N
H
CH_2R_1
CH_2
CH_2
RCH_2
H
N
Me_2N
H

(IX A)

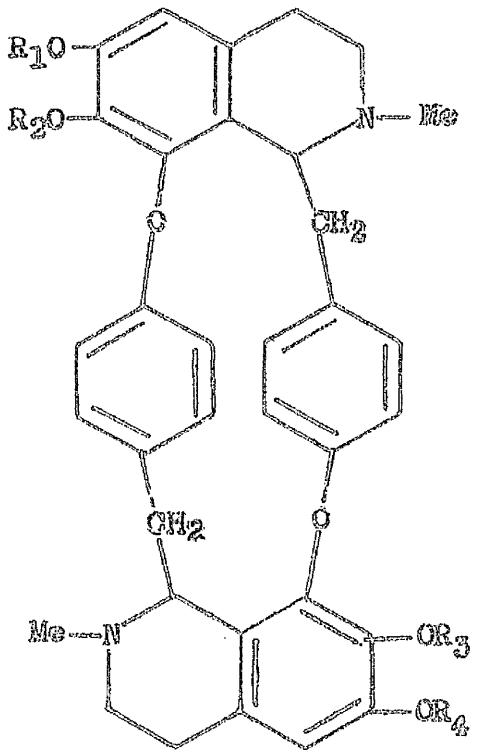

MeO
HO
N
H
O
CH_2
OH
CH_2
O
H
N
OMe

(VIII)

2. Pot Curare.

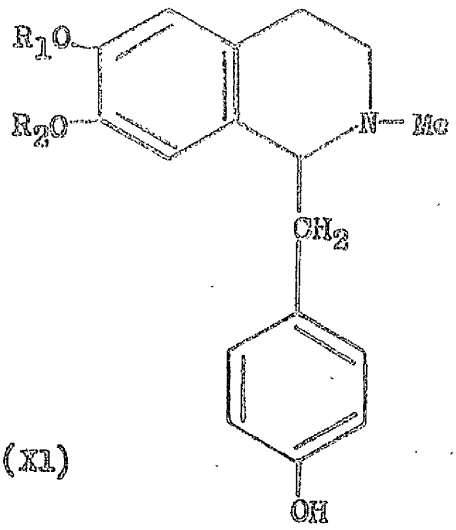
This type of curare was first investigated for chemical constituents by Buchner[10] in 1862 but he was unable to crystallise the active principles. In the latter part of the 19th century Boehm[11] reinvestigated pot curare and isolated the two crystalline bases protocurine and protocuridine.

King[12] in 1937 published his investigations in which he stated that the pot curare under examination contained many alkaloids, two of which, isoneoprotocuridine (X, $R_1=R_4=H$, $R_2=R_3=Me$ and protocuridine, (X, $R_2=R_4=H$, $R_1=R_3=Me$) were obtained in a crystalline condition. The latter was identical to protocuridine isolated by Boehm[11]. The bases were assigned the empirical



(X)

formula $C_{36}H_{38}O_6N_2$ by King[13], being double that previously suggested by Boehm. King further suggested that the alkaloids were based on a bisbenzyltetrahydroisoquinoline structure formed by the head-to-tail coupling of two norcoclaurine (Xl, R_1=R_2=Me) units.



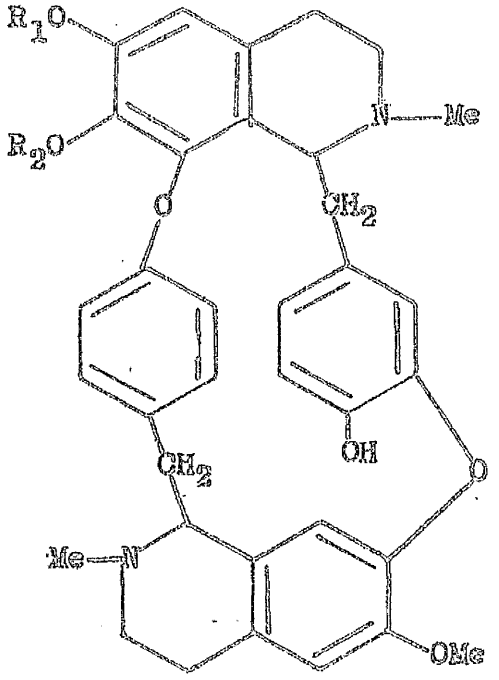
(xl)

The early belief that all curares were solely derived from various species of Strychnos was held until 1928 when Späth, Lefthe and Ladeck[14] and then later in 1935 King[15] cited Menispermaceous plants as a possible source of the drug. The botanical source of the pot curare investigated by King[15] would from the chemical constituents isolated, appear to be derived from Menispermaceous rather than from the Strychnos genus, a fact substantiated by the later work (q.v.) of King[15] and Dutcher[16] on tube curare.

3. Tube curare

Rather an abnormal situation occurred in the history of tube curare in that the important alkaloids had been isolated and chemically characterised and their structures elucidated before

their botanical source could be credibly established. The alkaloids referred to are the bases curine (XII, R_1=Me, R_2=H) chondocurine (XII, R_1=H, R_2=Me) and isochondodendrine (XIII) along with the quaternary bases tubocurarine (XIV, R_1=Me , R_2 = R_3=H) and chondocurarine (XIV, R_1=R_3=H, R_2=Me)



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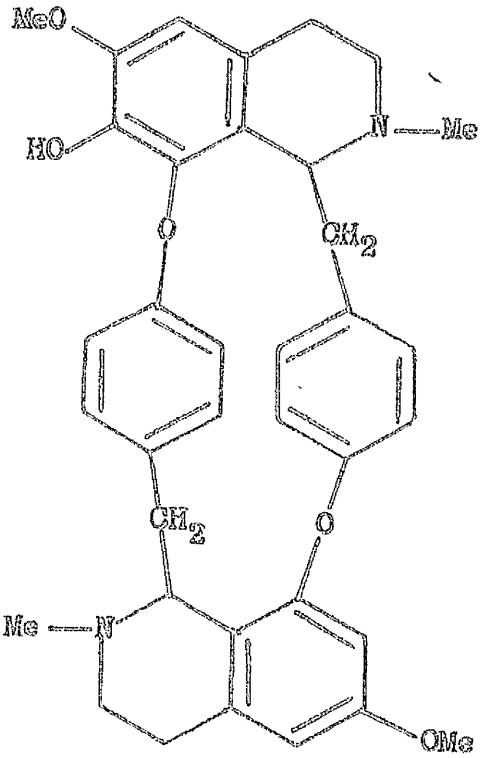

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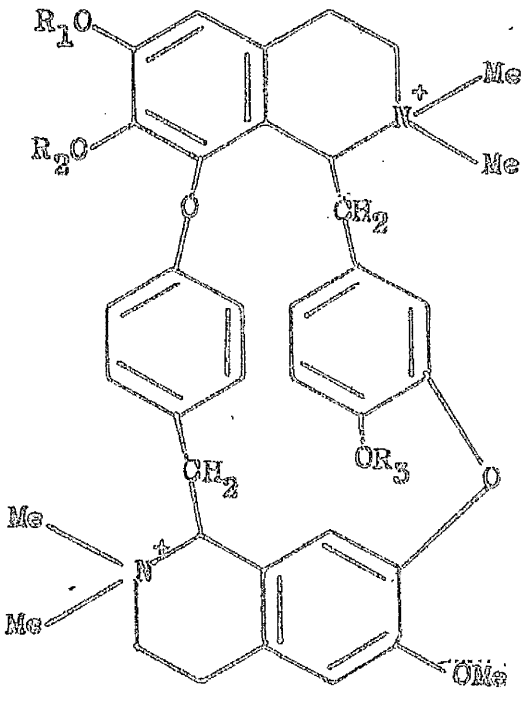
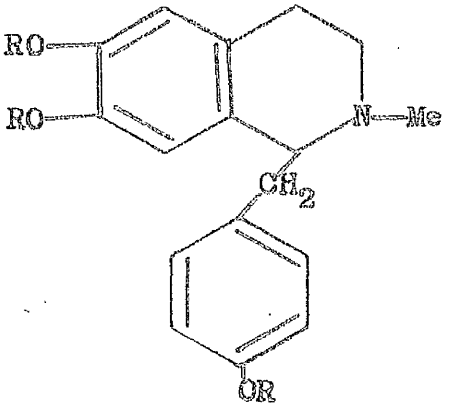


(XIV)

The only known source of the bases until about 35 years ago was the Brazilian drug radix pareirae bravae. In a reappraisal of a botanical review by Hanbury[17], Dells[18] expressed the view that pareira brava was the root of the Brazilian species Chondodendron platyphyllum (Miers). This was later reaffirmed by Krukoff and Moldenke[19] who included Ch. microphyllum (Eichl.) Moldenke as an additional source. A chemical examination of the roots and other parts of the two species by King[20] in 1940 confirmed this contention. Both species contained isochondodendrine (XIII) but in addition Ch. platyphyllum gave (-)-curine (XII, R_1=Me, R_2=H) whereas Ch. microphyllum yielded the dextro-enantiomorph. The only other material to have yielded (-)-curine up to the time of King was tube curare from which in 1897 Boehm[11] had isolated (-)-curine along with a quaternary amorphous base.

King[15] succeeded in 1935 in isolating the quaternary alkaloid (+)-tubocurarine, the active principle of tube curare, in crystalline form and further demonstrated the close structural similarity between it and curine. This fact made it extremely probable that the toxic botanical ingredient of tube curare was a Menispermaceous species of the genus Chondodendron. Ch. tomentosum was confirmed as the main source of the active constituents of tube curare when Wintersteiner and Dutcher[21] isolated (+)-tubocurarine from authenticated stems of Ch. tomentosum together with the alkaloids (-)-curine, (+)-isochondodendrine and its dimethylether, (+)-chondocurine and (+)-chondocurarine. With the exception of (-)-isococlaurine

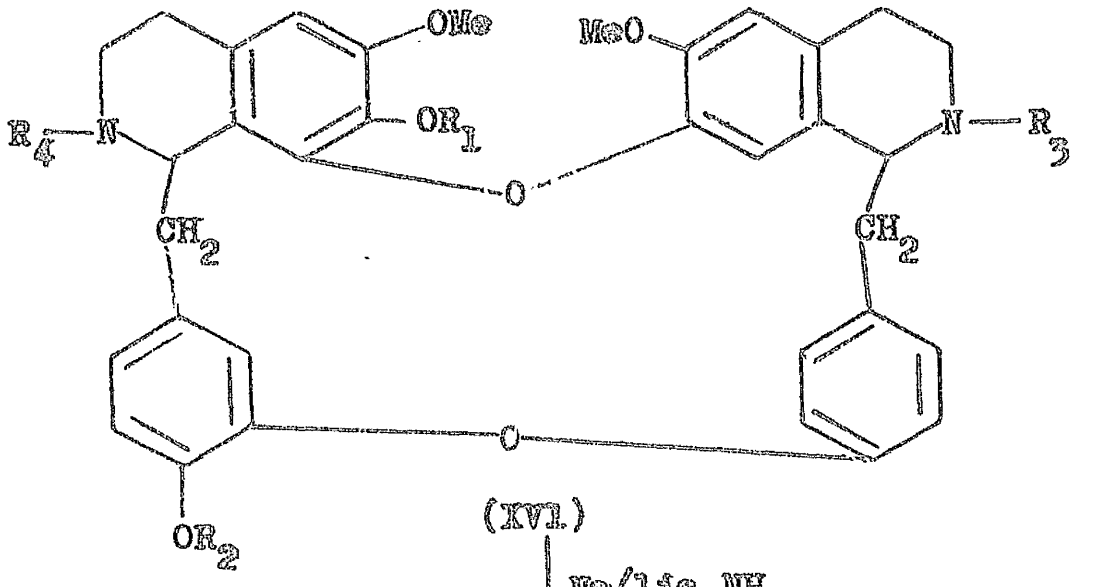
Chondodendron alkaloids are derived from a bisbenzyltetrahydroisoquinoline skeleton formed by the head-to-tail coupling of two coclaurine-type units(XV). In (+)-isochondodendrine (XIII)



(XV)

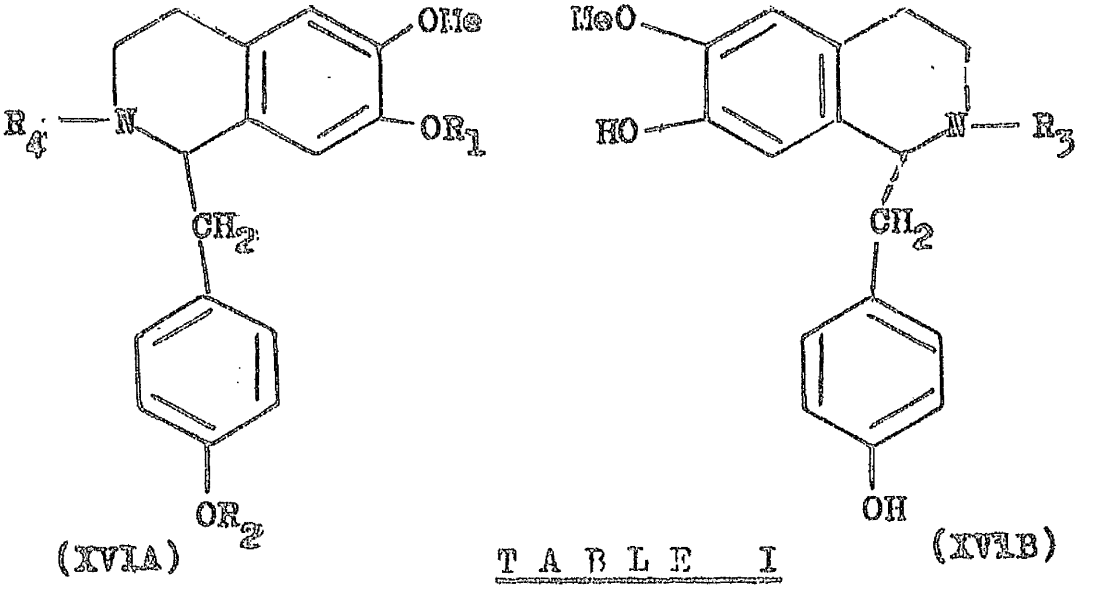
(+)-protocuridine (X, R_2=R_4=H, R_1=R_3=Me) and isoneoprotocuridine (X, R_1=R_4=H, R_2=R_3=Me) the two ether linkages form a centrosymmetric system whereas in curine (XII, R_1=Me, R_2=H), chondocurine (XII, R_1=H, R_2=Me), tubocurarine (XIV, R_1=Me, R_2=R_3=H), chondocurarine (XIV, R_1=R_3=H, R_2=Me), the ether bridges linking the two coclaurine structures are unsymmetrical.

An interesting comparison is provided by the alkaloids of certain other Menispermaceous plants in that two other possibilities of the arrangement of the diaryl ether are given on the one hand by isotetrandrine, berbamine, tetrandrine and phaeanthine[22] (Table I) and on the other hand by oxyacanthine, repandine, armoline, daphnoline and daphnandrine (Table II).



(XVI.)

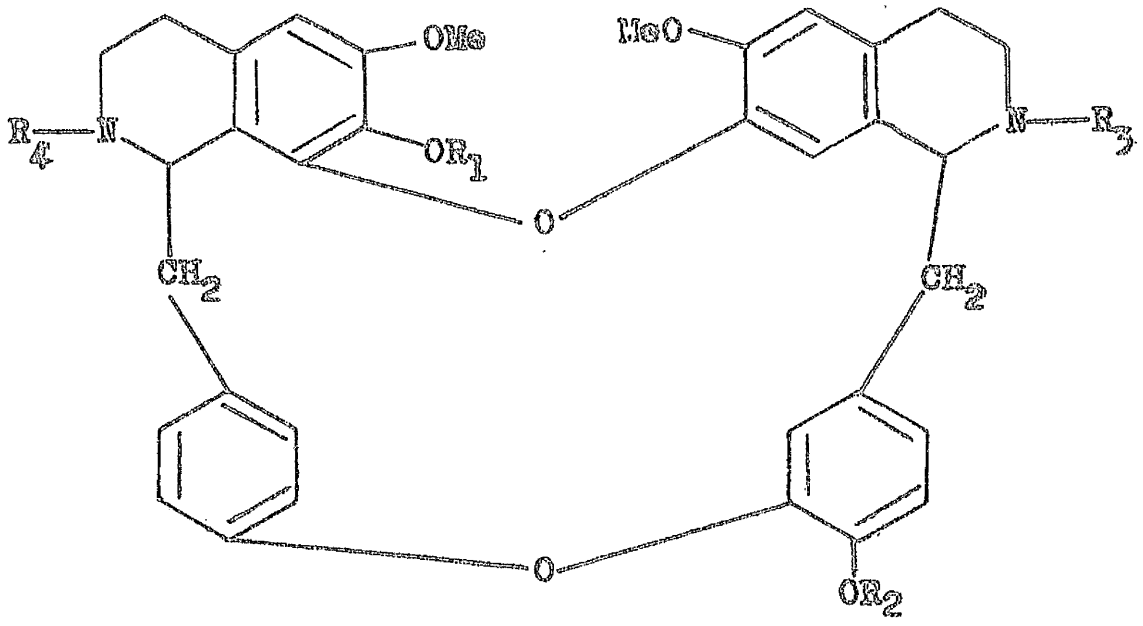
Na/liq NH_3



(XVIA) (XVIB)

T A B L E I

COMPOUND	STRUCTURE XVI			
	R_1	R_2	R_3	R_4
Isotetrandrine	Me	Me	Me	Me
Berbamine	Me	H	Me	Me
Tetrandrine	Me	Me	Me	Me
Phaeanthine	Me	Me	Me	Me



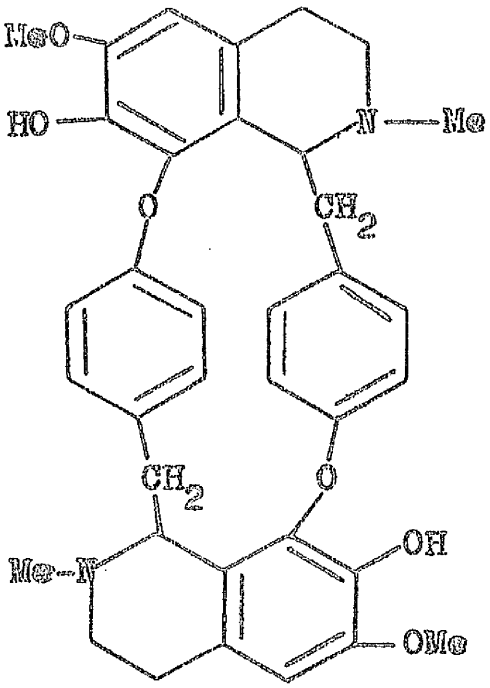
(XVII)

T A B L E II

COMPOUND	STRUCTURE XVII			
	R_1	R_2	R_3	R_4
Oxyacanthine	Me	H	Me	Me
Repandine	Me	H	Me	Me
Armoline	H	H	Me	Me
Daphnoline	H	H	H	Me
Daphnandrine	H	Me	H	Me

Although not constituents of curare, quaternary derivatives of these alkaloids do have appreciable curariform activity[23]. The main architects in the elucidation of the structures of the tetrandrine and oxyacanthine groups of alkaloids (Tables I and II) were Tomita and his coworkers[22]. Sodium and liquid ammonia cleaved the diaryl ether linkages of these alkaloids to produce a phenolic and a non-phenolic fragment corresponding to the two halves of the molecule. Thus, for example, tetrandrine (XVII, $R_1=R_2=R_3=R_4=Me$) on reductive fission yielded N-methylcoclaurine (XVIB, $R_3=Me$) and O-methylarmepavine (XVIA, $R_1=R_2=R_4=Me$). Examination of the products of fission and of the relative positions of the substituents on the phenolic and non-phenolic moieties permitted an unequivocal assignment of structure to these substances.

The elucidation of the structure of isochondodendrine (XVIII) was accomplished by Faltis, Wrann and Kühas[24] in 1932 and correlated with the two bases isolated from pot curare (+)-protocuridine (XIX) and neoprotocuridine, and by King when he demonstrated the identity of the dimethylether of (+)-protocuridine (X, $R_1=R_2=R_3=R_4=Me$) with O-dimethylisochondodendrine (XVIIIA). The difference in the two native alkaloids was in the relative positioning of the methoxyl and hydroxyl groups attached at positions 6 and 7 and at 6′ and 7′. On the basis of a positive Millon reaction both alkaloids have a free hydroxyl group at the 7 and 7′ positions. The most probable structure of isochondodendrine is (XVIII) in which case protocuridine must be



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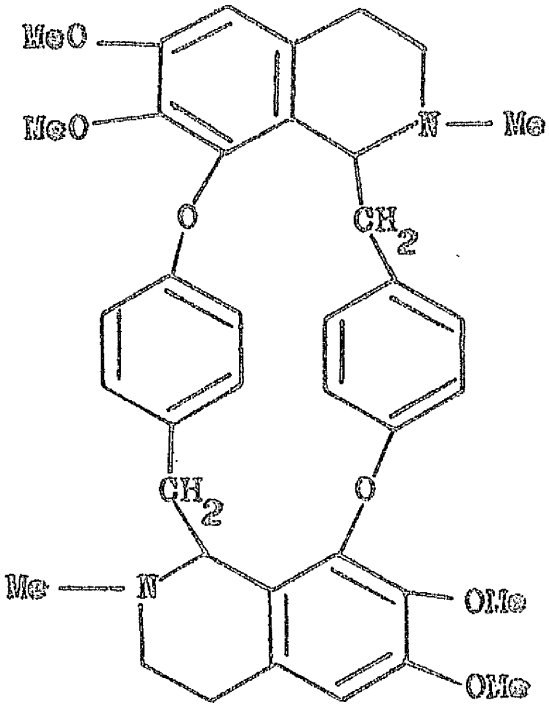
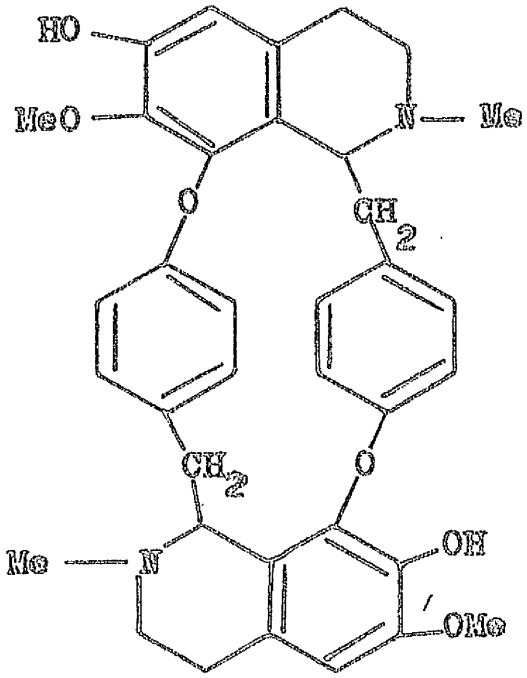
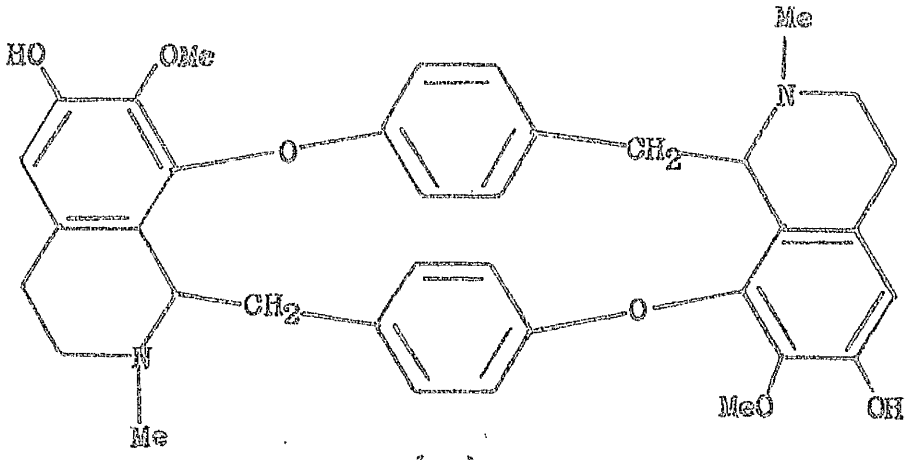


(XVIIIA)



(XIX)

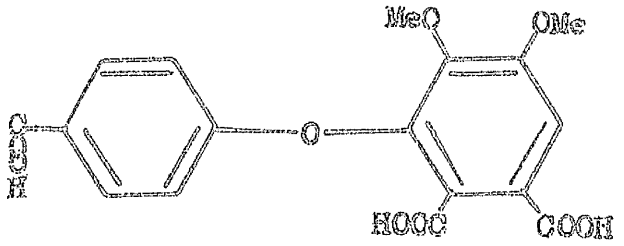
(XIX). Isoneoprotocuridine (XX) was related via a common degradation product to isochondodendrine (XVIII) and does not give a positive Millons reaction. It therefore followed that both its hydroxyl groups are in the 6 and 6′ positions as in (XX).



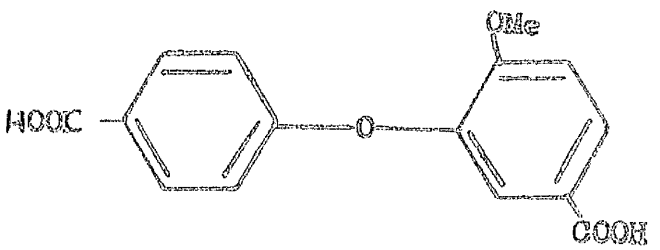
(XX)

Structure of Curine

Boehm and then later Späth, Leithe and Ladeck[14] proposed the empirical formula $C_{18}H_{19}O_3N$ for curine (XXI). However King[23] in 1933 suggested that curine should have double the empirical formula hitherto suggested assigned to it and that its structure was analogous to that proposed by Faltis, Wrann and Kühas[24] for isochondodendrine (XVIII). Almost simultaneously Späth and Kuffner[25] arrived at similar conclusions and demonstrated that oxidation of the methine obtained by means of a two stage Hofmann degradation of the dimethylether of curine yielded 2,3-dimethoxy-5,6,4'-tricarboxydiphenyl ether (XXA). This evidence showed that the structure of O, O-dimethylcurine (XVIII) was of the isochondodendrine type as opposed to the tetrandrine type, this latter on similar treatment yielding 2-methoxy-5, 4'-dicarboxy-diphenyl ether (XXB).

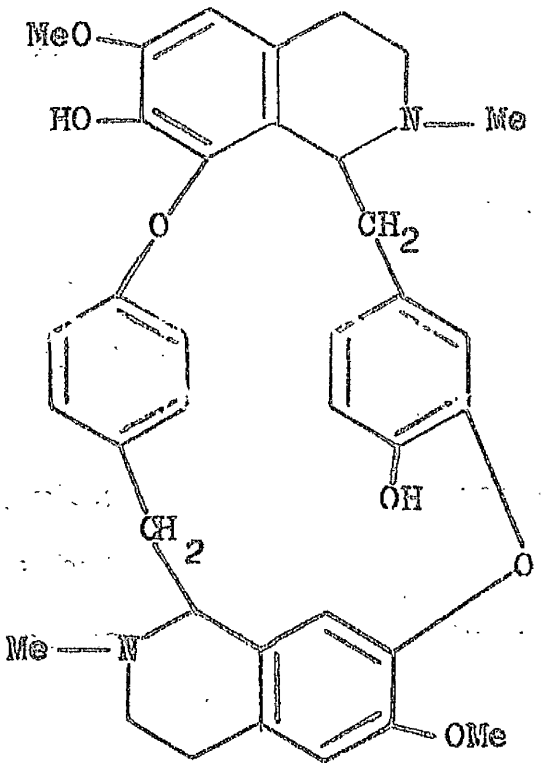


(XX A)



(XX B)

King[15] in 1935 adopted the structure (XXI) proposed by Späth and Kuffner[25] although not yet then proven for O,O-dimethylcurine and

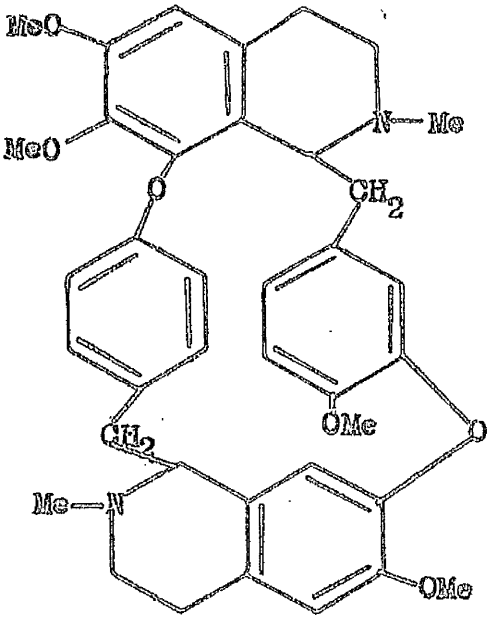


(XXI)

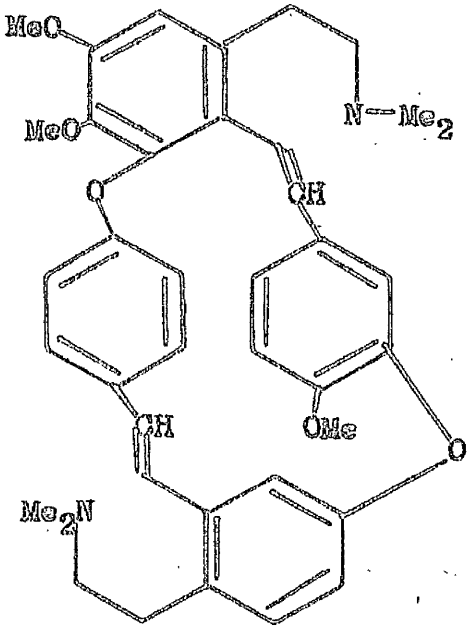
related its structure to that of (+)-tubocurarine (XIV, R_2=R_3=H, R_1=Me). Exhaustive methylation of curine with methyl iodide and methanolic potassium hydroxide gave O, O-dimethylcurine methosalts as previously established by Scholtz[26] and Späth, Leithe and Ladeck.[26]

Hofmann degradation of O,O-dimethyl-(+)- curine methochloride gave three different methine bases which were separated as the crystalline methiodides. A fourth methine was isolated in small amount but was not conclusively identified. Two of the methiodides were optically inactive and the third had a positive

rotation. These three methiodides were identified with three of the four methine methiodides isolated from a similar degradation of O, O-dimethyl-(+)-tubocurarine chloride the fourth compound in this latter instance being laevorotatory. King interpreted the above results as being compatible with structure (XXII) for O, O-dimethylcurine. The most likely explanation why there are only two optically active methine bases and not the expected three optically active bases is that the two inactive methine methiodides are devoid of centres of asymmetry the parent methine being (XXIIA) and that isomerism is due to a cis-trans arrangement of one of the two ethylene linkages or both.

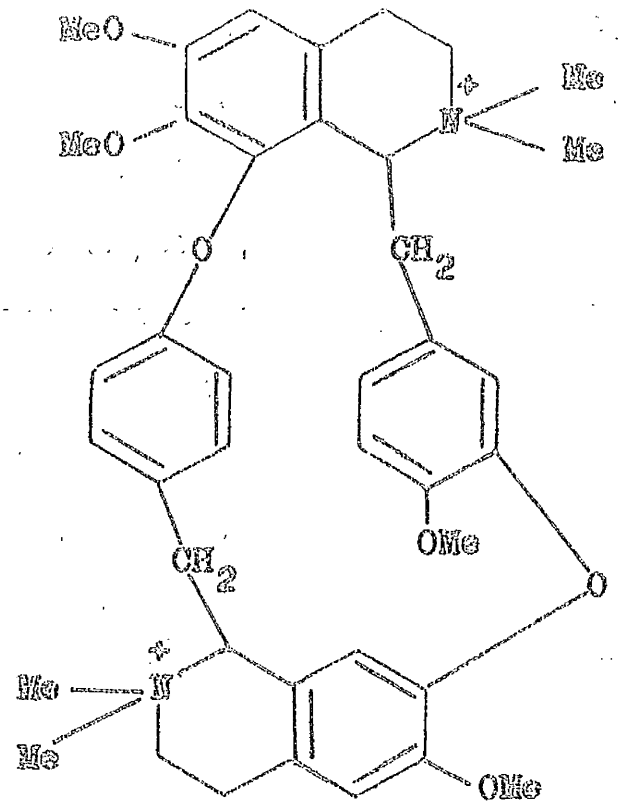


(XXII)



(XXII A)

O,O-dimethylcurarine chloride and O,O-dimethyltubocurarine chloride are on the same basis, represented by (XXIII)[28].

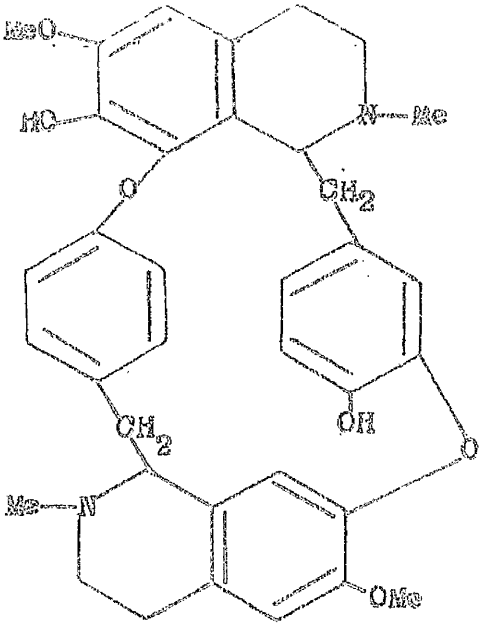


(XXIII)

This structure (XXIII) contains two asymmetric carbon atoms adjacent to the nitrogen atoms and thus four optical isomers are possible. Partial Hofmann degradation on each form could yield four different methine bases depending on which side of the nitrogen atom fission of the nitrogen-containing ring takes place.

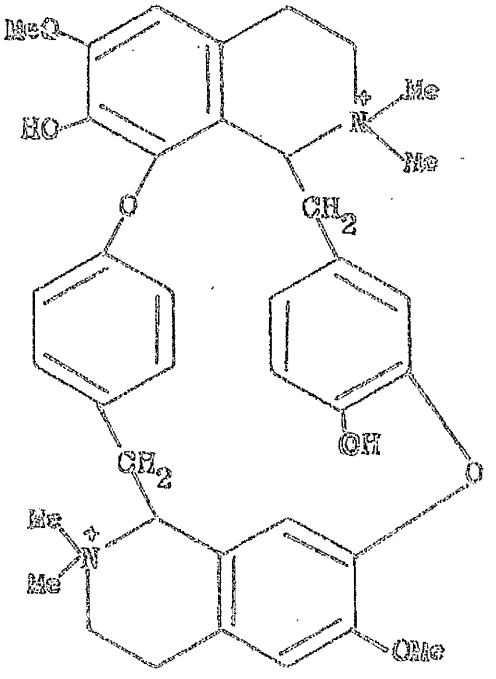
King[15] postulated both optical centres of (+)-curine as dextrorotatory and this was confirmed by Bick and Clezy[27] in 1953, whereas in (+)-tubocurarine one centre is laevorotatory and the other dextrorotatory.

This still left unsolved the relative positions of the methoxyl and hydroxyl groups at positions 6 and 7 of the tetrahydroisoquinoline nucleus. However, in 1939, King, by ethylating the two free phenolic groups followed by Hofmann degradation, determined the constitution of [illegible] (XXIV)[28]



(XXIV)

In a similar manner the structure of (+)-tubocurarine was established as (XIVA).



(XIV A)

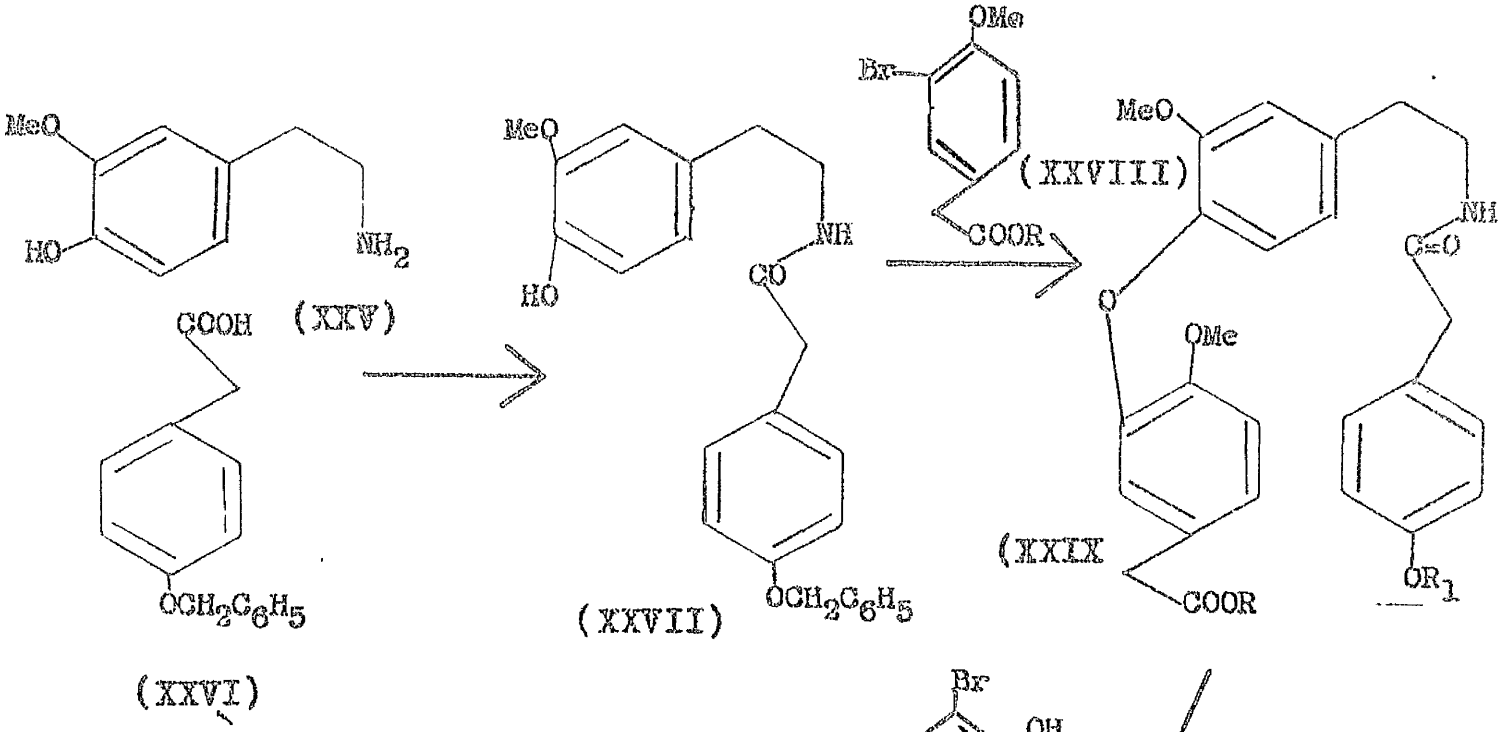
SYNTHESIS OF THE DIMETHYLETHER OF (±)-TUBOCURARINE IODIDE.

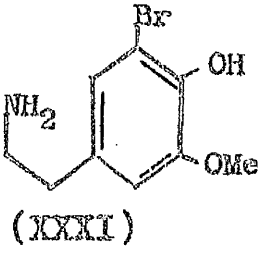
The total synthesis of the dimethylether of (±)-tubocurarine iodide was accomplished by Tolkachev, Voronin and Preobrazhenskii[2] in 1958. The basis of their route is a successive build up of a system having the elements of the natural alkaloid and the final stage is the formation of a second oxygen bridge.

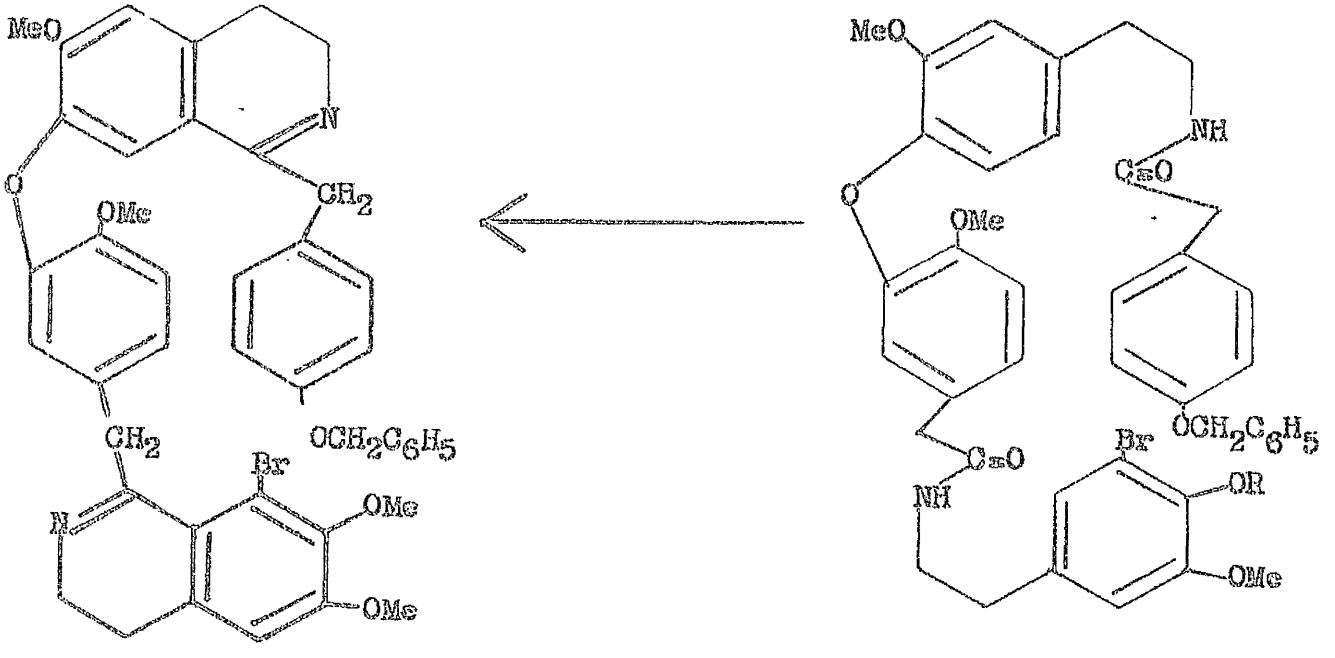
β-(3-Methoxy-4-hydroxyphenyl)-ethylamine (XXV), obtained by the catalytic reduction of the appropriate ω-nitrostyrene, was condensed with 4-benzyloxyphenylacetic acid (XXVI) at 190-200° to give the β-(3-methoxy-4-hydroxyphenyl)-ethylamide of 4′-benzyloxyphenylacetic acid (XXVII). Reaction of the potassium salt of the latter with (methyl or ethyl) esters of 3-bromo-4-methoxyphenylacetic acid (XXVIII, R=Me or C_2H_5) in the presence of copper powder at 190-200° gave the β-[3-methoxy-4-(2″-methoxy-5″-carbalkoxymethylphenoxyl)-phenyl]-ethylamide of 4′-benzyloxyphenylacetic acid (XXIX, R=Me or C_2H_5, R_1= $CH_2C_6H_5$).

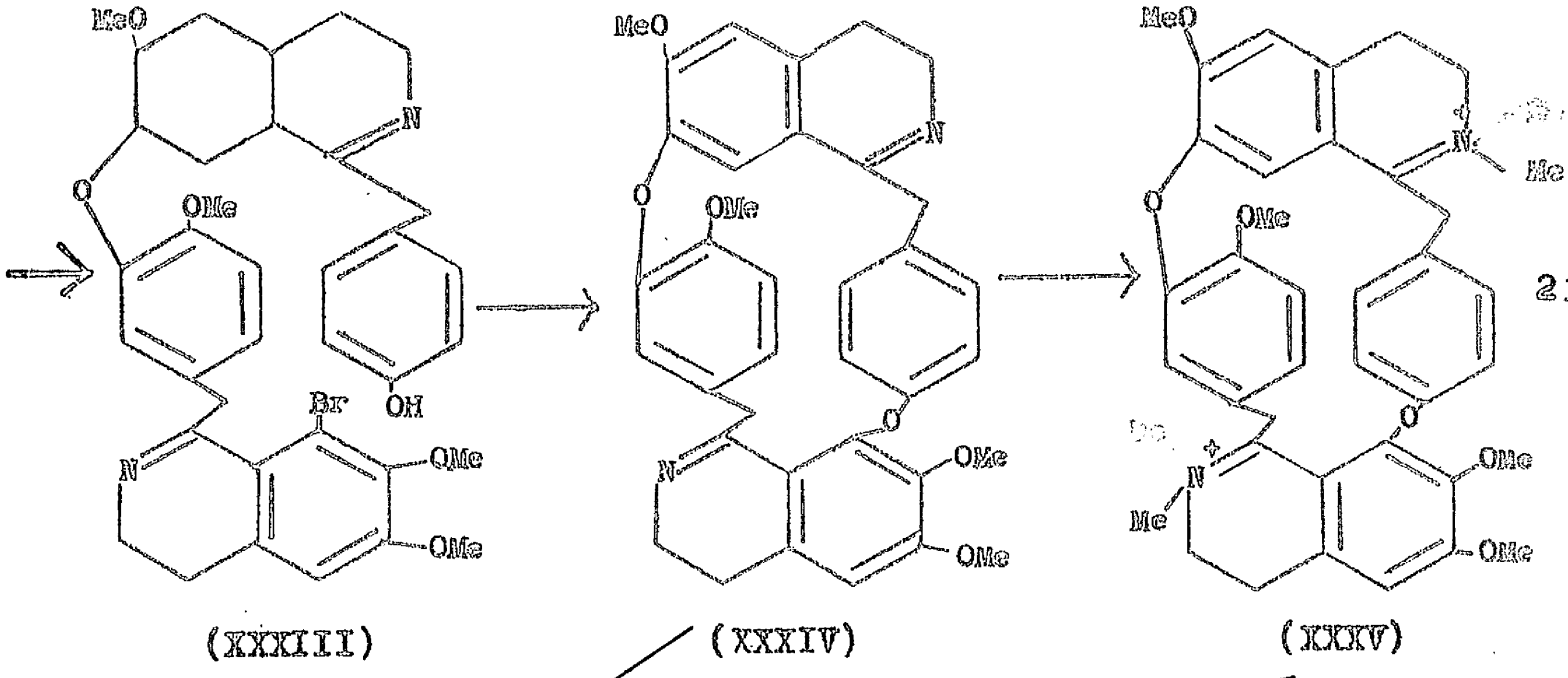
The substances obtained were hydrolysed with an aqueous alcoholic solution of potassium carbonate or sodium carbonate to the corresponding acid (XXIX, R=H, R_1=$CH_2C_6H_5$) and debenzylated with palladium to the β-[3-methoxy-4-(2″-methoxy-5″-carboxymethylphenoxy)-phenyl]-ethylamide of 4′-hydroxyphenylacetic acid (XXIX R=R_1=H). The β-3-methoxy-4-[2″-methoxy-5″-(β-(3‴-methoxy-4‴-hydroxy-5‴-bromophenyl)-ethylcarbamidomethyl)-phenoxy]-phenyl-ethylamide of 4′-benzyloxyphenylacetic acid (XXX, R=H) was obtained from (XXIX, R=Me or C_2H_5, R_1=$CH_2C_6H_5$) and β-(3-methoxy-4-hydroxy-5-bromophenyl)-ethylamine (XXXI) at 180° and from (XXIX R=H, R_1=$CH_2C_6H_5$) and (XXXI) at 190°.

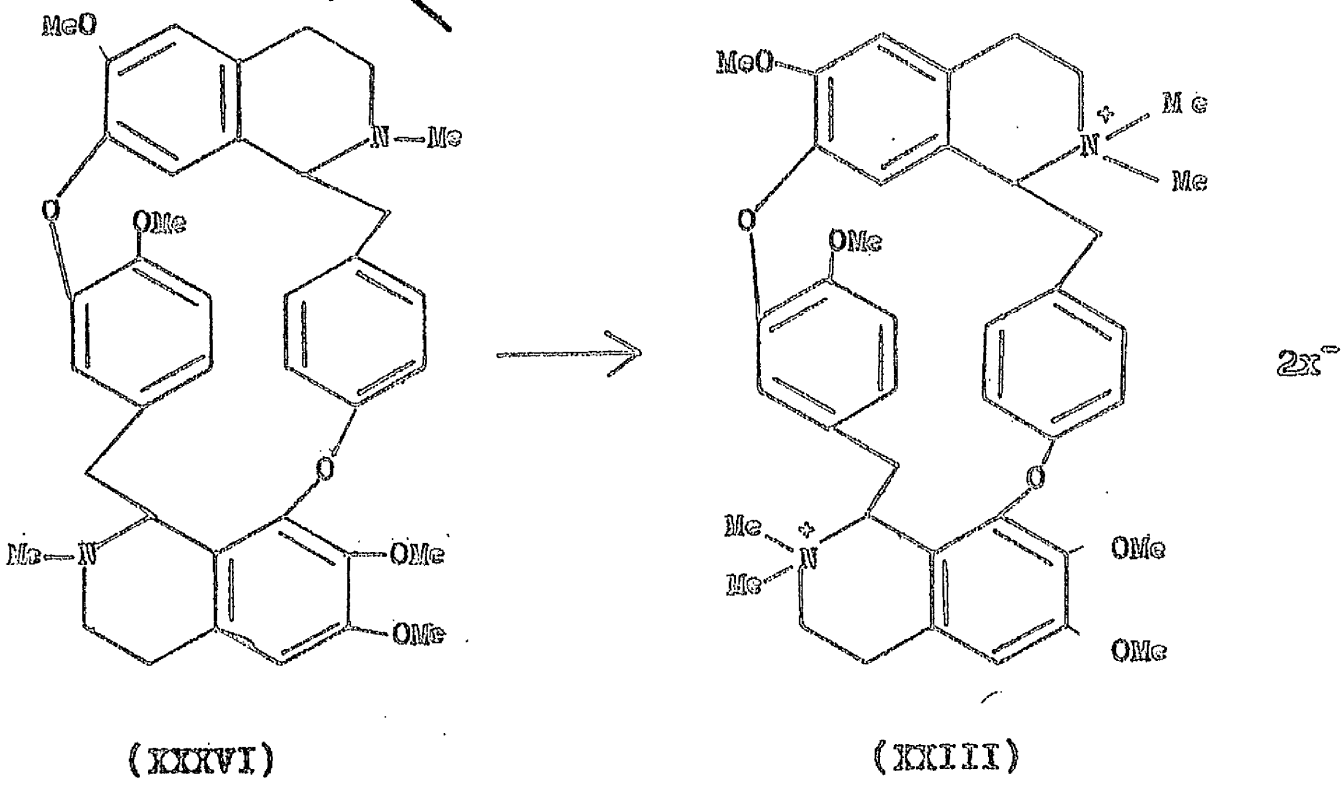
The compound (XXX, R=H) was methylated with methyl iodide to give the β-3-methoxy-4-[2''-methoxy-5''-(β'' '-(3''' ,4''' '-dimethoxy-5'' '-bromophenyl)-ethylcarbamido-methyl)-phenoxy]-phenyl-ethylamide of 4'-benzyloxyphenylacetic acid (XXX, R=Me).








MeO
N
O
OMe
Br
OH
OMe
OMe
(XXXIII)
(XXXIV)
(XXXV)
Me
2


MeO
N—Me
O
OMe
Me—N
OMe
OMe
(XXXVI)
(XXIII)
2x⁻

The compound (XXX, R = Me) was then cyclized with phosphorus oxychloride in chloroform solution forming a mixture of a phosphate and hydrochloride, from which the free base, 1-(4′ - benzyloxybenzyl)-6-methoxy-7-[2″-methoxy-5″-(6‴,7‴-dimethoxy-8‴- bromo-3‴,4‴-dihydroisoquinolyl-1‴-methyl)-phenoxy]-3,4-dihydroisoquinoline (XXXII) was isolated. The benzyloxy group of the latter was hydrolysed with 20% hydrochloric acid and finally the 1-(4′-hydroxybenzyl)-6-methoxy-7-[2″-methoxy-5″-(6‴,7‴-dimethoxy-8‴-bromo-3‴,4‴-dihydroisoquinolyl-1‴-methyl)-phenoxy]-3,4-dihydroisoquinoline (XXXIII) obtained was heated in the presence of copper, potassium carbonate and pyridine to give 1,2,1‴′,2‴′-tetradihydro-O-methylchondrofoline (XXXIV). After reduction with zinc dust in acetic acid, the latter was methylated to yield O, O-dimethylchondodendrine (XXXVI) which was also obtained from the dimethiodide of 1,2,1‴′,2‴′-tetradihydro-O-methylchondrofoline (XXX Then by the action of methyl iodide (XXXVI) was converted into the dimethylether of (±) - tubocurarine iodide (XXIII, X=I). The UV spect corresponded to that of the dimethyl ether of natural (+) - tubocurarine iodide and a mixed melting point was not depressed.

Pharmacology

It is now just over a century since Claude Bernard and Pelouze[30] demonstrated the importance of the neuromuscular junction in neuromuscular transmission, and at the same time, showed the paralytic action of crude extracts of curare.

In 1869 Crum-Brown and Fraser[31] cognisant of the fact that the crude extracts contained quaternary ammonium compounds examined several other quaternaries including the methiodides of atropine, brucine, strychnine, codeine, coniine morphine and thebaine and showed that they produced a paralytic action similar to that of the crude curare extracts.

Action of Acetylcholine

The arrival of the nerve impulse at the presynaptic membrane of the neuromuscular junction is thought to release acetylcholine from storage vesicles at the nerve endings. This acetylcholine is considered to diffuse across the synaptic gap where it causes depolarisation of the post-synaptic membrane. If the depolarisati reaches a critical level it gives rise to local currents of sufficient intensity to increase the sodium permeability of surrounding portions of the membrane and so initiates a propagated muscle action potential.

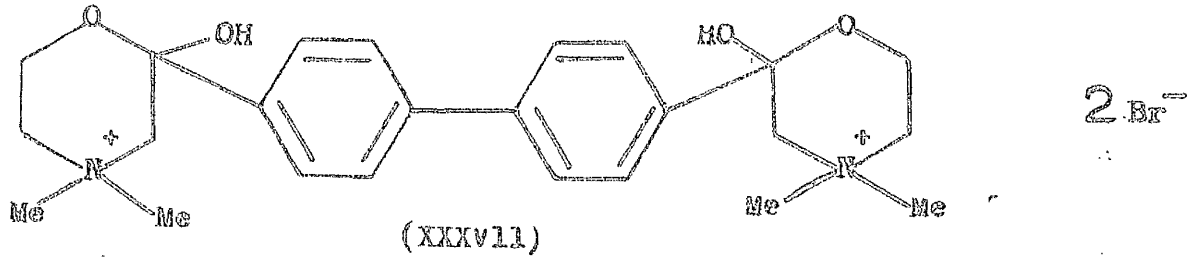
The nerves innervating skeletal muscle are therefore classified as cholinergic as are all preganglionic autonomic nerve fibres and the post-ganglionic nerve fibres of the parasympathetic system. The action of acetylcholine is not blocked by atropine at the neuromuscular junction nor mimicked by muscarine.

Nicotine however, produces a similar response and according to Dale's[32] classifications the action of acetylcholine at the neuromuscular junction is said to be nicotinic rather than muscarinic.

There are three methods by which the transmission described above can be blocked:[33]

1. by depressing acetylcholine synthesis.
2. by depressing acetylcholine release.
3. by depressing motor-end plate sensitivity.

1. Acetylcholine is synthesised by choline acetylase at the cholinergic nerve endings by the transfer of acetyl groups from acetyl-coenzyme A to choline. The most important group of compounds capable of inhibiting acetylcholine synthesis are the hemicholiniums of which HC-3 (XXXVII) is the most active member.[33a] These compounds are believed to inactivate the choline carrier mechanism across the nerve membranes to the intracellular site of acetylation.



(XXXVII)

2. Depression of acetylcholine release can be achieved by alteration of the concentration of calcium ions and magnesium ions in the extracellular fluid. Excess of both calcium and magnesium ions has a depressant effect on the direct excitability of the muscle fibre membrane. Depression of acetylcholine release is

also obtained by local anaesthetics and botulinus toxin.

3. Depression of the end-plate sensitivity can be achieved by one of two methods, the agents being depolarizing or non-depolarizi neuromuscular blocking agents.

A non-depolarizing block is produced by compounds such as (+) tubocurarine and gallamine which compete with acetylcholine for its receptors and thus prevent depolarization of the post-synaptic membrane. Their action is specific in that they combine with the acetylcholine receptors and do not interfere with the release of acetylcholine on stimulation of the nerve endings.[34]

The feasibility of this mechanism of action is supported by the fact that the paralysis produced is relieved by measures which increase local concentration of acetylcholine at the motor-end plate region. This reversibility is of importance clinically as the effects of non-depolarizing neuromuscular blocking agents including (+) - tubocurarine and gallamine are rapidly counteracted by administration of the anticholinesterases neostigmine and edrophonium.

A depolarising block is produced by compounds which act in a way similar to an excess of acetylcholine. After an initial stimulation they produce electrical inexcitability in the region of the motor-end plate, though this happens only in certain species including man. In the majority of animal species however, the above mechanism changes in some way during the blocking process and the actual block produced is due to the raising of the threshold to acetylcholine and is known as a "dual block".[35] Compounds acting by "dual block" cause a rapid decrease in the sensitivity of the

muscle to repeated doses which is characteristic of neither non-depolarizing nor depolarizing agents. However Hodges and Foldes[36] suggested that the difference between non-depolarizing and depolarizing action in neuromuscular blocking agents may be one merely of degree.

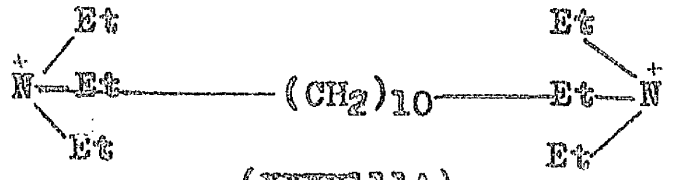
It has been postulated that the mechanism of neuromuscular block is ionic in nature and that the cationic centre of the blocker involves itself with the complementary anionic site of the receptor associated with the so-called nicotinic effects of acetylcholine.[37-39] There are however a variety of responses exhibited by different quaternary compounds on a single tissue preparation indicating that for a specific action at the neuromuscular junction other factors including chain length, onium substitution, charge density and van der Waals forces are important.

1. Chain Length. From the fact that the tubocurarine molecule contains two nitrogen atoms separated by a distance considered from molecular models to be most likely between 9 Å and 15 Å,[46] a large number of compounds of similar interonium distance were developed. That the interonium distance may well be in this range is partly substantiated by the finding from X-ray crystallography that in toxiferine it is 9.7 Å.[41] The dual effects of chain length and onium group substitution on the potency of polymethylenebisquaternaries has recently been investigated by Elworthy.[42] Hexamethonium bromide and iodide (XXXVIII R=Me, n=6) decamethonium bromide and iodide (XXXVIII R=Me, n=10) and decaethonium iodide (XXXVIII R=Et, n=10) were found by Elworthy to have in aqueous solution internitrogen distances of 6.3Å, 9.5Å and 10.2-13.5Å respectively.

$R_3\overset{+}{N}.(CH_2)_{10}\overset{+}{N}R_3$

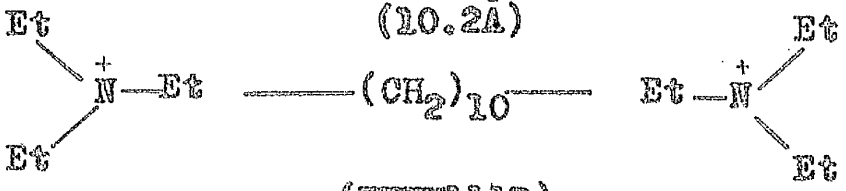
(XXXVIII)

13.1Å



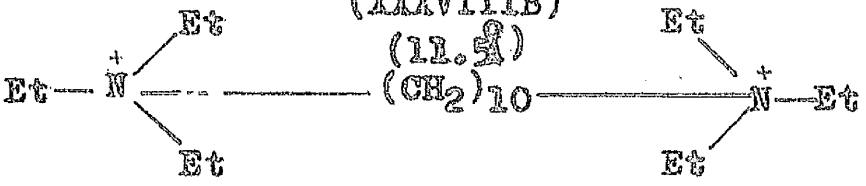
(XXXVIIIA)

(10.2Å)



(XXXVIIIB)

(11.5Å)



(XXXVIIIC)

There are several possible arrangements of the ethyl groups on the nitrogen atom of decaethonium. The ethyl groups can be alongside the main part of the hydrocarbon chain (XXXVIIIA) or point outwards in the X direction (XXXVIIIB). To produce the minimum interfacial energy between the water and the hydrocarbon chain it would be expected that as many groups as possible would be in the first position resulting in 13.1 Å for the N-N distance With two ethyl groups close to the hydrocarbon chain and one directed outwards 11.5 Å (XXXVIIIC) between N-N is obtained and with one ethyl group close to the chain and two directed outwards 10.2 Å is found for N-N. The remaining arrangement does not seem possible as the molecular models indicate that there is insufficient space on one side of a nitrogen atom to accomodate all three ethyl groups.

These internitrogen distances are larger than those found fo decamethonium and the results imply that a measure of protection

is given to the hydrocarbon chain by introducing large substituents on to the nitrogen atom.

The N-N distance for hexamethonium, 6.3 Å, is slightly shorter than the mean N - N distance calculated by Gill[40] (6.9 Å) but falls within the range of his probability calculations i.e. 6-7.8 Å: it is about 3 Å shorter than that measured on a Catalin model arranged in a regular configuration, this being due to interfacial energy effects in solution.

The 9.5 Å N - N distance obtained for decamethonium is about 4 Å shorter than the distance found from measurements on a Catalin model but is in agreement with the 9-10 Å suggested by Caray, Edwards, Lewis and Stenlake in 1959.[46]

When an ethyl group is substituted for a methyl group the N - N increases though only if the chain linking the nitrogen atoms is flexible.

In the bistriethylammonium series peak curariform activity occurs in the tridecyl compound which has an internitrogen distance of 12-15 Å, a value found by interpolation on a graph of N - N distance against the number of carbon atoms joining the nitrogens. This value exceeds the 9.5 Å found for decamethonium[43-45] which has the peak curariform activity of the methonium series thus supporting the view that other factors have to be considered along with the internitrogen distance of the molecule.

The chain separating the two quaternary nitrogen centres can be composed of several entities, the simplest being a polymethylene chain.

Replacement of the methylene groups by ester linkages (XXXIX) and reversed ester linkages (XL) results in the compounds

exhibiting a similar overall pattern of activity to the corresponding polymethylene compounds, suxamethonium (XL , n= m=2, R=Me) being the most effective of the ester-linked compounds. [47-49]

$$Me_3\overset{+}{N}\cdot(CH_2)_n\cdot CO\cdot O\cdot(CH_2)_m\overset{+}{N}Me_3 \qquad 2I^-$$

(XXXIX)

$$R_3\overset{+}{N}\cdot(CH_2)_n\cdot O\cdot CO\cdot(CH_2)_m\cdot CO\cdot O\cdot(CH_2)_n\cdot\overset{+}{N}R_3 \qquad 2I^-$$

(XL)

If one or more of the methylene groups of decamethonium are replaced by ether groups as in (XLI) and (XLII) maximum activity of these compounds appears to be at a greater interonium distance than in the corresponding compounds in the polymethylene bisonium series. These observations are difficult to reconcile with the fact

$$R_3\overset{+}{N}\cdot(CH_2)_n\cdot O\cdot(CH_2)_n\cdot O\cdot(CH_2)_n\cdot\overset{+}{N}R_3 \qquad 2I^-$$

(XLI)

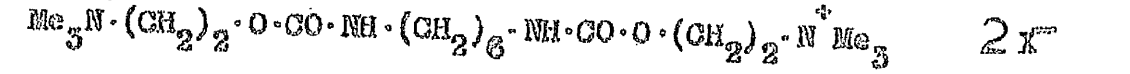
$$Me_2\overset{+}{N}\cdot(CH_2)_2\cdot O\cdot(CH_2)_{10}\cdot O\cdot(CH_2)_2\cdot\overset{+}{N}Me_2 \qquad 2Br^-$$
$$\quad CH_2\cdot CO\cdot OPr \qquad\qquad\qquad CH_2\cdot CO\cdot OPr$$

(XLII)

that an ether link compared with a methylene group extends the chain by only 0.13 Å. [50]

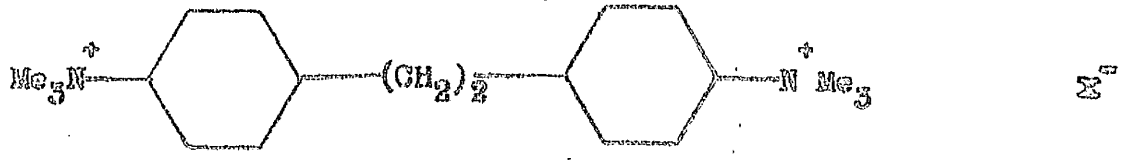
Substitution of the methylene units of decamethonium by ureido and carbamoyl linkages results in a change similar to that observed

with ether substituted compounds, the maximum activity again occurring in the longer chain compounds. The premier example of this type is the carbolinium compound[5/-59] (XLIII, n=6)

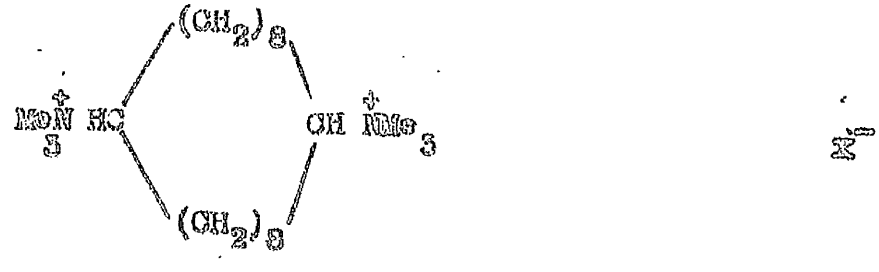


(XLIII)

If however, methylene chain units are substituted by alicyclic rings the resulting compounds show reduced potency and modified activity as in the case of the cyclohexane derivative[60] (XLIV) and cyclomethone[51,62,63] (XLV)

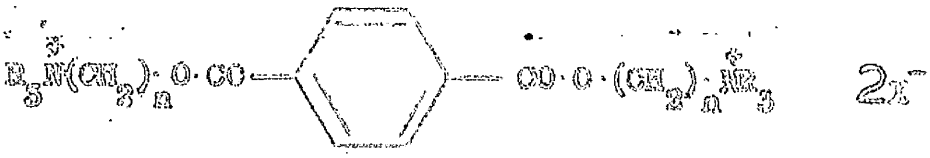


(XLIV)



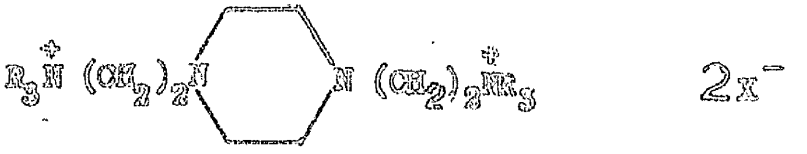
(XLV)

The introduction of an aromatic ester function (XLVI) does not seriously modify the type of block and these compounds share the property of the other linked compounds in exerting their maximum potency at a somewhat greater interonium distance when compared to the corresponding polymethylene chain compounds.[64-67]

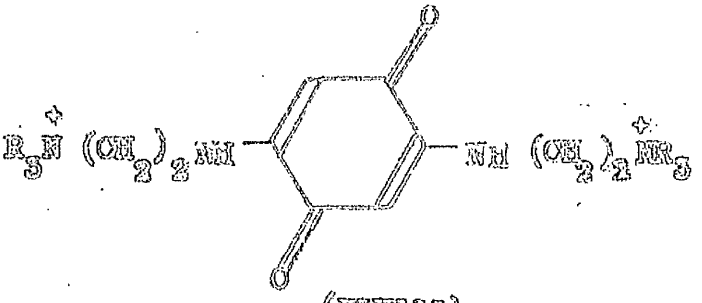


(XLVI)

Many other types of interquaternary chains have been examined including piperazine[68] (XLVII) and benzoquinone linked compounds[69,70] (XLVIII) but their clinical usefulness has been limited as the



(XLVII)



(XLVIII)

neuromuscular block produced is only weakly antagonised by neostigmine.

2. Variation in End-Groups

The accumulated data on substitution in the quaternary ammonium head of the bis-onium compounds would seem to indicate that the effect is partly dependent on the interonium distance.

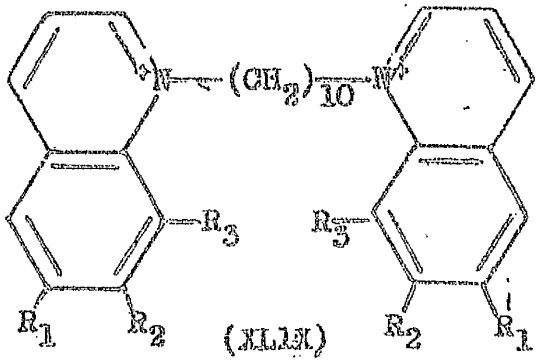
In the decamethonium and suxamethonium series replacement of N - methyl by N - ethyl groups resulted in a fall in potency. It was illustrated by Rossum and Arïens[71] that an increase in alkyl chain length led not only to a fall in potency but also to a change in the mode of action. Decamethonium and suxamethonium both act as depolarizing agents but on substitution of the methyl groups by propyl groups the action is mixed depolarizing and non-depolarizing and on substitution with still higher alkyl substituents the action becomes completely non-depolarizing.

This can be rationalised by the conductivity measurements of Elworthy[42], which showed that the N-N distance in polymethylene bis-onium salts was increased by the substitution of ethyl groups for methyl groups (see page 28).

The incorporation of the quaternary nitrogen into a hetero-aromatic nucleus (XLIX) and (L) causes a reduction in potency. The introduction of nuclear methoxyl substitue-nts (XLIX) into the decamethylene bis-quinolinium compounds (XLIX) restores the activity. The greater formal structural similarity between these latter compounds and (+) - tubocurarine chloride is obvious. (TABLE III).

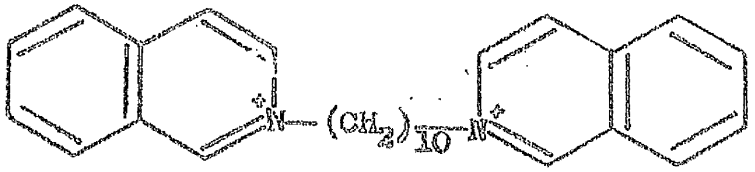
T A B L E III

Potency of compounds with heterocyclic onium groups.

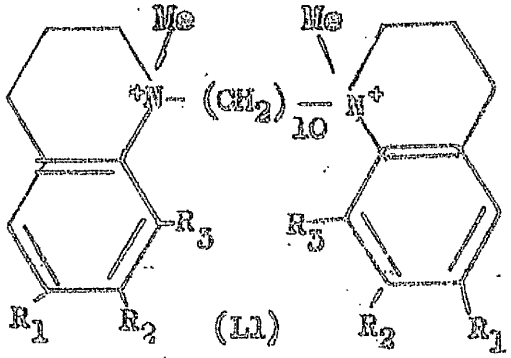


(XLIX)

R_1	R_2	R_3	Rabbit ED_{50} (mg/kg)
H	H	H	4.5
MeO	H	H	4.4
H	H	MeO	0.2
H	H	EtO	> 0.3
MeO	MeO	-	0.15

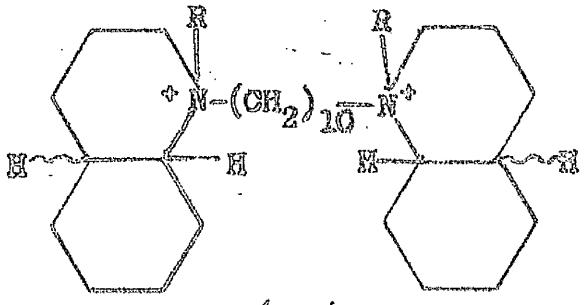


Rabbit ED_{50} (mg/kg)
4.0



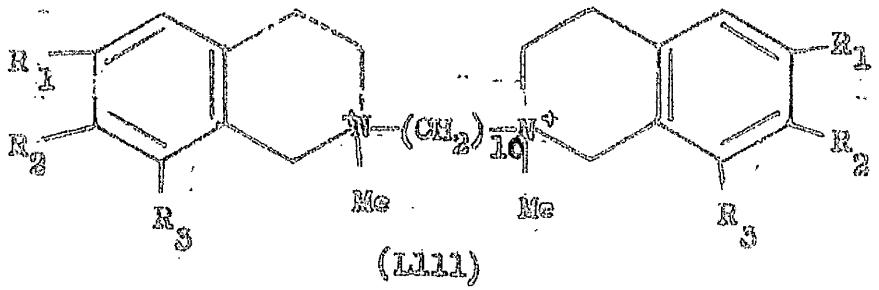
(L1)

R_1	R_2	R_3	Rabbit ED_{50} (mg/kg)
H	H	H	0.75
MeO	H	H	0.2
H	H	MeO	0.1



(LII)

Ring Fusion	R	Rabbit ED_{50} (mg/kg)
cis	Me	0.12
trans	Me	0.1
trans	Et	0.4

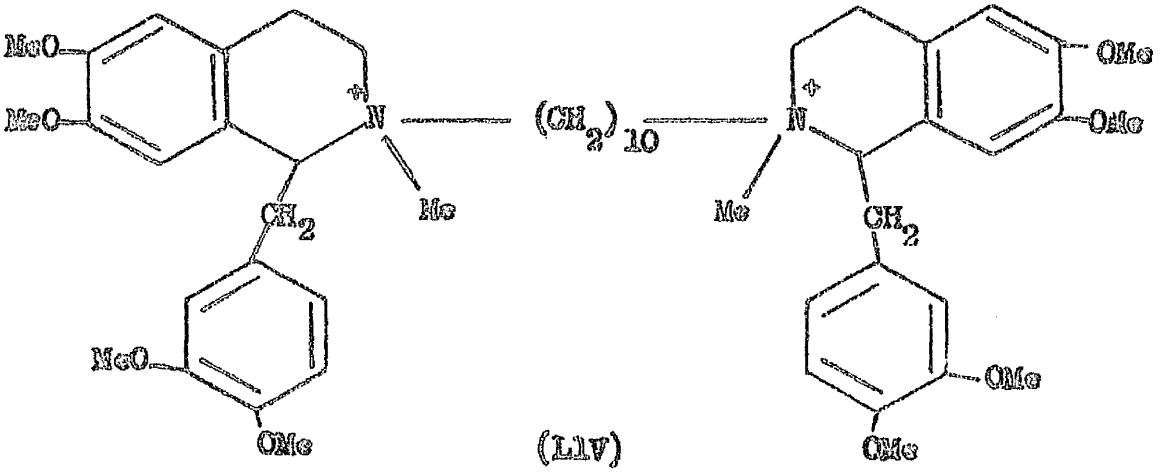


(LIII)

R_1	R_2	R_3	Rabbit ED_{50} (mg/kg)
H	H	H	1.5
MeO	H	H	0.2
MeO	MeO	H	0.05
MeO	MeO	MeO	0.02

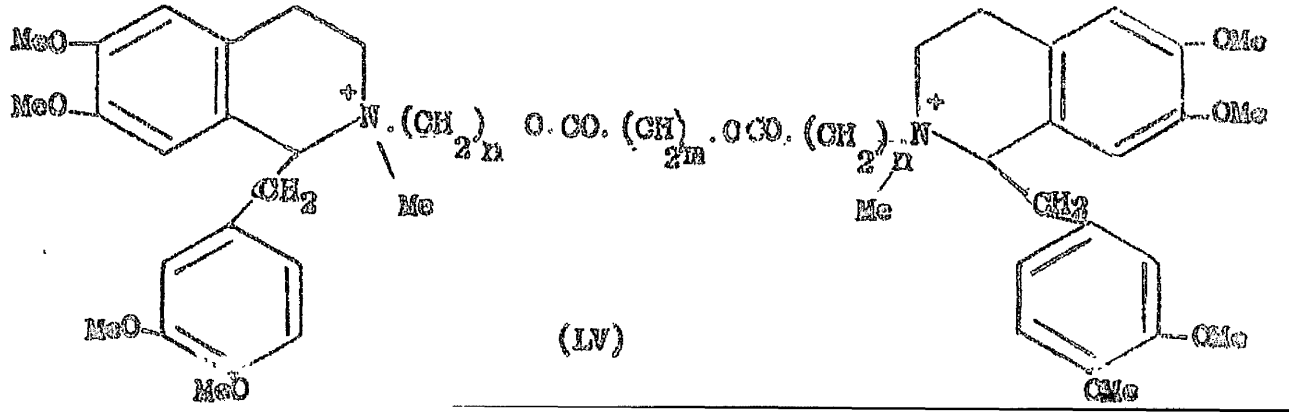
In the related decamethylenebistetrahydroquinolinium (LI), decamethylenebisdecahydroquinolinium (LII) and decamethylene-bistetrahydroisoquinolinium (LIII) salts potency is still further increased.[72]

The most useful compound which has so far emerged from these heterocyclic onium substituted compounds is laudexium (LIV)[73-78]. It is more potent than (+)-tubocurarine in the cat and rabbit and its action is readily reversed by neostigmine[79]. The main disadvantage is that it has a long duration of action.



(LIV)

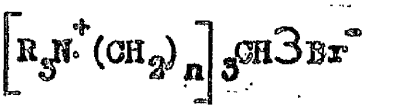
A logical extension of these studies was the replacement of the trimethylammonium groups of suxamethonium by large saturated heterocyclic groups as in the benzyltetrahydroisoquinolinium compound (LV, n=2, m=2) which has been examined in some detail[80]. It produces a tubocurarine-like block in the cat which is antagonised by neostigmine, but its potency is low being only 1/30 - 1/40 that of suxamethonium[80-80a].



(LV)

Polyonium Compounds

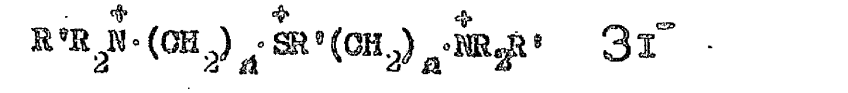
Kensler, Zirkle, Matallana and Condouris examined a series of tris-onium compounds (LVI, n=2, 3 or 4, R=Me or Et) and found that



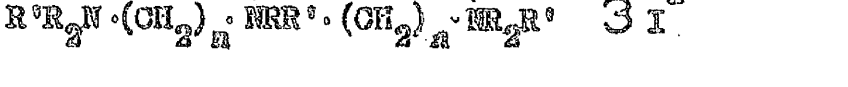
(LVI)

the maximum potency occurred in (LVI, n=4, R=Et)[81].

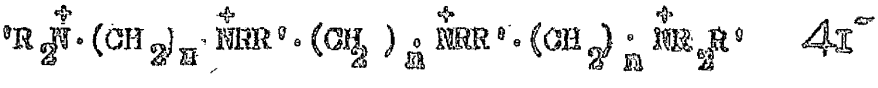
Stenlake et al.[46,82-85] examined an extensive series of linear polyonium compounds including NSN -(LVII), NNN - tris-onium (LVIII), NNNN, -tetra-onium (LIX), NNSNN, - penta-onium (LX) and the NNNNNN,- hexa-onium compounds (LXI).



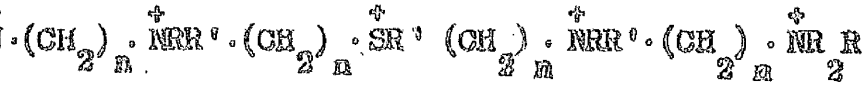
(LVII)



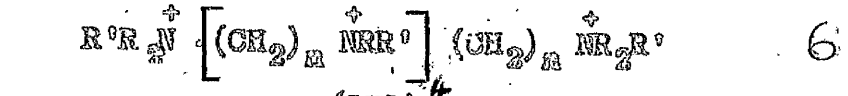
(LVIII)



(LIX)



(LX)



(LXI)

It was found that ethonium compounds with the onium groups separated by 5 or 6 methylene groups were predominantly tubocurarine-like in action whilst those compounds with 10 methylene groups separating the onium centres acted like decamethonium. The overall chain length is unimportant in determining the type of block as is illustrated by Table (IV).

T A B L E IV

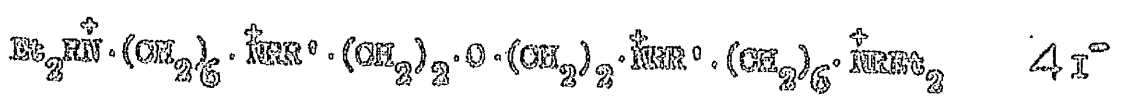
Comparison of chain length and interonium distance of polyonium groups with the type of neuromuscular block exhibited.

(Tc= Tubocurarine, C10= Decamethonium)

Compound	Type of Block.	No. of atoms separating terminal onium groups.
$Et_3\overset{+}{N}(CH_2)_6\overset{+}{N}Et_2(CH_2)_6\overset{+}{N}Et_2(CH_2)_6\overset{+}{N}Et_3$	Tc-like	20
$Et_3\overset{+}{N}(CH_2)_{10}\overset{+}{N}Et_2(CH_2)_{10}\overset{+}{N}Et_3$	C10-like	21
$Et_3\overset{+}{N}(CH_2)_6\overset{+}{N}Et_2(CH_2)_8\overset{+}{N}Et_2(CH_2)_6\overset{+}{N}Et_3$	Tc-like	22
$Et_3\overset{+}{N}(CH_2)_8\overset{+}{N}Et_2(CH_2)_6\overset{+}{N}Et_2(CH_2)_8\overset{+}{N}Et_3$	Tc-like	24
$Et_3\overset{+}{N}(CH_2)_6\overset{+}{N}Et_2(CH_2)_{10}\overset{+}{N}Et_2(CH_2)_6\overset{+}{N}Et_3$	Tc-like	24
$Et_3\overset{+}{N}(CH_2)_8\overset{+}{N}Et_2(CH_2)_8\overset{+}{N}Et_2(CH_2)\overset{+}{N}Et_3$	'Transitional'	26
$Et_3\overset{+}{N}(CH_2)_6\overset{+}{N}Et_2(CH_2)_6\overset{+}{S}Et(CH_2)_6\overset{+}{N}Et_2(CH_2)_6\overset{+}{N}Et_3$	Tc-like	27
$Et_3\overset{+}{N}(CH_2)_{10}\overset{+}{N}Et_2(CH_2)_6\overset{+}{N}Et_2(CH_2)_{10}\overset{+}{N}Et_3$	C10-like	28
$Et_3\overset{+}{N}(CH_2)_6\overset{+}{N}Et_2(CH_2)_6\overset{+}{N}Et_2(CH_2)_6\overset{+}{N}Et_2(CH_2)_6\overset{+}{N}Et_3$	Tc-like	34
$Et_3\overset{+}{N}(CH_2)_{10}\overset{+}{N}Et_2(CH_2)_{10}\overset{+}{N}Et_2(CH_2)_{10}\overset{+}{N}Et_3$	C10-like	32

Substitution of a methylene group in the interonium polymethylen chain by an ether link (LXII)and (LXIII)produced a marked decrease in activity.[86-89] This is in contrast to the potentiating effect of aromatic ether links i.e. methoxyl substitution in (+) - tubocurarine and its isomers and in decamethylene bistetrahydroquinolinium and tetrahydroisoquinolinium compounds (LI) and (LXII).

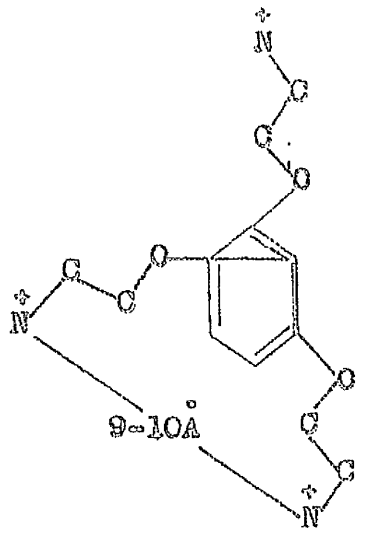
(LXII)



(LXIII)

It would appear that ether substitution in proximity to the quaternary centre in aliphatic compounds reduces the charge on the nitrogen atom and so lowers its capacity to bind at the receptor surface; conversely it is reasonable to suppose that the influence of methoxyl and other ether links in (+) - tubocurarine and related molecules is concentrated in the aromatic rings and the charge on the nitrogen is not similarly affected.

Based on a knowledge of the potency observed in aliphatic choline ethers and the contribution of ether links to the activity of (+) - tubocurarine and related compounds the tris-onium compound, gallamine was synthesised in 1946 by Bovet, Depierre and de Lestrange.[90] It is tubocurarine-like in action but has only one fifth of the potency in man.



(LXIV)

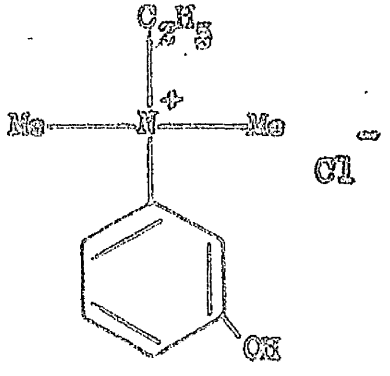
Successive substitution of ethyl groups by methyl groups results in decreased potency[91]. The most probable structure (LXI results from the natural repulsion of like charges (LXIV). This staggered orientation places the onium groups at a distance of 9-10 Å units apart.

MEDICINAL USES OF NEUROMUSCULAR BLOCKING AGENTS

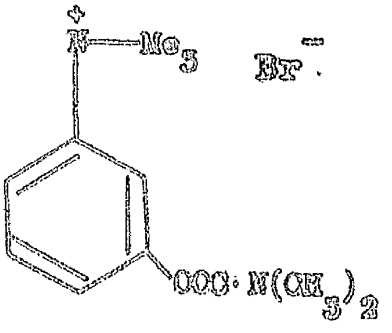
Neuromuscular blocking agents are used in surgery to achieve muscular flaccidity especially in the abdominal region. Safe control of the neuromuscular block in anaesthesia and surgery is essential and the choice of the type of muscle relaxant drug is based on the duration of the operation and the condition of the patient. The main muscle relaxants used in surgery are shown in Tables (V) and (VI)[92]. Brief muscular relaxation of 5-10 minutes duration is most often achieved by suxamethonium. Where a protracted operation in conjunction with a muscle relaxant is anticipated a continuous intravenous drip of a solution of suxamethonium may be used. The main advantage of this method of administration is that no antagonistic drug such as neostigmine i

required as suxamethonium is rapidly hydrolysed in the body; a disadvantage exists however in the rigid control which must be maintained in the rate of infusion.

Synergism is exhibited between some neuromuscular blocking drugs and anaesthetics and this enables the anaesthetist to achieve a deeper plane of unconsciousness with less anaesthetic than he would normally require with the consequent lessening of risk to the patient. Tubocurarine and dimethyltubocurarine are particularly indicated for use with ether due to the large synergistic effect exhibited in the presence of each other, though care must be taken to ensure that apnoea does not develop. Apnoea and respiratory depression can be countered by administration of such substances as edrophonium (LXV) which acts within seconds of administration and neostigmine (LXVA), which though not as quick in onset has a more prolonged action.



(LXV)



(LXV A)

$$HOOC\cdot(CH_2)_2\cdot CO\cdot O\cdot(CH_2)_2\cdot \overset{+}{N}Me_3$$

(LXVI)

The non-depolarizing relaxants like tubocurarine and gallamine have a marked antagonistic effect on the depolarizing compounds like suxamethonium. In order to produce a neuromuscular block with a depolarizing substance after prior administration of a subparalytic dose of tubocurarine requires a dose of 3 to 4 times greater than the normal paralytic dose of suxamethonium. The intensity and length of the block when finally induced may be excessive and the resulting apnoea not easily reversed. However, in the reverse case the neuromuscular junction is not unduly sensitized to (+) - tubocurarine by a previous dose of suxamethonium. This method has been utilized to prevent the accumulation of succinylmonocholine (LXVI) the primary breakdown product of suxamethonium which itself may produce severe respiratory depression. After 30-45 minutes of continuous drip infusion of suxamethonium small doses of tubocurarine or gallamine can be administered and at the termination of surgery respiration will not be depressed or if depressed easily reversed by neostigmine.

T A B L E V

Neuromuscular blocking agents used in surgery.[92]

Generic name	Trade Name
(+)-Tubocurarine chloride pentahydrate	
Dimethyltubocurarine chloride	Mecostrin
Dimethyltubocurarine iodide	Metubine
C-toxiferine Iodide	———
C-Curarine Iodide	———
Gallamine triethiodide	Flaxedil
Benzoquinonium chloride	Mytolon
Laudexium methylsulphate	Laudolissin
Decamethonium bromide	Syncurine
Succinylcholine chloride	Anectine
	Scoline
Succinylcholine iodide	Celocurine
Suxethonium bromide	Brevedil E
Succinylmonocholine Iodide	
———	Imbretil
———	Prestonal

T A B L E VI

Comparative potency of neuromuscular blocking agents for the production of surgical relaxation [92]

Agent	On weight basis of Cation	On weight basis of Salt.	On molar basis.
(+)-Tubocurarine	1.0	1.0	1.0
Dimethyltubocurarine chloride.	3.2	3.7	3.4
Dimethyltubocurarine iodide.	3.2	2.9	3.4
Toxiferine	8.5	8.6	7.5
Gallamine	0.3	0.2	0.2
Benzoquinonium	1.0	1.1	0.9
Laudexium	0.5	0.5	0.7
Decamethonium Bromide	7.7	6.0	3.1
Succinylcholine Chloride	1.6	1.8	0.9
Succinylcholine Iodide	1.8	1.2	0.9
Imbretil	5.7	5.0	3.4

Despite the number of compounds synthesised for use as neuromuscular blocking agents the clinically useful ones (Table V) which have arisen from synthetic procedures are comparatively few and none is ideal. Rather than direct synthesis a second approach to an ideal neuromuscular blocking agent is exemplified by the modifications of existing well-tried blocking agents.

There are four stereoisomers of tubocurarine and they exhibit marked differences in potency from each other in the rabbit-head drop and other tests (Table VII).

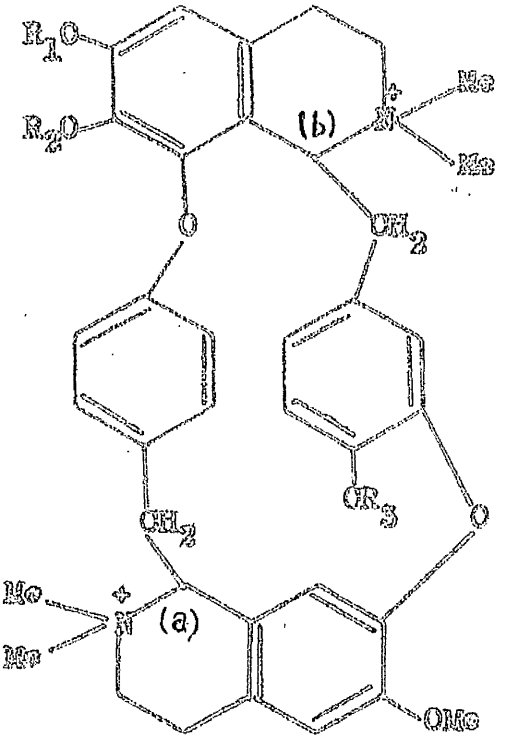
T A B L E VII

Relative potencies of tubocurarine isomers[93,94]

(rat diaphragm test)

Isomers.	Optical Rotation at centres. a	b (LXVII)	Potency
(+)-Tubocurarine	+	-	1.0
(-)-Tubocurarine	-	+	negligible
(+)-Curarine	+	+	3.5
(-)-Curarine	-	-	1.3

Complete methylation of tubocurarine (LXVII, R_1=Me, R_2=R_3=H) affords dimethyltubocurarine (LXVII, R_1=R_2=R_3=Me) which is 8.7 times as potent as (+)-tubocurarine and similarly (-)-dimethylcurarine (LXVII, R_1=R_2=R_3=Me) is more than twice as potent as (-)-curarine (LXVII, R_1=Me, R_2=R_3=H). From these results it was concluded that the number and position of the methoxyl groups considerably affects the activity.



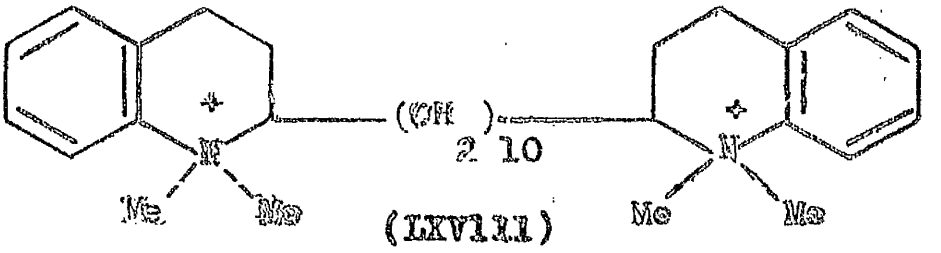
(LXVII)

Cavallito suggested as a possible explanation that zwitterion formation arose from the presence of a phenolic hydroxyl group in (+) - tubocurarine and might cause the lower activity. In support for this theory Kalow showed that as the pH was increased a reduction in potency of (+) - tubocurarine was observed, the pH being associated with the dissociation of one phenolic group. The activity of chondocurarine (LXVII , R_2 =Me, R_1=R_3 =H) also added weight to the theory. It is nearly three times as potent as (+) - tubocurarine and has the hydroxyl and methoxyl groups in positions 6 and 7 of the isoquinoline nucleus, the reverse of (+) - tubocurarine,with the result that the phenolic hydroxyl group is removed by one carbon atom from the influence of the onium group as compared with the situation in (+) - tubocurarine.

In the case of the di-n-butyl (LXVII, R_1=Me, R_2 =R_3 =Bu) and the dibenzyl ethers (LXVII, R_1=Me, R_2 =R_3 =Bz) which have a

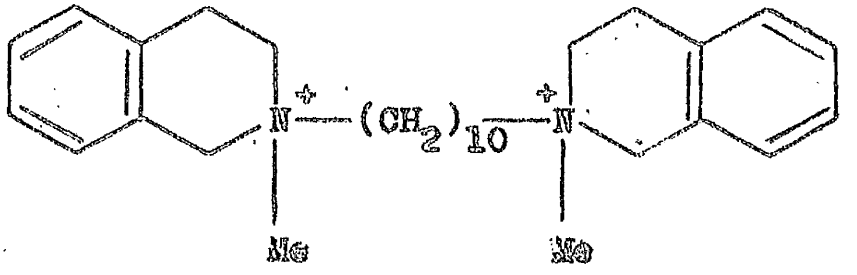
T A B L E VIII

Potencies of methoxytetrahydroquinolines and methoxytetrahydroisoquinolines (Minimal effective dose in rabbits [12]).



(LXVIII)

Substituents.	Dose. mg/kg.
None	0.75
6 - Methoxy	0.2
8- Methoxy	0.1



(LXIX)

Substituents	Dose. mg/kg.
None	0.75
6-Methoxy	0.2
6,7-Dimethoxy	0.05
6,7,8-Trimethoxy	0.02
(+)-tubocurarine	0.1

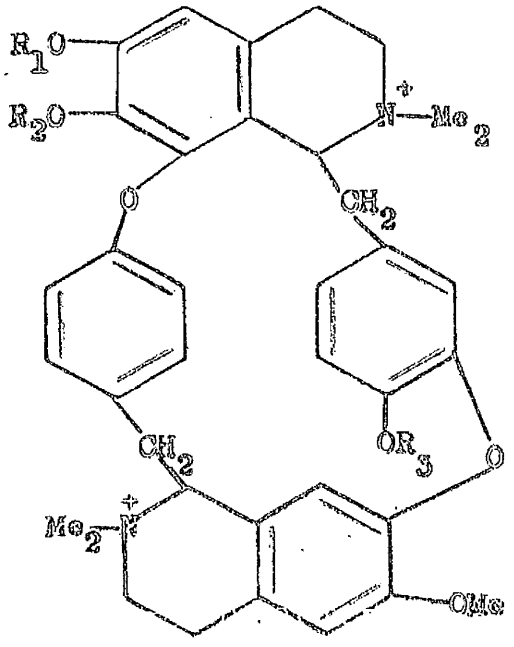
low order of activity the explanation of Kalow does not suffice. The increase in potency of N,N-dimethyl-1,10-decamethylene-bistetrahydroquinolinium (LXVIII) and the related tetrahydroisoquinolinium di-iodides (LXIX) supports the view that the number and position of the methoxyl substituents in this type of molecule plays a vital part in the activity of the molecule (Table VIII).

The increase in potency of O,O-dimethyl-(+)-tubocurarine iodide over (+)-tubocurarine and the increase in potency when methoxyl substituents are added to compounds (LXIX) and (LXVIII) may also be due in part to an increase in lipoid solubility[97].

DISCUSSION

DISCUSSION

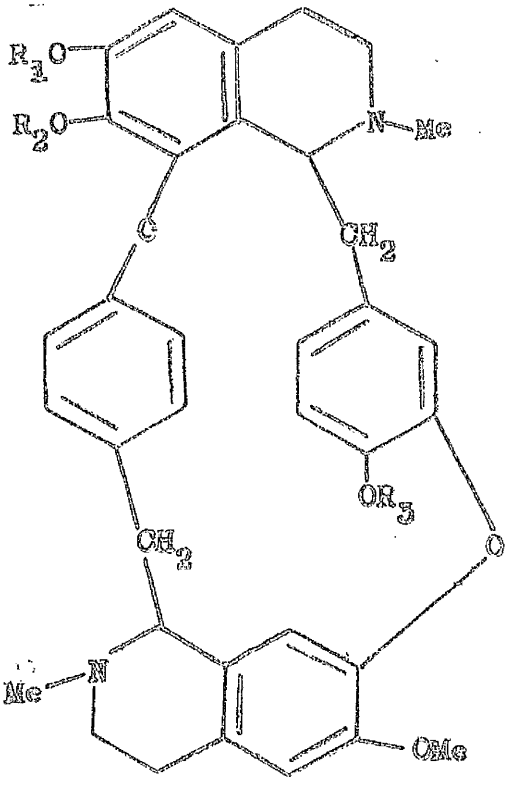
When the present research on (+)-tubocurarine chloride (LXX, R_1=Me, R_2=R_3=H) was instigated in 1960 it was already known that curine (LXXI, R_1=Me, R_2=R_3=H) and chondocurine (LXXI, R_1=R_3=H, R_2=Me) were isomeric[98]. The stereochemical relationship between these two tertiary bases had been recently determined by Bick and Clezy[99], who used sodium in liquid ammonia to cleave the dimethyl ethers of the two isomers. The fission of O,O-dimethyl-(+)-chondocurine (LXXI, R_1=R_2=R_3=Me) and O,O-dimethyl-(-)-curine (LXXI, R_1=R_2=R_3=Me) afforded in both cases two known optically active coclaurine bases, O-methylarmepavine (LXXII, R_1=R_2=R_3=Me, R_4=H) and N-methyl-coclaurine (LXXII, R_1=Me, R_2=R_3=R_4=H). From O,O-dimethyl-(+)-



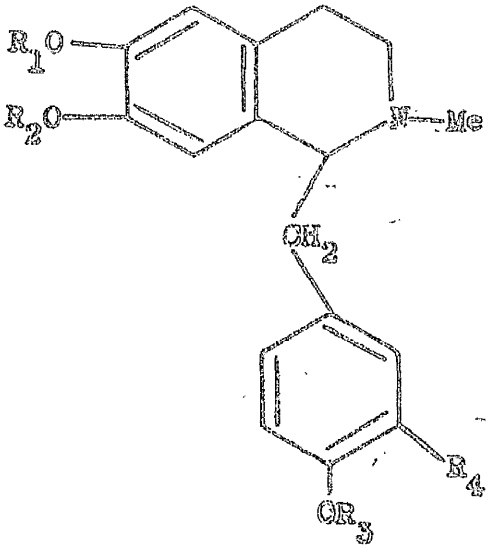
$2x^-$

(LXX)

chondocurine, (-)-O-methylarmepavine (LXXII, $R_1=R_2=R_3=Me$, $R_4=H$) and (+)-N-methylcoclaurine (LXXII, $R_1=Me$, $R_2=R_3=R_4=H$) were obtained while both the O-methylarmepavine (LXXII, $R_1=R_2=R_3=Me$, $R_4=H$) and the N-methylcoclaurine (LXXII, $R_1=Me$, $R_2=R_3=R_4=H$)



(LXXI)



(LXXII)

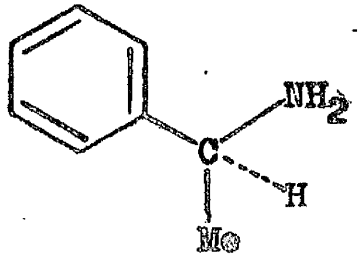
obtained from O, O-dimethyl(-)curine (LXXI, R_1=R_2=R_3=Me, R=H) exhibited a negative rotation.

(+)-Chondocurine (LXXI, R_1=R_3=H, R_2=Me) when quaternised yields (+)-chondocurarine (LXX, R_1=R_3=H, R_2=Me) and since the dimethyl ether of the quaternary base has been proved identical to the dimethyl ether prepared from (+)-tubocurarine[16] (LXX, R_1=Me, R_2=R_3=H) the configurational relationships ascertained by Bick and Clezy[99] for the tertiary base chondocurine must also hold for the quaternary alkaloid tubocurarine (LXX, R_1=Me, R_2=R_3=H).

The absolute configuration was not known and from a biological point of view the conformation of the tubocurarine molecule was important in that it might help further understanding of the action at the receptor sites at the neuromuscular junction. During the course of the present study Kunimoto and Tomita[103] in 1962 deduced the absolute configuration of the molecule (vide infra).

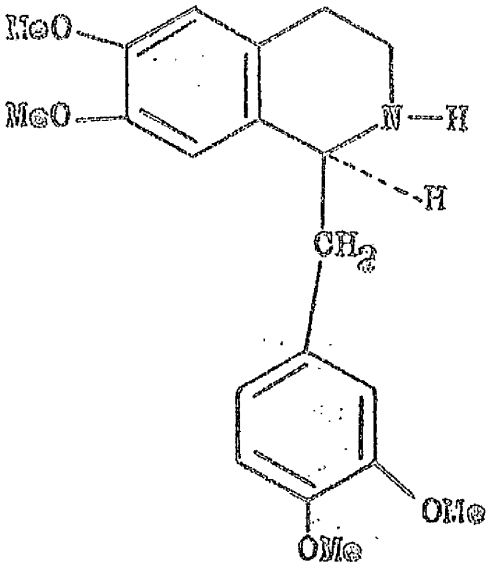
In 1934 Leithe compared the optical rotations of laudanosine methiodide (LXXIII) and synthetically prepared (-)-α-phenylethylamine (LXXIV) in solvents of different polarity and concluded that these two compounds probably had the same absolute configuration[100].

Leithe's conclusion was confirmed by Corrodi and Hardeggar[101]

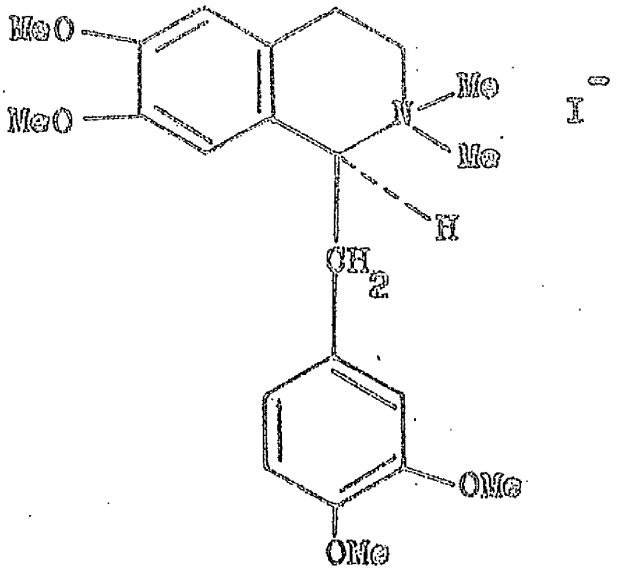


(LXXIV)

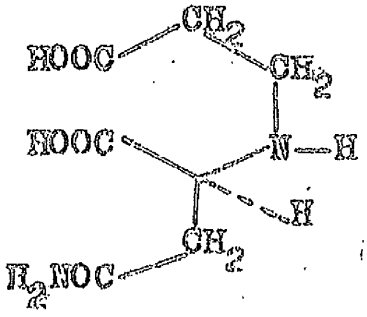
who converted (-)-tetrahydropapaverine (LXXV) by methylation to (+)-laudanosine methiodide (LXXIII) and thence by ozonolysis to N-β-carboxyethylasparagine (LXXVI) of known absolute configuration. In a somewhat analogous manner Battersby and Edwards[102]



(LXXV)

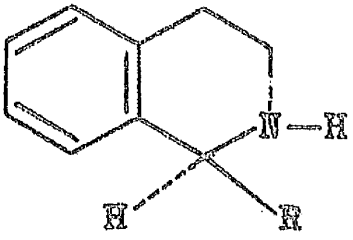


(LXXIII)

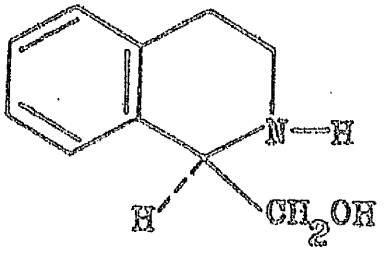


(LXXVI)

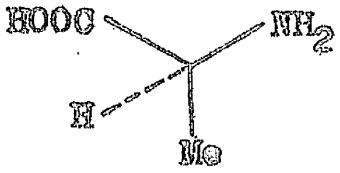
derived the absolute configurations of the isoquinoline alkaloids salsolidine (LXXVII, R=Me), salsoline (LXXVII, R=H) and calycotamine (LXXVIII) by degrading (-)-salsolidine base to N-2-carboxyethyl-L-alanine (LXXIX) which had been synthesised from L-alanine for comparison. In 1962 Tomita and Kunimoto,[103] using the previous results of Corrodi and Hardeggar,[101] deduced



(LXXVII)

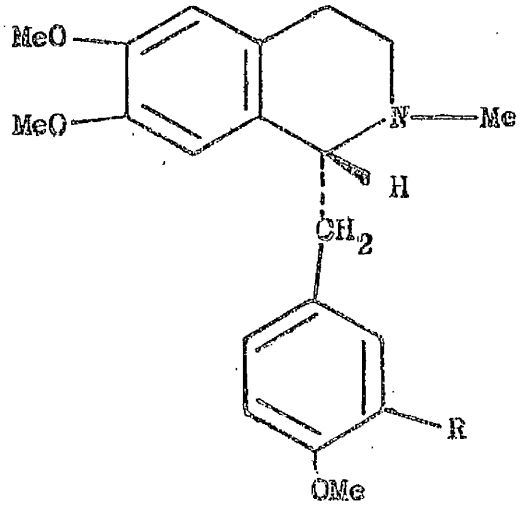


(LXXVIII)



(LXXIX)

the absolute configuration of a number of coclaurine alkaloids. D(-)-laudanosine (LXXX, R=OMe) was demethylated by sodium in liquid ammonia to D(-)-laudanidine (LXXX, R=OH) and this product then converted to D(-)-laudanidine phenyl ether (LXXX, R=OC_6H_5) by an Ullmann reaction with bromobenzene. The phenyl ether (LXXX, R=OC_6H_5) was cleaved with sodium in liquid ammonia to yield (-)-O, O, N-trimethylcoclaurine (LXXX, R=H) (i.e. (-)-O-methylarmepavine).



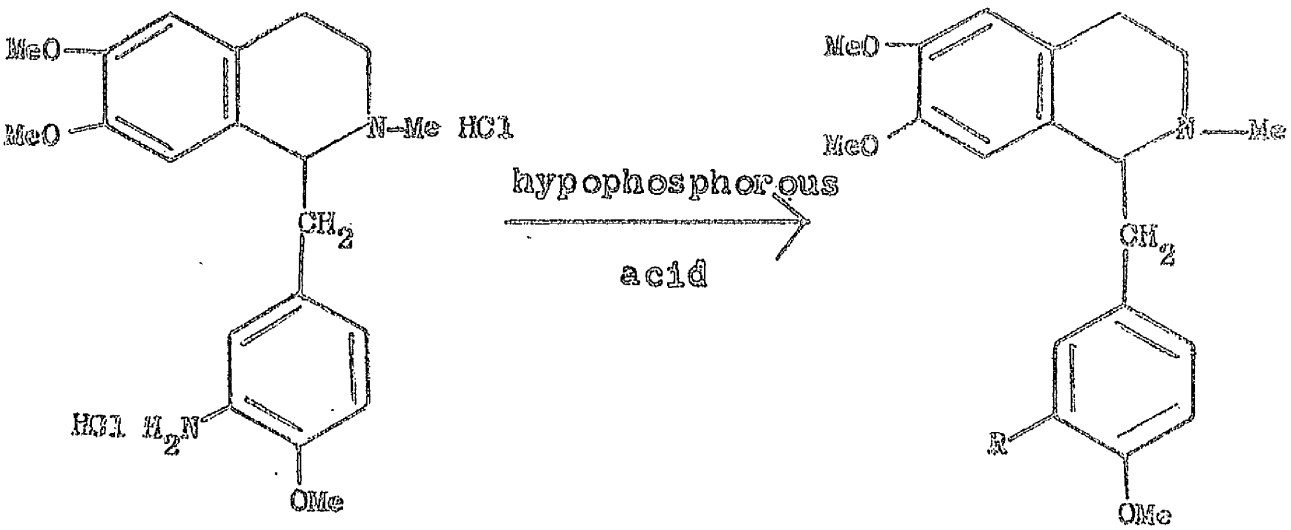
(LXXX)

It was thus proved that D(-)-laudanosine (LXXX, R=OMe) and (-)-O, O, N-trimethylcoclaurine (LXXX, R=H) had the same absolute stereochemistry and similarly shown that L(+)-laudanosine and (+)-O,O, N-trimethylcoclaurine had the same absolute configuration.

Almost simultaneously Ferrari and Deulofeu[104] arrived at a similar conclusion with regard to L(+)-laudanidine (LXXX, R=OH) and (+)-O-methylarmepavine (LXXX, R=H) which were prepared from a common optically active intermediate. Diazotised (+)- and (-)-1-(3-amino-4-methoxybenzyl)-1,2,3,4-tetrahydro-6,7-

dimethoxy-2-methylisoquinoline dihydrochloride (LXXXI) was treated with hypophosphorus acid to give (+) and (-) O-methyl-armepavine respectively. Partial substitution of the diazonium moiety by hydroxyl (but always with simultaneous substitution by hydrogen), was effected by preparing the fluoroborate of the diazonium cation followed by decomposition of this salt with acetic acid.

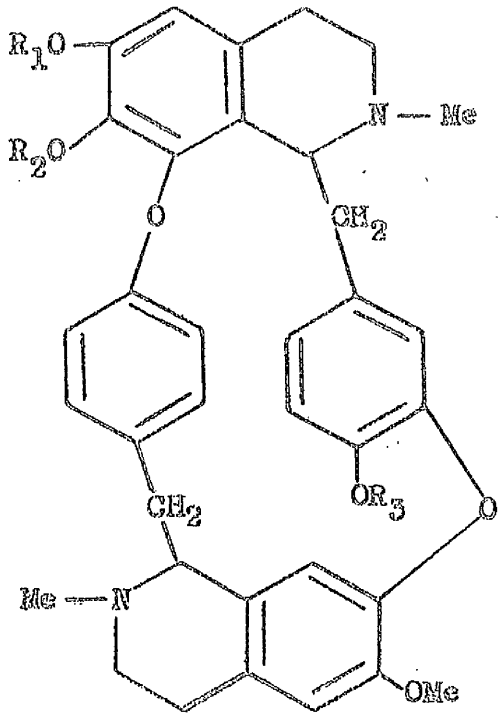
Separation of the two products afforded (+)-O-methyl-armepavine (LXXX, R=H) and (+)-laudanidine (LXXX, R=OH). These authors therefore concluded that natural (-)-armepavine had the R(D) configuration.



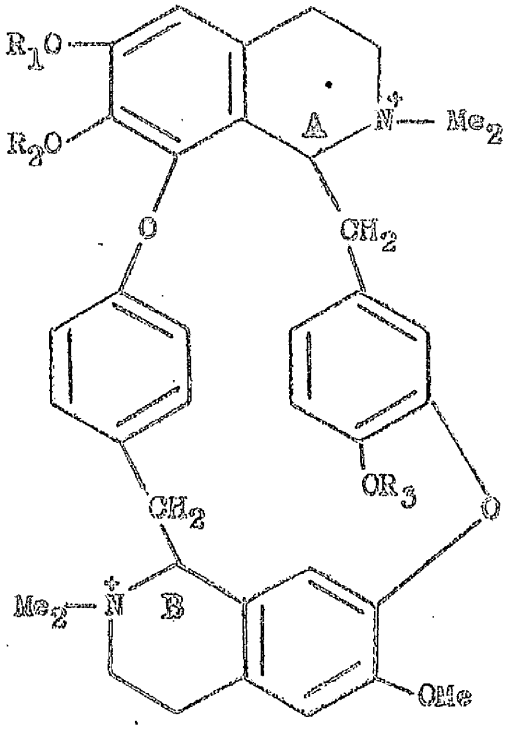
(LXXXI) (LXXX)

The demonstration that the laevorotatory bases of the coclaurine type had the D configuration and that the dextro-rotatory coclaurine bases had the L configuration led Tomita and his associates to examine the absolute configurations of the two asymmetric centres in certain biscoclaurine bases

including curine (LXXI, R_1=Me, R_2=R_3=H), chondocurine (LXXI, R_2=Me, R_1=R_3=H) and the quaternary derivative tubocurarine (LXX, R_1=Me, R_2=R_3=H). From the known relationship between curine, chondocurine, chondocurarine and tubocurarine (p.49) Tomita on consideration of the known fission products deduced that centre A was D(R) and centre B was L(S) in (LXX).



(LXXI)



(LXX)

In this laboratory the problem was approached from a different point of view, namely the derivation of the base tubocurine or preferably its O,O-dimethyl derivative from the naturally occurring quaternary salt tubocurarine. Fission of the dimethyl base with sodium in liquid ammonia would then be expected to yield on the basis of literature evidence (*vide infra*) two bases of the coclaurine type, each base representing one optical centre of the original molecule. It was further expected that measurements of the optical rotation of the two products of fission in solvents of varying polarity would provide evidence of

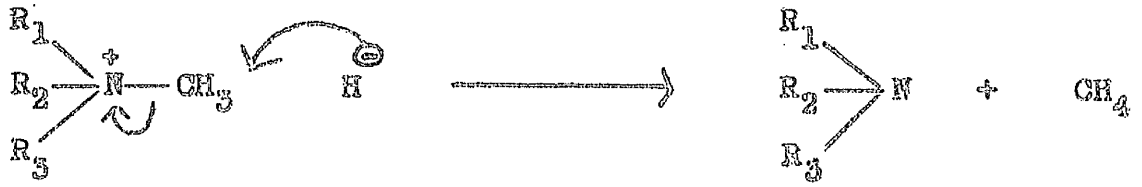
the absolute configuration of the coclaurine bases.

Preparation of O, O-dimethyltubocurine.

Three main methods are known for the removal of a methyl group from a quaternary ammonium salt, namely reduction with lithium aluminium hydride[105], treatment with potassium thiophenolate[106] in triethylene glycol at 150-200° and treatment with ethanolamine[117] at elevated temperatures.

Reductive dealkylation of O, O-dimethyltubocurarine by lithium aluminium hydride.

The reduction of a quaternary ammonium salt to a tertiary amine by lithium aluminium hydride was first reported by Kenner and Murray[105] who reduced strychnine methosulphate to strychnidine. It was suggested by these workers that this reacti on account of the greater inherent susceptibility of methyl groups compared to higher alkyl groups to S_N2 reactions, proceeds by a bimolecular displacement of the N-methyl group by the hydride ion.

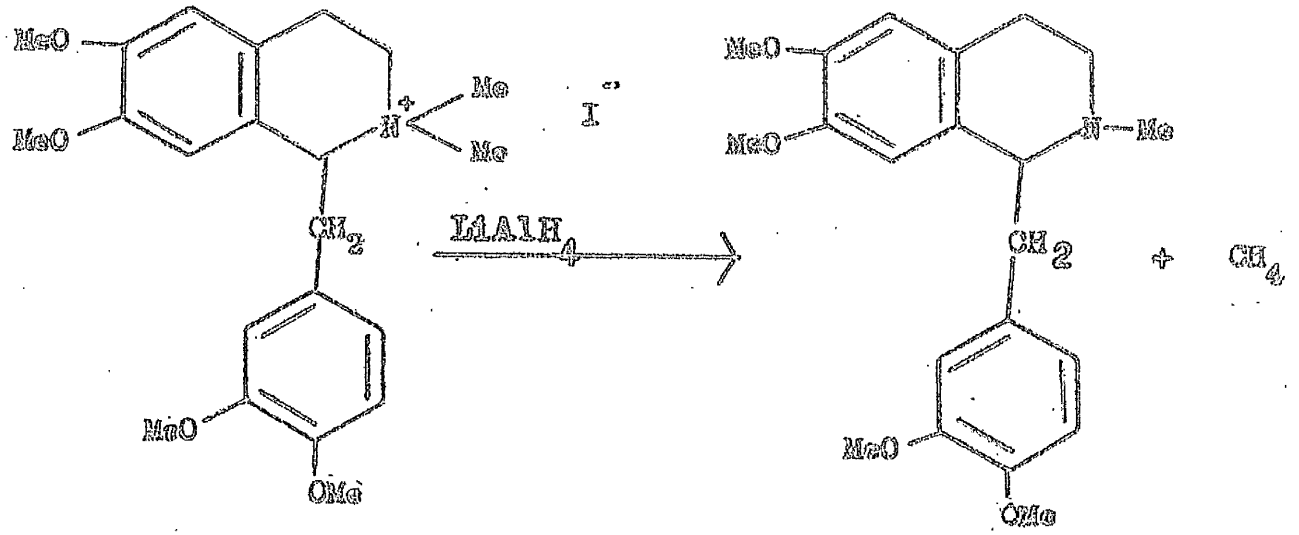


(LXXXII)

The identification of methane on the basis of its mass spectrum serves to substantiate the mechanism proposed for this reaction (LXXXII). The progress of this type of reaction may be followed easily by measurement of the amount of gas evolved.

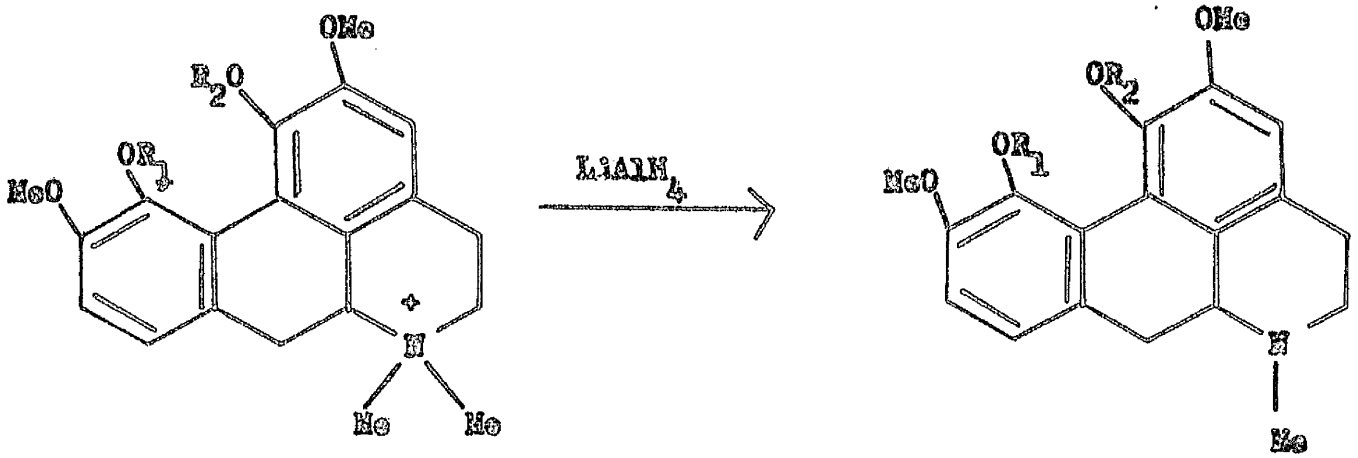
O, O-Dimethyltubocurarine iodide, obtained in high yield from tubocurarine chloride[16], when reduced with lithium aluminium hydride gave only a minute quantity of the expected tertiary base O, O-dimethyltubocurine. Variation in the reaction conditions failed to increase the yield. These results parallel those of Tomita and Ibuka[107] who found that, whereas for certain simpler alkaloids including (±)-laudanosine methiodide (LXXXIII), (±)-O, O-dimethylcorytuberine methiodide (LXXXIV, R_1=R_2=Me) and canadine methiodide (LXXXV), the corresponding tertiary bases were obtained in high yield. The more complex alkaloids including a number of biscoclaurine quaternary alkaloids only afford small amounts of the tertiary derivatives.

Attempted reductive dealkylation on the aporphine type phenolic quaternaries corytuberine methiodide (LXXXIV, R_1=R_2=H) and isocorydine methiodide (LXXXIV, R_1=Me, R_2=H) with lithium aluminium hydride resulted in almost complete recovery of the starting material.



(LXXXIII)

Dealkylation of the quaternary tubocurarine compounds had been satisfactorily achieved using ethanolamine. Potassium thiophenolate has been reported by Trumbell, Härberli and Ammon[106] to smoothly convert quaternary ammonium compounds to tertiary bases and although the low basicity of the anion is alleged to avoid Hofmann elimination in certain cases dealkylation of phenylethylamine derivatives has been accompanied with a small percentage of olefin. The dealkylation of bisbenzyltetrahydroisoquinoline quaternary compounds with potassium thiophenolate was not investigated because of the facile dealkylation obtained with ethanolamine and the ready availability of the reagent.



I^-

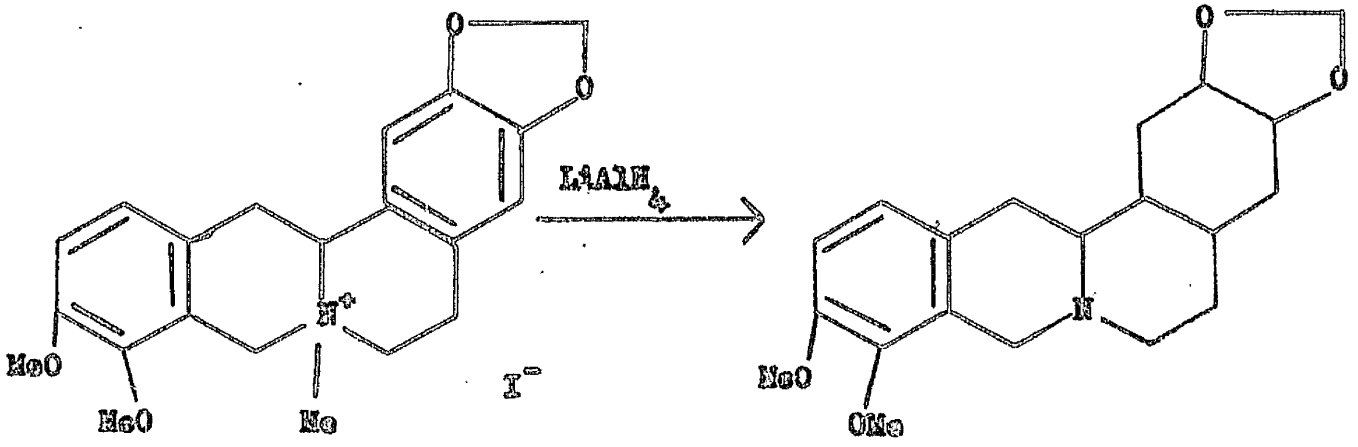
(LXXXIV)

Dealkylation of Quaternary Salts.

Pyrolytic decomposition of quaternary ammonium compounds usually follows one of two pathways:

(a) the Hofmann elimination[108] which occurs in the presence of strong bases and leads to the formation of an olefin ;

(b) the decomposition of quaternary salts with weak bases of which the classic example is the pyrolytic decomposition of quaternary ammonium chloride, first studied by Collie and Schryver[109], which results in the formation of the tertiary base and an alkyl halide.



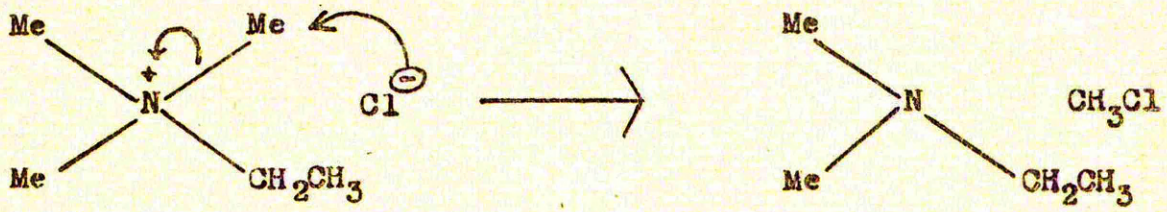
(LXXXV)

The decomposition of mixed quaternary salts of the type $Me_3\overset{+}{N}RCl^-$ (LXXXVI) is of special interest and leads in general to dissociation as follows:

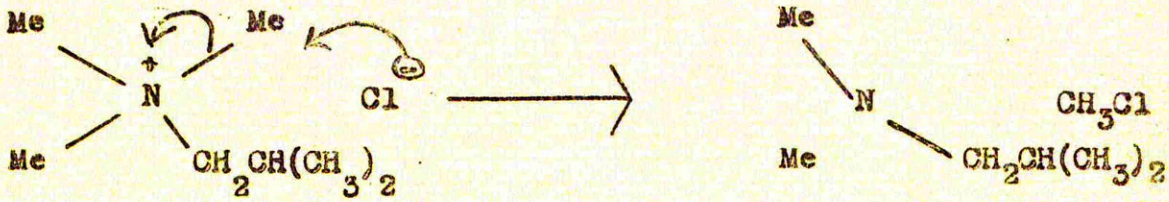
$$Me_3\overset{+}{N}RCl^- \longrightarrow Me_2NR + MeCl$$

(LXXXVI)

If the reaction is conceived as a nucleophilic displacement on carbon the course of the reaction can be explained by the expectation that delocalisation of positive charge from nitrogen to the adjacent α-carbon atom[110] will be unequal and greatest in methyl substituents. Nucleophilic attack will thus be favoured at one of the methyl groups rather than at the alkyl groups R, as in the following examples recorded by Collie and Schryver[109] (LXXXVII) and (LXXXVIII).



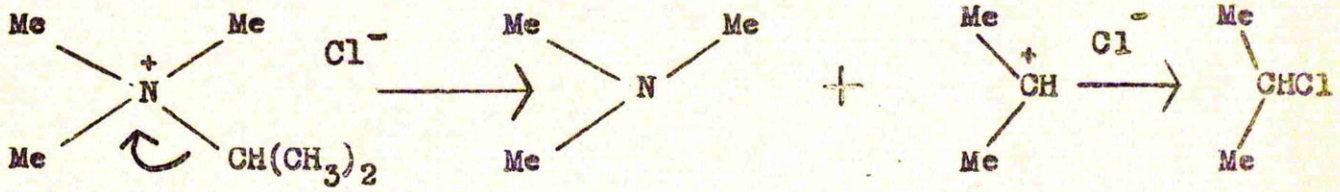
(LXXXVII)



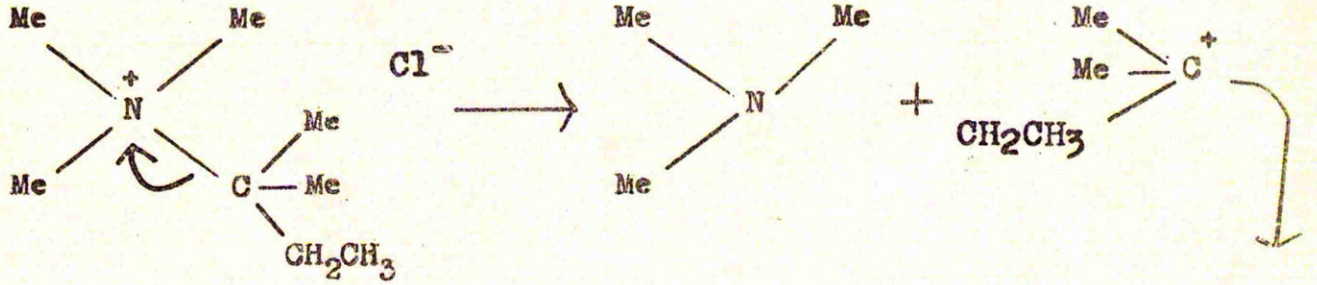
(LXXXVIII)

Although multiple branching on the α-carbon atom should on this basis still further inhibit displacement of the groups R, their effect is clearly outweighed by the enhanced resonance stabilisation which the intermediate carbonium ion will activate by hyperconjugation if the reaction proceeds by an S_N1 mechanism.

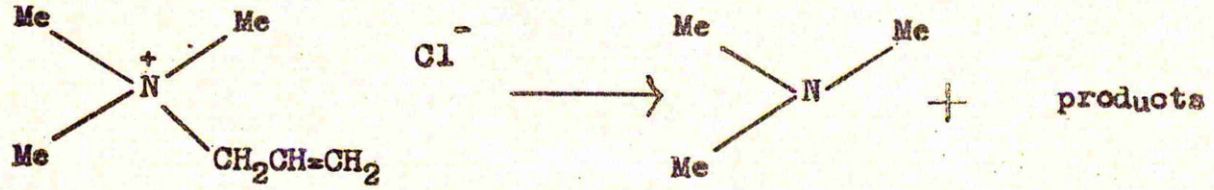
That this appears to be so is evident from the following decompositions also reported by Collie and Schryver[109], (LXXXIX), (XC) and (XCI).



(LXXXIX)



(XC)



(XCI)

The pyrolytic decomposition of $(CH_3)_3\overset{+}{N}BzCl^-$ (XCII), however, is apparently anomalous proceeding as follows with the fission of a methyl rather than the benzyl group. On the other

$$(CH_3)_3\overset{+}{N}BzCl^- \longrightarrow (CH_3)_2NBz + MeCl.$$

(XCII)

hand benzyl is lost in preference to ethyl from $Et_3\overset{+}{N}BzCl^-$.

The dissociation of quaternary halides forms the basis of their use as alkylating agents. In this respect they rank in efficiency after alkyl chlorides and sulphonium salts[111]. Quaternary salts have been used for C-alkylation, O-alkylation, S-alkylation and, in particular, N-alkylation by the use of appropriate reagents which promote nucleophilic displacement of a tertiary amine from the quaternary salt[112].

The competition between the nucleophilic displacements which form the basis of these reactions and the alternative Hofmann elimination was first studied in detail by Hanhart and Ingold[113] with a series of propyltrimethylammonium salts (XCIII).

$$\underset{\text{(XCIII)}}{Pr\overset{+}{N}Me_3\bar{X}} \xrightarrow{a} PrNMe_2 + MeX$$

$$\xrightarrow{b} Me_3N + CH_3{\cdot}CH = CH_2 + H_2O$$

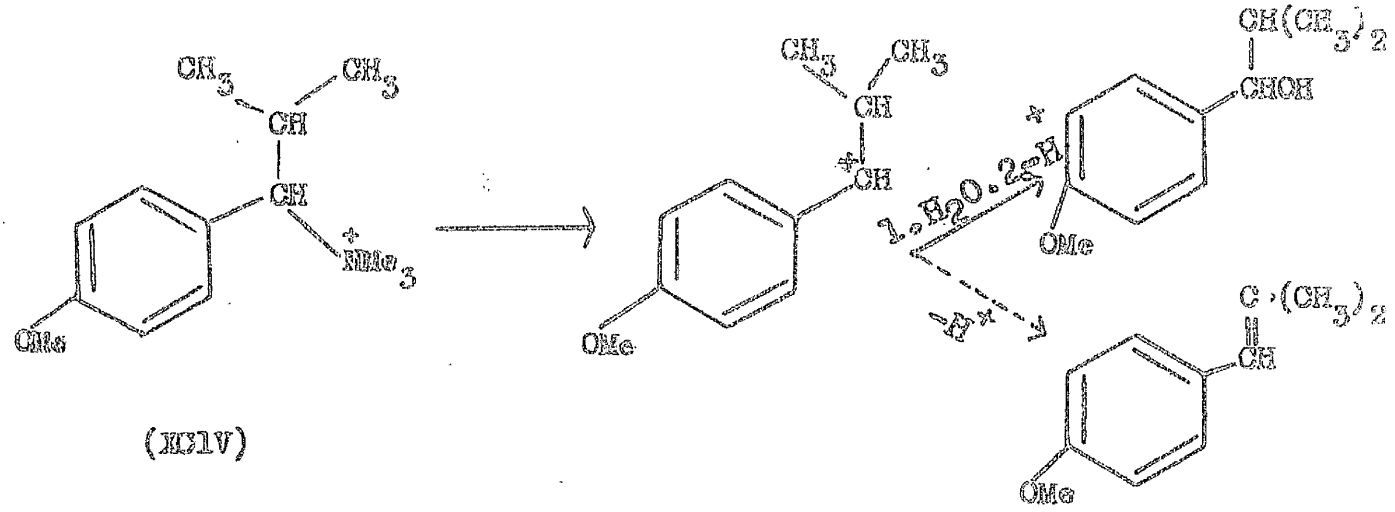
They found that the nucleophilic displacement reaction (a) became increasingly favoured where the anion is in the order of decreasing basicity,

$$HO^- < PhO^- \equiv CO_3^{2-} < \text{acetate} \equiv \text{p-}NO_2C_6H_4O^-$$

and that the alternative Hofmann elimination is correspondingly increasingly less favoured.

The decomposition of a series of α-arylalkyltrimethylammonium iodides in water at 100° was also studied by Norcross and Openshaw[114] and similarly shown to proceed mainly by displacement according to the findings of Hanhart and Ingold.

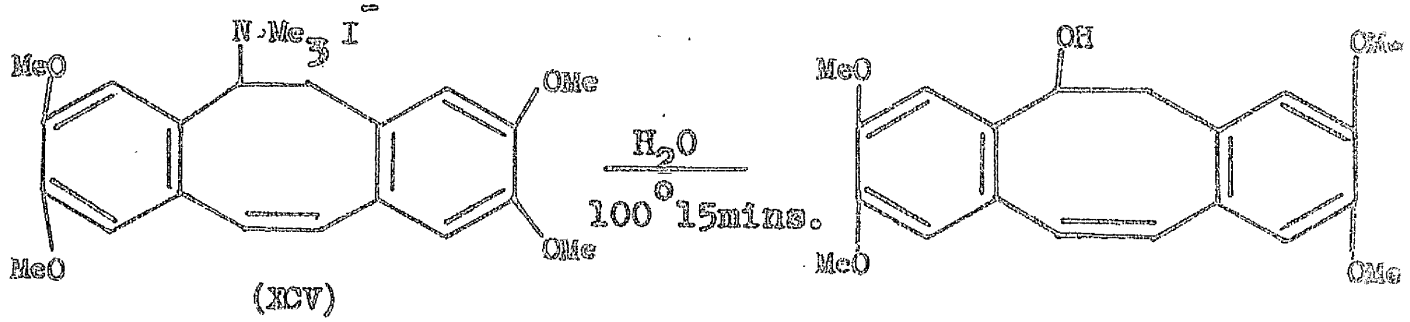
The decomposition occurs only when a methoxyl group is present in the p-position in the ring and is further facilitated by the presence of an alkyl substituent on the β-carbon of the side-chain, as in the following example (XCIV):



(XCIV)

The reaction is arrested as a result of the stabilisation of the carbonium ion by the mesomeric effect of the p-methoxy groups, and its separation is facilitated by the inductive effects of the alkyl substituents on the β-carbon atom. In aqueous solution, the reaction proceeds largely as shown by displacements, but in diethyl ketone elimination predominates.

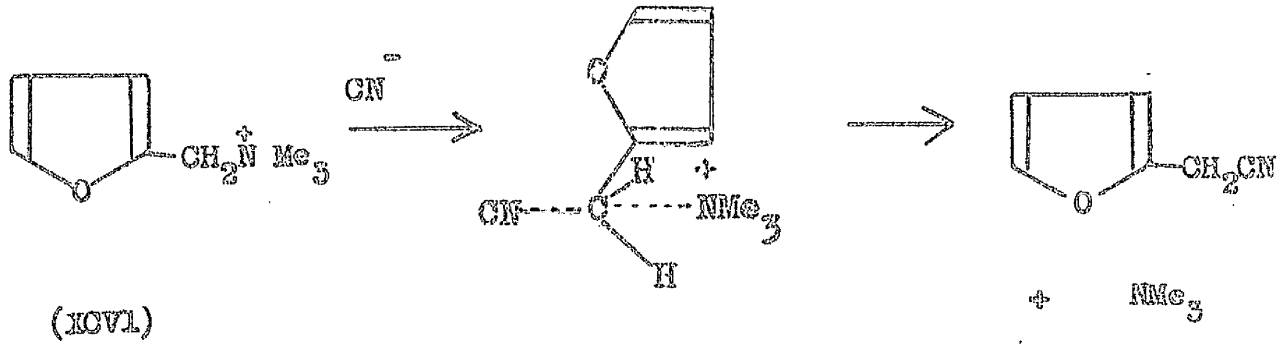
Battersby and Binks[115] have similarly studied the decomposition of N-methylpavine methiodide (XCV) which similarly proceeds in water at 100° by displacement to give the corresponding alcohol (and traces of the iodide) through the intermediate carbonium ion (XCV).



(XCV)

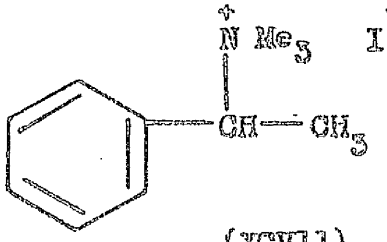
That displacement of ammonium groups does not always proceed by an S_N1 (or E1) mechanism is evident from the experiment of Eliel and Peckham[116]. These show that whereas nucleophilic displacement of Cl^- by CN^- on treatment of furfuryl chloride

with sodium cyanide leads to a rearranged product 2-methyl-5-cyanofuran, implying a carbonium ion intermediate, no such rearrangement occurs in the corresponding displacement of NMe_3 by CN^-, thus implicating reaction by an S_N2 mechanism proceeding via the transition state (XCVI).

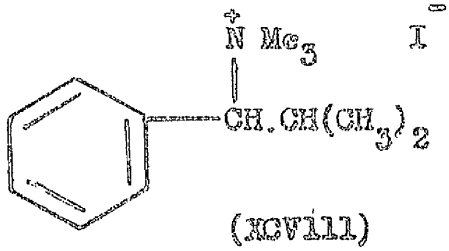


(XCVI)

Two observations of Norcross and Openshaw[114] are of interest to the present study. Firstly the absence of a p-methoxy substituent in α-alkylbenzylamines inhibits the nucleophilic displacement reaction. Thus trimethyl-1-phenylethylammonium iodide (XCVII), trimethyl-1-phenylisobutylammonium iodide (XCVIII) are both stable in water at 100°.



(XCVII)



(XCVIII)

This stability under the conditions reported is in contrast to the decomposition of benzyltrimethylammonium chloride previously reported by Collie and Schryver[109] though the latter admittedly was submitted to more drastic reaction conditions and no direct comparison has been made.

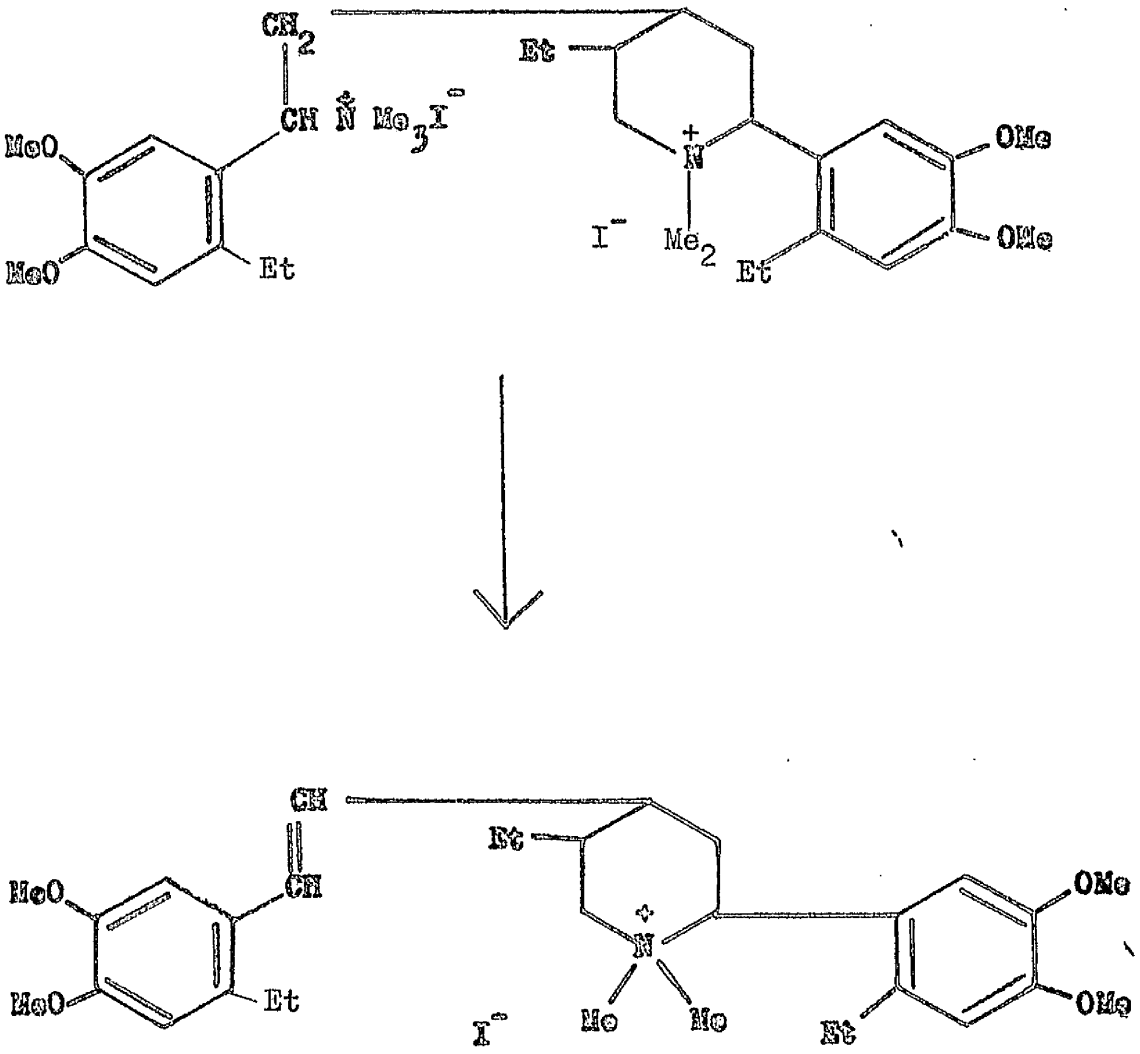
Secondly, Norcross and Openshaw[114] clearly established the enhanced stability in nucleophilic displacement (and elimination) reactions of quaternary ammonium salts in which the quaternary

group is part of a heterocyclic ring. Thus dimethyl-2-p methoxyphenylpiperidinium iodide is stable in water at 100° and only the non-heterocyclic quaternary groups of N-methyl-emetinetetrahydromethine dimethiodide is decomposed (by elimination) under these conditions (XCIX).

The stability of these cyclic quaternaries is ascribed to inherent reversibility of the C-N fission process, it being suggested that in the case of the open-chain compound it is possible for the two fission products to separate quickly to a distance sufficient to prevent recombination, but with the fission of cyclic C-N links there is a restraint on such separation.

The use of the thiophenoxide ion and ethanolamine for the dealkylation of quaternary ammonium salts clearly depends on the high nucleophilicity and low basicity of these reagents which according to Hanhart and Ingold[113] favour nucleophilic displacement at the expense of the competing elimination reaction. The use of ethanolamine in the dealkylation of quaternary salts (XCX) has been studied in detail by Hunig and Baron[117]. In addition to its high nucleophilicity and low basicity, it offers the further advantages of being a good solvent for quaternary salts and possessing a reasonably high boiling point.

In accordance with the foregoing discussion the decomposition of alkyltrimethylammonium halides occurs principally



(XCIX)

with the elimination of the methyl groups, unless the departing alkyl group is capable of forming a stable carbonium ion intermediate, as for example if unsaturation is present in the alkyl chain. (Table IX).

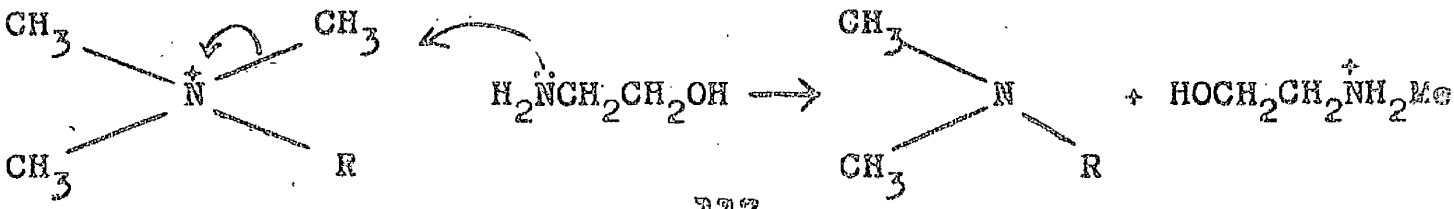
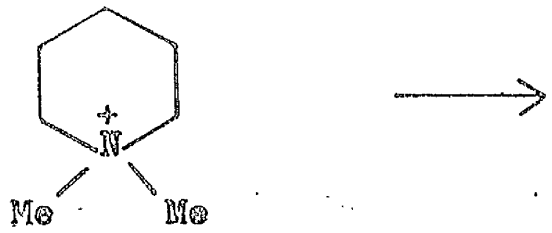


Table IX.[117]

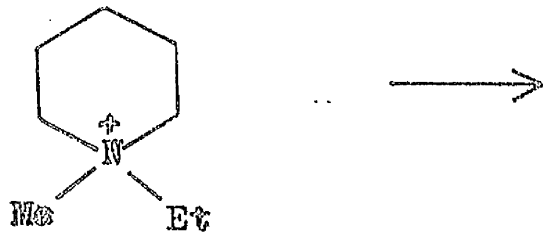
$(CH_3)_3\overset{+}{N}-R$ (R)	%$(CH_3)_3N$ formed (splitting of R)	%$(CH_3)_2N-R$ (Splitting of Me)
$-CH_2CH_2CH=CH_2$	99	1
$-CH_2CH=CH.CH_3$	68	32
$-CH_2CH=CH_2$	66	34
$-CH_3$	25	75
$-CH(CH_3)_2$	12	88
$-CH_2CH_3$	5	95
$-CH_2CH_2CH_3$	5	95
$-CH_2CH_2CH_2CH_3$	2	98
$-CH_2CH(CH_3)_2$	1	99

Similarly, in accordance with the experience of Norcross and Openshaw[114] heterocyclic quaternary ammonium groups tend to resist ring fission, displacement of an alkyl group being favoured. In general methyl groups are displaced in preference to larger alkyl groups in accordance with the expected degree of delocalisation of positive charge from nitrogen (Table X).

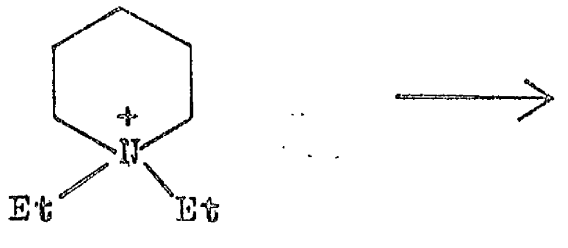
TABLE X



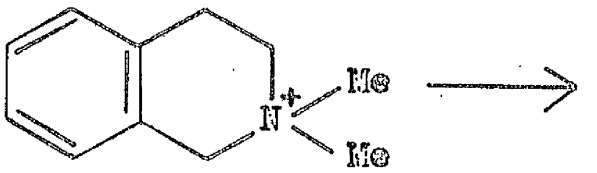
96%



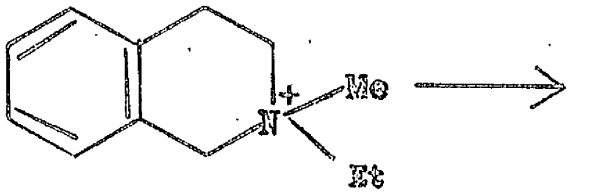
9% 86%



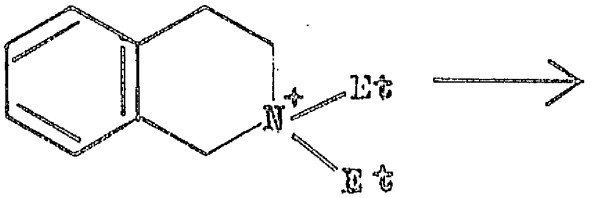
97%



92%

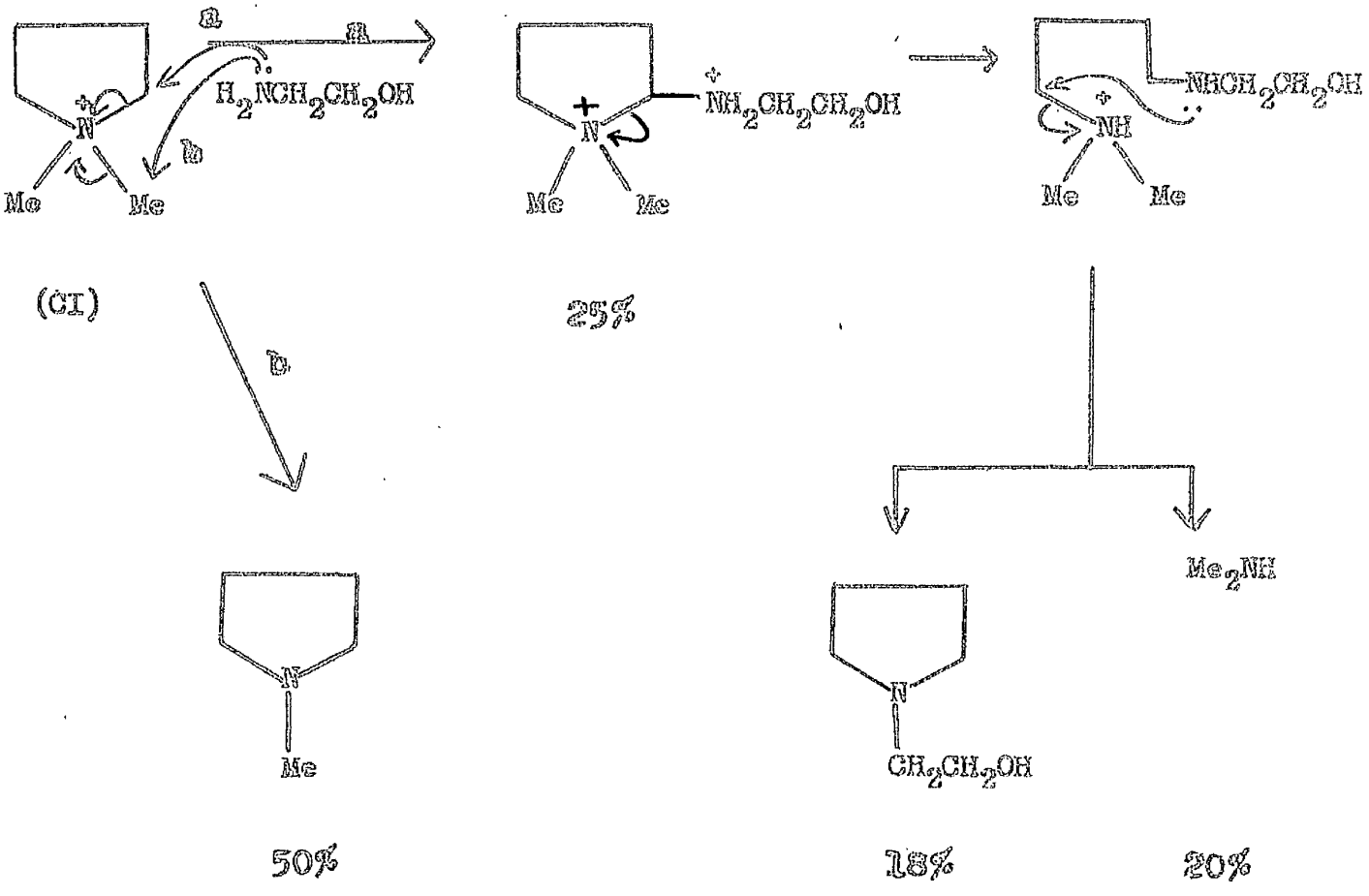


12% 78%

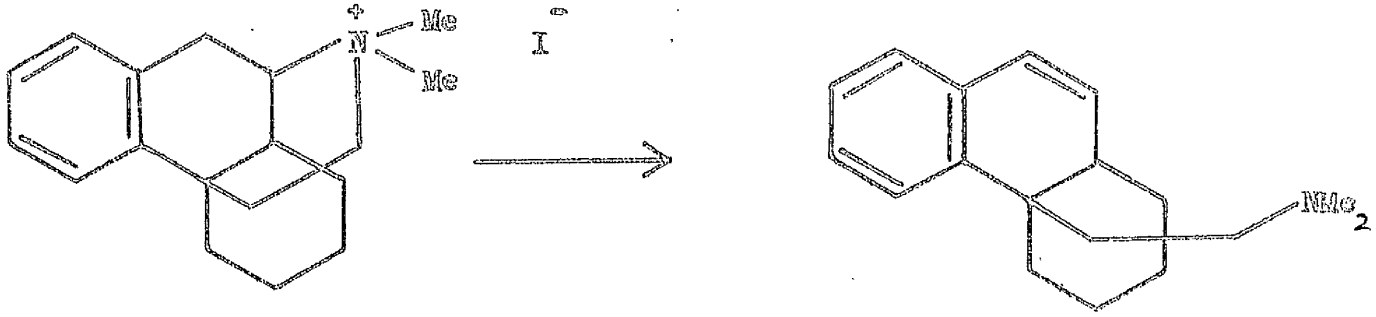


75%

The behaviour of pyrrolidinium salts (CI), however, is somewhat anomalous in that considerable ring fission occurs as a result of displacement at the ring C_2-N bond as opposed to the N-CH_3 bond even in the dimethylammonium salt. These may result partly from steric hindrance to the approach of the attacking reagent to the N-CH_3 bonds which in the five membered ring structure will be in the eclipsed (rather than the staggered as in six membered rings) orientations with respect to the hydrogen atoms of the α- carbon atoms. Further the use of ethanolamine in large molar excess promotes displacement by ethanolamine at the expense of displacement by I^- thus further inhibiting the possibility of reversal of the reaction by recyclisation as proposed by Norcross and Openshaw[114].



Hunig and Baron[117] also reported the decomposition of N-methylmorphinan methiodide (CII) in ethanolamine in which unexpected Hofmann elimination is favoured. The apparent anomaly



(CII)

of this reaction is possibly accounted for by the unusual stereochemistry of the morphinans, though clearly the acidic nature of the benzylic hydrogen is an important factor since Hunig and Baron[117] report a similar elimination for phenethyltrimethylammonium iodide (CIII) though in this case elimination is accompanied by displacement.

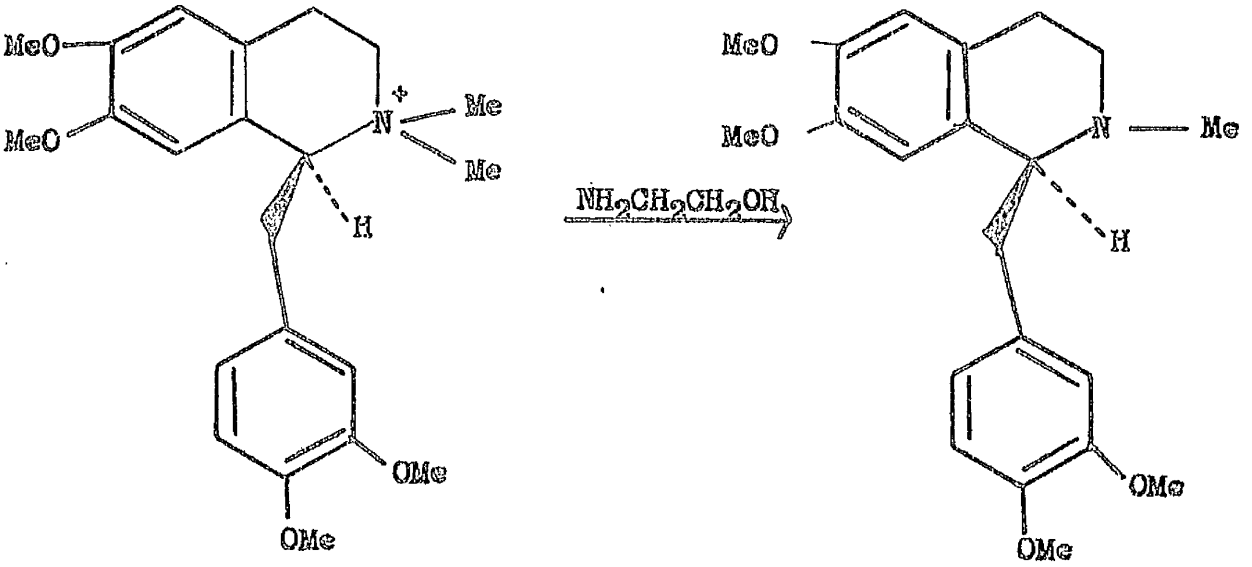
$$C_6H_5CH_2CH_2\overset{+}{N}(CH_3)_3I^- \begin{cases} \rightarrow C_6H_5CH = CH_2 + (CH_3)_3N & 68\% \\ \rightarrow C_6H_5CH_2CH_2NMe_2 & 28\% \end{cases}$$

(CIII)

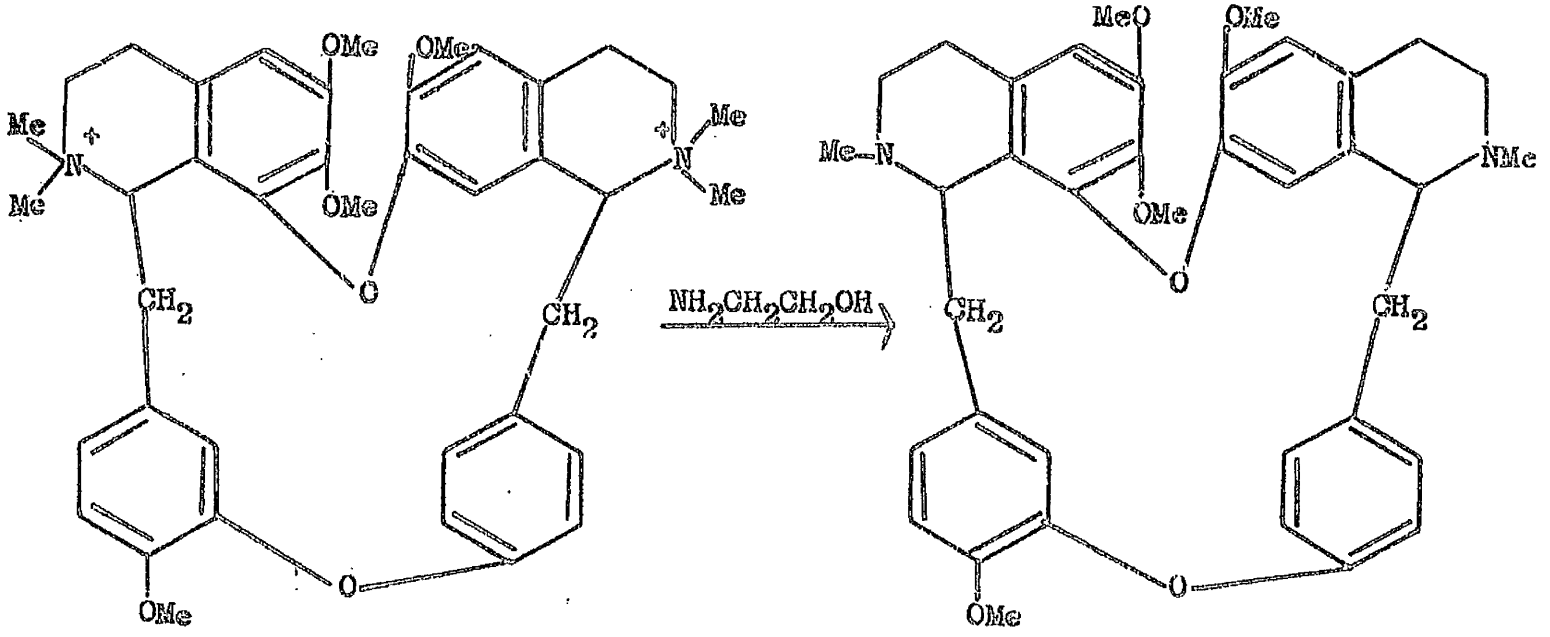
The stereochemistry of the morphinan is particularly favourable to an elimination since the departing β-hydrogen and ammonium groups are trans and antiparallel, and the steric environment of the reacting centres are both unfavourable to the establishment of the necessary transition state leading to displacement and favourable to a relief of steric hindrance by elimination.

These studies of Hunig and Baron[117] have been extended by Tomita and Takano[118] who have shown that in accordance with

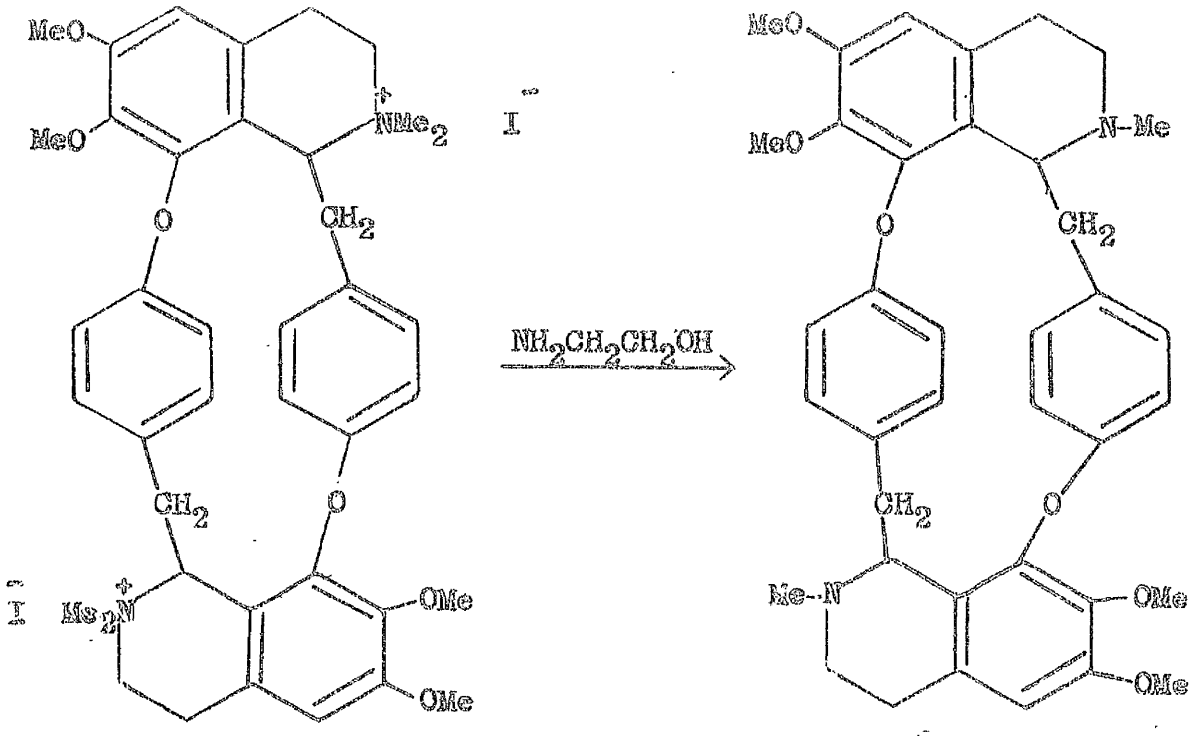
expectation dealkylation of (±)-laudanosine methiodide (CIV) gave (±)-laudanosine in 80% yield. In the case of more complex biscoclaurine alkaloids the yields of the tertiary bases corresponding to isotetrandrine methiodide (CV), cycleanine methiodide (CVI) and insularine methiodide (CVII) were in the 60% region.



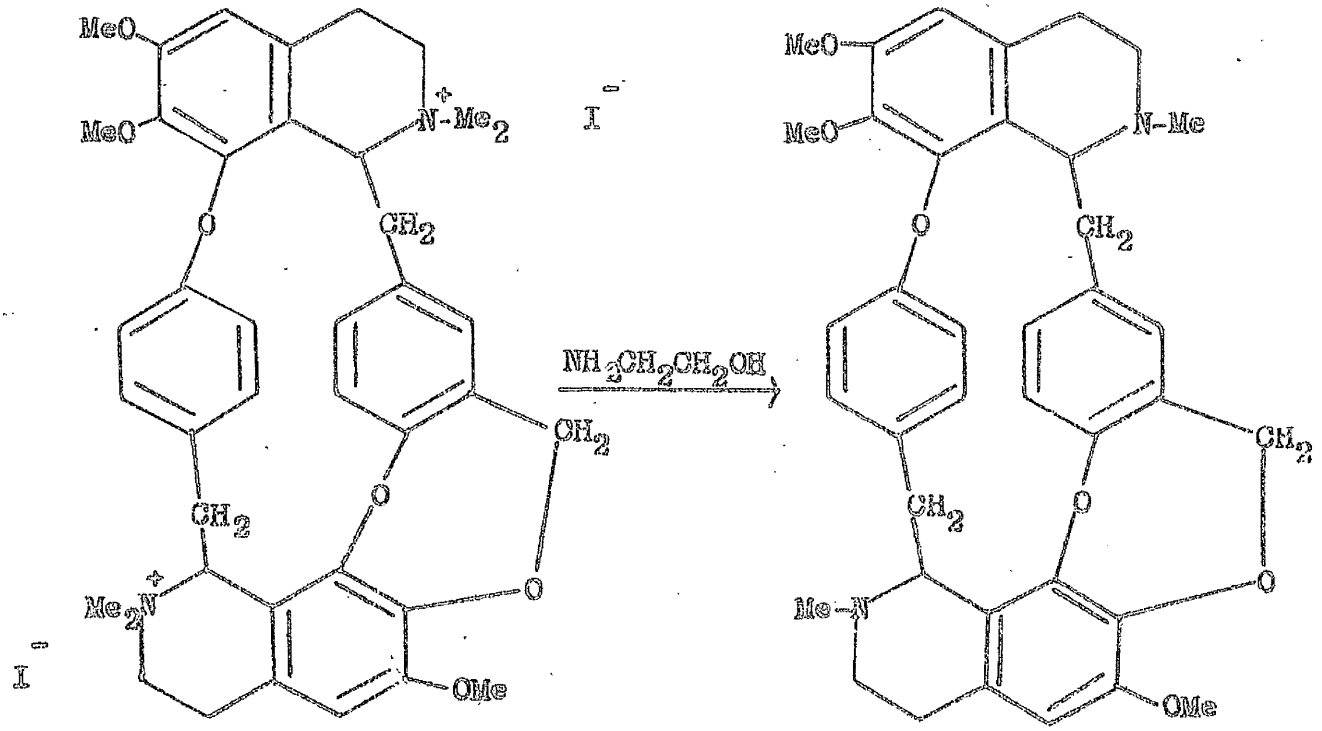
(CIV)



(CV)



(CVI)



(CVII)

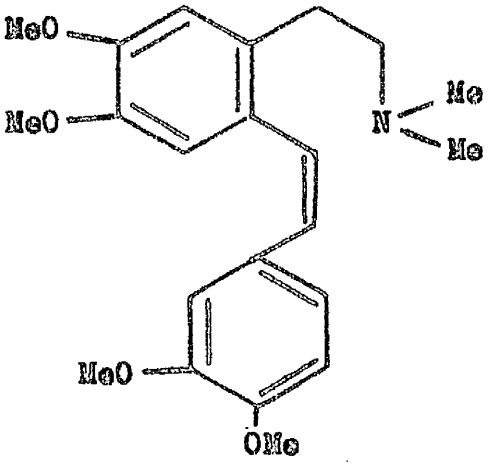
Preparation of O, O-dimethyltubocurine using ethanolamine

The reaction proceeded smoothly and O, O-dimethyltubocurine was obtained in approximately 60% yield.

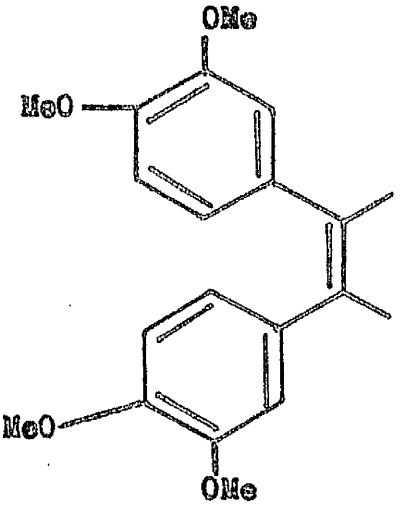
To ascertain that fission of the ring had not taken place when O, O-dimethyltubocurarine iodide was refluxed with ethanolamine

an ultra-violet spectrum of the tertiary base O,O-dimethyl-(+)-tubocurine showed peaks λ max. of 215 mμ and 286 mμ in the same ratio (ca 5:1) as the peaks in tubocurarine chloride λmax. 215 mμ and 286 mμ.

The cis-methine base (CVIIA) resulting from a Hofmann elimination in laudanosine shows λ max. at 215 mμ and 294 mμ in the intensity ratios of 2:1 as does 3,3',4,4'-tetramethoxystilbene (CVIII) λ max. 214 mμ and 303 mμ.



(CVIIA)



(CVIIB)

Preparation of (+)-tubocurine by ethanolamine.

(+)-Tubocurine was obtained without difficulty in high percentage yield.

Preparation of O,O-dimethyltubocurine from (+)-tubocurine with diazomethane.

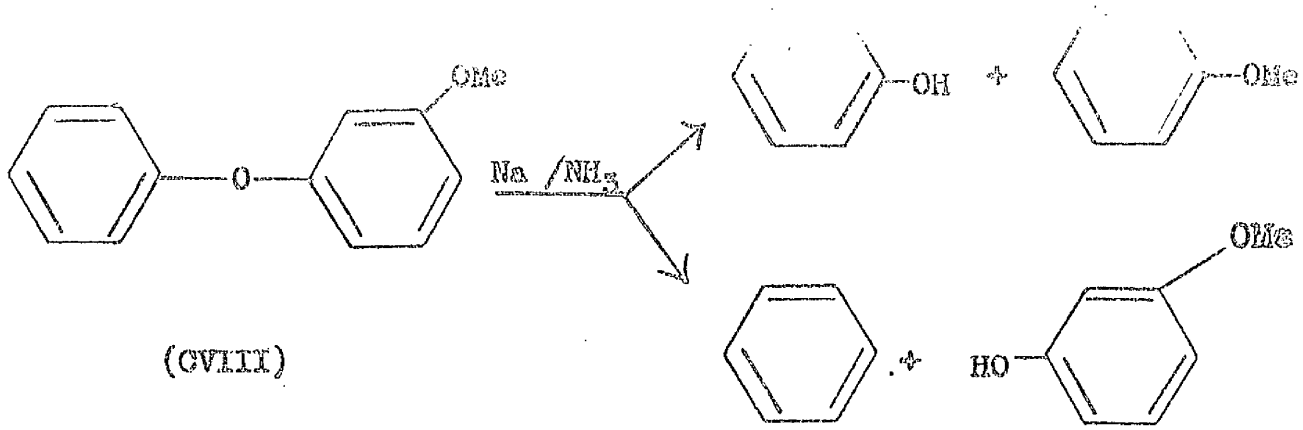
To complete the cycle and obtain further confirmation of the identity of (+)-tubocurine, the latter obtained from the ethanolamine reduction of (+)-tubocurarine chloride was treated with diazomethane in an attempt to produce O,O-dimethyltubocurine.

Excess of diazomethane over a period of six days did not convert all the (+)-tubocurine to its dimethyl ether. The separation of the two components was effected on alumina with elution by petroleum ether (40/60°) giving O,O-dimethyltubocurine identical to that produced by the action of ethanolamine on O,O-dimethyltubocurarine iodide.

Quaternisation of O,O-dimethyltubocurine, prepared from (+)-tubocurine and diazomethane, with methyl iodide by the general method described for the preparation of quaternary halides yielded O,O-dimethyl-(+)-tubocurarine iodide, the melting point and optical rotational values of which were slightly lower than those of authentic material. However, infra-red spectra were superimposable on infra-red spectra of the authentic material. The difficulty of preparing bisbenzyltetrahydroisoquinoline quaternary compounds has been recorded by several workers and the material showed optical rotational values sufficient to exclude, along with ultra-violet evidence, the possibility of either Hofmann elimination or more than slight racemization.

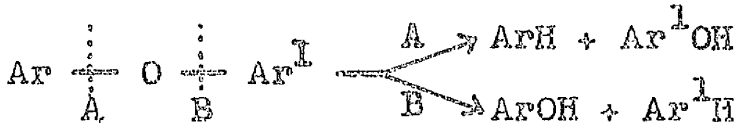
Fission of Diaryl Ethers with Sodium and Liquid Ammonia.

Sowa[119-121] and his coworkers in 1937 demonstrated that diaryl ethers (CXIII) were cleaved by sodium and liquid ammonia to yield a hydrocarbon and a phenol. It was established that in general only diaryl ether linkages are readily cleaved, alkyl-aryl ether linkages being much more resistant to attack because one of the groups is saturated and in this case the relevant charge is not on the carbon but on the oxygen.



(CVIII)

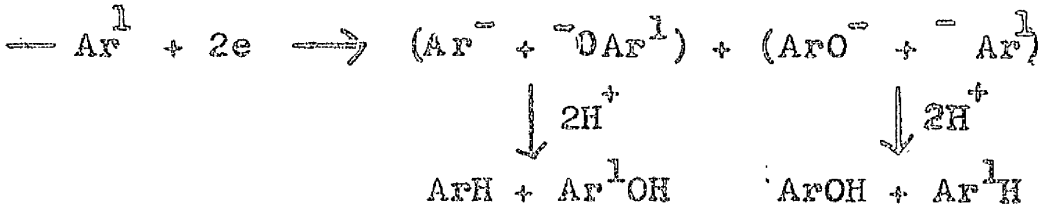
The reaction may proceed by alternative pathways expressed as:-



(CIX)

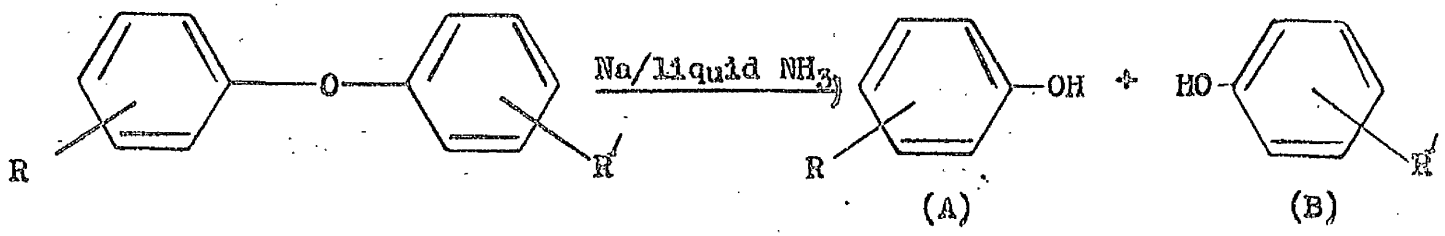
The nature of the products depends upon whether fission occurs at A or B (CIX) and in certain cases fission at both A and B (CIX) occurs simultaneously (vide infra).

Birch [132] has proposed the following reaction mechanism for the fission:-



The direction of cleavage is decided by whichever transition state has the lower energy. From the fact that ArH (pK about 37-40) is a much weaker acid than ArOH (pK about 16-18) Birch postulates the greater part of the energy will be required to form the carbanion Ar^- or Ar^{1-}, or in other words the greater part of the energy of the transition state will be consumed by the carbon system. The relative stabilities of the carbanion intermediates (Ar^- and Ar^{1-}) (which of course determines the ultimate course of the reaction) should be largely

determined by the substituents present on the respective aromatic moieties. In practice this is found to be true; the aryl groups containing the greater number of electron-withdrawing groups or containing the lesser number of electron-donating groups almost invariably makes a greater contribution to the formation of the hydrocarbon. Results pertinent to the effect of substituents on the direction of fission have been obtained by the examination of the products of cleavage of unsymmetrical diaryl ethers (Table XI)[119-121].



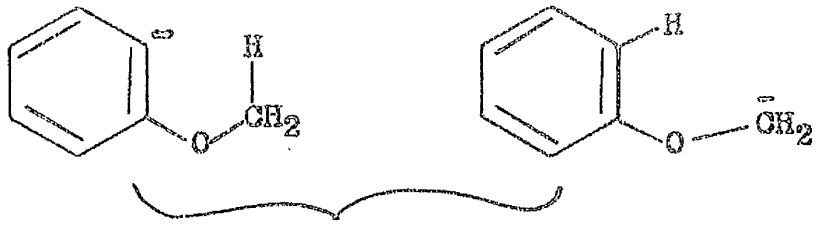
(CX) (A) (B)

Table XI Cleavage products of substituted diphenyl ethers.

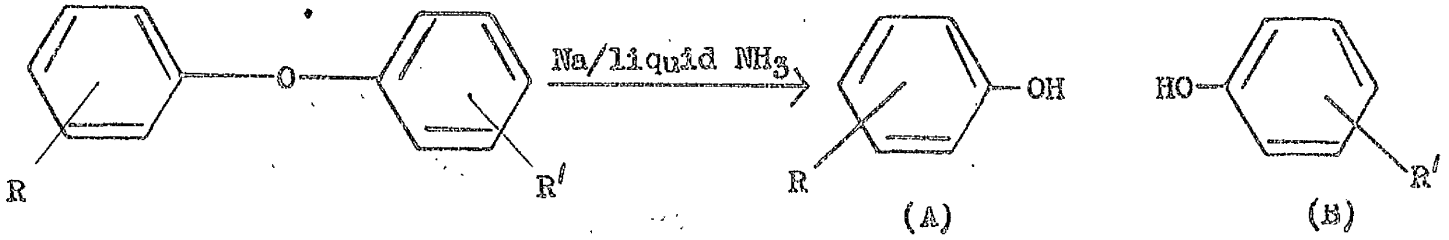
Substituent (Structure CX)		Yield% (Structure CX)	
R	R^1	A	B
H	H	100	0
p-CH_3	H	75	25
o-CH_3	H	53	47
o-CH_3	p-CH_3	39	61
H	p-NH_2	0	100
H	o-NH_2	1	99
H	p-COOH	100	0
H	o-COOH	90	-
H	m-NH_2	28	72

R	R^1	A	B
H	m-CH_3	38	62
H	m-COOH	64	36
o-CH_3	m-CH_3	47	53
m-CH_3	p-CH_3	23	77
p-CH_3	p-OCH_3	21	79
p-CH_3	p-NH_2	0	100
p-CH_3	p-t-C_4H_9	48	52
p-OCH_3	p-NH_2	8	92

The methoxyl group is the one substituent group so far examined (Table XI) whose influence on reductive fission is not immediately obvious from its known electronic character, its action on diaryl ether fissions and on aryl-alkyl fissions depending on its position. An o-methoxyl is activating (charge-stabilising relative to hydrogen) and a p-methoxyl is strongly deactivating. This double effect may be related to the known dual electronic character of the group, but this would mean that in an o-position the inductive effect would predominate C $\rightarrow$ OMe and in a p-position the mesomeric effect C $\leftarrow$ OMe which is unlikely. A more plausible explanation of this unexpected result is that hyperconjugation occurs with o-methoxyl substituents which stabilises the charge by transferring it partially to the carbon of the methoxyl group as shown (CXI).



(CXI)

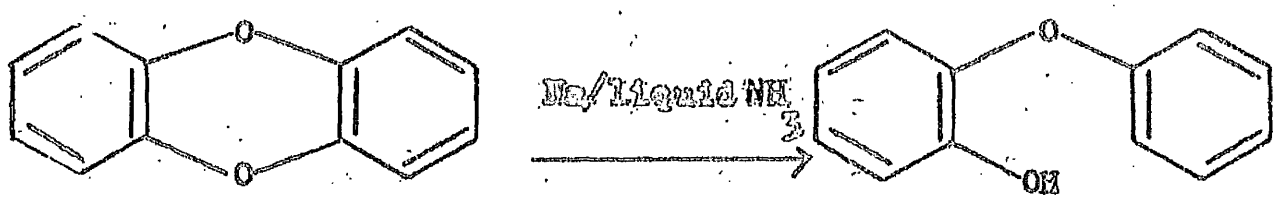


(CXII) (A) (B)

Table X. Cleavage products of meta substituted diphenyl ethers.

Substituents (Structure CXII)		Yield %	
R	R^1	A	B
H	m-OCH_3	53	47
H	o-OCH_3	55	45
o-OCH_3	m-OCH_3	24	76
m-OCH_3	p-OCH_3	8	92
o-OCH_3	p-OCH_3	1	99

In 1951 Tomita[122] showed that the presence of a phenolic hydroxyl substituent in the para or ortho positions to the ether link increases greatly the resistance of the ether link to cleavage by sodium in liquid ammonia.

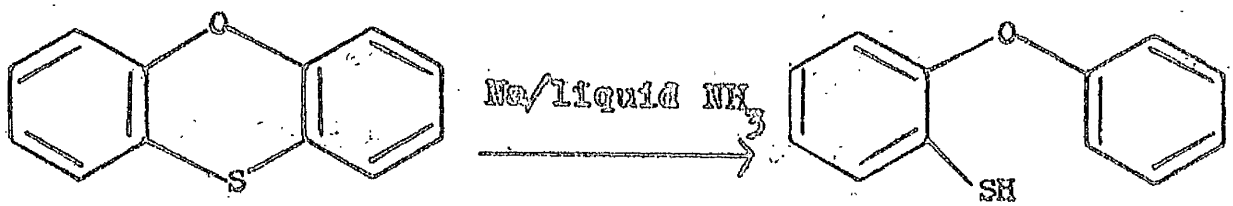


(CXIII) (CXIV)

He attempted cleavage of diphenylene dioxide (CXIII) with sodium in liquid ammonia but found that even with excess of sodium the reaction would proceed no further than the production of 2-hydroxydiphenyl ether (CXIV).

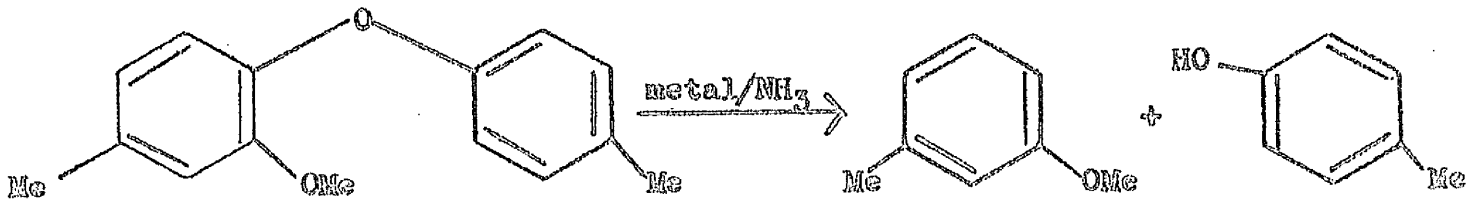
As a result, Tomita[122] then applied this reaction to 2-hydroxydiphenyl ether and 4-hydroxydiphenyl ether and in both cases almost quantitative recovery of starting material resulted.

As a parallel the cleavage reaction was applied by Tomita[122] to phenoxthine (CXV) when the oxygen linkage similarly remained unbroken and only the sulphur linkage was cleaved yielding 2-mercaptodiphenyl ether (CXVI).



(CXV) (CXVI)

Replacement of sodium by potassium for the cleavage of 4,4'-dimethyl-2-methoxydiphenyl ether (CXVII) in liquid ammonia produced identical results with both alkali metals except that the reaction was more vigorous with potassium.

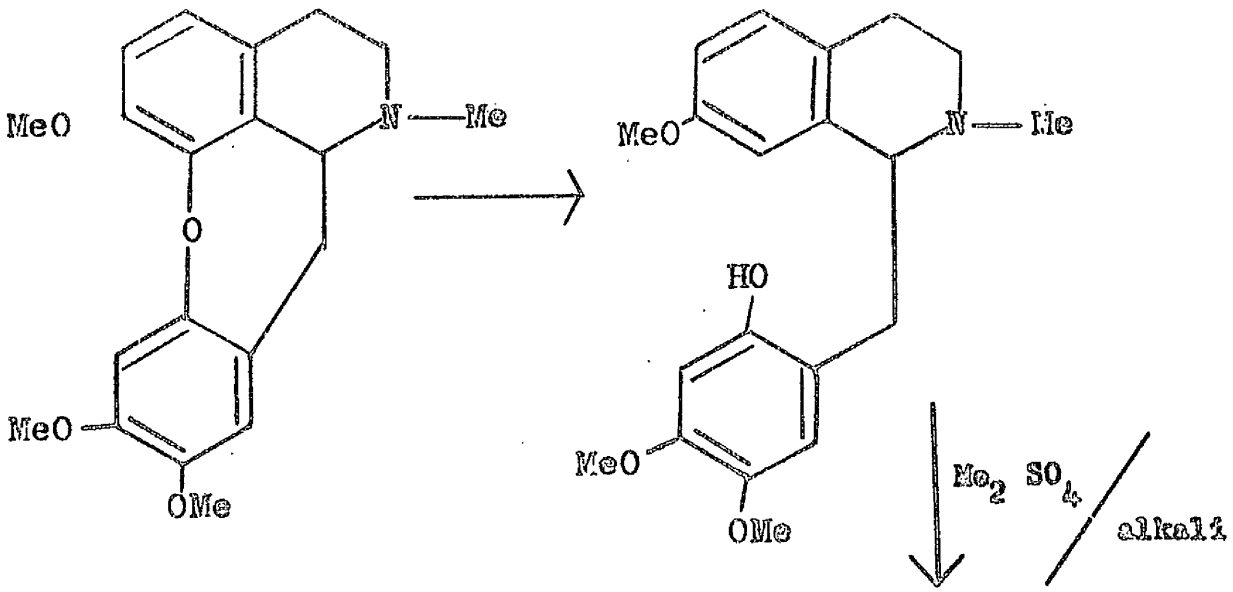


(CXVII)

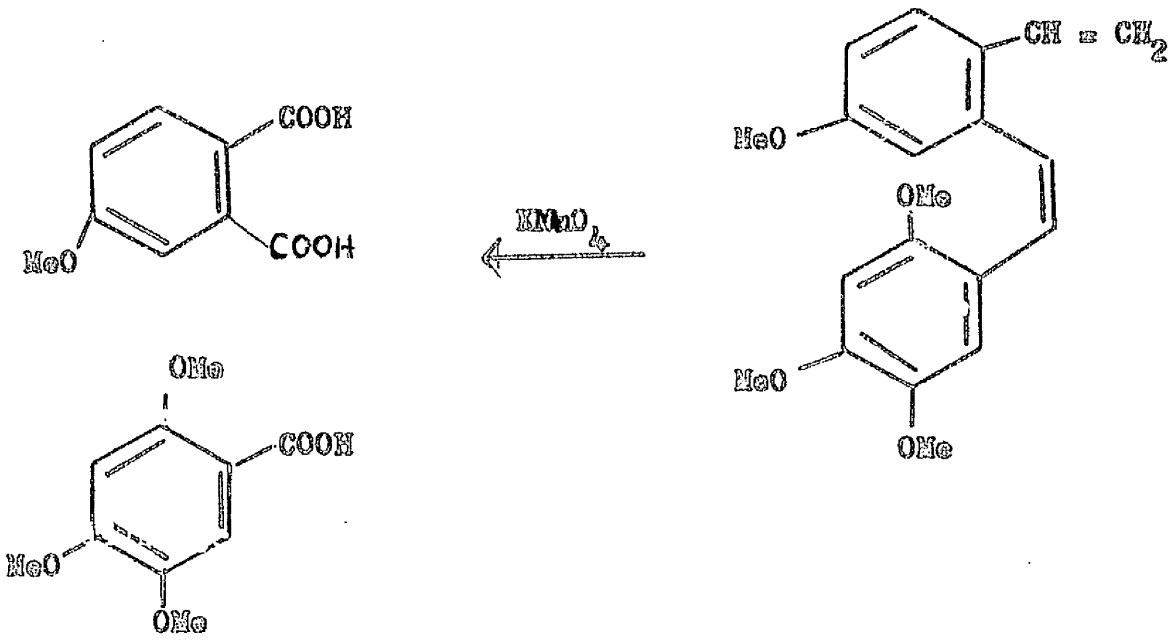
Substituents can be classified into two groups[122] according to whether they strengthen or weaken the linkage between oxygen and the substituted phenyl groups against cleavage. The substituents arranged in order of their increasing bond strengthening effect are: o-Me, m-NH_2, p-Me, p-OMe, o-NH_2 and p-NH_2. Substituents arranged in the order of their decreasing bond weakening effect are: m-OMe, o-OMe, m-COONa and p-COONa.

Manske[123] recognised the suitability of the metal/ammonia reaction, especially for elucidation of the structure of alkaloids with diaryl ether linkages, and in 1950 confirmed the structure of cularine (CXVIII) by this method, (see over).

In the following year Tomita[124] and his coworkers applied the metal/ammonia reagent to two bisbenzyltetrahydroisoquinoline alkaloids which in general are cleaved into two benzylisoquinoline fragments of known or easily determined structure thus permitting the elucidation of the structures of a number of these compounds. The optical rotation of the cleavage products gives the relative orientation of the asymmetric centres. Four distinct groups of biscoclaurine alkaloids with



(CXVIII)

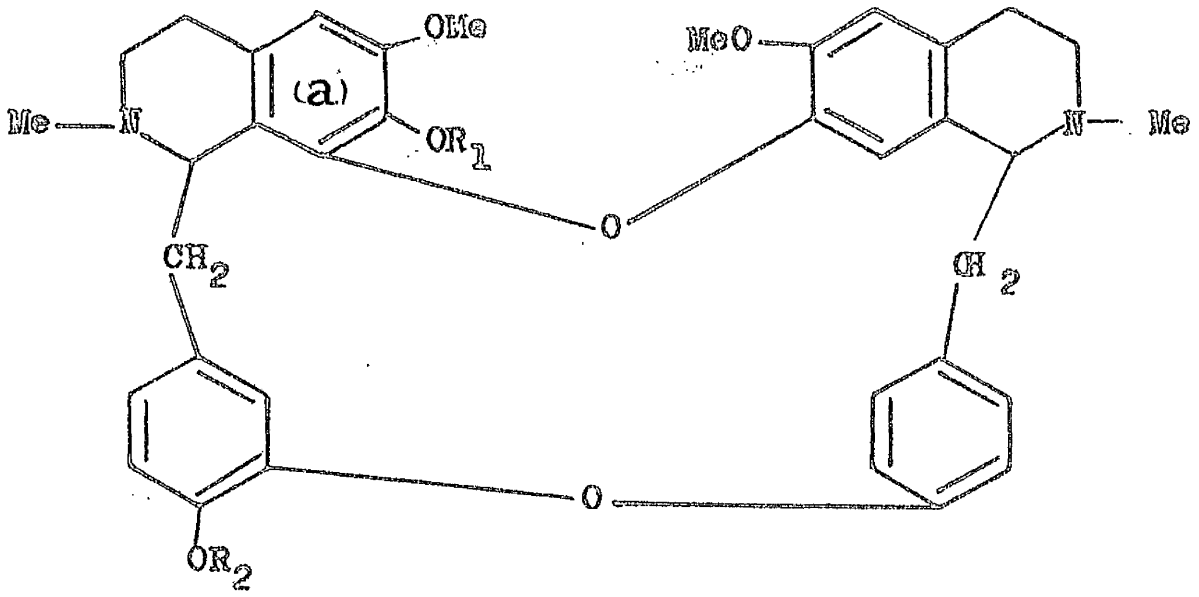


diaryl linkages have been studied in this way[125].

I <u>The Tetrandrine Group</u>

Alkaloids of the tetrandrine group have the general structure (CXIX). The structure of representative alkaloids of this group is shown in Table XIII.

Cepharanthine also belongs to this group but has a 6,7-methylenedioxy group in the tetrahydroisoquinoline (a), the remaining oxygen substituents being fully methylated.

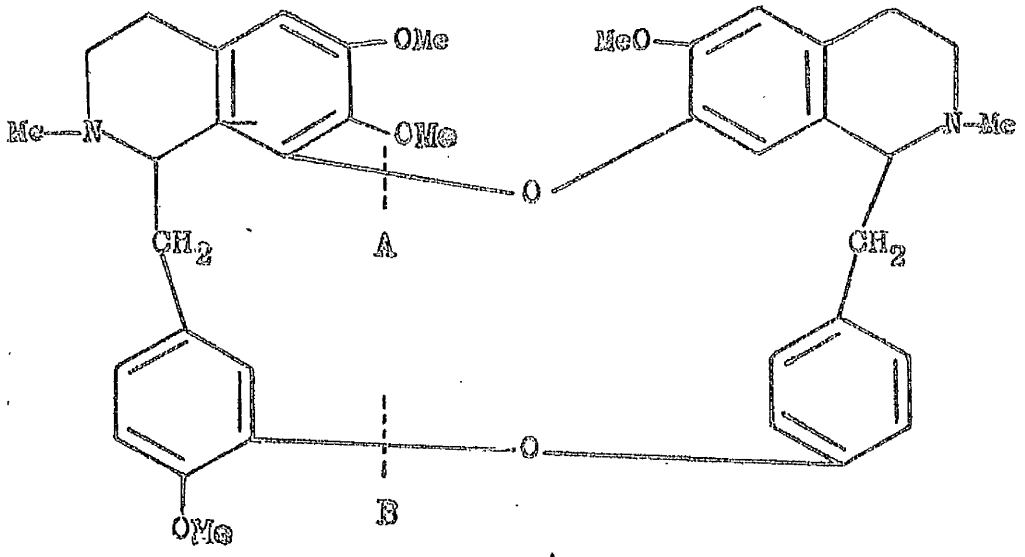


(CXIX)

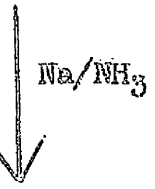
TABLE XLII

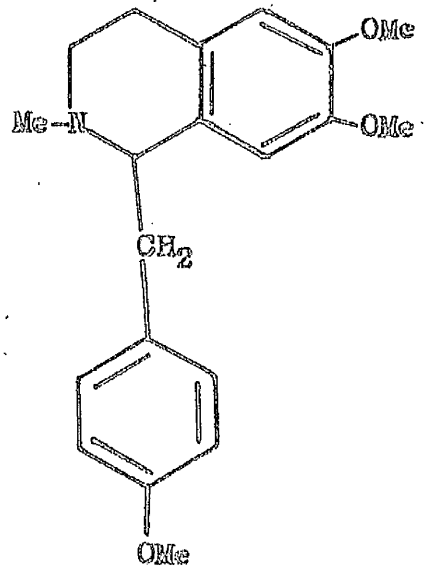
Structure (CXIX) R_1	R_2	Alkaloids of the tetrandrine group
Me	Me	isotetrandrine
Me	H	berbamine
Me	Me	tetrandrine
Me	Me	phaeanthine
H	Me	fangchinoline

With the exception of fangchinoline, berbamine and cepharanthine, alkaloids of this group yield only two products, N-methyl-coclaurine (CXX) and O-methylarmepavine (CXXI) thus showing that fission occurred exclusively at A and B in formula (CXXII).

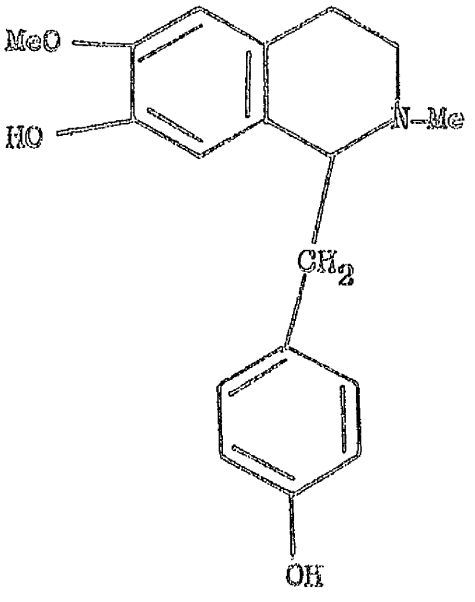


(CXXII)



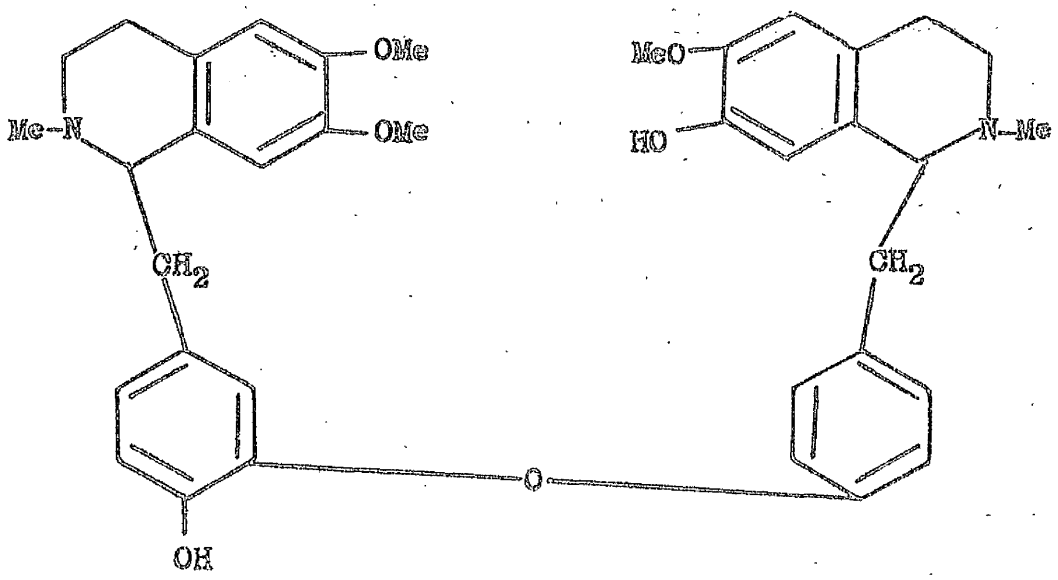


(CXXI)



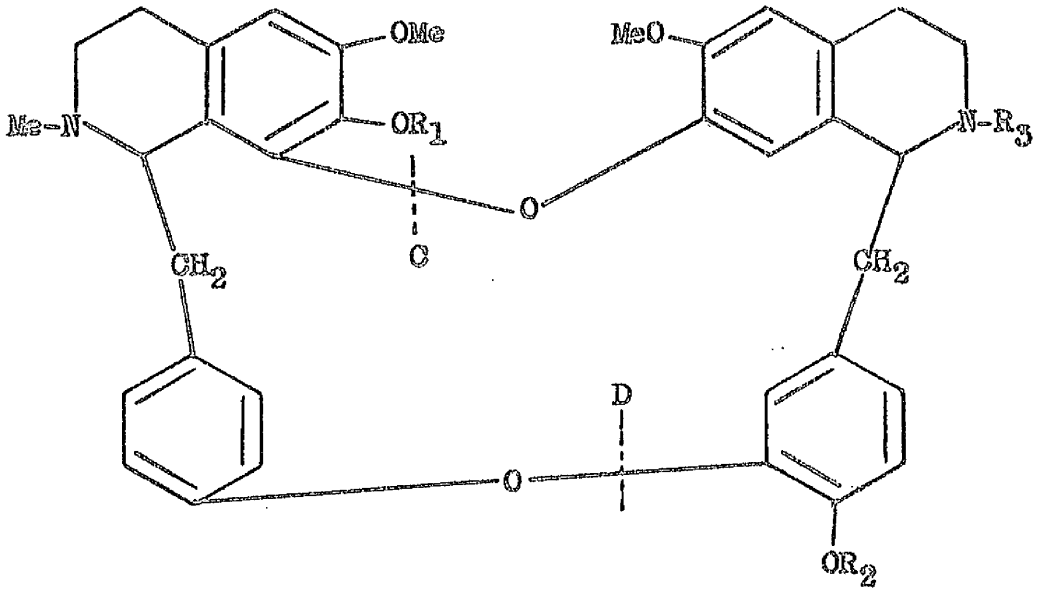
(CXX)

Alkaloids containing free phenolic hydroxyl groups, like the simpler compounds (*vide supra*), are more difficult to cleave because of phenoxide formation. Thus fangchinoline (CXIX, R_1=H, R_2=Me) is unaffected by metal/ammonia reagents and in the berbamine (CXIX, R_1=Me, R_2=H) one diphenyl ether linkage is protected by the adjacent free phenolic group so that fission with sodium and liquid ammonia produces a base of the dauricine type (CXXIII). O-Methylberbamine is cleaved into the two expected benzylisoquinoline fragments.



(CXXIII)

II The Oxyacanthine Group.



(CXXIV)

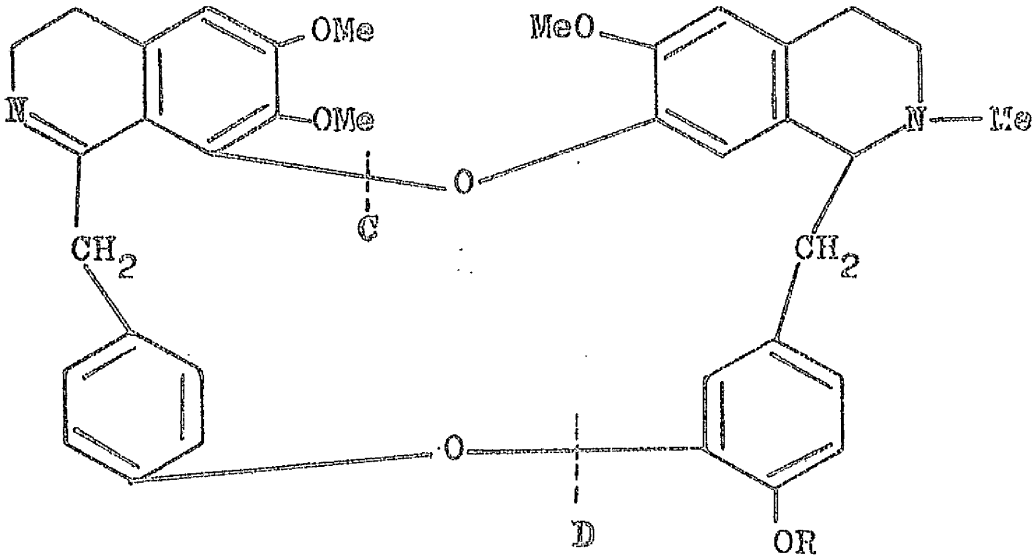
As shown in the general structure (CXXIV) there is a close relationship between the alkaloids of the oxyacanthine group and those of the tetrandrine group involving primarily a change in the position of the ether linkage connecting the benzyl moieties of the two coclaurine units.

Alkaloids of this group are listed in Table XIV. This group also includes the closely related epistephanine (CXXV, R=H) and hypoepistephanine (CXXV, R=Me).

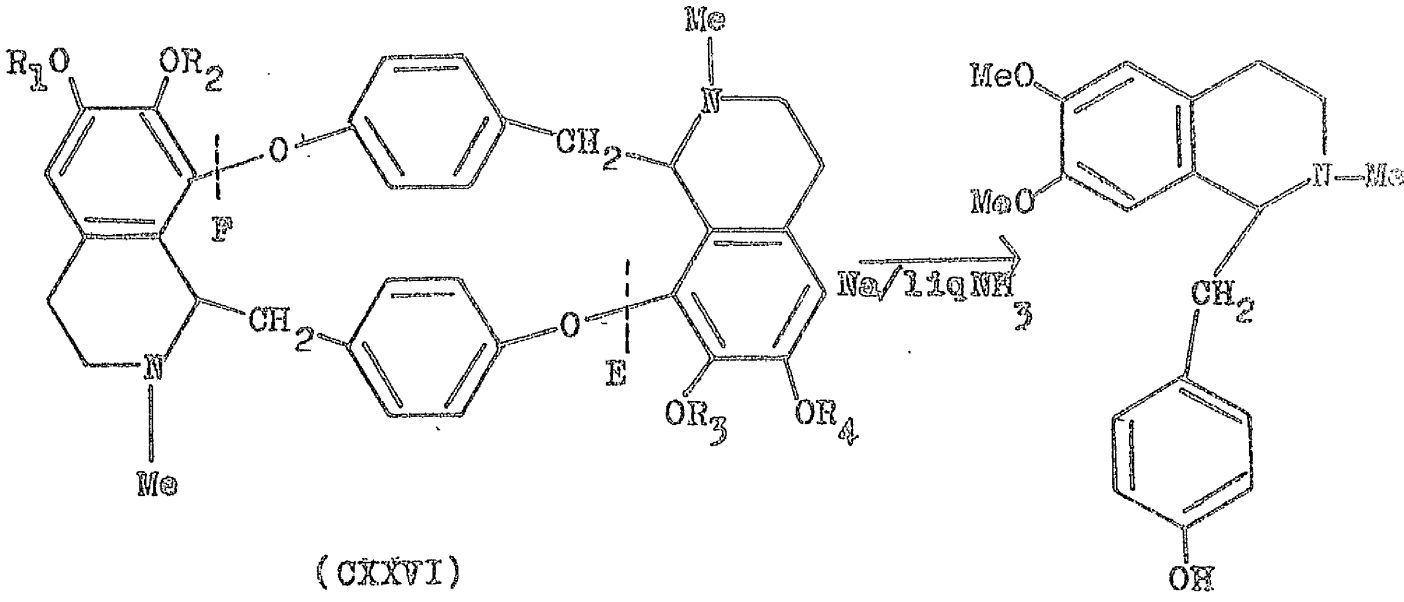
Fission of the fully methoxylated oxyacanthine alkaloids (CXXIV) with sodium and liquid ammonia results, as might be predicted, in cleavage exclusively at C and D (CXXIV).

TABLE XIV

Structure (CXXIV) R_1	R_2	R_3	Alkaloids of the oxyacanthine group
Me	H	Me	oxyacanthine
Me	H	Me	repandine
H	H	Me	armoline
H	H	H	daphnoline
H	Me	H	daphnandrine



(CXXV)



(CXXVI)

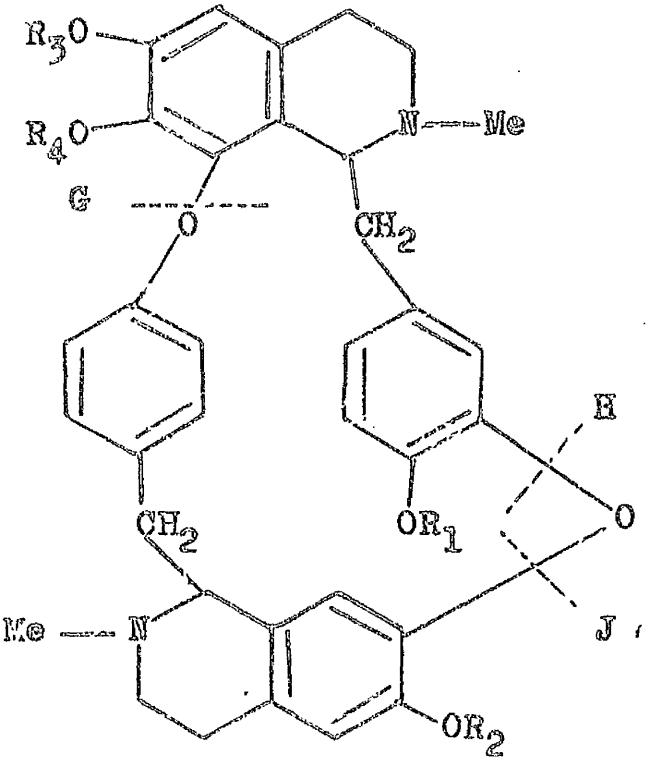
III. The Isochondodendrine Group.

This group includes the alkaloids cycleanine (CXXVI, $R_1=R_2=R_3=R_4=Me$), protocuridine (CXXVI, $R_2=R_4=H$, $R_1=R_3=Me$), isochondodendrine (CXXVI, $R_2=R_3=H$, $R_1=R_4=Me$) and isoneo-protocuridine (CXXVI, $R_1=R_4=H$, $R_2=R_3=Me$).

The cleavage of the fully methylated cycleanine with sodium and liquid ammonia produced armepavine (CXXVIA) as the sole product consistent with fission having occurred at E and F (CXXVI). Methylated isochondodendrine (CXXVI, $R_1=R_2=R_3=R_4=Me$) the only other alkaloid of this group to have been examined on similar treatment yielded the same product.

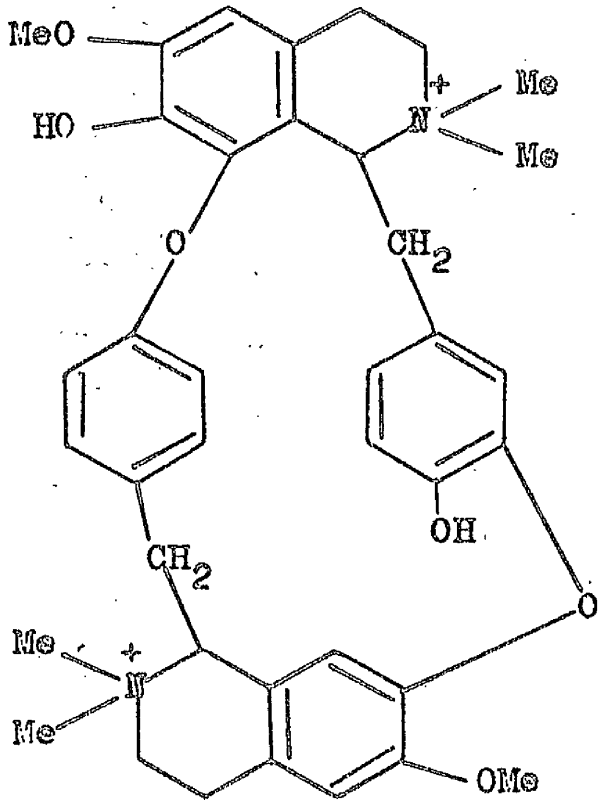
IV. The Curine Group.

Representative alkaloids of this group are



(CXXVII)

curine (CXXVII, $R_1=R_4=H$, $R_2=R_3=Me$), chondocurine (CXXVII, $R_2=R_4=$ Me, $R_1=R_3=H$) and the quaternary derivative tubocurarine (CXXVIII).



(CXXVIII)

O,O-Dimethyl-(-)-curine and O,O-dimethyl-(+)-chondocurine[125] with sodium and ammonia gave O-methylarmepavine (CXXI) as the non-phenolic product and as the phenolic product N-methyl-coclaurine (CXX) as a result of fission at G and H in structure (CXXVI) respectively. These observations are in accordance with an empirical rule formulated by Tomita[133], which stated that cleavage of bisbenzylisoquinoline alkaloids always yields

bases of the coclaurine type. That this should be so in O,O-dimethylcurine (CXXVII, $R_1=R_2=R_3=R_4=Me$) and O,O-dimethylchondocurine (CXXVII, $R_1=R_2=R_3=R_4=Me$) is not immediately obvious since the known effect of ortho-methoxyl substituents OR_1 and OR_2 should make cleavage at H and J equally probable.

Reductive fission of O,O-dimethyl-(+)-tubocurine.

In the present work fission of a benzene-toluene solution of O,O-dimethyl-(+)-tubocurine (CXXVII, $R_1=R_2=R_3=R_4=Me$) with sodium-liquid ammonia and separation into non-phenolic and phenolic portions afforded brown syrups, neither of which could be induced to crystallise.

Thin-layer chromatography showed the presence of two constituents in the non-phenolic fraction and three in the phenolic fraction, a result which is at variance in general with the empirical rule of Tomita and in particular with the results of Kidd and Walker[125] (vide supra) from sodium-ammonia cleavage of O,O-dimethyl-(-)-curine and O,O-dimethyl-(+)-chondocurine in benzene solution.

Since O,O-dimethyltubocurine and O,O-dimethylchondocurine are identical and further since both these substances differ from O,O-dimethylcurine only in the arrangement about the optical centres, O,O-dimethyltubocurine would have been expected a priori to yield solely (+)-O-methylarmepavine and (-)-N-methylcoclaurine as the respective non-phenolic and phenolic products of fission.

Criticism could perhaps be levelled at Bick and Clezy[99] on their cleavage with sodium and liquid ammonia of O,O-dimethyl-(-)-curine and O,O-dimethyl-(+)-chondocurine which

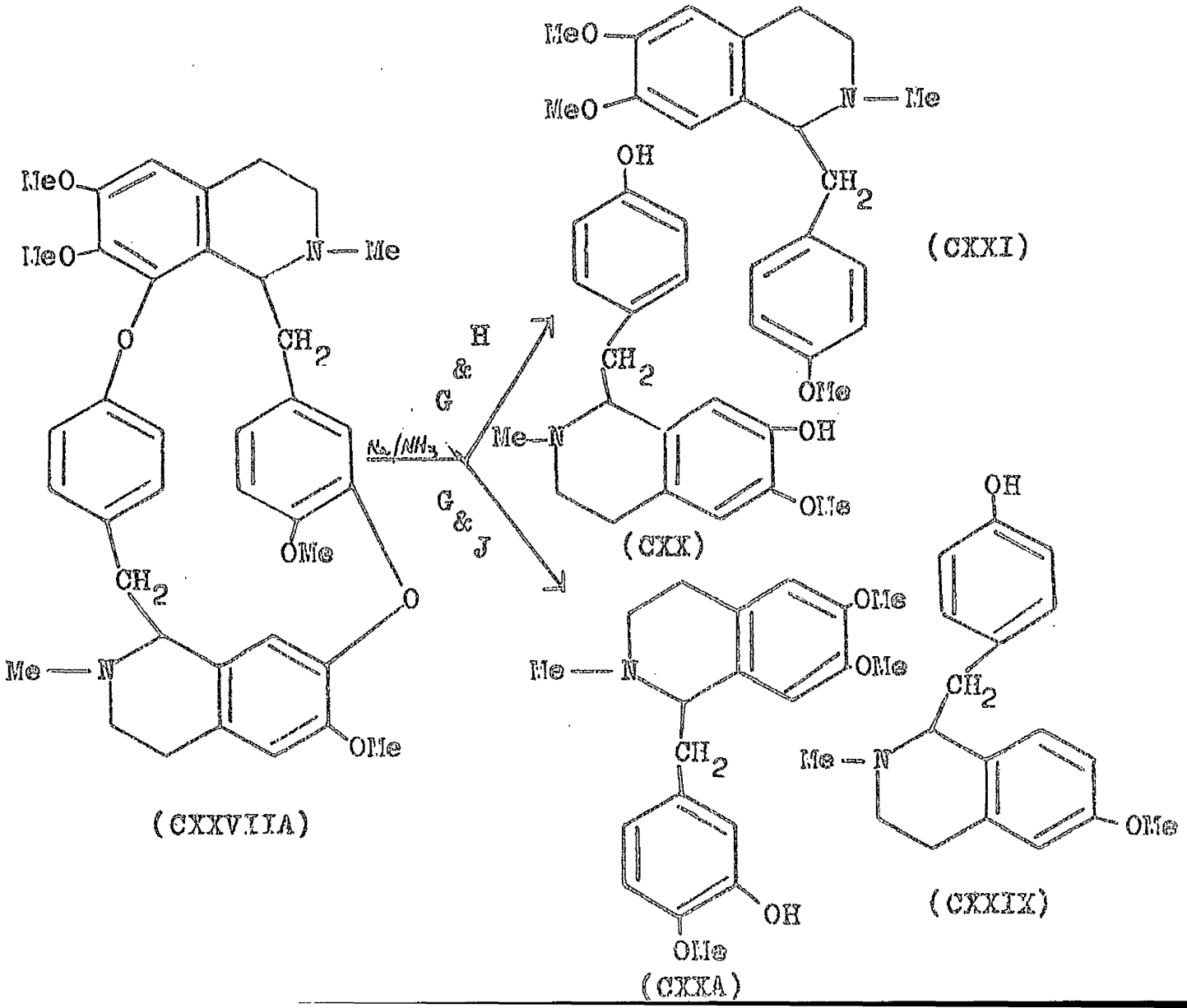
were prepared by methylation of curine and chondocurine respectively with diazomethane producing in both cases oils, which could not be crystallised and which were not examined for purity. Further of the cleavage products only crystalline material was reported and the remainder was not examined. Again there was no report of any unreacted starting material which should be present as the total weight of phenolic and non-phenolic methiodides was less than the weight of the O,O-dimethylcurine initially used. Finally, no mention is made of the yield of phenolic material from the reductive cleavage of O,O-dimethylchondocurine.

Similar criticism is not applicable to Kidd and Walker[125] who showed their starting materials to be chromatographically homogeneous by paper-chromatography. They were also cognisant of the fact that inhomogeneity of cleavage could also occur. Thus, on careful examination of the reaction products from the cleavage of O,O-dimethylcurine in benzene-toluene, they noted the presence in the non-phenolic fraction of unreacted starting materials, and reported the phenolic fraction to be homogeneous by paper chromatography.

In a similar reductive cleavage of phaeanthine (CXIX, R_1=R_2=M in toluene-benzene both the non-phenolic fraction, a non-crystalline brown syrup, and the phenolic fraction which on recrystallisation from benzene gave N-methylcoclaurine, were proved by paper-chromatography to be homogeneous.

The phenolic portion which was obtained by the fission of O,O-dimethyltubocurine (CXXVIIA) was chromato-graphed on alumina using benzene and 1% methanol in benzene as

eluants. A fraction was isolated having properties agreeing closely with those of laudanidine (CXXA); N-methylcoclaurine (CXX) was also isolated with difficulty and identified by comparison with authentic material. The third phenolic compound was not obtained crystalline but by deduction could only be the complementary component to laudanidine, 1-(4-hydroxybenzyl)-2-methyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline (CXXIX). The yields were very low possibly due to adsorption on the column. A further difficulty in effecting separation was elution of the second component before the previous one has been completely removed from the column.



O,O-dimethyl-(+)-tubocurine along with (-)-O-methylarmepavine. Attempts to separate the two components by column chromatography resulted in isolation of only the O,O-dimethyltubocurine, the O-methylarmepavine being always contaminated with O,O-dimethyltubocurine.

Separation was then attempted on thick thin-layer chromatography using 10% methanol in chloroform as the developing solvent and plating out the non-phenolic syrup heavily. Trailing of the upper component interfered with separation of the component with R_F=0.38 which was identified as O-methylarmepavine by comparison on thin-layer chromatography with the non-phenolic fraction obtained from the sodium and liquid ammonia cleavage of phaeanthine.

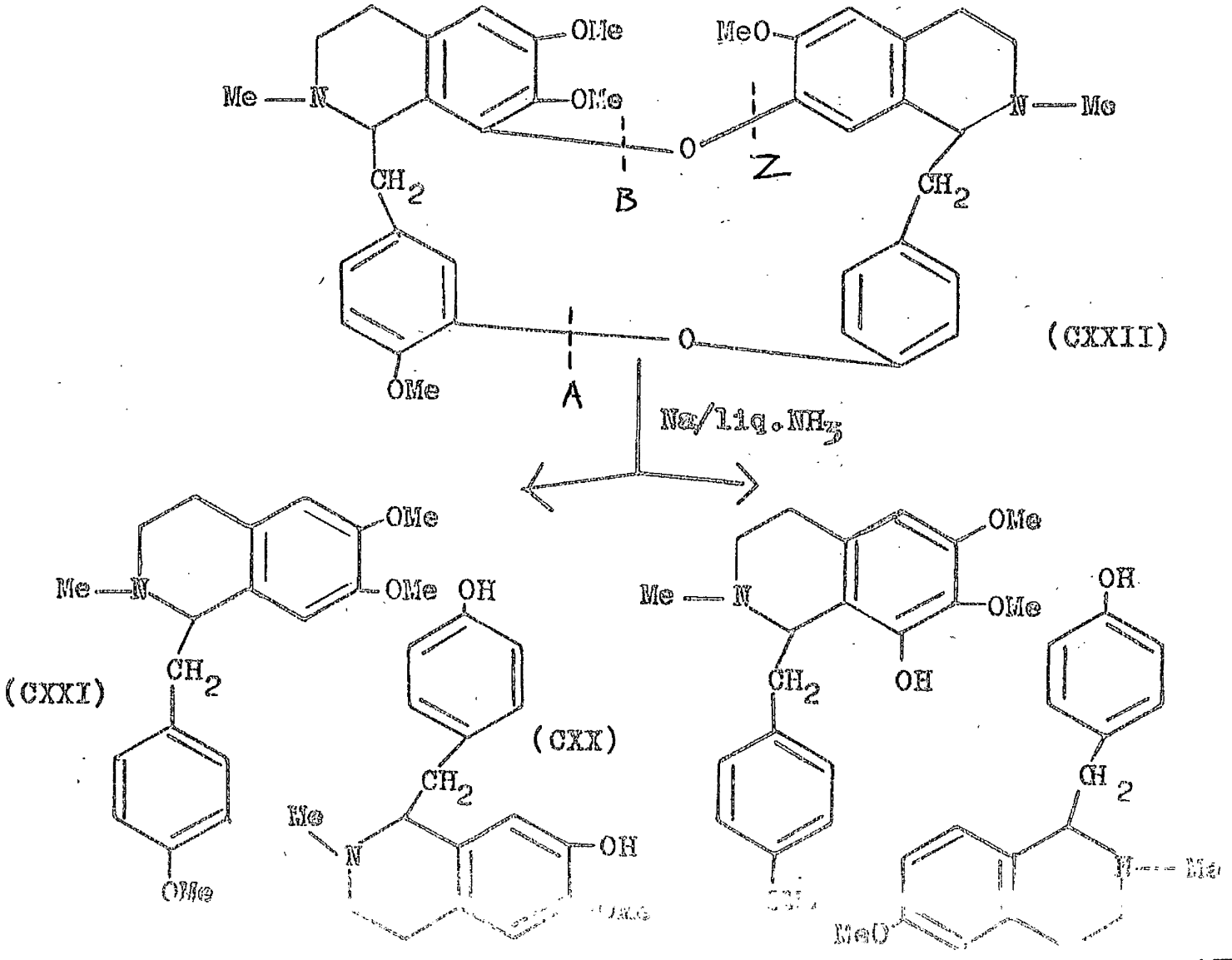
Evidence for the non-homogeneous fission (i.e. fission at G and at both H and J)(CXXVII) is provided by Kidd and Walker[125]. When O,O-dimethyl-(-)-curine was similarly reduced in dioxan solution they obtained one non-phenolic product which on treatment with methyl iodide gave crystalline (-)-O-methylarmepavine methiodide. Paper chromatography of the phenolic products showed two compounds which were successfully separated by fractional crystallisation and column chromatography and identified as (-)-N-methylcoclaurine (CXX) and (-)-laudanidine (CXXX, R_1=H, R_2=OH). No trace of the phenolic material 1-(4-hydroxybenzyl)-2-methyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline (CXXIX) complementary to laudanidine was observed.

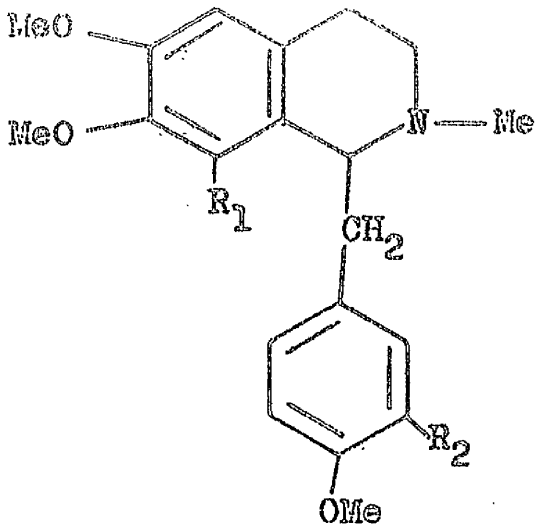
Cleavage of phaeanthine by sodium and liquid ammonia.

Phaeanthine was subjected to cleavage by sodium and liquid ammonia to obtain O-methylarmepavine which it was intended to

use to authenticate O-methylarmepavine obtained from the cleavage of O,O-dimethyltubocurine.

Kidd and Walker cleaved phaeanthine (CXXII) in dioxan with sodium and liquid ammonia and obtained (-)-O-methylarmepavine in crystalline condition. The phenolic fraction afforded two compounds one of which was (-)-N-methylcoclaurine (CXX). The second phenolic fragment could not be positively identified though it was proved to be neither corpaverine (CXXX, R_1=OH, R_2=H) nor laudanosine (CXXX, R_1=H, R_2=OH) and by exclusion could only have been the dihydric phenol 1-(3-hydroxy-4-methoxybenzyl)-2-methyl-6,7-dimethoxy-8-hydroxy-1,2,3,4-tetrahydroisoquinoline (CXXX, R_1=R_2=OH).





(CXXX)

In the present work phaeanthine was reduced in toluene-benzene by sodium and liquid ammonia. Two non-phenolic materials and three phenolic substances were observed on thin-layer chromatography of the products, the intensity of two spots of the phenolic components being very much less than that of the third spot. The non-phenolic material was identified by thin-layer chromatography as consisting of starting material as shown by comparison chromatographically with an authentic sample of phaeanthine and by exclusion O-methylarmepavine (CXXI) although satisfactory separation could not be achieved on a quantitative scale.

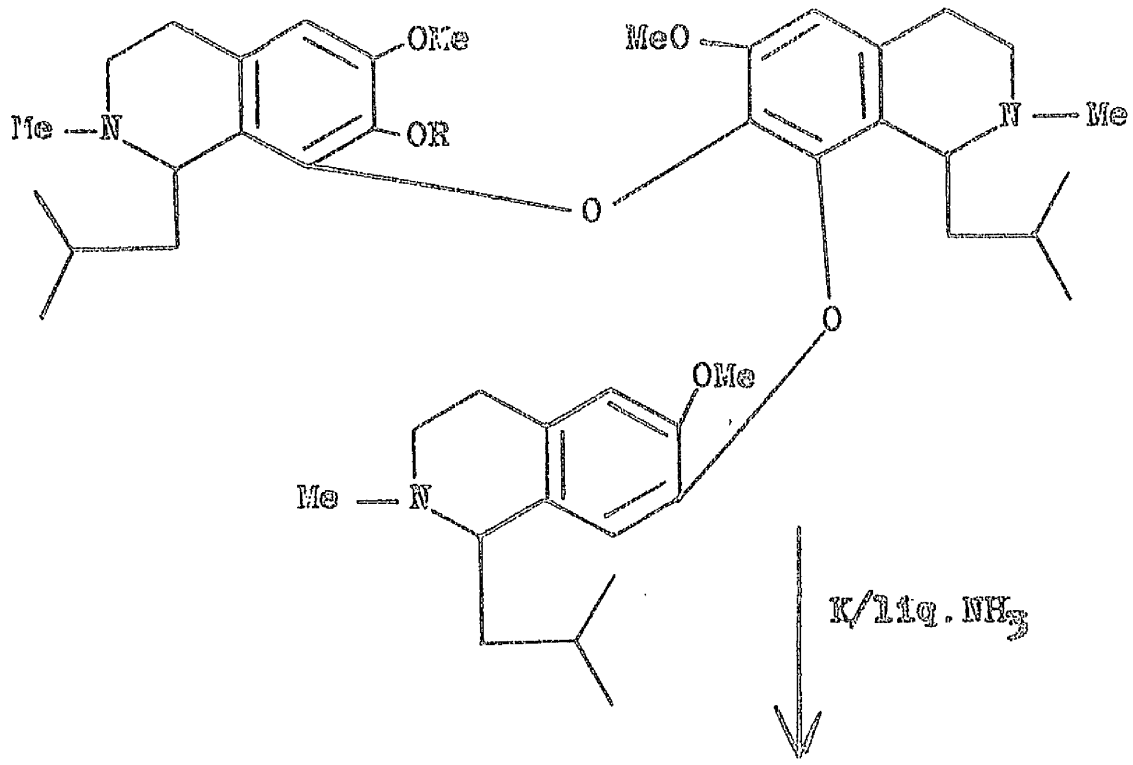
The phenolic portion, dissolved in benzene, on standing deposited crystals identified as N-methylcoclaurine (CXX). This material was then used to positively identify the N-methylcoclaurine (CXX) obtained from fission of O,O-dimethyl-(+)-tubocurine (CXXVII). The experiment also proved that one ether link in phaeanthine underwent non-homogeneous cleavage (i.e. fission at A, B and A, Z (CXXII)) in agreement with Sowa[119-121] that the meta-methoxyl has no effect on the point of cleavage (see Table XI) and contrary to the results of Tomita[130] and Kidd and Walker[125].

When O,O-dimethylisochondodendrine (cycleanine) (CXXVI, $R_1=R_2=R_3=R_4=Me$) was reduced by Kidd and Walker[125] in dioxan solution with sodium and liquid ammonia the product, in agreement with Fujita and Murai[126] was essentially (-)-armepavine (CXXVI A).

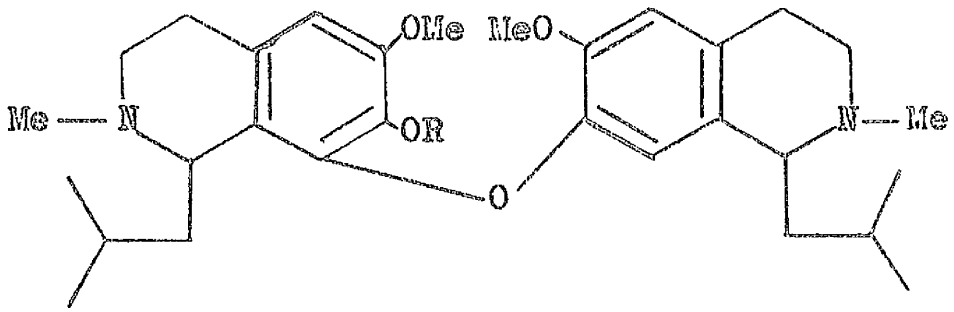
Evidence of two further substances was also reported as shown by two spots on paper chromatograms. However, no satisfactory Gibb's reaction was observed, although the product of any other mode of fission than that giving (-)-armepavine would have been unsubstituted in the para-position to a phenolic hydroxyl group.

Why, therefore, we should obtain similar results when the tertiary base is dissolved in benzene-toluene to the results obtained by others using dioxan as solvent is at present not obvious.

Further proof that diaryl ether links split on both sides of the oxygen has recently been obtained by Djerassi[127] and his coworkers and by Bessho[128]. It was shown that several fractions resulted from the potassium-liquid ammonia cleavage of O-methylpilocereine (CXXXI, R=Me). The substitution of potassium for sodium was shown to have no other effect on the reduction than that of making the reaction more vigorous. Cleavage of the ethyl ether (CXXXI, R=Et) produced isopilocereine (CXXXII) and the fragments a, b, c and d (CXXXIII).

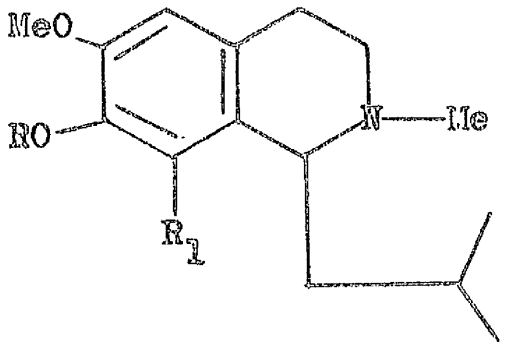


(CXXXI)



+

(CXXXII)



(CXXXIII)

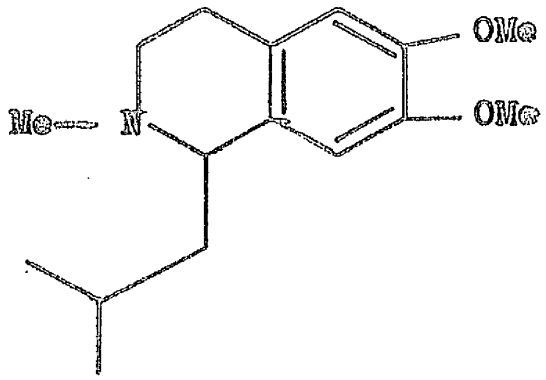
a $R=R_1=H$

b $R=OEt$, $R_1=H$

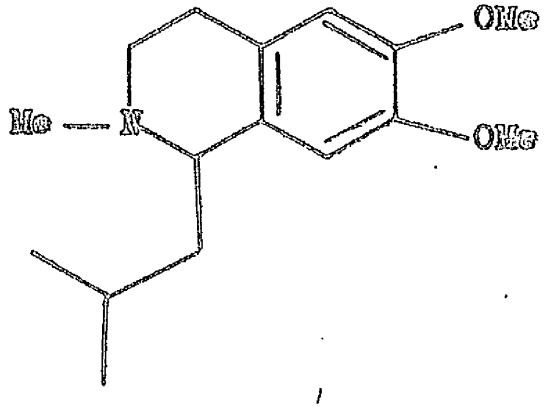
c $R=OEt$, $R_1=OH$

d $R=H$, $R_1=OH$

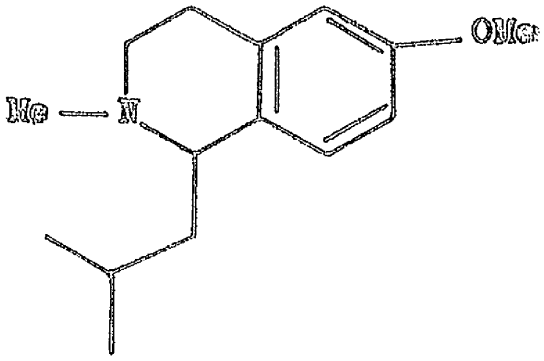
Djerassi[127] also cleaved isopilocereine (CXXXII) in ether with sodium and liquid ammonia and found that the ether link cleaved on both sides of the oxygen atom resulting in three compounds, 1-isobutyl-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (CXXXIV), 1-isobutyl-2-methyl-6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline (CXXXV), and 1-isobutyl-2-methyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline (CXXXVI). No mention is made, however, of 1-isobutyl-2-methyl-6,7-dimethoxy-8-hydroxy-1,2,3,4-tetrahydroisoquinoline (CXXXVII), the product complementary to (CXXXVI).



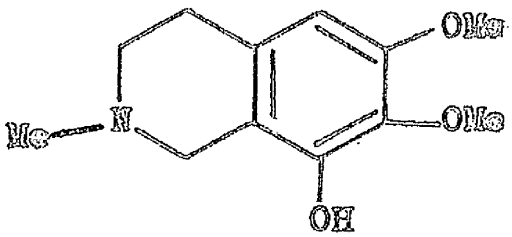
(CXXXIV)



(CXXXV)



(CXXXVI)

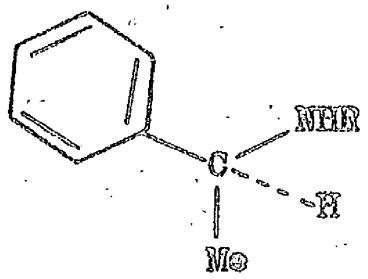


(CXXXVII)

Optical Rotation Studies.

Leithe[100] in 1934 observed that the molecular rotation of 1,2,3,4-tetrahydroisoquinoline bases and α-phenyl-benzylamines (CXXXVIII) were affected by the polarity of the solvent in which the observations were made and further that for a series of solvents of increasing polarity the optical rotations of the bases having the same absolute configuration were shifted in the same direction. Thus a convenient method for the establishment of the absolute configuration of related compounds of unknown stereochemistry was introduced.

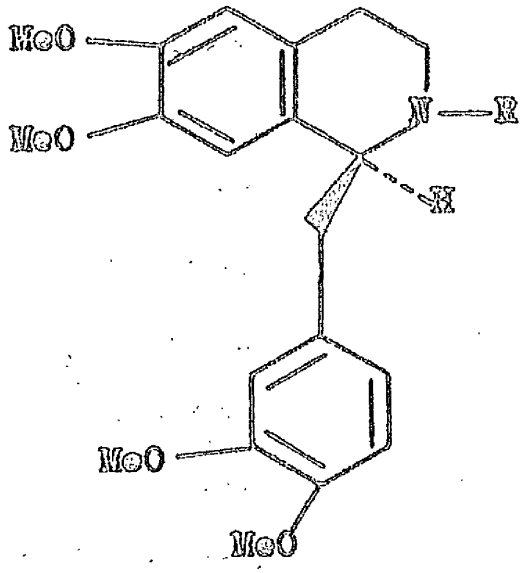
The absolute configuration of α-methylbenzylamine (CXXXVIII, R=H) and (-)-N-ethyl-α-methylbenzylamine (CXXXVIII, R=Et) were established by chemical means and from optical measurements it was found that both showed a marked positive shift in the direction of rotation as solvent polarity was increased.



(CXXXVIII)

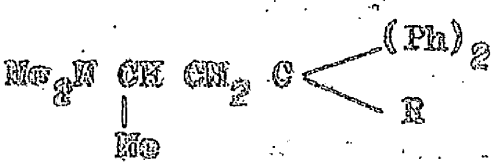
(-)-Tetrahydropapaverine (CXXXIX, R=H) and (+)-laudanosine (CXXXIX, R=Me) also exhibited the same direction of shift and their absolute configuration was shown by Corrodi and Hardegger[101] to correspond with the absolute configuration of (-)-α-phenylethylamine (CXXXVIII, R=H). Thus a positive shift in rotation brought about by increasing the polarity of the solvent was taken as a strong indication that the base in hand had the absolute stereochemistry shown (D) and a negative shift

was taken as evidence for the antipodal structure (L).

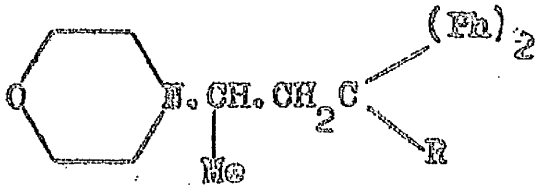


(CXXXIX)

The determination of conformation by this method was not so successful when applied to more complex structures such as the morphine-codeine bases and Beckett and Casy[129] have questioned the validity of assigning the configurations of certain molecules on the basis of this method. From optical rotation measurements (Table XV) on compounds of the methadone (CL) and phenadoxone (CLI) series they showed the dissimilarity in rotational change with polarity of solvent which occurred in these compounds, in which only the substituents of the carbon atom β to the asymmetric centre differed. Opposite effects occur for compounds of not too dissimilar group types, e.g. ketone-amide and ketone-ester (Table XV).



(CL)



(CLI)

However, it would seem reasonable to use dependence of molecular rotation on polarity of solvent (using one wavelength) in relating configurations of closely related compounds of type (CL) and (CLI) in which only a basic group is altered, provided a number of corresponding members of each series are available, configurational identity within each series is established and a consistency of patterns observed.

To prove the usefulness of the method, Battersby and Edwards[102] obtained the results shown in Table XVI and deduced, as did Beckett and Casy[129], that the method was feasible if kept to the following limitations: (1) a number of corresponding members of each series are available; (2) configurational identity within each series is established;

Table XV. Molecular Rotations at 19° ± 1° (c in parenthesis).

R	(−)-Methadone (CL) series in C_6H_{12}	C_6H_6	EtOH	H_2O	Direction of Displacement	(−)-Phenaźzone (CLI) series in C_6H_{12}	C_6H_6	EtOH	H_2O	Direction of Displacement
COEt	−106	−74.2	−80.4	−386	⟶	+247	+179	+245	+345	⟶
	(1.2)	(1.3)	(1.5)	(1.5)	−	(0.8)	(0.8)	(0.8)	(0.8)	+
CN	−181	−165	−142	+16.7	⟶	+183	+154	+211	−48	⟶
	(0.9)	(0.8)	(0.8)	(5.0)	+	(1.0)	(1.0)	(1.0)	(1.0)	−
H	−168	−127	+30.4	+153	⟶	+145	+123	+54.6	−96.6	⟶
	(4.8)	(4.7)	(3.4)	(1.0)	+	(1.0)	(1.0)	(1.0)	(1.0)	−
$CONH_2$	−497	−536	−332	−249	⟶	420	491	431	256	⟶
	(0.5)	(0.9)	(0.9)	(0.9)	+	(0.9)	(0.9)	(0.9)	(0.9)	−
CO_2Et	−278	−240	−163	−135	⟶	−	−	−	−	
	(2.0)	(1.8)	(1.8)	(1.0)	+					
SO_2Et	−	+41.4	0	−109	⟶	−	−	−	−	
		(2.0)	(2.0)	(5.0)	−					
OH	+99	+87.7	−73.5	−121	⟶	−	−	−	−	
	(0.8)	(1.9)	(0.8)	(1.0)	−					
NMe_2CHMe	+321	+309	+427	+614	⟶	−	−	−	−	
$Ph_2C{=}HC$	(1.0)	(1.0)	(1.0)	(0.9)	+					

(3) a consistency of rotational change pattern is observed. These views are confirmed by the independent information on the absolute stereochemistry of certain members of this series obtained by chemical degradation to compounds of known absolute stereochemistry

Table XVI Molecular Rotations in different solvents.

Compound	Solvent			
	C_6H_6	$CHCl_3$	EtOH	N-HCl
D-(-)-Salsolidine	-133°	-	-123°	-57°
D-(-)-N-Methylsalsolidine	-115°	-	-55°	+17°
D-(-)-Dihydro-N-methyl-salsolidinemethine	-244°	-	-175°	-20°
D-(+)-N-Ethylsalsolidine	+14°	+18°	+20°	+30°
D-(+)-Calycotamine	-	≈ 0°	+6°	+25°
D-(-)-Anhalonine	-	-124°	-	-108°
D-(-)-Lophophorine	-	-111°	-	-44°

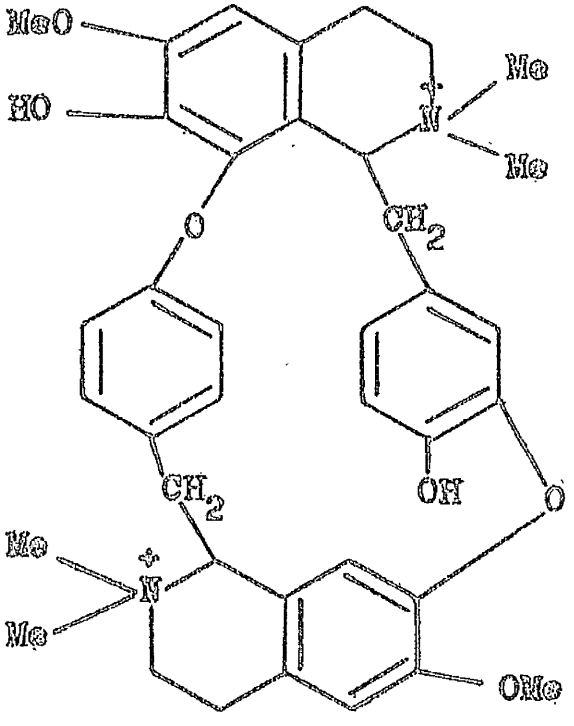
Optical Rotation measurements on the products from sodium/liquid ammonia cleavage of O,O-dimethyl-(+)-tubocurine.

Optical rotation measurements on laudanidine, obtained from fission of O,O-dimethyl-(+)-tubocurine, in benzene, chloroform and methanol, show a positive shift in rotation in solvents of increasing polarity (Table XVII) indicative of the D-configuration. N-methylcoclaurine from the same source on the other hand showed a negative shift when polarity of the solvent was increased indicating the L-configuration. These results correspond with the fact that centre A (CLII) has the D-configuration and that centre B (CLII) has the L-configuration.

TABLE XVII Specific Rotations in different solvents

Compound	Solvent		
	C_6H_6	$CHCl_3$	MeOH
Laudanidine	-111°	-98°	$+40^{\circ}$
N-Methylcoclaurine	-	$+73^{\circ}$	$+66^{\circ}$

The assignment of absolute stereochemistry to the two optical centres of O,O-dimethyl-(+)-tubocurine by cleavage to D-(-)-laudanidine and L-(+)-N-methylcoclaurine therefore provides independent experimental confirmation of the absolute stereochemistry assigned to the (+)-tubocurarine molecule by Tomita and Kunimoto[103], which therefore may be correctly represented as (CLII).



(CLII)

Preparation of Quaternary Halides from (+)-Tubocurarine and (-)-Curine.

The synthesis of O-alkylated derivatives of tubocurarine and its isomers has been reported and their potencies compared with the corresponding quaternary compounds . The availability of the naturally occurring tertiary base (+)-chondocurine has permitted the preparation of quaternary salts substituted on the N-atom with ethyl and benzyl groups instead of methyl. The relative potencies of these ethers derived from (+)-tubocurarine and its isomers are summarised in Table XVIII.

Table XVIII Relative potencies of ethers derived from tubocurarine and its isomers (rat diaphragm test).

Compound	Structure (CXIII)			Rotation of optical centre		Potency
	R_1	R_2	R_3	(a)	(b)	
(+)-Tubocurarine	Me	H	H	+	-	1.0
O, O-Dimethyl-(+)-tubocurarine	Me	Me	Me	+	-	8.7
O, O-Diethyl-(+)-tubocurarine	Me	Et	Et	+	-	1.9
O, O-Di-n-butyl-(+)-tubocurarine	Me	n-Bu	n-Bu	+	-	0.09
O, O-Dibenzyl-(+)-tubocurarine	Me	CH_2Ph	CH_2Ph	+	-	0.07
(+)-Curarine	Me	H	H	+	+	3.5
O, O-Dimethyl-(+)-curarine	Me	Me	Me	+	+	-
(-)-Curarine	Me	H	H	-	-	1.3
O, O-Dimethyl-(-)-curarine	Me	Me	Me	-	-	3.3

(Methylbisisoquinolinium)

Compound	Structure (CLIII) R_1	R_2	R_3	Rotation of optical centre (a)	(b)	Potency
(+)-Chondocurarine	H	Me	H	+	-	4.3
N,N-Diethylchondocurine	H	Me	H	+	-	0.5
N,N-Dibenzylchondocurine	H	Me	H	+	-	0.12

The position of methoxyl substituents appears significant since (+)-chondocurarine is three times as potent as (+)-tubocurarine. Similarly, the number of methoxyl substituents appears to influence activity as is demonstrated by a comparison of the potencies of O,O-dimethyl-(+)-tubocurarine and (+)-tubocurarine and O,O-dimethyl-(-)-curarine and (-)-curarine.

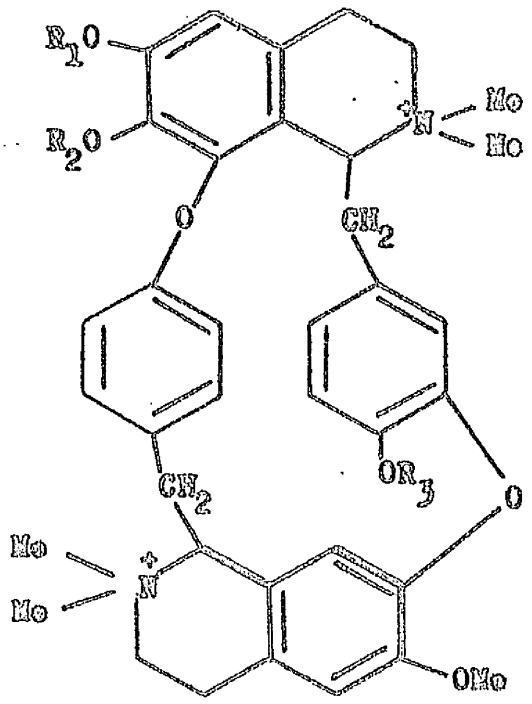
Kalow[96] has demonstrated a reduction in the potency of (+) tubocurarine as pH increased and Cavallito[93] has therefore suggested that the lower activity of (+)-tubocurarine as compared with its dimethyl ether may be ascribed to zwitterion formation in the former arising from the presence of the phenolic hydroxyl group in the molecule; this however fails to explain the low potency of the di-n-butyl and dibenzyl ethers of (+)-tubocurarine.

The effect of variation of onium group substitution has only been investigated in (+)-chondocurine and this study has been limited to the ethyl and benzyl quaternaries. The N-ethyl derivative is equipotent and the benzyl one fifth as potent as (+)-tubocurarine.

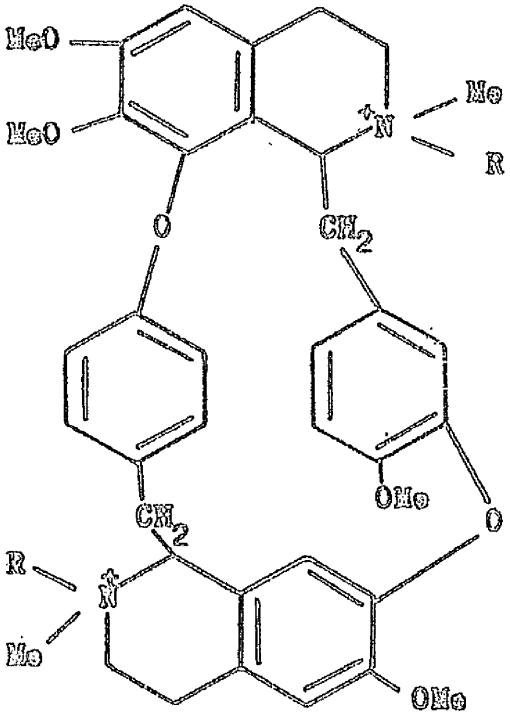
The effect of variation of onium group substitution has only been investigated in (+)-chondocurine and this study has been limited to the ethyl and benzyl quaternaries, the N-ethyl derivative being equipotent and the benzyl one fifth as potent as (+)-tubocurarine. Alkylation changes in decamethonium and acetylcholine result in a change in the mode of action. However, the number of compounds related to the bisbenzyltetrahydroisoquinoline series investigated is too small to attempt any rationalisation. No attempt has yet been made to study the combination of onium substitution and O-alkylation nor to study the effects of these two modifications divorced from each other. Towards this end a series of quaternaries derived from (+)-tubocurine, O,O-dimethyl-(+)-tubocurine, (-)-curine and O,O-dimethyl-(-)-curine were prepared.

Preliminary syntheses which involved heating the tertiary base with the required alkyl halide at 65°, using as solvent the alkyl halide, for 1½ - 3 hrs. resulted in the production of quaternary halides hygroscopic in nature and in very low yield except in the case of alkyl iodides which rapidly formed the appropriate quaternary.

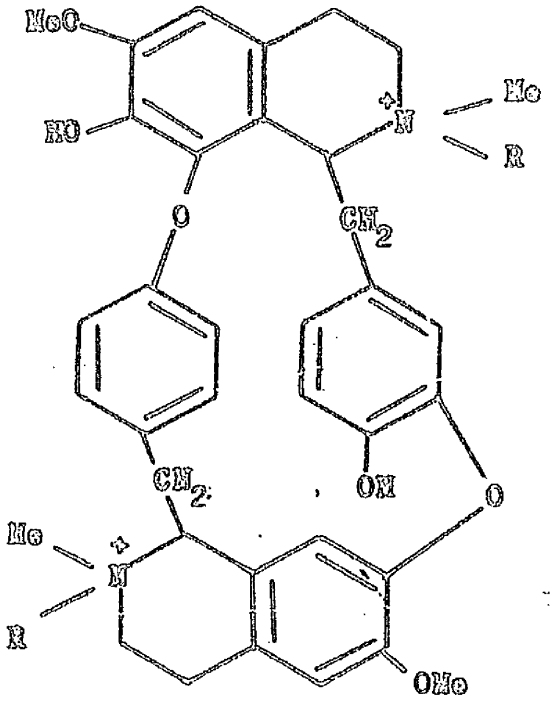
Preparation of the four series of quaternary halides from the above tertiary bases was accomplished by heating the tertiary base at 65° for 2 - 4 days with the requisite alkyl halide as solvent. The resultant quaternary halide was filtered off and washed copiously with ether to remove any unreacted tertiary base



(CLIII)



(CLIV)



(CLV)

EXPERIMENTAL

EXPERIMENTAL

Ultra-violet spectra were recorded on an Optica C.F. 4.

Infra-Red spectra were recorded on a Perkin Elmer Infracord 327 and Infracord 237.

Thin Layer Chromatography.

Thin layer chromatography was used to identify and to detect the products of the sodium and liquid ammonia fission of biscoclaurine alkaloids.

The plates were coated with aluminium oxide (Camag) (0.33 mm.) using a Camag spreader. The quantity of alumina was 10 g. per plate slurried with 10 ml. of water. After spreading, the plates were allowed to dry for 20 min. in the atmosphere before activation by heating for 8-10 hr. at 150°-160°.

The developing solvent was 10% methanol in chloroform both solvents being dried. The developing tank (23 cm. high, 11.5 cm. diameter) was lined with Whatman® No. 1 paper and the solvent added till a depth of 0.5 cm. was attained and the tank then sealed and allowed to equilibrate. The ascent of the solvent was limited by a trough drawn across the plate 10 cm. from the spot of the material which in turn is 1 cm. from the base of the plate. The time taken for the solvent to reach the 10 cm. mark was 30-35 minutes.

The plate on removal from the developing tank was dried in the atmosphere and sprayed with a modified Dragendorff's Reagent* the spots appearing as a bright orange.

* Dragendorff's Reagent:- Bismuth oxynitrate (0.85 g.) was dissolved in water (10 ml.) and glacial acetic acid (10 ml.).

potassium iodide (25 g.) in water (50 ml.) was added and the mixture shaken. An aliquot (5 ml.) of this solution was added to glacial acetic acid (10 ml.) and made up to 50 mls. with water.

Alumina Columns.

The alumina used was Woehlm neutral alumina (Activity Grade 1).

Liquid Ammonia was obtained commercially in high pressure cylinders and in most cases used directly without further purification. Redistillation where indicated was effected by allowing the liquid ammonia to evaporate at room temperature and recondensing in U-tubes placed in a mixture of solid carbon dioxide and acetone.

(+)-Tubocurarine chloride was obtained from Burroughs Wellcome and used without further purification.

Phaeanthine was obtained commercially m.p. 161° (literature gives 161°). On thin layer chromatography it showed as a single spot on spraying with Dragendorff's Reagent and was used without further purification.

Diazomethane was prepared from p-tolylsulphonylmethylnitrosamide (4.93 g.) dissolved in ether 70 ml.) followed by the addition of potassium hydroxide (0.8 g.) in 96% ethanol (25 ml.). A further 25 ml. of 96% ethanol was added to remove the precipitate which formed. The ethereal solution of diazomethane (50ml.) was distilled over and used when freshly prepared.

Melting points were taken on a hot-stage microscope and are uncorrected.

O,O-Dimethyltubocurarine iodide[16].

(+)-Tubocurarine chloride (5.28 g.) was dissolved in a mixture of ethanolic potassium hydroxide (55 ml., 0.5N) and methyl iodide (7 ml.) and heated for 2 hours at 70°. The solution was reduced to small bulk (ca 10 ml.), cooled and the resultant solid (6.7 g.) collected by filtration. The solid material was redissolved in water (200 ml.) and reprecipitated by the addition of potassium iodide (0.9 g.) yielding O,O-dimethyltubocurarine iodide (6.27 g.)

m.p. 266° $[\alpha]_D$ + 179° (c 1.3 in MeOH) Literature

m.p. 266° $[\alpha]_D$ + 172° (c 1.02 in MeOH)[16].

O, O-Dimethyltubocurine from O,O-dimethyltubocurarine iodide.

1. Reduction with lithium aluminium hydride.[105]

Lithium aluminium hydride (2.087 g.) in tetrahydrofuran (75 ml.) was refluxed for 1 hour and O,O-dimethyltubocurarine iodide (5.27 g.) was added in powder form. The mixture was refluxed with continuous stirring until the evolution of methane ceased (ca 1 hour). The reaction mixture was cooled in an ice-bath and the excess of lithium aluminium hydride decomposed by the addition of water (9 ml.) and 20% sodium hydroxide (1.5 ml.) and the solution filtered. The filtrate was acidified with hydrochloric acid, reduced to small volume (ca 15 ml.) and the tertiary amine liberated by addition of sodium hydroxide and extracted with ether (3 x 50 ml.). The combined extracts were dried over Na_2SO_4, filtered and evaporated to a brown syrup (98 mg.) which on purification by elution on an alumina column (12 g., 1 x 10 cm.) with

benzene, afforded a yellow material (80 mg.), m.p. 90°.

2. Dealkylation by ethanolamine[117].

O,O-Dimethyltubocurarine iodide (1.18 g.) in ethanolamine (60 ml.) was refluxed for 45 minutes, the solution cooled and an aqueous solution of potassium hydroxide (1.14 g. in 30 ml.) added. The alkaline solution was extracted with petroleum ether (b.p. 40 - 60°, total volume 1.5 L.). The combined extracts were dried (Na_2SO_4) and evaporated to dryness to yield O,O-dimethyltubocurine. (0.402 g., 58%), m.p. 98°.

$[\alpha]_D$ + 168° (c 1.25 in MeOH). R_F = 0.89

Ultra-violet λ max. 215 mμ and 286 mμ

Found: C, 72.4; H, 6.5; N, 4.49 %

$C_{38}H_{42}O_6N_2\frac{1}{2}H_2O$ requires C, 72.4; H, 6.8; N, 4.43 %

Preparation of (+)-tubocurine by dealkylation by ethanolamine.

Tubocurarine chloride (0.8 g.) in ethanolamine (10 ml.) was refluxed for 1 hour, the solution cooled and solid carbon dioxide added. The resultant precipitate was extracted with ether, dried (Na_2SO_4), evaporated to dryness and then dried in a vacuum pistol over phosphorus pentoxide at 62° to yield (+)-tubocurine (0.51 g., 84%), m.p. 164°.

$[\alpha]_D^{17}$ + 153° (c 1 in CH_3OH). $[\alpha]_D^{17}$ + 144 (c 0.5 in 0.1N HCl) $[\alpha]_D^{17}$ + 72° (c 0.67 in pyridine)

Found: N, 4.9% Equivalent (titration) 298

$C_{36}H_{38}N_2O_6$ requires N, 4.7% Equivalent (titration) 297.5

O,O-Dimethyltubocurine from (+)-tubocurine.

Tubocurine (0.153 g.) was dissolved in ether (15 ml.) and methanol (5 ml.) and to the solution was added an

[illegible] diazomethane (21 ml. 0.5 g. of nitroso-

methylurea) and the mixture was left standing 3 days at room temperature. A similar quantity of ethereal diazomethane was added and the solution left for a further 3 days. The excess of diazomethane was evaporated to leave a yellow powder which showed two components on thin layer chromatography.

Separation of the two components was achieved by chromatography on a column of alumina (12 g., 1 cm. x 10 cm.) in petroleum ether (b.p. 40 - 60°) and using the same solvent as eluant 12 fractions of 10 ml. were taken, 1 - 8 showing one spot on thin layer chromatography (R_F = R_F of O,O-dimethyltubocurine, i.e. 0.89). When fractions 1 - 8 were bulked and evaporated to dryness the material obtained was identical to O,O-dimethyltubocurine in m.p. and infra-red spectrum. The remaining fraction showed two components; R_F = 0.89 and R_F=0.78, the second spot being identical to tubocurine (R_F = 0.78)

Cleavage of O,O-dimethyl-(+)-tubocurine by sodium in liquid ammonia.

O,O-Dimethyltubocurine (1.2g.) in toluene/benzene (1:1, 40 ml.) was added dropwise to liquid ammonia (400 ml.) containing a small piece of sodium, and more sodium was added whenever the blue colour faded, until the reaction was complete as judged by the blue colour being relatively stable for 30 - 40 minutes (total sodium = 1.2 g.). After spontaneous evaporation of the ammonia, water was added and the aqueous layer separated from the organic layer. The organic phase was washed with 2% sodium hydroxide (2 x 25 ml.) dried (Na_2SO_4) and the solvent removed to yield the brown non-phenolic syrup (0.585 g.).

The combined alkaline solutions were carbonated with solid carbon dioxide and the precipitate which formed extracted with ether (4 x 250 ml.). The ethereal extracts were dried (Na_2SO_4) and evaporated to dryness to yield the phenolic fragment (0.444 g.) as a yellow oil.

Attempted separation of phenolic products from sodium/liquid ammonia cleavage of O,O-dimethyl-(+)-tubocurine.

Initial attemps at separation involved elution through an alumina column (10 x 1 cm.) prepared by mixing 12 g. of alumina with benzene to form a slurry which was poured into the column and allowed to settle before the excess of benzene was run off.

The pale brown phenolic syrup (250 mg.) was dissolved in a minimum of benzene and added to the column and elution commenced with 1% methanol in benzene. Forty fractions (10 ml.) were collected, each evaporated to small bulk and examined by thin layer chromatography. Fraction 1 contained only one substance R_F =0.85, fractions 2 - 15 were likewise composed of a single component R_F =0.68 and fractions 16 - 40 were non-homogeneous having distinct spots R_F = 0.68 and 0.39 on thin layer chromatography. The column was then washed with methanol (50 ml.) which was collected and added to fractions 16 - 40 which had been bulked and the resultant solution evaporated to dryness, the material on thin layer chromatography showing three spots.

A fresh column was prepared identical to the first and the material obtained from the methanol washings along with fraction 16 - 40 was dissolved in a minimum of benzene and put on to the column. Elution proceeded with benzene and of the

first eight fractions 1 and 2 on thin layer chromatography showed only one spot R_F = 0.85 and fractions 3 - 8 showed no spots. The eluant was then changed to 1% methanol in benzene resulting in fractions 8 - 16 which also showed no spots on thin layer chromatography. The concentration of methanol was increased to 5% and fractions 17 - 31 resulted in one spot R_F = 0.69 and as a result were bulked along with fractions 2 - 15 of previous column, evaporated to small volume (ca 3 ml.) and on standing deposited crystals of laudanidine (10 mg.), m.p. 184°.

With concentrated sulphuric acid the crystals gave a faint pink colour darkening rapidly to purple red on heating[134] and a methanolic solution was rendered pale green by a drop of aqueous ferric chloride[135], $[\alpha]_D^{17}$ - 111° (c 0.07 in benzene). $[\alpha]_D^{17}$ + 40° (c 0.07 in MeOH). $[\alpha]_D^{17}$ = -98° (c 0.07 in chloroform), literature reports $[\alpha]_D^{16}$ - 99.2° (c 0.11 in chloroform)[125], m.p. 182° - 183°.

The concentration of methanol was further increased to 10% and fractions 32 - 40 which had been bulked and evaporated to small volume showed the presence of a single spot on thin layer chromatography R_F = 0.39. On standing crystals of N-methylcoclaurine (ca 8 mg.) separated $[\alpha]_D^{17}$ + 66° (c 0.07 in MeOH), $[\alpha]_D^{17}$ = + 73° (c 0.07 in chloroform), m.p. 175°. Literature reports $[\alpha]_D^{23}$ + 69.6° (c 0.85 in $CHCl_3$)[125], m.p. 176 - 177°.

Separation of phenolic material from a further cleavage of O,O-dimethyltubocurine.

The phenolic syrup(0.300 g.) was dissolved in benzene

with a minimum of methanol and alumina (0.5 g.) added to the flask and the mixture evaporated to dryness. The phenolic syrup was adsorbed by the alumina which was then removed from the flask and added to the alumina column (15 g., 12 x 1 cm.) prepared as before. The column was attached to a fraction collector (Locarte, fitted with an automatic drop counter) and eluted with dry benzene. On investigation by thin layer chromatography fractions (5 ml.) shown by this preliminary examination to be of similar constitution were then bulked and reduced to small volume (ca 3 ml.) and again examined by thin layer chromatography.

The variation of eluting solvent with fraction number is shown in Table XVIII; the final order in which the fractions were bulked together with the number of constituents and their respective R_F values are presented on Table XIX

Table XVIII. Variation of eluting solvent with fraction number.

Fractions	Eluant
1 - 60	Benzene
61 - 80	1% methanol in benzene
81 - 100	Benzene
101 - 600	0.7% methanol in benzene
601 - 675	0.75% -do-
676 - 790	1.0% -do-
791 - 950	1.3% -do-
951 - 1600	2.0% -do-
1601 —	Methanol

Table XIX Variation of components with fraction number.

Fraction number	Number of components shown by thin-layer chromatography	R_F Value
1 - 298	1	0.89
299 - 596	2	0.89 and 0.65
597 - 705	1	0.65
706 - 950	2	0.89 and 0.65
951 - 1010	1	0.65
1011 - 1200	2	0.65 and 0.34
1201 - 1600	1	0.34
1601 -	1	0.34

To another alumina column (20 g., 1 cm. x 20 cm.) prepared as previously described the phenolic syrup (0.3 g.) was added and the components separated by elution. The results are tabulated on a similar basis to that described above. Table XX and table XXI

Table XX Variation of eluting solvent with fraction number.

Fractions	Eluant
1 - 3100	Benzene
3101 - 3210	0.1% methanol in benzene
3211 - 3300	0.2% methanol in benzene
3301 - 3815	0.25% methanol in benzene
3816 - 3845	0.3% methanol in benzene
3846 - 3902	0.35% methanol in benzene
3903 - 4016	0.5% methanol in benzene
4017 -	methanol

Table XXI. Variation of components with fraction number.

Fractions	Number of components shown by thin-layer chromatography	R_F Value	Fraction Weights
1 - 670	1	0.89	3 mg.
671 - 1845	1	0.89	5 mg.
1846 - 2413	2	0.89; 0.64	7 mg.
2414 - 2810	1	0.64	11 mg.
2811 - 3085	1	0.64	10 mg.
3086 - 3297	1	0.64	13 mg.
3298 - 4016	2	0.64; 0.34	15 mg.
4017 -	1	0.34	35 mg.

The last fractions 4017 - deposited crystals of N-methylcoclaurine

Attempted separation of the non-phenolic product from sodium/liquid ammonia cleavage of O,O-dimethyltubocurine.

The non-phenolic syrup (0.42 g.) was placed on a neutral alumina column prepared by slurrying 20 g. of alumina in 1% methanol in benzene, the eluting solvent being of similar composition. A series of fractions (10 ml.) were collected and of these the first six were shown by thin layer chromatography to contain only one constituent of R_F value 0.89. These fractions were combined, evaporated to small volume (ca 5 ml.) and on cooling yielded O, O-dimethyltubocurine (20 mg.), m.p. 98° (undepressed on admixture with authentic O,O-dimethyltubocurine), R_F = 0.89 corresponded with R_F = 0.89 of authentic material and the infra-red spectrum of both materials were superimposable.

The remaining fractions (7 - 25) all contained two components as shown by thin layer chromatography.

The following adsorbents and eluting solvents proved unsatisfactory and failed to effect a separation of the non-phenolic product. Table XXII

Table XXII. Adsorbents and eluants used in attempted separation of non-phenolic components.

Adsorbent	Eluant
Alumina (Camag DS-5)	10% methanol in chloroform
Neutral Woelm Alumina (Camag DS-5) 3:1	10% ethanol in chloroform
Neutral Woelm Alumina	ether
Neutral Woelm Alumina	petroleum ether (b.p. 40°-60°)
Neutral Woelm Alumina	benzene
Neutral Woelm Alumina	benzene/ethanol
Neutral Woelm Alumina	benzene/chloroform
Neutral Woelm Alumina	benzene/methanol

Cleavage of phaeanthine by sodium/liquid ammonia.

(a) with undistilled liquid ammonia.

Phaeanthine (0.77 g.) in a solution of toluene and benzene (40 ml. 1:1) was added dropwise to stirred liquid ammonia (700 ml.) containing a small piece of sodium and more sodium (total 1.0g.) was added whenever the blue colour faded until the reaction was complete (blue colour relatively stable 1 hr.). After spontaneous evaporation of the ammonia, water was added and the aqueous layer separated from the organic layer. The latter was washed with aqueous sodium hydroxide (5%, 3 x 25 ml.) dried (Na_2SO_4) and solvent removed to yield a brown syrup (0.348 g.) which on thin-layer chromatography showed starting material (R_F 0.9) present together with one other component (R_F 0.4).

The combined alkaline solutions were carbonated with solid carbon dioxide, extracted with ether (3 x 500 ml.) and the combined extracts dried (Na_2SO_4). Evaporation of the solvent yielded a brown syrup which on thin-layer chromatography showed the presence of three constituents of R_F 0.95, 0.65 and 0.36, the colour intensity of those of R_F values 0.95 and 0.65 being considerably less than that of R_F = 0.36. A solution of the brown phenolic syrup in benzene on standing deposited prismatic crystals (55 mg.) of N-methylcoclaurine, m.p. 177°. $[\alpha]_D^{17}$ + 84° [c 0.28 in $CHCl_3$]. Literature records $[\alpha]_D^{23}$ - 69.6 (c 0.85 in $CHCl_3$)[125] m.p. 176°-177°: Tomita, Fujita and Murai[130] record m.p. 137-138.5°, $[\alpha]_D^{16}$ +88.51° (c 0.14 in $CHCl_3$) for hemihydrate and Fujita and Murai[126] record 138°-139°, $[\alpha]_D^{16}$ +87.33° (c 0.11 in $CHCl_3$).

(b) with distilled liquid ammonia.

The above experiment was repeated using dry ammonia and clean sodium at -60° and using dry ammonia and clean sodium at ca -30°

i.e. without an ice-bath surrounding the reaction vessel. In both cases the results obtained were identical to the first experiment.

Analysis of quaternary salts.

A full analysis for carbon, hydrogen and nitrogen was completed on one quaternary compound from each series of quaternaries prepared from the four tertiary bases, O,O-dimethyltubocurine, curine, tubocurine and O,O-dimethylcurine.

The difficulty of obtaining correct analytical values for the quaternary salts of curine, chondocurine and tubocurine even when pure has been noted by Dutcher[16], King[15] and Faltes and Neumann[120].

Loss of water of crystallisation.

To confirm the analysis four quaternaries, one from each series from the quaternisation of the four tertiary bases O,O-dimethyltubocurine, curine, tubocurine and O,O-dimethylcurine were heated at 105° over phosphorus pentoxide in high vacuum to constant weight.

Confirmation that the quaternary salts from curine and O,O-dimethylcurine existed as dihydrates and trihydrates respectively was obtained. Drying to constant weight the quaternary salts of O,O-dimethyltubocurine and tubocurine resulted in the loss of four molecules of water of crystallisation and three molecules of water of crystallisation respectively.

Halide determination of quaternary salts.

The Mohr titration method was used to determine the percenta halogen. The weight of the quaternary salt used was in most case very small (ca 5-10 mg.) due to the limited amount of material available and the titre with N/50 $AgNO_3$ was also small. These two factors combined to make it extremely likely that the experimental error incurred in the determination is greater than would normally be tolerated.

General method of production of quaternary salts.

The tertiary base (ca 200 mg.) was added to the alkyl halide (5-7 ml.) and the solution was heated at 65° for 1 - 4 days. The solid which separated was collected by filtration, washed copiously with ether, dried and weighed.

Compound	Structure Number (CLIV) R	% Yield	m.p.	Reaction Time (hr.)	Formula	Found %		Required%	
						N	Halide	N	Halide
N,N^1-diethyl-O,O-dimethyltubocurine iodide pentahydrate	C_2H_5-	81	210°	2	$C_{42}H_{62}I_2N_2O_{11}$	2.5	25.4	2.8	24.8
N,N^1-dipropyl-O,O-dimethyltubocurine bromide pentahydrate	C_3H_7-	60	202°	2.5	$C_{44}H_{66}Br_2N_2O_{11}$	3.1	18.4	3.1	19.4
N,N^1-di-isopropyl-O,O-dimethyltubocurine iodide pentahydrate	iso-C_3H_7-	69	218°	96	$C_{44}H_{66}I_2N_2O_{11}$	2.9	23.7	2.8	24.6
*N,N^1-dibutyl-O,O-dimethyltubocurine bromide pentahydrate	C_4H_9-	69	203°	48	$C_{46}H_{70}Br_2N_2O_{11}$	3.0	17.9	2.8	16.9
N,N^1-dipentyl-O,O-dimethyltubocurine bromide pentahydrate	C_5H_{11}	53	195°	24	$C_{48}H_{74}Br_2N_2O_{11}$	2.9	15.4	2.8	15.7
N,N^1-dioctyl-O,O-dimethyltubocurine bromide pentahydrate	C_8H_{17}	71	173°	90	$C_{54}H_{86}Br_2N_2O_{11}$	2.5	14.7	2.6	14.5
N,N^1-didecyl-O,O-dimethyltubocurine bromide pentahydrate	$C_{10}H_{21}$-	56	172°	48	$C_{58}H_{94}Br_2N_2O_{11}$	3.3	14.1	2.5	13.8
N,N^1-dibenzyl-O,O-dimethyltubocurine bromide pentahydrate	C_6H_5-	60	189°	48	$C_{50}H_{62}Br_2N_2O_{11}$	2.9	17.5	2.7	15.6
N,N^1-diallyl-O,O-dimethyltubocurine bromide pentahydrate	CH_2=$CHCH_2$-	75	198°	1	$C_{44}H_{62}Br_2N_2O_{11}$	3.0	17.2	2.9	16.7
N,N^1-(2-ethoxyethyl)-O,O-dimethyltubocurine bromide pentahydrate	$C_2H_5OCH_2CH_2$-	100	211-213°	72	$C_{46}H_{70}Br_2N_2O_{13}$	2.6	15.6	2.8	16.0
N,N^1-(3-phenylpropyl)-O,O-dimethyltubocurine bromide pentahydrate	$C_6H_5CH_2CH_2CH_2$-	58.5	197°	42	$C_{56}H_{74}Br_2N_2O_{11}$	2.6	14.6	2.5	14.4
N,N^1-(2-phenylethyl)-O,O-dimethyltubocurine bromide pentahydrate	$C_6H_5CH_2CH_2$-	63	200°	10	$C_{54}H_{70}Br_2N_2O_{11}$	3.0	15.5	2.6	14.7

O,O-dimethyltubocurarine iodide. m.p. 230° OR +154° (c. 0.24 in MeOH.)

* Found C, 55.4; H, 6.8 $C_{46}H_{70}Br_2N_2O_{11}$ requires C, 56.0; H, 7.1%

Compound	Structure Number (CLV) R	% Yield	m.p.	Reaction Time (hr.)	Formula	Found%		Required%	
						N	Halide	N	Halide
N,N'-dipropyl-tubocurine bromide	C_3H_7-	50	232°	1¼	$C_{42}H_{52}Br_2N_2O_6$	3.2	19.7	3.0	17.2
N,N'-di-isopropyl-tubocurine iodide pentahydrate	iso-C_3H_7	70	238°	24	$C_{42}H_{62}I_2N_2O_{11}$	3.0	23.7	2.7	24.3
N,N'-dibutyl-tubocurine bromide pentahydrate	C_4H_9-	63	224°	24	$C_{44}H_{66}Br_2N_2O_{11}$	3.4	13.5	2.9	16.7
*N,N'-dipentyl-tubocurine bromide pentahydrate	C_5H_{11}-	77	229°	90	$C_{46}H_{70}Br_2N_2O_{11}$	3.2	16.4	2.8	16.4
N,N'-dihexyl-tubocurine bromide pentahydrate	C_6H_{13}-	69	200°	93	$C_{48}H_{74}Br_2N_2O_{11}$	2.6	16.4	2.8	15.8
N,N'-dioctyl-tubocurine bromide pentahydrate	C_8H_{17}-	65	193°	90	$C_{52}H_{82}Br_2N_2O_{11}$	3.4	13.6	2.6	14.9
N,N'-didecyl-tubocurine bromide pentahydrate	$C_{10}H_{21}$-	73	184°	140	$C_{56}H_{90}Br_2N_2O_{11}$	2.9	13.2	2.6	14.2
N,N'-diallyl-tubocurine bromide pentahydrate	$CH_2:CH.CH_2$	72	218°	1½	$C_{42}H_{58}Br_2N_2O_{11}$	3.0	18.6	3.0	17.2
N,N'-(2-ethoxyethyl)-tubocurine bromide pentahydrate	$C_2H_5OC_2H_4$-	95	217°	90	$C_{44}H_{66}Br_2N_2O_{13}$	4.1	14.9	2.8	16.0
N,N'-(3-phenylpropyl)-tubocurine bromide pentahydrate	$C_6H_5C_3H_6$-	82.3	214°	42	$C_{54}H_{70}Br_2N_2O_{11}$	3.2	15.6	2.9	16.2
N,N'-(2-phenylethyl)-tubocurine bromide pentahydrate	$C_6H_5C_2H_4$-	78	222°	60	$C_{52}H_{66}Br_2N_2O_{11}$	2.96	16.6	2.9	16.6

* Found C, 54.7, H, 6.7. $C_{46}H_{70}Br_2N_2O_{11}$ requires C, 55.6; H, 7.7%

Compound	Structure Number (CLV) R	% Yield	m.p.	Reaction Time (hr.)	Formula	Found %		Required %	
						N	Halide	N	Halide
N,N′-diethyl curine iodide	C_2H_5-	80.6	238°	46	$C_{40}H_{48}I_2N_2O_6$	3.0	28.4	2.9	26.9
N,N′-dipropyl curine bromide dihydrate	C_3H_7-	77	220°	10	$C_{42}H_{56}Br_2N_2O_8$	2.9	13.7 14.0	3.2	18.1
N,N′-di-isopropyl curine iodide dihydrate	iso-C_3H_7-	65	240°	6	$C_{42}H_{56}I_2N_2O_8$	2.9	26.6	2.9	26.1
N,N′-dibutyl curine bromide dihydrate	C_4H_9-	58	222-223°	48	$C_{44}H_{60}Br_2N_2O_8$	3.2	16.5	3.1	17.5
N,N′-dipentyl curine bromide dihydrate	C_5H_{11}-	52	219°	65	$C_{46}H_{64}Br_2N_2O_8$	2.8	15.5	3.0	17.1
N,N′-dioctyl curine bromide dihydrate	C_8H_{17}-	60	202°	67	$C_{52}H_{76}Br_2N_2O_8$	3.2	15.2	2.9	16.4
N,N′-didecyl curine bromide dihydrate	$C_{10}H_{21}$-	62	177°	140	$C_{56}H_{84}Br_2N_2O_8$	2.9	14.3	2.6	14.9
N,N′-(2-phenylethyl)-curine bromide dihydrate	$C_6H_5CH_2CH_2$-	81	226°	95	$C_{52}H_{60}Br_2N_2O_8$	3.0	16.6	2.9	16.0
N,N′-(3-phenylpropyl)-curine bromide dihydrate	$C_6H_5CH_2CH_2CH_2$-	86.5	222°	90	$C_{54}H_{64}Br_2N_2O_8$	3.0	16.1	2.7	15.5
N,N′-diallyl curine bromide dihydrate	CH_2=CH.CH_2-	99	210°	90	$C_{42}H_{52}Br_2N_2O_8$	2.9	19.5	3.3	19.2
N,N′-(2-hydroxyethyl)-curine bromide dihydrate	HO.CH_2CH_2-	85	242-244°	60	$C_{40}H_{48}Br_2N_2O_{10}$	2.8	17.2	3.1	18.0
N,N′*-(2-ethoxyethyl)-curine bromide dihydrate	$C_2H_5OCH_2CH_2$-	76	225°	50	$C_{44}H_{60}Br_2N_2O_{10}$	3.3	16.4	3.0	17.1

* Found C, 56.1; H, 6.3. $C_{44}H_{60}Br_2N_2O_{10}$ requires C, 56.1; H, 7.0%

Compound	Structure Number (CLIV) R	% Yield	m.p.	Reaction Time (hr.)	Formula	Found %		Required %	
						N	Halide	N	Halide
N,N'-diethyl-O,O-dimethylcurine iodide trihydrate	C_2H_5-	91	216°	91	$C_{42}H_{58}I_2N_2O_9$	3.3	27.6	2.8	25.7
*N,N'-dipropyl-O,O-dimethylcurine bromide trihydrate	C_3H_7-	90	213°	92	$C_{44}H_{62}Br_2N_2O_9$	3.4	17.8	3.2	18.4
N,N'-di-isopropyl-O,O-dimethyl-curine iodide trihydrate	iso-C_3H_7-	75	205°	92	$C_{44}H_{62}Br_2N_2O_9$	2.5	26.3	2.7	25.2
N,N'-dibutyl-O,O-dimethylcurine bromide trihydrate	C_4H_9-	90	214°	91	$C_{46}H_{66}Br_2N_2O_9$	3.1	16.8	2.9	16.8
N,N'-(2-phenylethyl)-O,O-dimethyl-curine bromide trihydrate	$C_6H_5 \cdot CH_2CH_2$-	84	216°	92	$C_{54}H_{66}Br_2N_2O_9$	2.9	17.6	2.7	15.3
N,N'-(3-phenylpropyl)-O,O-dimethyl-curine bromide trihydrate	$C_6H_5CH_2CH_2CH_2$-	76.6	201°	92	$C_{56}H_{70}Br_2N_2O_9$	2.6	16.2	2.6	15.0

* Found: C, 57.2; H 6.8. $C_{44}H_{58}Br_2N_2O_9$ requires C, 57.1; H, 6.9%

BIBLIOGRAPHY

REFERENCES

1. Karrer, Curare and Curare-Like Agents (Ed. Bovet, Bovet-Nitti and Marini-Bettolo): Elsevier, London, 1959, p.125.
2. Wieland, Konz and Sonderhoff, Ann. 1937, 527, 160.
3. Wieland and Pistor, Ann. 1938, 536, 68.
4. Wieland, Pistor and Bähr, Ann. 1941, 547, 140.
5. Wieland, Bahr and Witkop, Ann. 1941, 547, 156.
6. King, J. chem. Soc. 1949, 955.
7. Schmid, Kebrle and Karrer, Helv. chim. acta 1952, 35, 1864.
8. Battersby, Binks, Hodson and Yeowell, J. chem. Soc. 1960, 1848.
9. Karrer, Curare and Curare-Like Agents (Ed. Bovet, Bovet-Nitti and Marini-Bettolo): Elsevier, London, 1959, p.127.
10. Buchner, Arch. Pharm. 1862, 160, 19.
11. Boehm, Abhandl. Kgl. sachs. Ges. Wiss. 1897, 24, 22.
12. King, J. chem. Soc. 1937, 1472.
13. King, Ann. Reports 1933, 30, 242.
14. Späth, Leithe and Ladek, Ber. 1928, 61, 1698
15. King, J. chem. Soc. 1935, 1381.
16. Dutcher, J. Amer. chem. Soc. 1946, 68, 419.
17. Hanbury, Pharm. J. [3], 1873, 4, 81.
18. Diels, Pflanzenreich. (A. Engler) 1910, 4, 41.
19. Krukoff and Moldenke, Brittonia 1938, 3, 1.
20. King, J. chem. Soc. 1940, 737.
21. Wintersteiner and Dutcher, Science 1943, 97, 467.
22. Tomita, Fujita and Murai, J. Pharm. Soc. Japan 1951, 71, 23
23. Marsh and Herring, Experentia 1950, 6, 34.
24. Faltis, Wrann and Kuhas, Ann. 1932, 497, 69.
25. Spath and Kuffner, Ber. 1934, 67, 55.

26. Scholtz, Ber. 1896, 2054; Arch. Pharm. 1898, 236, 530; 1899, 237, 199, Späth, Leithe and Ladek, Ber. 1928, 61, 1698.

27. Bick and Clezy, J. chem. Soc. 1953, 3893.

28. King, J. chem. Soc. 1939, 1157.

29. Tolkachev, Voronin and Preobrazhenskii, J. Gen. Chem. (U.S.S.R. 1959, 29, 1163.

30. Bernard, Lecons sur les effets des substances toxiques et medicamenteuses, Paris 1857, 238-353.

31. Crum-Brown and Fraser, Trans. Roy. Soc. Edinburgh 1869, 25, 151, 693.

32. Dale, J. Pharmacol. 1914, 6, 147.

33. Bowman, Progress in Medicinal Chemistry Vol. 2 (Ed. Ellis and West) Butterworth, London, 1962, p.88.

33a. Long and Schueler, J. Amer. Pharm. Assoc. (Sci Ed.) 1954, 43, 79.

34. Dale, Feldberg and Vogt, J. Physiol. 1936, 86, 353.

35. Zaimis, J. Physiol. 1953, 122, 238.

36. Hodges and Foldes, Lancet 1 1957, 272, 373.

37. Taylor, Pharmacol. Rev. 1951, 3, 412.

38. Stenlake, Progress in Medicinal Chemistry Vol. 3 (Ed. Ellis and West) Butterworth, London 1963, p.7.

39. Barlow, Introduction to Chemical Pharmacology (chap. 3). Methuen, London, 1955.

40. Gill, Proc. Roy. Soc. 1959, B150, 381.

41. Battersby and Hodson, Q. Rev. chem. Soc. 1960, 14, 77.

42. Elworthy, J. Pharm. London 1963, 15, 137T.

43. Paton and Zaimis, Brit. J. Pharmacol. 1949, 4, 381.

44. Barlow and Ing, Nature, London 1948, 161, 718; Brit. J. Pharmacol. 1948, 2, 298.

45. Paton and Zaimis, Nature, London 1948, 161, 718.

46. Carey, Edwards, Lewis and Stenlake, J. Pharm. London 1959, 11, 70T.

47. Bovet, Bovet-Nitti, Guarino, Longo and Morotto R.C. Ist. sup. Sanit. 1949, 12, 106.

48. Hunt and De Taveau, Brit. med. J. 1906, ii, 1788.

49. Phillips, J. Amer. chem. Soc. 1949, 71, 3264.

50. Lewis, Martin-Smith and Muir, Brit. J. Pharmacol. 1963, 20, 307.

51. Cheymol, Bull. Acad. Nat. Med. 1953, 137, 83.

52. Cheymol, Delaby, Chabrier, Najer and Bourillet, Arch. int. Pharmacodyn. 1954, 98, 161.

53. Cheymol, Delaby, Najer and Gray, C.R. Acad. Sci., Paris 1952, 235, 1711.

54. Delaby, Chabrier and Najer, Bull. Soc. chim. Fr. 1955, 1616.

55. Delaby, Chabrier and Najer, Bull. Soc. chim. Fr. 1956, 212.

56. Klupp, Kraup, Storman and Stumpf, Arch. int. Pharmacodyn. 1953, 96, 161.

57. Klupp and Kraup, Arch. int. Pharmacodyn. 1954, 98, 340.

58. Najer, Chabrier and Delaby, Bull. Soc. chim. Fr. 1956, 689.

59. Cheymol, Chabrier, Giudicelli, Najer and Bourillet, C.R. Acad. Sci. Paris 1957, 245, 2560.

60. Randall, Giuliano, Kappell and Allen, J. Pharmacol. 1952, 105, 7.

61. Lüttringhaus, Kerp and Preugschas, Arzneimitt.-Forsch. 1957, 7, 222.

62. Protiva and Borovička, Czech. Pat. 88,309.

63. Votava and Metyšova-Šrankova, Physiol. Bohemoslov. 1955, 8, 431.

64. Fusco, Palazzo, Chiavarelli and Bovet, Gazz. chim. ital. 1958, 88, 1293.

65. Rosnati, Gazz. chim. ital. 1957, 87, 215.

66. Barstad, Skramstad and Øksne, Arch. int. Pharmacodyn. 1959, 121, 395.

67. Scognamiglio, Arch. ital. sci. Farmacol. 1959, 9, 191.

68. Philippot and Dallemagne, Arch. int. Pharmacodyn. 1954, 96, 485.

69. Hoppe, Curr. Res. Anesth. 1951, 30, 262.

70. Hoppe, Funnell and Lape J. Pharmacol. 1955, 115, 106.

71. Rossum and Ariëns, Arch. int. Pharmacodyn. 1959, 118, 393.

72. Collier and Taylor, Nature, London 1949, 164, 491.

73. Bodman, Morton and Wylie, Lancet 1952, 11, 517.

74. Binning, Anaesthesia 1953, 8, 268.

75. Lederman, Sinai Hosp. J. 1953, 2, 25.

76. Dundee, Gray and Riding, Brit. J. Anaesth. 1954, 26, 13.

77. Wyant and Sadove, Curr. Res. Anesth. 1954, 33, 178.

78. Hunter, Brit. J. Anaesth. 1955, 27, 73.

79. Collier and Macauley, Brit. J. Pharmacol. 1952, 7, 409.

80. Collier, Gladych, Macauley and Taylor, Nature, London 1959, 183, 542.

80a. Collier, Gladych, Macauley and Taylor, Nature, London 1958, 182, 1424.

81 Kensler, Zirkle, Natallana and Condouris, J. Pharmacol. 1954, 112, 210.

82. Carey, Edwards, Lewis and Stenlake, J. Pharm., London 1959, 11, 70T.

83. Edwards, Lewis, Stenlake and Zoha, J. Pharm., London 1957, 9, 1004.

84. Edwards, Lewis, Stenlake and Zoha, J. Pharm., London 1958, 10, 106T.

85. Edwards, Lewis, Stenlake and Zoha, J. Pharm., London 1958, 10, 122T.

86. Protiva and Pliml, Coll. Czech. chem. Comm. 1953, 18, 836.

87. Vanecek and Votava, Physiol. Bohemsloven. 1955, 4, 220.

88. Edwards, Lewis, McPhail, Muir and Stenlake, J. Pharm. London 1960, 12, 137T.

89. Lewis, McPhail, Muir and Stenlake, J. Pharm., London 1961, 13, 543.

90. Bovet, Depierre and De Lestrange, C.R. Acad. Sci., Paris 1947, 225, 74.

91 Rike~~R~~s and Wescoe, Ann. N.Y. Acad. Sci., 1951, 54, 373.

92 Foldes, Clinical Pharm. and Therap., 1960, 1, 345.

93 Holiday and Varney, quoted by Wintersteiner Curare and Curare-like Agents (Ed. Bovet, Bovet-Nitti and Marini-Bettôlo): Elsevier, London, 1959, p. 160.

94 King, Nature, London 1946, 158, 515; J. chem. Soc. 1947, 936.

95 Cavallito, Curare and Curare-like Agents (Ed. Bovet, Bovet-Nitti and Marini-Bettôlo): Elsevier, London 1959, p. 292.

96 Kalow, J. Pharmacol. 1954, 110, 433.

97 Ehrenpreis, Biochem. Biophys. Acta 1960, 44, 561.

98 Dutcher, J. Amer. chem. Soc. 1952, 74, 2221.

99 Bick and Clezy, J. chem. Soc. 1953, 3893.

100 Leithe, 1. Oester. Chem. Ztg. 1932, 35, 133.
2. Ber. 1934, 67, 1261.

101 Corrodi and Hardeggar, Helv. chim. Acta 1956, 39, 889.

102 Battersby and Edwards, J. chem. Soc. 1960, 1214.

103 Tomita and Kunimoto, J. Pharm. Soc. Japan 1962, 82, 734.

104 Ferrari and Deulofou, Tetrahedron 1962, 419.

105 Kenner and Murray, J. chem. Soc. 1950, 406.

106 Trumbull, Haberli and Ammon, Abstr. Pap. Am. chem. Soc. Sept. 1958, 76.

107 Tomita and Ibuku, J. Pharm. Soc. Japan 1962, 82, 12, 1652.

108 Hofmann, Ann. 1851, 78, 253, 79, 11.
Ber. 1881, 14, 494.

109 Collie and Schryver, J. chem. Soc. 1890, 57, 767.

110 Thomas, J. Med. Pharm. Chem. 1961, 3, 309.

111 Ingold, Structure and Mechanism in Organic Chemistry: G. Bell & Sons Ltd., 1953.

112 Brewster and Ebel, Organic Reactions VII, 138

113 Hanhart and Ingold, J. chem. Soc. 1927, 997.

114 Norcross and Openshaw, J. chem. Soc. 1949, 1174.

115 Battersby and Binks J. chem. Soc. 1955, 2888.

116. Ebel and Peckam, J. Amer. Chem. Soc. 1950, 72, 1209.

117. Hunig and Baron, Ber. 1957, 90, 395, 403.

118. Tomita and Takano, J. Pharm. Soc. Japan 1960, 80, 301.

119. Sartoretto and Sowa, J. Amer. Chem. Soc. 1937, 59, 603.

120. Kranzfelder and Sowa, J. Amer. Chem. Soc. 1937, 59, 1488.

121. Weber and Sowa, J. Amer. Chem. Soc. 1938, 60, 94.

122. Tomita, Inubushi and Niwa, J. Pharm. Chem. Japan 1951, 72, 206.

123. Manske, J. Amer. Chem. Soc. 1950, 72, 55.

124. Tomita, Fujita and Murai, J. Pharm. Chem. Japan 1950, 71, 226.

125. Kidd and Walker, J. chem. Soc. 1954, 669.

126. Fujita and Murai, J. Pharm. Soc. Japan 1951, 71, 1043.

127. Djerassi, Brewer, Clarke and Durham, J. Amer. chem. Soc. 1962, 84, 3210.

128. Bessho, Chem. Pharm. Bull. Japan 1963, 11, 1504.

129. Beckett and Casy, J. chem. Soc. 1957, 3077.

130. Tomita, Fujita and Murai, J. Pharm. Soc. Japan 1951, 71, 1035.

131. Faltis and Neumann, Monatsh. 1922, 42, 311.

132. Birch, Q. Rev. chem. Soc. 1950, 4, 69.

133. Tomita, Fortsch. Chem. Org. Naturstoffe, 1952, 9, 175.

134. Hesse, Annalen, Suppl., 1872, 8, 273

135. Hesse, Annalen, 1870, 153, 54