THE ALKALOIDS OF THE AMARYLLIDACEAE:

The Isolation and Structures of Two New Alkaloids from *Haemanthus natalensis* and *Nerine krigeii* and Contributions to the Chemistry of Coccinine. The Absolute Configuration of Alkaloids based on the 5:10b-Ethanophenanthridine Nucleus.

BY

P.W. JEFFS, A.R.I.C.

A Thesis

submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Department of Chemistry and Chemical Engineering, University of Natal.

Department of Chemistry
and Chemical Engineering,
University of Natal,
Pietermaritzburg,
South Africa.

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CONTENTS.

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SUMMARY.

An investigation of the alkaloid content of several species of the Amaryllidaceae has been undertaken. Two new alkaloids, 3-epihaemanthidine and krigenamine have been isolated and a complete stereostructure is proposed for the former. Krigenamine has been studied and although conclusive evidence is lacking a structure is proposed. The alkaloid coccinine has been isolated from a new source and a contribution made to its chemistry.

Haemanthus natalensis has yielded in addition to haemanthamine, previously isolated from this plant, the known alkaloid haemanthidine and a new alkaloid 3-epihaemanthidine. 3-Epihaemanthidine, $C_{17}H_{19}O_5N$, has been shown to contain a methylenedioxy group, two hydroxyl groups, an aliphatic methoxyl and one double bond. Treatment of 3-epihaemanthidine or its methiodide with alkali resulted in a molecular rearrangement affording products which were shown to be related to tazettine. Furthermore 3-epihaemanthidine on treatment with hydrochloric acid gave apohaemanthidine which

indicated that the alkaloid contained the 5:10b-ethano-phenanthridine skeleton and that it belonged to the (+)-crinane series. These results in conjunction with other experiments lead to the structure of 3-epi-haemanthidine.

The application of Mills's rule for allylic alcohols to a number of alkaloids based on the 5:10b-ethanophenanthridine skeleton has allowed the prediction of their absolute configurations.

A new alkaloid, krigenamine $C_{18}H_{23}O_5N$, has been isolated from the bulbs of Nerine krigeii and it has been shown to contain a methylenedioxy group, an aromatic methoxyl, an N-methyl group and one hydroxyl group. The hydroxyl group and the remaining oxygen atom are located in a hemi-acetal since oxidation of the alkaloid gave a lactone. All evidence suggests that krigenamine is an analogue of lycorenine yet conclusive proof is lacking.

Coccinine, $C_{17}H_{19}O_4N$, has been isolated from the bulbs of Haemanthus makenii. The presence of a methylene-dioxy group, one hydroxyl group, a tertiary nitrogen atom and one double bond has been established confirming the results of previous workers. The previously reported

non-crystalline hydrogenation product has been found to consist of three compounds. One of these compounds is shown to be a hydrogenolysis product since it forms an O:N diacetate. The structure of the O:N diacetate is deduced on the basis of the recently reported 5,11 methanomorphenanthridine nucleus for coccinine.

A review of some representative classes of Amaryllidaceae alkaloids is presented with an emphasis on their stereochemistry in Part I of this thesis. Part II is devoted to a discussion of alkaloid biogenesis with particular reference to the alkaloids of the Amaryllidaceae.

PART I.

SECTION I.

A REVIEW
OF
THE STEREOCHEMISTRY
OF
LYCORINE AND CARANINE
AND
THE STRUCTURES OF TWO
NEW ALKALOIDS CONTAINING THE
PYRROLO[de]PHENANTHRIDINE SKELETON.

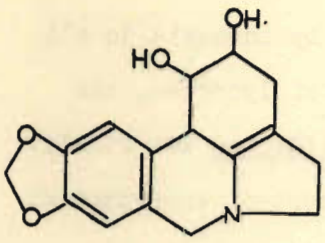
After a considerable effort by chemists in all parts of the world the structure of lycorine, the principal alkaloid of the Amaryllidaceae was finally elucidated in 1954. A large amount of experimental data had been accumulated since its isolation by Gerard in 1877, and the main contributer was the Japanese chemist Kondo. (Kondo's early structure is shown in Chart I/1 together with the structural proposals of other investigators). It is befitting, in view of his long study of lycorine, that Kondo's name appears as an author of the paper in which the structure was finally concluded - though some modification of the stereochemistry proposed has been necessary.

This review of lycorine and the alkaloids containing the pyrrolo[de]phenanthridine nucleus will be confied mainly to a discussion of the stereochemistry of lycorine and caranine. However a brief account is presented of the chemistry of two alkaloids in this group which have been isolated very recently.

The Stereochemistry of Lycorine.

Humber, Kondo, Taylor, and Uyeo[1] et al., proposed a complete stereostructure (I) for lycorine after a

CHART I/1



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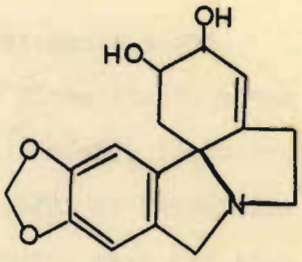

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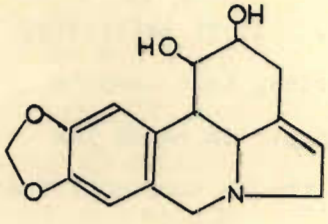

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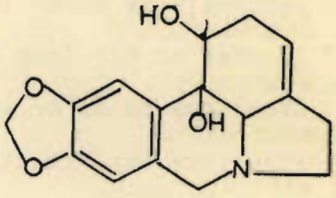
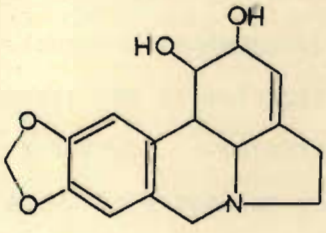


WENKERT 1954



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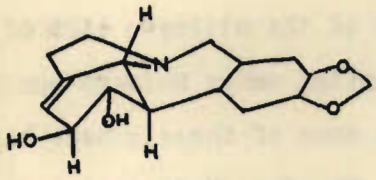
STRUCTURAL PROPOSALS FOR LYCORINE.



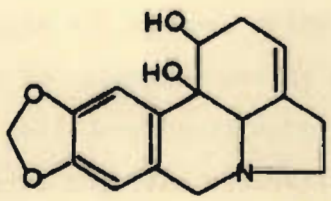
I

careful study of the Hofmann degradation and the synthesis of key degradation products. The course of the Hofmann degradation is rather unusual and is worth mentioning. Methylation of lycorine yields two diastereoisomeric methiodides, both of which undergo the Hofmann degradation with concomitant loss of two molecules of water to furnish the methine base, lycorine anhydromethine. Both the double bond and a quarternary nitrogen atom are necessary for this reaction since both dihydrolycorine methohydroxide and lycorine itself are unaffected by the condition under which lycorine methohydroxide forms lycorine anhydromethine (see Chart I/2).

These authors recovered lycorine, and dihydrolycorine, unchanged after treatment with a). potassium amyloxide at 170°, b). refluxing 10% ethanolic sulphuric acid. The absence of ready elimination of the allylic hydroxy

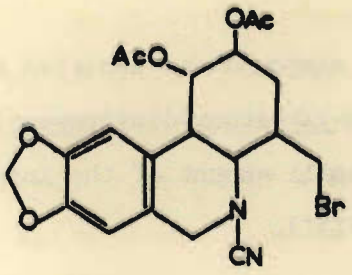
group or of the hydroxyl group β to the aromatic nucleus, coupled with the stability of the nitrogen atom of the hetrocyclic ring to elimination under Hofmann conditions, was taken to indicate that none of these groups had trans coplanar hydrogen atoms. The four hydrogen atoms concerned were therefore assigned a pseudo-axial configuration.

Wenkert[2] as the result of a reinterpretation of Kondo's work and biogenetic reasons submitted that lycorine possessed a secondary-tertiary glycol system and represented it as structure (II).



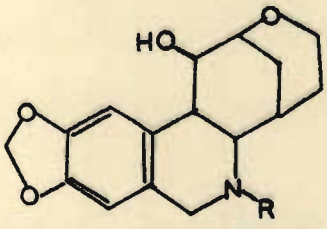
II

Taylor and Uyeo[3] et al., presented further support for their structure for lycorine from a study of the product obtained by treating diacetyldihydrolycorine with cyanogen bromide. The reaction had been carried out some sixteen years earlier by Kondo and structure (III), diacetyl-ω-bromo-N-cyanodihydrosecolycorine, was assigned to the product.



III

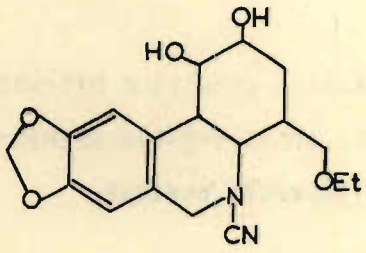
Treatment of (III) with ethanolic potassium hydroxide gave four compounds in addition to anhydro-N-cyanodihydro-secolycorine (IV) ehich had been reported earlier.



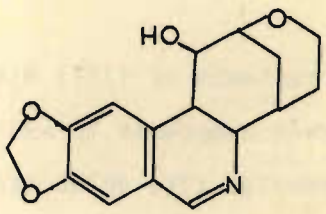
IV; R=CN
V; R=H
VI; R= C(=NH)OEt

One of these compounds was found to be anhydrodihydro-secolycorine (V) since it could be obtained by treating (IV) with dilute acid. The imidate (VI) was identified as a product since it could be obtained by treating (IV) with hot ethanolic potassium hydroxide.

A neutral compound was isolated and identified as anhydro-N-cyano-ω-ethoxydihydrosecolycorine (VII). In addition a small amount of the known anhydrodihydrosecolycorine (VIII).

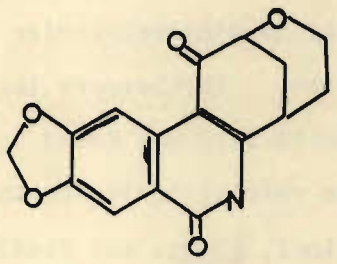


VII



VIII

Chromic acid oxidation of (IV) afforded a keto-lactam which was soluble in alkali and precipitated unaltered after acidification. It could be N-methylated with dimethyl sulphate and alkali after which it was found to be insoluble in alkali. This behaviour was recognised to be that of an isocarbostyril and structure (IX) was proposed.



IX

For ether formation to occur in the above compounds the ethyl residue in structure (III) must *cis* to the C_2-oxygen function. If the B:C ring fusion is *trans* and the hydroxyl group equatorial it would require an improbable *trans* fusion of ring C:D in dihydrolycorine. In view of this these authors assumed, incorrectly, that the hydroxyl groups were *trans* equatorial and therefore dihydrolycorine possessed a *cis* B:C ring juncture and a *trans* C:D ring fusion.

A series of reactions carried out by Takeda and Kotera[4] resolved many of the uncertainties of the stereochemistry of lycorine and dihydrolycorine.

Tosylation of dihydrolycorine gave a monotosylate (X), which was readily converted by methanolic potassium hydroxide into the epoxide (XI) together with a small amount of the methyl ether (XII). The epoxide was hydrolysed

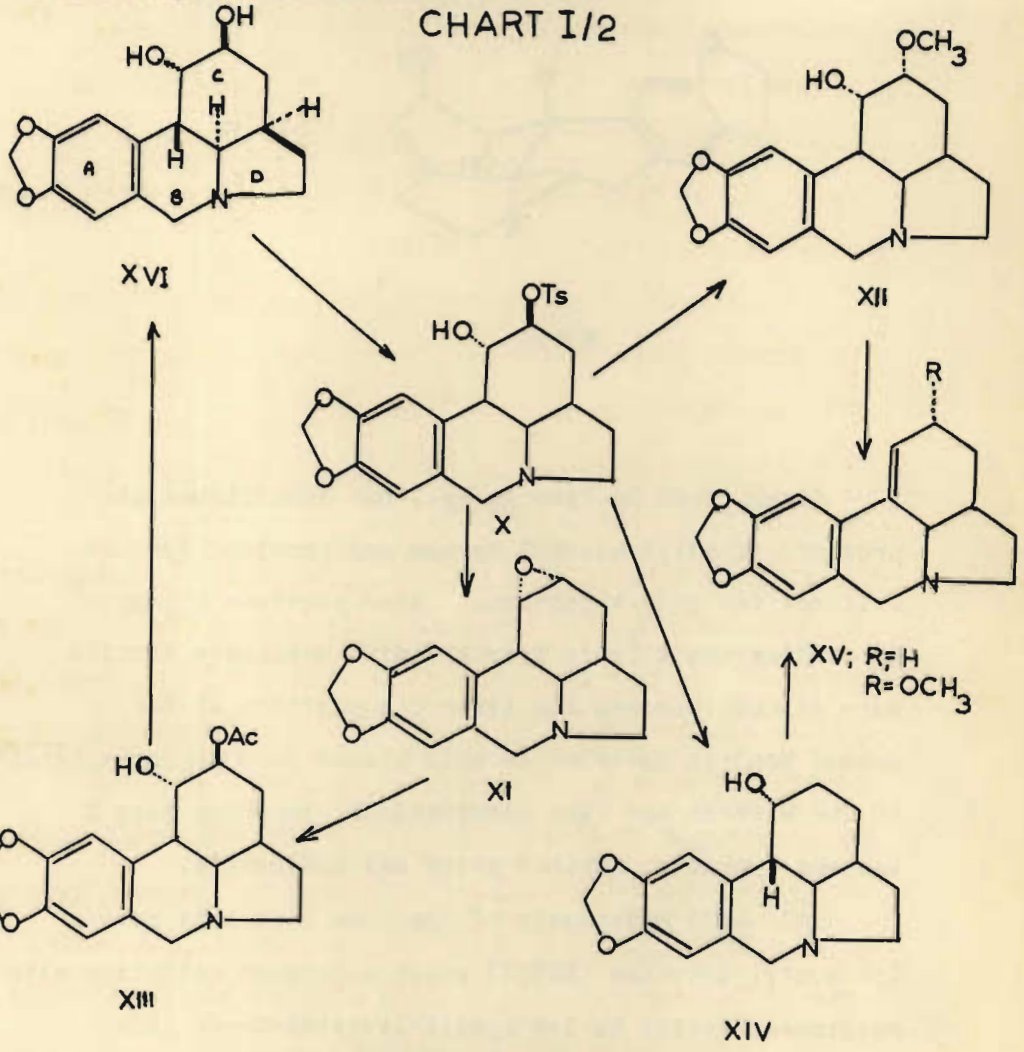
with acetic acid to 2-O-acetyldihydrolycorine (XIII) which could be hydrolysed to dihydrolycorine or acetylated to diacetyldihydrolycorine. Furthermore the epoxide on treatment with sulphuric acid was found to give dihydrolycorine. These results indicated that the hydroxyl groups are vicinal, trans, and diaxial. Reduction of the tosylate (X) or the epoxide (XI) afforded monodesoxydihydrolycorine (XIV).

Treatment of monodesoxydihydrolycorine (XIV) and the methyl ether (XII) with phosphorus oxychloride gave the corresponding $\Delta^{1(11b)}$ olefins (XV; R,H) and (XV; R, OCH_3) the structures of which were evident from their ultra-violet absorption spectra.

The formation of $\Delta^{1(11b)}$ olefins indicates that the hydrogen atom at 11b is axial and trans to the C_1-hydroxyl group. The previous observation regarding the formation of the cyclic ethers from diacetyl-ω-bromo-N-cyanoseco-dihydrolycorine under alkaline conditions were now reinterpreted by these authors. The C_2-hydroxyl is axial, and since the ethyl residue in ring D must lie on the same side of ring C it must also be linked in an axial manner to ring C. Since trans diaxial fusion of rings C

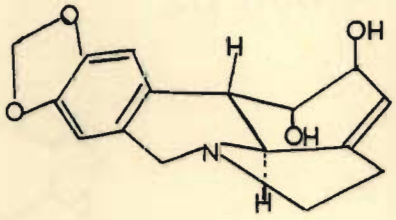
and D is impossible the C_{11c}—N bond must be equatorial and rings B and C are <u>trans</u>-fused. Hence dihydrolycorine can be represented by structure (XVI).

CHART I/2



ELUCIDATION OF THE STEREOCHEMISTRY OF DIHYDROLYCORINE.

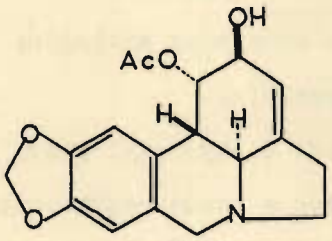
Therefore on the basis of these results lycorine may be assigned the stereostructure (XVII).



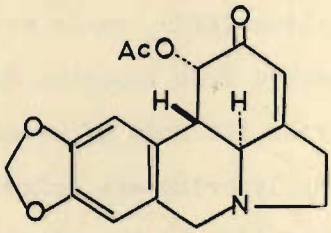
XXVII

Recent work by Uyeo et al.[5], has established the presence of allyl alcohol system and provided further evidence for this structure. Some previous attempts[6] to oxidise the allylic hydroxyl with manganese dioxide were without success and since the position of the double bond in lycorine is only placed from an interpretation of the Hofmann and Fmde degredations, in which ring C becomes aromatic, further proof was desireable.

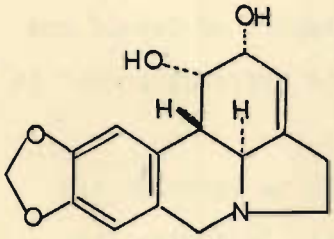
Mild acid hydrolysis of lycorine diacetate gave 1-O-acetyl-lycorine (XVIII) which underwent oxidation with manganese dioxide to 1-O acetyl-lycorine-2-one (XIX)



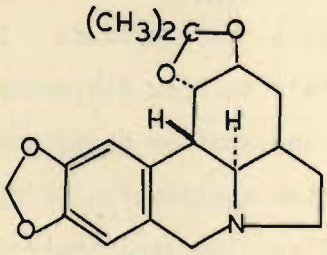
XVIII



XIX



XX



XXI

which was characterised from its ultra-violet and infrared spectra. It is interesting to note that the acetoxyl group was not epimerised in this reaction since (XIX) could be converted to 1-O-acetyldehydrolycorine. This is rather unusual since one would expect it to invert to the more stable equatorial isomer as the result of keto-enol tautomerism. The possibility of migration of the double bond was excluded since reduction of 1-O-acetyl-lycorine-2-one with sodium borohydride followed by hydrolysis yielded lycorine along with its epimer, 2-epilycorine (XX). 2-Epilycorine, which was also obtained as the only isolable product from treating 1-O-acetyl-lycorine-2-one with

lithium aluminium hydride, gave an isopropylidene derivative (XXI), while attempts to obtain an analogous derivative from lycorine were unsuccessful.

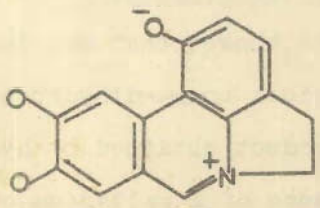
From its mode of preparation the C_2-hydroxyl group in 2-epilycorine was inferred to have a pseudo-equatorial conformation and therefore the cis-1-hydroxyl must have an axial conformation. Thus in support of Takeda and Kotera's work on dihydrolycorine the hydroxyl groups in lycorine must be trans and diaxial.

The application of Mills's rule to lycorine and 2-epilycorine (and their derivatives) indicates that lycorine possesses the absolute configuration as represented by formula (XVII).

The hydrogenation of 2-epilycorine in contrast to lycorine afforded two hydrogenation products α-dihydro-2-epilycorine and β-dihydro-2-epilycorine differing only in their manner of ring C:D fusion. The many reductions of lycorine have always yielded only one hydrogenation product and it may be concluded that the pseudo-equatorial group in 2-epilycorine is less effect in directing the entering hydrogen atoms to the double bond than the pseudo-axial 2-hydroxyl groups in lycorine.

The Stereochemistry of Caranine and its Reduction Products.

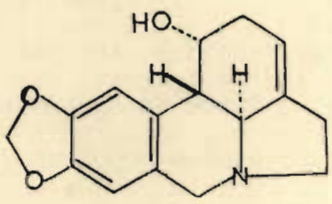
Caranine[7], $C_{16}H_{17}O_3N$, was shown by Wildman et al., to contain a methylenedioxy group, one hydroxyl and a tertiary nitrogen atom. The absence of N-methyl and methoxyl groups was proved by analysis. Hydrogenation with platinum catalyst gave ∝-dihydrocaranine, and palladium catalyst afforded β-dihydrocaranine. Since an Oppenauer oxidation of caranine gave a phenanthridinium compound (XXII) identical to one of the products obtained from a similar oxidation of lycorine it was assigned the lycorine ring system and the hydroxyl group was tentatively placed in the 1-position.



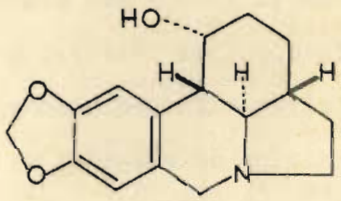
XXII

The double bond in caranine was assigned 3,3a position by Warnhoff and Wildman in preference to the alternative 3a,4 position on the basis of its oxidation to the

phenanthridinium compound (XXII) and caranine was represented as (XXIII) on the basis of the work of Takeda and Kotera mentioned below.



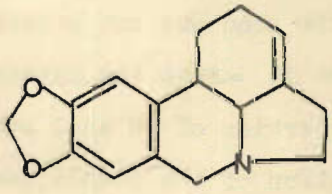
XXIII



XXIV

Its relationship to lycorine was first established by Takeda and Kotera[4] who showed that monodeoxydihydrolycorine (XIV) was identical to α-dihydrocaranine. Thus β-dihydrocaranine the product obtained by hydrogenation of caranine in the presence of a palladium catalyst, is represented as structure (XXIV) which has a <u>trans</u> C:D ring junction.

These results indicated that caranine must possess the stereochemistry as represented in structure (XXIII) and in view of the recent determination of the absolute configuration of lycorine this also represents its absolute configuration. The conversion of lycorine to caranine has been achieved[8] by another route. Lycorine or its 0:0 diacetate on treatment with sodium and amyl alcohol afforded two major crystalline products one of which was caranine, which is produced by hydrogenolysis of the C_2-oxygen function. The other product was assigned the structure (XXV) and was given the trivial name lycorene.



XXV

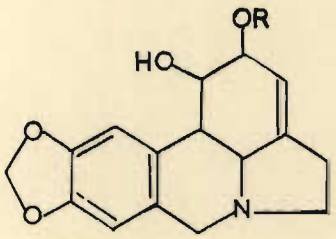
The Structures of Two New Alkaloids Containing the Pyrrolo[de]Phenanthridine Skeleton.

PARKAMINE.

In a recent study of the alkaloids of the plant Amaryllis parkeri Boit[9] isolated a new alkaloid which was named parkamine, $C_{18}H_{21}O_5N$. It was shown to contain a methylenedioxy group, one hydroxyl, two methoxyl groups and a tertiary nitrogen atom. One methoxyl group was linked to the aromatic ring since the infrared spectrum showed a strong absorption at 1616 $cm.^{-1}$.[10] Hydrogenation with platinum catalyst gave a dimorphic dihydro compound. Its failure to undergo oxidation with manganese dioxide and the absence of a C=N in the infrared spectrum of the perchlorate indicated the double bond was not present in an allyl alcohol system nor was it $\alpha\beta$-to the nitrogen atom. Parkamine did not show the properties of an enol ether and therefore the most likely position of the double bond was either 3,3a or 3a,4.

Treatment of parkamine with sodium and pentyl alcohol gave a mixture of lycorene (deoxycaranine), α-dihydrocaranine and caranine. This result demonstrated that parkamine was a methoxy caranine and that the methoxy group was present in an allyl methyl ether system with the methoxyl attached to the 2-or 4- position. The latter position

was excluded since the action of hot mineral acid on parkamine or vacuum pyrolysis yielded anhydrofalcatine-lactam[11]. (XXVI). Thus structure (XXVII, R = OCH_3) was proposed for parkamine.



XXVII

AMARYLLIDINE.

Amaryllidine, $C_{17}H_{19}O_5N$, was isolated from Amaryllis belladonna in low yield by Boit et al.[12], and the structure of this alkaloid has recently been proposed[9]. It was shown to possess one methoxyl group, a methylenedioxy group two hydroxyl groups and a double bond. The infrared

spectrum indicated that the methoxyl group was present in the aromatic ring. The hydroxyl groups were shown to be present in an ∝-glycol by oxidation with periodic acid. The infrared spectrum closely resembled that of parkamine and on the evidence presented above amayllidine was assigned structure (XXVII, R = OH).

PART I.

SECTION II.

A REVIEW OF THE CHEMISTRY AND STEREOCHEMISTRY OF THE LACTONE AND HEMIACETAL ALKALOIDS OF THE AMARYLLIDACEAE.

Homolycorine and Lycorenine. —

The alkaloid homolycorine[13] was isolated in 1929 from the herb Lycoris Radiata in Japan. Kondo ascribed the formular $C_{19}H_{23}O_4N$ to homolycorine and in addition the presence of two methoxyl groups and an N-methyl group was indicated by the usual analytical procedures. Little value was placed on the N-methyl determination at the time since lycorine which has a bridge-head nitrogen also gave an apparent N-methyl determination by the method of Hertzig and Meyer.

Homolycorine was isolated by Boit[14] from two other species of the Amaryllidaceae Leucojum vernum L and Narcissus poeticus var. ornatus and the molecular formular revised to $C_{18}H_{21}O_4N$.

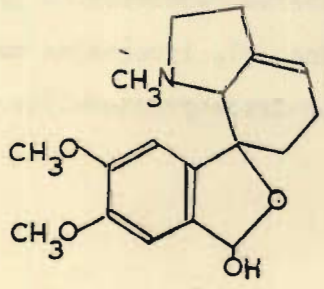
The combined Japanese and Canadian school of Uyeo and Taylor[15] confirmed Boit's formular and established the presence of two methoxy groups and N-methyl group.

Attempted acetylation of the base failed so the nature of the two remaining oxygen still remained to be elucidated. It was found that although the base was insoluble in cold alkali it dissolved in hot sodium hydroxide to yield a salt which could not be extracted with organic solvents.

Re-acidification with hydrochloric acid and extraction with chloroform afforded homolycorine hydrochloride in quantitative yield. This result indicated the two oxygen atoms were present as a lactone. Reduction of homolycorine by lithium aluminium hydride gave tetrahydro-homolycorine which on treatment with hot dilute sulphuric acid gave anhydrotetrahydrohomolycorine. The latter compound was found to be identical with deoxylycorenine obtained by Kondo et al., on electrolytic reduction of lycorenine, a base isolated also from Lycoris Radiata.

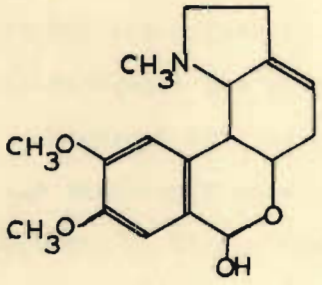
The close relation between homolycorine and lycorenine was demonstrated almost simultaneously and independently by the German workers, Boit et al., and the Japanese-Canadian school of Taylor and Uyeo, when lycorenine was found to undergo oxidation with chromic acid to homolycorine.

Wenkert and Hansen[16] on biogenetic grounds and on a reinterpretation of Kondo's experimental data had previously proposed a benzylic hemi-acetal structure (I).

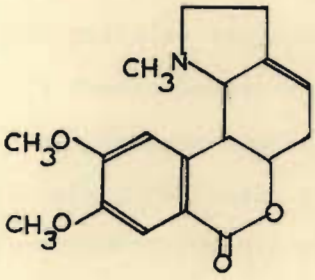


I

to account for the observed ether formation on reduction of lycorenine. Boit supported this structure for lycorenine, by representing homolycorenine as a γ-lactone. The fact that homolycorine showed carbonyl absorption at 1712 $cm.^{-1}$ which indicated that it was a δ-lactone was pointed out by Taylor and Uyeo[15] and other workers[17] and the structures of lycorenine and homolycorine were revised to (II) and (III) respectively.



II



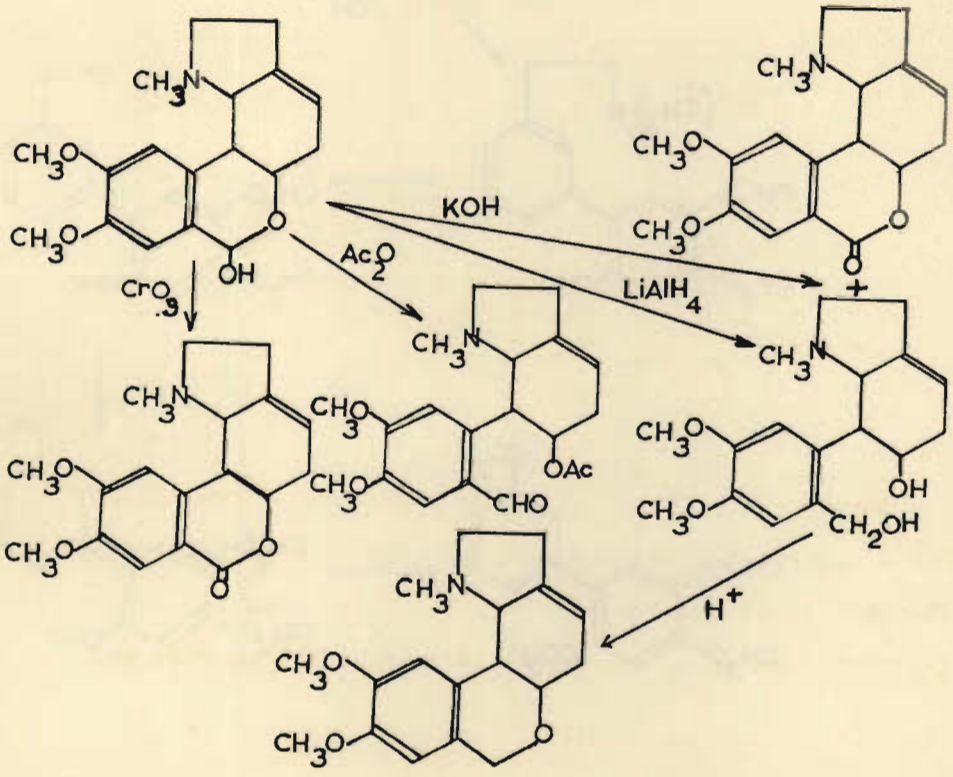
III

In support of their structural proposals Uyeo showed that a), lithium aluminium hydride reduction of lycorenine afforded tetrahydrohomolycorine, b), lycorenine was disproportionated by alkali to tetrahydrohomolycorine and

homolycorine. Wenkert's structure was also critisised on the grounds that the potential tertiary hydroxyl group should have been eliminated in a number of degradation products of lycorenine, e.g. on acetylation. Acetylation of lycorenine yields the acetate of the open form rather than the acetate of the hemi-acetal as indicated on the similarity of its ultra-violet spectrum with veratraldehyde.

The above reaction are summarised in Chart I/3.

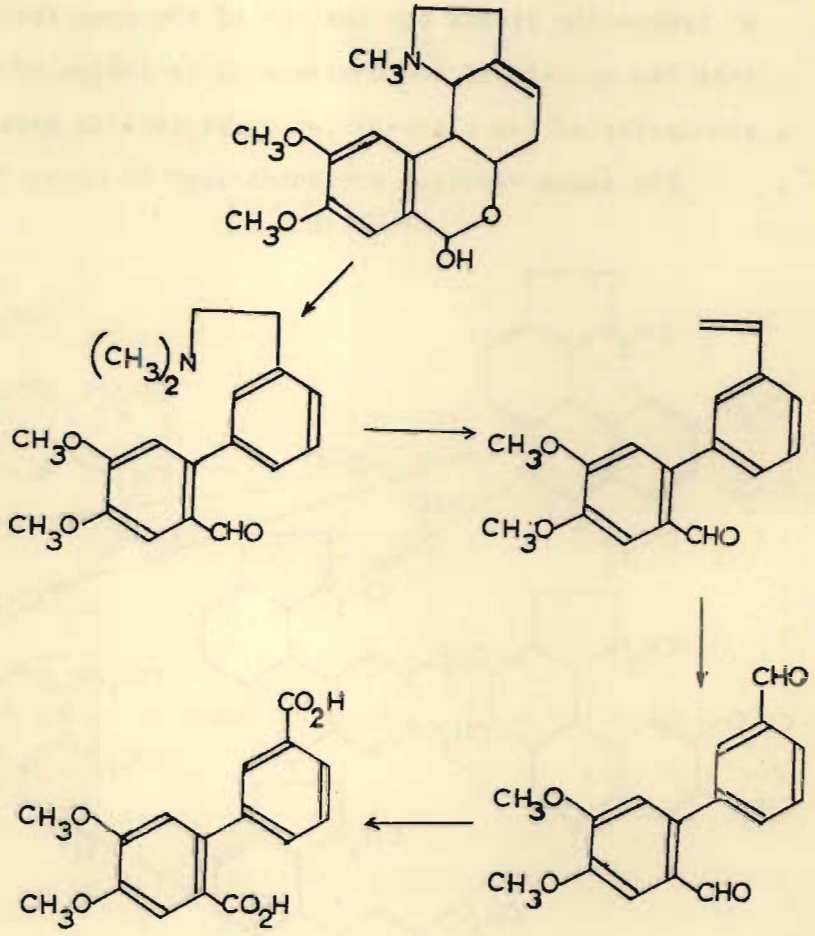
CHART I/3



REACTIONS OF LYCORENINE

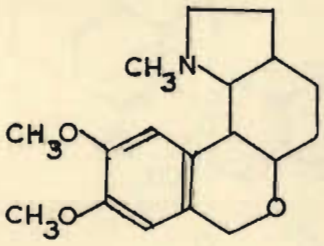
The Hofmann degradations of Kondo and Ikeda may now be interpreted as shown in ChartI/4.

CHART I/4

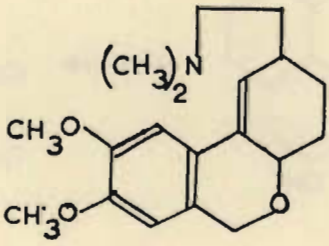


HOFMANN DEGRADATION OF LYCORENINE

The position of the nitrogen atom had not been determined with certainty and a further paper by Uyeo [18] and Taylor firmly established their structural proposals. The position of the nitrogen atom was ascertained from the ultra-violet absorption spectrum of the Hofmann degradation product of ∝-dihydrodeoxylycorenine (IV) which showed the presence of a double bond conjugated with the aromatic nucleus (V).



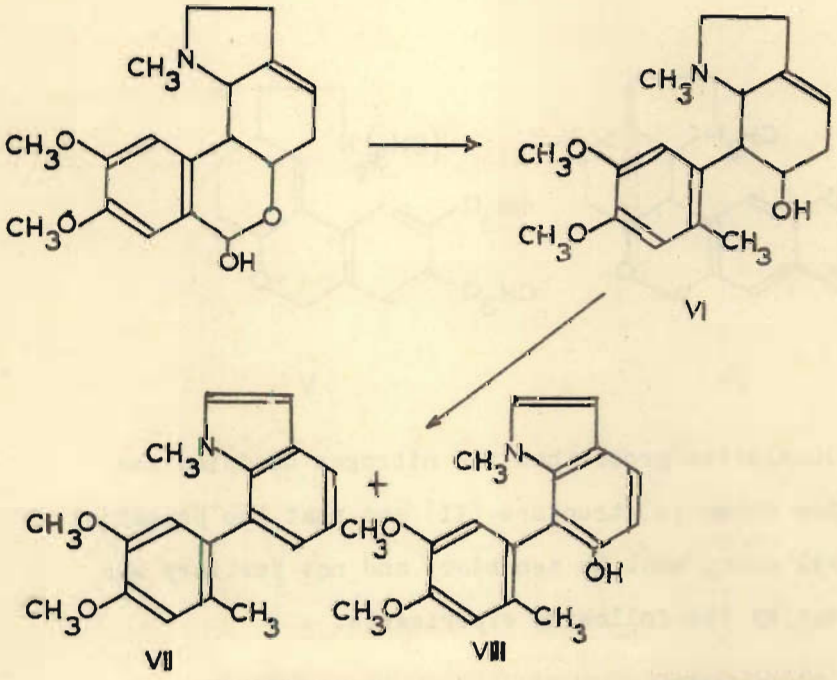
IV



V

Conclusive proof that the nitrogen occupies the position shown in structure (II) and that the potential hydroxyl group must be secondary and not tertiary was provided by the following experiments.

Wolff-Kishener reduction of lycorenine afforded the dehydrodeoxy compound (VI) which on dehydrogenation with palladium on charcoal gave two compounds. A neutral compound which exhibited typical indole colour reactions and analysed correctly for the expected 7-(3:4-dimethoxy-6 methylphenyl)-1-methylindole (VII). The second product was phenolic and also an indole and was assigned structure (VIII) on the basis of analytical data.

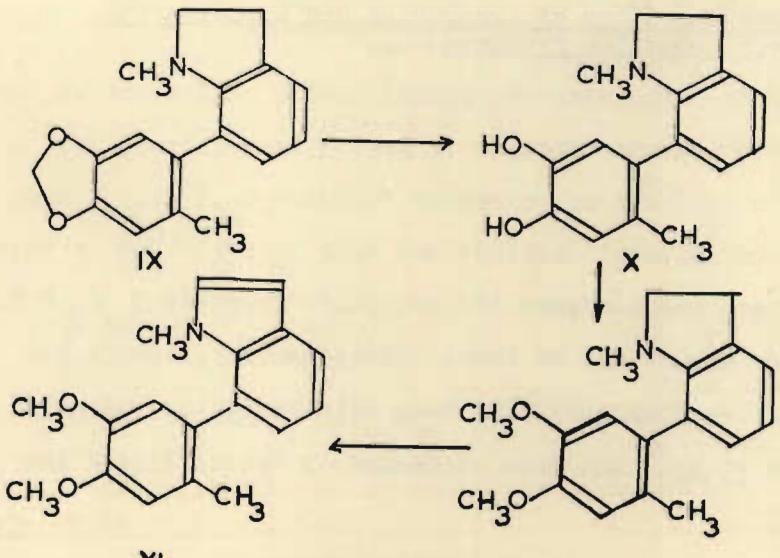


REACTIONS ESTABLISHING THE NATURE OF THE POTENTIAL HYDROXY GROUP AND THE POSITION OF NITROGEN ATOM IN LYCORENINE.

Thus Wenkert's structure, containing potential tertiary hydroxyl, may be excluded since it would be unlikely that a phenolic compound would be obtained unless some unusual rearrangement is involved.

The neutral product (VII) is closely related to the Ende reduction product of lycorine anhydrohydromethine (IX) which had been previously synthesised. Lycorine anhydrohydromethine with aluminium chloride gave a phenolic base (X) which in treatment with ethanol diazomethane followed by dehydrogenation gave a neutral product (XI) identical with that obtained from lycorenine (see Chart I/5).

CHART I/5



THE RELATIONSHIP BETWEEN LYCORINE AND LYCORENINE.

The position of the double bond in lycorenine and homolycorenine has been assigned by indirect methods. Stability of the double bond towards lithium aluminium hydride excludes it being $\alpha\beta$- to the nitrogen and since two isomers are produced on catalytic reduction it must be at least trisubstituted. The ultra-violet spectra eliminate the conjugated positions and unsaturation at positions 5,5a is untenable since deoxylycorenine does not have the properties of an enol ether. Of the two remaining positions 3a,4 was preferred to 3,3a by analogy with neronine and krigeine and its recent conversion to the lycorine type alkaloid pluviine has confirmed this assignment.

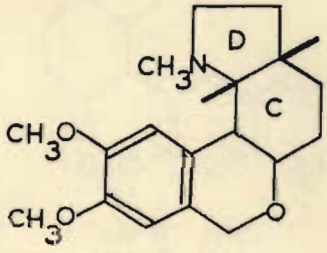
The Stereochemistry of Lycorenine and Homolycorine and their Reduction Products. ——

Kondo[19] studying the hydrogenation of lycorenine found that with Adams's catalyst in acetic acid it gave two epimeric products of molecular formular $C_{18}H_{25}O_3N$; when palladium charcoal catalyst was used they obtained a third base which was assigned the molecular formular $C_{18}H_{27}O_4N$.

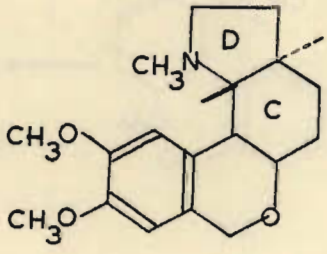
The structures of these hydrogenation products and their stereochemistry have been reinvestigated recently by Uyeo *et al.*[20], who have succeeded in rationalizing the

early work of Kondo. The structures of the two reduction products obtained when using Adams's catalyst became obvious when it was discovered that they could be obtained from the catalytic hydrogenation of deoxylycorenine which was known to be identical with anhydrotetrahydrohomolycorine.

Therefore they must be represented by the structures (XII) and (XIII)



XII

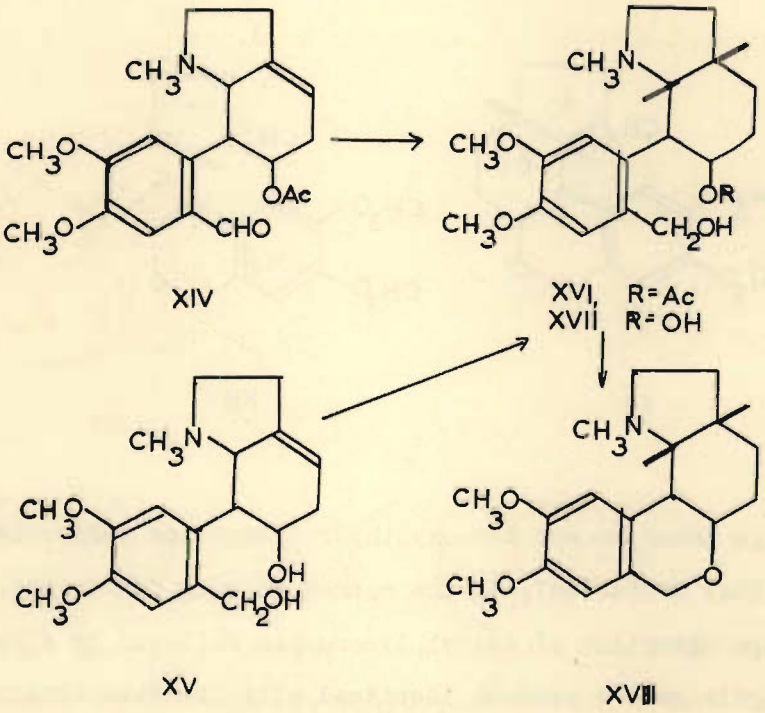


XIII

and were named ∝- and β-deoxydihydrolycorenine respectively since they differ only in the manner of ring C:D fusion.

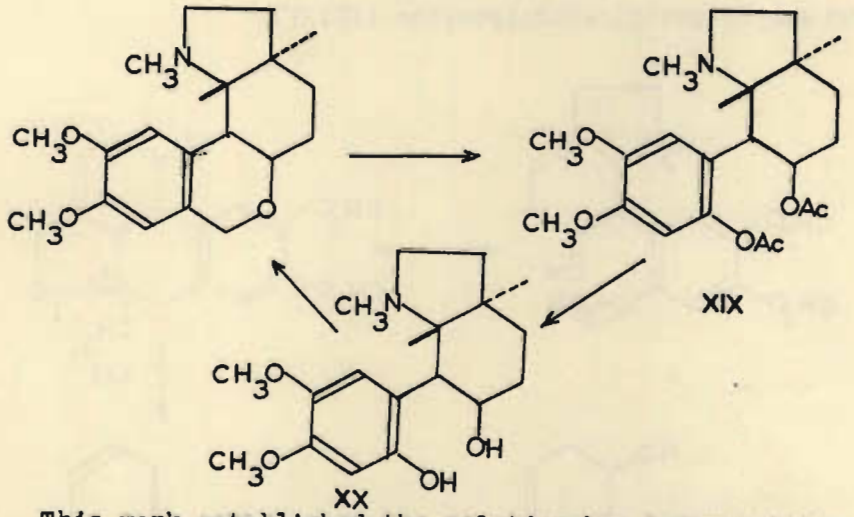
Hydrogenation of acetyl lycorenine followed by alkaline hydrolysis gave a product identical with the base obtained

from the hydrogenation of lycorenine with palladium-charcoal catalyst. On the basis of structure (XIV) for acetyl lycorenine the structures of the hydrogenation product and its hydrolysis product, which was also obtained by hydrogenating tetrahydrohomolycorine (XV) may be expressed as (XVI) and (XVII) respectively.



Treatment of the hexahydrohomolycorine (XVII) with dilute sulphuric acid gave ∝-deoxydihydro lycorine (XVIII). It must therefore possess the same manner of ring C:D fusion as in ∝-deoxydihydrolycorine and hence belong to the ∝-series.

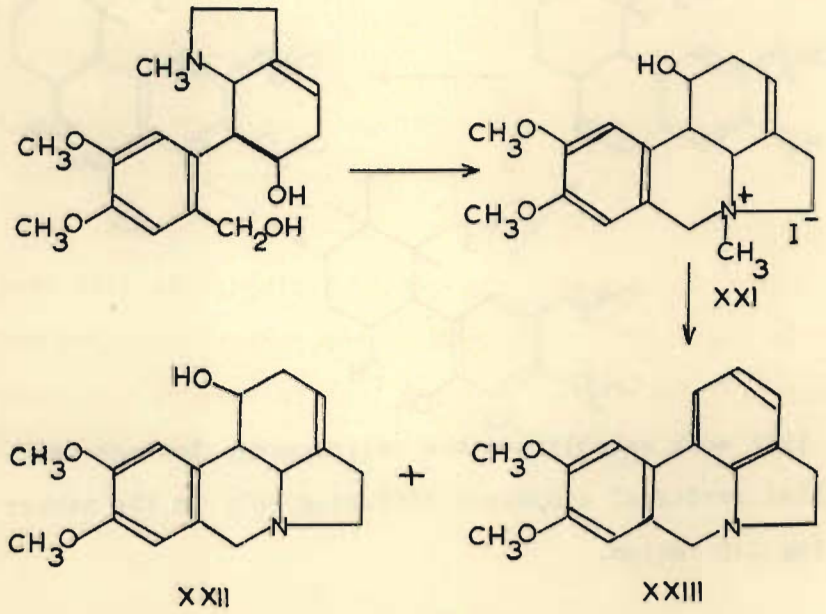
Corresponding compounds in the β-series were obtained by acetolysis of β-deoxydihydrolycorenine to the diacetate (XIX) and hydrolysis to β-hexahydrohomolycorine (XX). The latter compound reverted back to β-deoxydihydrolycorenine and treatment with dilute sulphuric acid.



This work established the relationship between two parallel series of compounds differing only in the manner of ring C:D fusion.

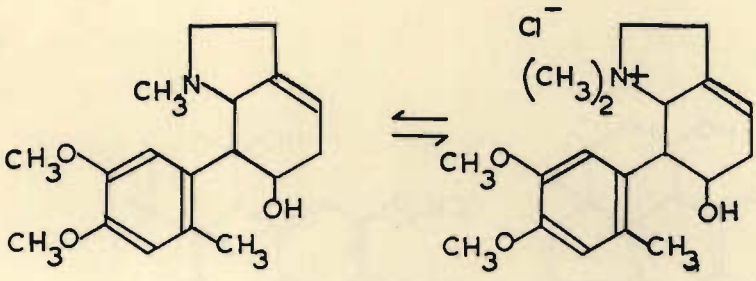
An interesting series of reactions were carried out on tetrahydrohomolycorine in which it was converted to the alkaloid pluviine which is based on the pyrrolo[de] phenanthridine skeleton. This conversion related both the structures and stereochemistry of lycorenine and homolycorine with pluviine.

Tetrahydrohomolycorine with toluene-p-sulphonyl chloride in pyridine furnished a quarternary salt, isolated as its crystalline iodide. This salt was diasterioisomeric with the known pluviine methiodide, since pyrolysis of the corresponding methochloride gave both pluviine (XXII) and anhydromethylpseudolycorine (XXIII).



XXII XXIII

The possibility of a change in structure and configuration during pyrolysis was ruled out since it was found that deoxolycorenine (XXIV) obtained by Wolff-Kishner reduction of lycorenine was regenerated from its methochloride (XXV) by distillation in high vacuum.

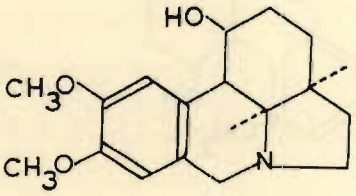


XXIV XXV

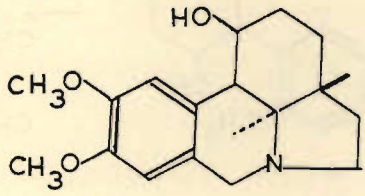
A similar series of reactions carried out with ∝- and β-hexahydrohomolycorine afforded ∝- and β-dihydropluviine respectively.

Since the preparations of ∝- and β-dihydropluviine from pluviine was exactly analogous to the preparation of ∝- and β-dihydrocaranine it was reasoned that in view of the close

similarity between pluviine and caranine the configurations of their reduction products should be comparable. On this basis ∝-dihydropluviine was represented by structure (XXVI) containing a cis C:D ring fusion as in ∝-caranine and β-dihydropluviine by structure (XXVII with the trans.C:D ring fusion in accord with the trans system in β-dihydro-caranine.



XXVI

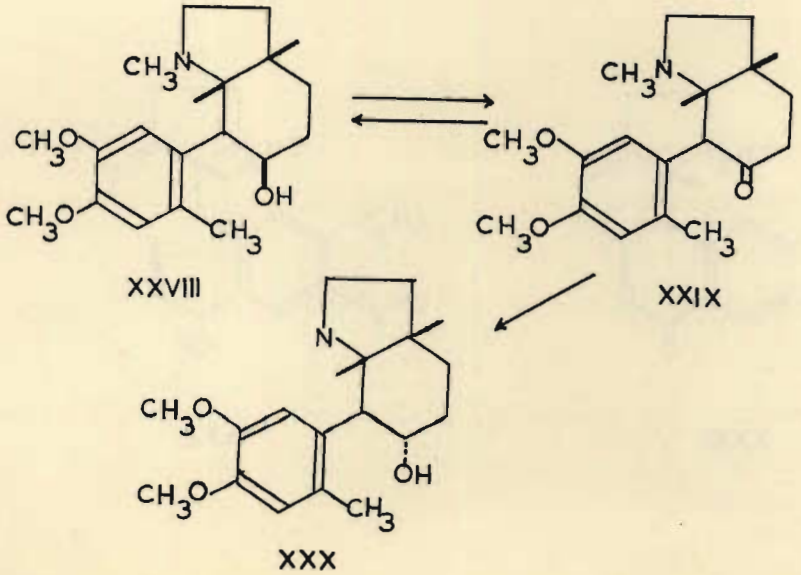


XXVII

The assignments for ∝- and β-dehydropluviine therefore defined the manner of ring C:D fusion in the ∝- and β-series of the reduction products of lycorenine and homolycorine as cis and trans respectively.

The nature of the ring B:C fusion in homolycorine and lycorenine was determined from a study of the oxidation

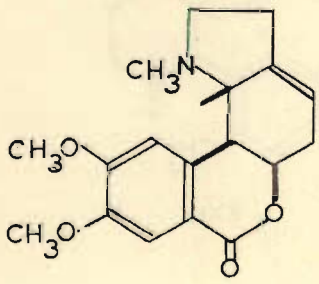
of ∝-deoxodehydrolycorenine (XXVIII) obtained catalytic reduction of deoxolycorenine (XXIX). The product of this oxidation was ∝-deoxodehydrolycorenone (XXIX) which on reduction with lithium aluminium hydride or sodium borahydride gave an epimer of ∝-deoxodehydrolycorenine (XXX). Catalytic reduction of ∝-deoxodehydrolycorenone with Adams's catalyst in an acid medium furnished ∝-deoxodehydrolycorenine. These results indicated that the hydroxyl group in ∝-deoxodehydrolycorenine possessed the axial configuration since metal-hydride reductions of cyclohexanones usually give the more stable equatorial alcohol and catalytic hydrogenation in acid medium the axial compound.



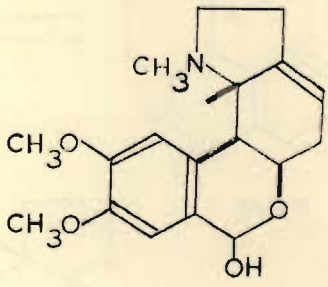
Thus the oxygen atom (in ring B) in lycoreneine is linked to ring C configuration by an axial bond. It is sterically impossible for two six-membered rings to be joined by two diaxial linkages and therefore the bond from the aromatic ring to ring C must be equatorial and necessitating a cis B:C junction.

Since a pair of diastereoisomers are produced on saturating the olefinic double bond in lycorenine and homolycorine the C-N bond must be equatorial otherwise only one product, the cis ring C:D isomer is possible.

These observations completed the relative stereochemistry of homolycorine and lycorenine and their structures are represented as (XXXI) and (XXXII) respectively.



XXXI

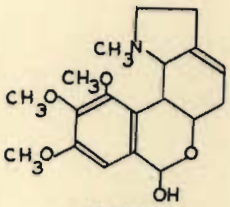


XXXII

CHART I/6

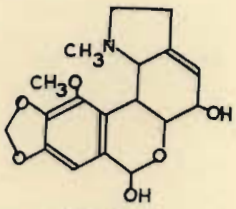
THE STRUCTURES OF THE LACTONE AND HEMI-ACETAL ALKALOIDS

LYCORENINE

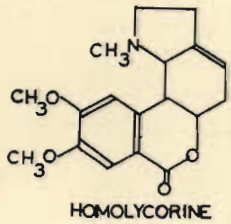


NERININE

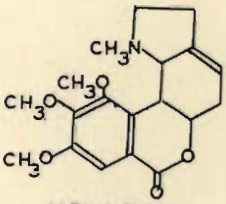
URECOLINE



KRIGEINE

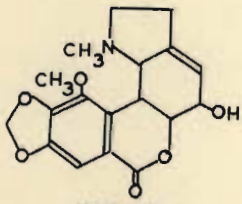


HOMOLYCORINE

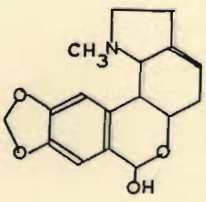


ALBOMACULINE

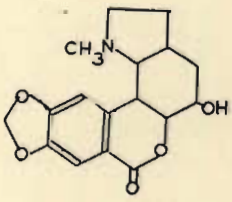
URMININE



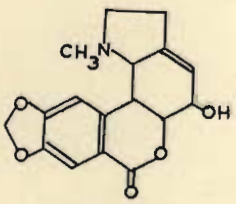
NERONINE



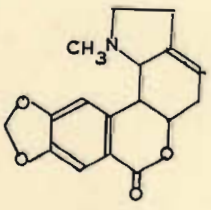
ODULINE



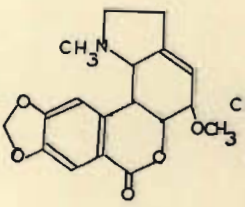
CLIVONINE



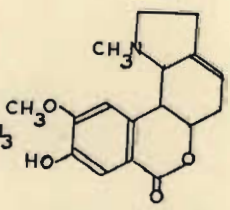
HIPPEASTRINE



MASONINE

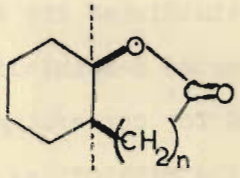


NIVALINE



9 DEMETHYLHOMOLYCORINE

The absolute configuration of the alkaloids have been proposed by Uyeo et al.[20], from a consideration of Klyne's extension of Hudson's Lactone rule. Klyne has shown that the molecular rotation of lactones of the general formular (XXXIII) are more positive than the corresponding hydroxy acids or their equivalents.



XXXIII

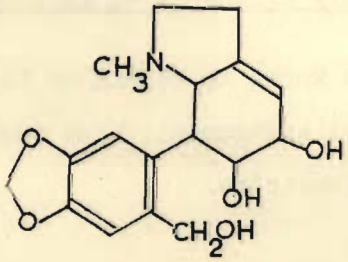
The application of this method to homolycorine and lycorenine indicated that they possess the absolute configuration as represented by the structures (XXXI) and (XXXII).

The Structure and Stereochemistry of the known Lactone and Hemi-acetal Alkaloids. —

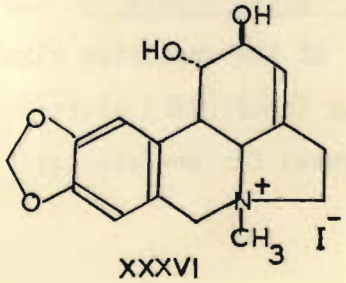
Of the remaining alkaloids known to belong to this group (Chart I/6.) stereochemical assignments have only been proposed for one alkaloid, hippeastrine.

HIPPEASTRINE.

Hippeastrine was characterised as a tertiary base $C_{17}H_{17}O_5N$ by Boit et al.[21] Analytical and spectral data revealed the presence of one hydroxyl group, a lactone function, a double bond, a methylenedioxy group and no methoxyl groups. These authors proposed structure (XXXIV) from a consideration of a possible biogenetic pathway originating from lycorine. Uyeo et al.[20], have confirmed this structure and established its stereochemistry by converting it to lycorine β-methiodide. The method was the same as that used for converting homolycorine (or lycorenine) to pluviine methiodide. Hippeastrine was reduced by lithium aluminium hydride to tetrahydrohippeastrine (XXXV). Quarterization of tetrahydrohippeastrine with toluene p-sulphonyl chloride and isolating the salt as the iodide gave a product identical with lycorine β-methiodide (XXXVI).

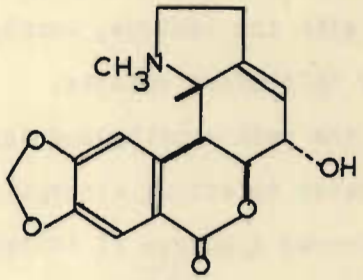


XXXV



XXXVI

The stereochemistry and absolute configuration of lycorine had been established (see Part I, section I) and hippeastrine was therefore represented as (XXXVII).



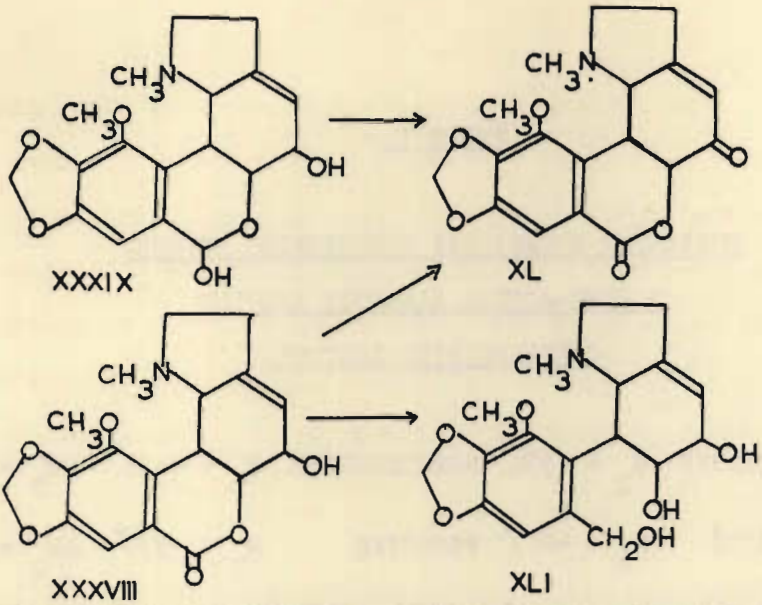
XXXVII

Of the remaining alkaloids of this group only Neronine and Krigeine will be discussed in detail since the plant Nerine krigeii[22] from which they were isolated has been investigated by the author.

Krigeine, $C_{18}H_{21}O_6N$ and Neronine $C_{18}H_{19}O_6N$ were isolated from Nerine krigeii and shown by analysis and spectral data to contain one methoxyl, one N-methyl group at least one hydroxyl group, methylenedioxy group and in the case of neronine a carbonyl group.

The ultra-violet spectrum of neronine with maxima at 228 mμ and 285 mμ and in conjunction with a carbonyl maximum in the infrared at 1706 $cm.^{-1}$ indicated that it was an aromatic δ-lactone. Confirmation of this was obtained when it was found that a solution of neronine in warm alkali could not be extracted with an organic solvent. In common with the lactone, homolycorine the hydrochloride was chloroform soluble.

The presence of the methoxymethylenedioxyphenyl chromophore was indicated by strong absorption at 1616 $cm.^{-1}$ in the infrared spectrum of tetrahydroneronine (XLI) obtained by lithium aluminium hydride reduction of neronine. Tetrahydroneronine was found to be a 1:2 glycol by periodate oxidation. Oxidation of neronine with manganese dioxide to an α,β-unsaturated ketone demonstrated that neronine must be an allyl alcohol. The above evidence was sufficient to assign neronine the structure (XXXVIII). Krigeine possessed only low intensity maxima in the ultra-violet spectrum, at 279 mμ and 287 mμ and the infrared spectrum showed the presence of methoxymethylenedioxyphenyl chromophore. Its structure was assigned as (XXXIX) on the basis of its oxidation with manganese dioxide to oxoneronine (XL).



The evidence for the structures of the remaining hemiacetal alkaloids is rather thin and is based on oxidation to the lactone and analytical data. Similarly little experimental data is available for the lactone types though they are readily identified by spectral means. The lactone alkaloids possess an unusual property in that their hydrochlorides are chloroform soluble.

The structures of urceoline and urminine are considered[23] as being stereoisomers of nerine and albomaculine respectively though the evidence is scant and in view of the rather unusual molecular rotational values reported for these alkaloids they should be viewed with reserve. (See Table I.).

TABLE I.

MOLECULAR ROTATIONAL DIFFERENCES BETWEEN A HEMI-ACETAL ALKALOID AND THE CORRESPONDING LACTONE.

LYCORENINE	$M_D = +570^\circ$	HOMOLYCORINE	$M_D = +368^\circ$	$\Delta M_D = -202^\circ$
KRIGEINE	$M_D = +813^\circ$	NERONINE	$M_D = +559^\circ$	$\Delta M_D = -254^\circ$
NERININE	$M_D = +538^\circ$	ALBOMACULINE	$M_D = +245^\circ$	$\Delta M_D = -293^\circ$
ODULINE	$M_D = +690^\circ$	MASONINE	$M_D = +421^\circ$	$\Delta M_D = -269^\circ$
URCEOLINE	$M_D = +625^\circ$	URMININE	$M_D = -34.5^\circ$	$\Delta M_D = -659^\circ$

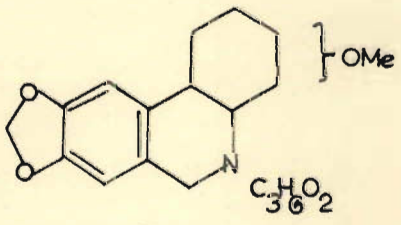
PART I.

SECTION III.

A REVIEW OF THE CHEMISTRY OF TAZETTINE.

THE STRUCTURE OF TAZETTINE.

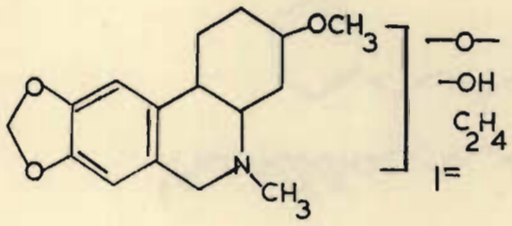
Tazettine is found to occur in many species of the Amaryllidaceae and has been widely studied throughout the world. It was first isolated by Spath and Kahovec in 1934 [24] and they made the first investigation of its chemistry. In addition to the isolation of hydrastic acid by permanganate oxidation and phenanthridine from zinc dust distillation of tazettine, Hofmann degradation of tazettine gave a methine which on further degradation afforded 6-phenylpiperonyl alcohol. On the basis of these results the methine was given a partial structure (I).



I

Further contributions to the chemistry of tazettine were made by Kondo, [25] in 1945, when the position of the methoxyl and the presence of an N-methyl group were

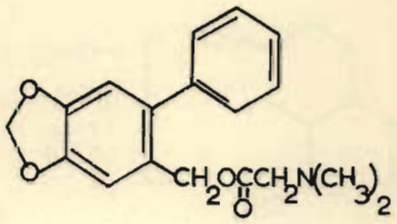
established. Shortly following this Clemo and Felton [26] established the presence of a hydroxyl group, a saturated ether linkage and an ethylenic double bond in tazettine; and a partial structure (II) was suggested.



II

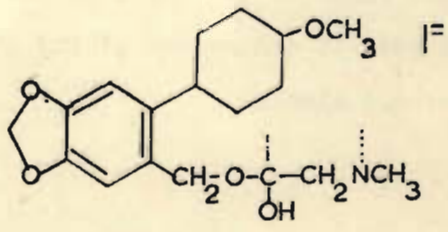
Considerable difficulty was experienced in attempts to elucidate the structure of tazettine methine and it was not until 1954 when Clemo and Hogarth [27] succeeded in preparing crystalline tazettine methine methiodide that any significant results were obtained. The tazettine methine methiodide was found to be very unstable and when heated with mineral acid it decomposed giving derivatives of 6-phenylpiperonyl alcohol.

Taylor and Uyeo [28] reinterpreted this work and showed that tazettine methine is 6-phenylpiperonyl-N,N-dimethyl glycinate (III) and confirmed this by synthesis.



III

The methine contains all the carbon atoms of tazettine except for that of the methoxyl lost in the aromatisation of ring C. Thus structure (II) may be extended to the partial structure (IV).

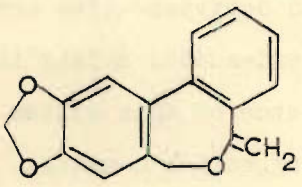


IV

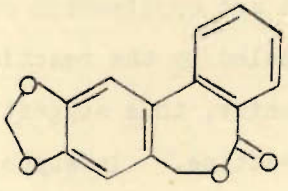
Although tazettine methine contains a carbonyl group, the infrared spectrum of tazettine showed no carbonyl absorption and it was concluded that the carbonyl was either masked or formed under Hofmann conditions. Tazettine was found [29] to undergo reduction with lithium aluminium hydride to tazettadiol, $C_{18}H_{23}O_5N$. Tazettadiol was found to have two hydroxyl groups which indicated that its formation from tazettine must have occurred by ring fission with the concomitant addition of hydrogen. Tazettadiol on treatment with hot mineral acid underwent dehydration to yield on ether, deoxytazettine. The reduction of tazettine by lithium aluminium hydride to a diol and cyclisation of the diol to deoxytazettine are paralleled by the reactions of the hemi-acetal moiety in lycorenine, thus suggesting the presence of this system in tazettine. In support of this suggestion O-methyl tazettine was found to be stable towards lithium aluminium hydride.

A study of the Hofmann degradation of deoxytazettine proved to be of great value in the elucidation of the structure of tazettine. It was found that in dilute mineral acid the true methine readily rearranges to yield an

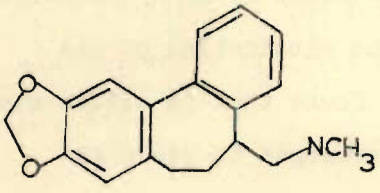
optically inactive neomethine. The structure of the neomethine was proved as follows. Hofmann degradation afforded a nitrogen free compound (V) which although it did not contain a carbonyl group gave a 2:4-dinitrophenylhydrozone. Oxidation of this compound (V) with potassium permanganate gave a lactone (VI) which could be oxidised further to 4,5-methylenedioxydiphenyl-2,2-dicarboxylic acid (VII). The structures of the last two compounds were confirmed by Ulmann syntheses. These results lead to the structure of neomethine as represented by structure (VIII).



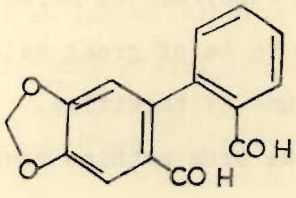
V



VI

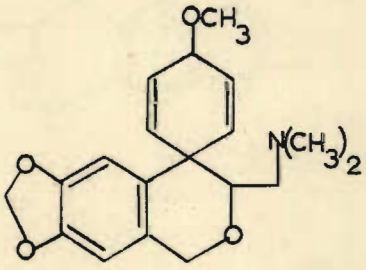


VIII

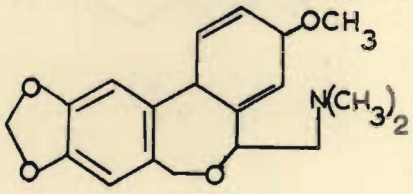


VII

On the basis of structure (VII) for the neomethine Taylor and Uyeo et al., considered that there were two possible structures (IX) and (X) for the true methine.



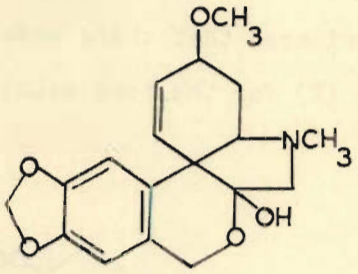
IX



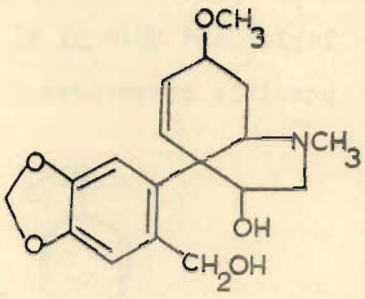
X

Structure (X) was critisised since it would be expected to aromatise with concomitant loss of methanol under the alkaline conditions of the Hofmann. Furthermore the true methine in aqueous acid affords 6-phenylpiperonyl alcohol in addition to the neomethine and this is also not explicable on the basis of structure (X).

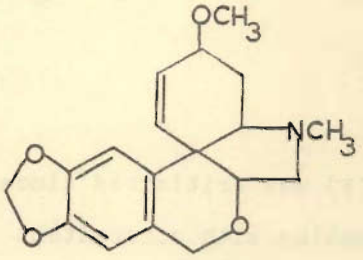
Structure (IX) for the methine indicates that structures (XI), (XII), and (XIII) may be assigned to tazettine, tazettadiol and deoxytazettine respectively.



XI



XII



XIII

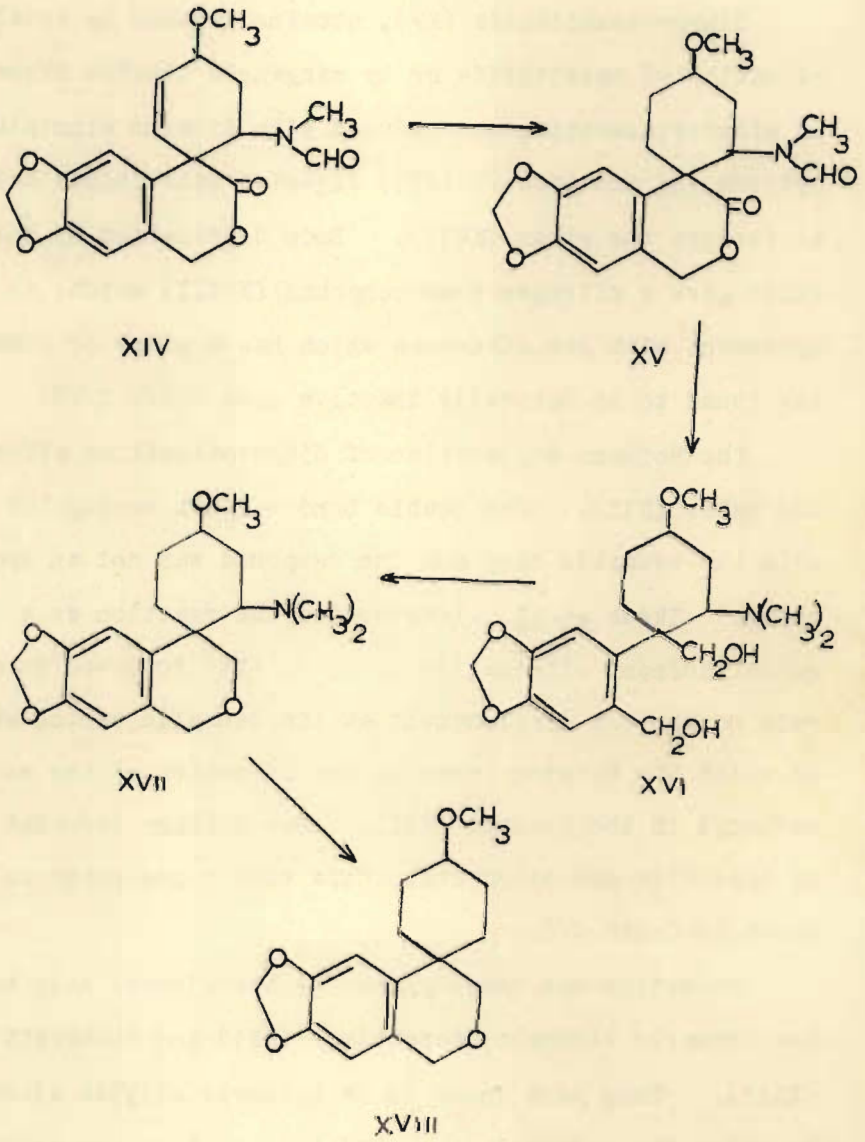
Wildman [30] had shown that tazettine on treatment with manganese dioxide gave a product, tazettamide (XIV) which was characterised as a lactone-amide. Degradation of this compound by Ikeda et al., provided additional evidence for their structural proposals for tazettine.

Dihydrotazettamide (XV), obtained either by catalytic reduction of tazettamide or by manganese dioxide oxidation of dihydrotazettine, was reduced with lithium aluminium hydride and the product (XVI) digested with dilute acid to furnish the ether (XVII). Emde degradation of the ether gave a nitrogen free compound (XVIII) which, in agreement with its structure which has a plane of symmetry, was found to be optically inactive (see Chart I/7).

The Hofmann degradation of dihydrotazettine afforded the ester (XIX). The double bond was not conjugated with the aromatic ring and the compound was not an enol ether. Ikeda et al., interpreted the reaction as a normal Hofmann elimination to yield (XX) followed by a rate controlled displacement at the benxylic carbon atom in which the driving force is the formation of the ester carbonyl in the product (XIX). The Hofmann degradations of tazettine and dihydrotazettine were represented as shown in Chart I/8.

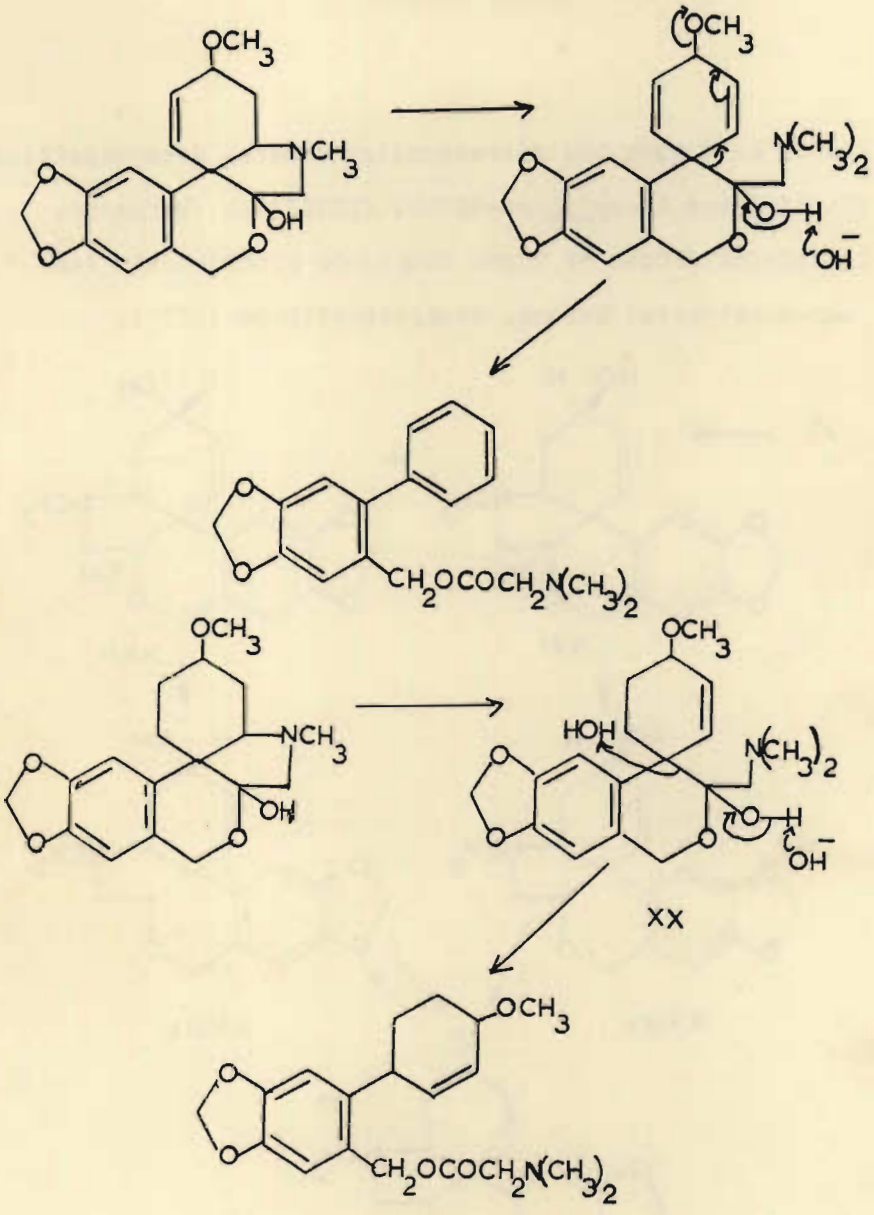
Tazettine was demethylated by hot mineral acid to two isomeric alcohols, tazettinol (XXI) and isotazettinol (XXII). They were shown to be epimeric allylic alcohols in which the methoxyl group had been replaced by hydroxyl. Reduction of both tazettinol and isotazettinol with lithium aluminium hydride followed by cyclisation with

CHART I/7



DEGRADATION OF TAZETTAMIDE TO THE EMDE BASE (XVIII)

CHART 1/8

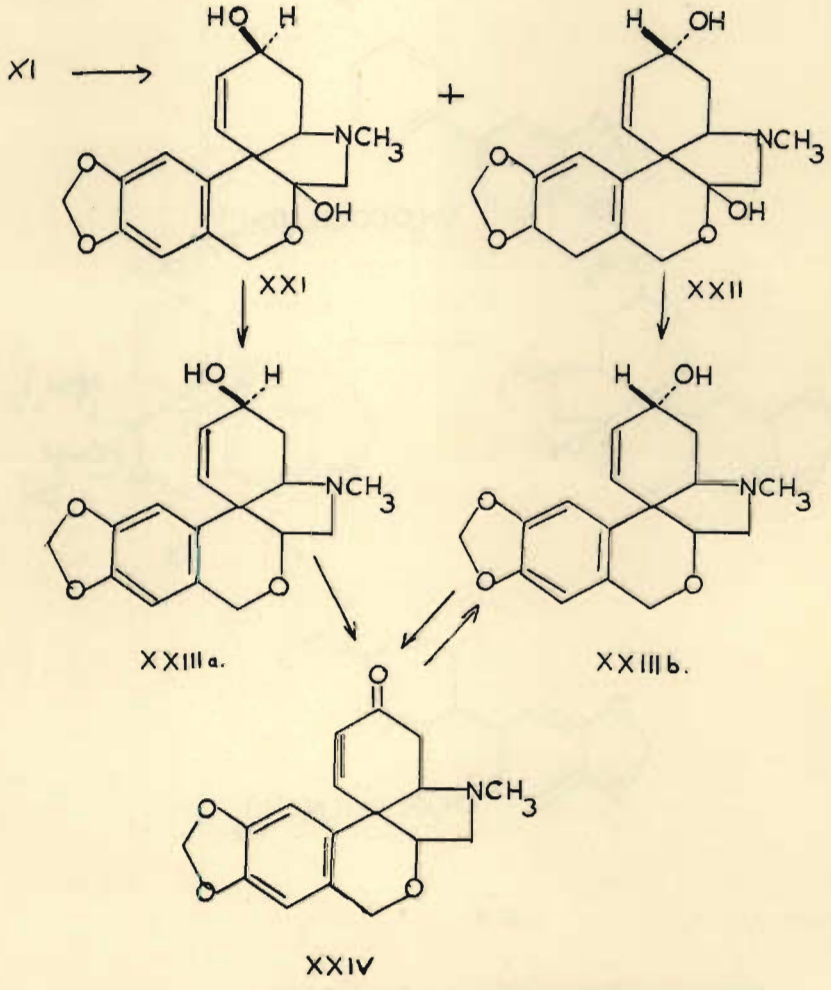


XX

XIX

HOFMANN DEGRADATION OF TAZETTINE AND DIHYDROTAZETTINE.

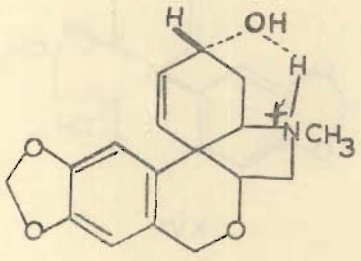
dilute acid gave the corresponding ethers, deoxytazettinol (XXIIIa) and deoxyisotazettinol (XXIIIb). Manganese dioxide oxidation of these compounds afforded the same $\alpha\beta$-unsaturated ketone, deoxytazettinone (XXIV).



XXIV

Metal hydride reduction of deoxytazettinone gave as the major product deoxy*iso*tazettinol. This result, in conjunction with the observation that *iso*tazettionol is the major product from the demethylation of tazettine, indicated that the hydroxyl group in the *iso*-series is in the thermodynamically more stable position i.e., equatorial.

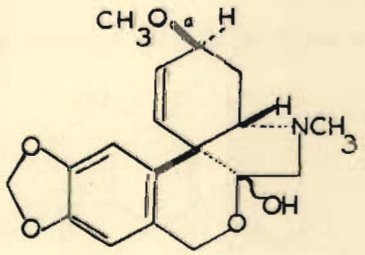
Further information on the stereochemistry of the molecule was obtained from a study of the dissociation constants of deoxytazettinol and deoxy*iso*tazettinol. Deoxy*iso*tazettinol was found to be the stronger base and this was attributed to hydrogen bonding of the proton in the conjugate acid with the hydroxyl group (XXV).



XXV

This requires that the substituents in ring C in deoxy*iso*tazettinol are *cis*. Therefore in tazettine the

C_{4a}-N bond is <u>trans</u> to the C_3-methoxyl and the ready elimination of the amino side chain in tazettine and its derivatives suggests that it is axial. The above requirements necessitate that the five membered D ring is <u>cis</u> fused to ring C. A comparison of the molecular rotations of compounds in the normal-series (tazettine) with those in the <u>iso</u>-series showed that those of the <u>iso</u>-series were always more positive and on the basis of Mills's rule tazettine was assigned the partial stereo-structure (XXVI).

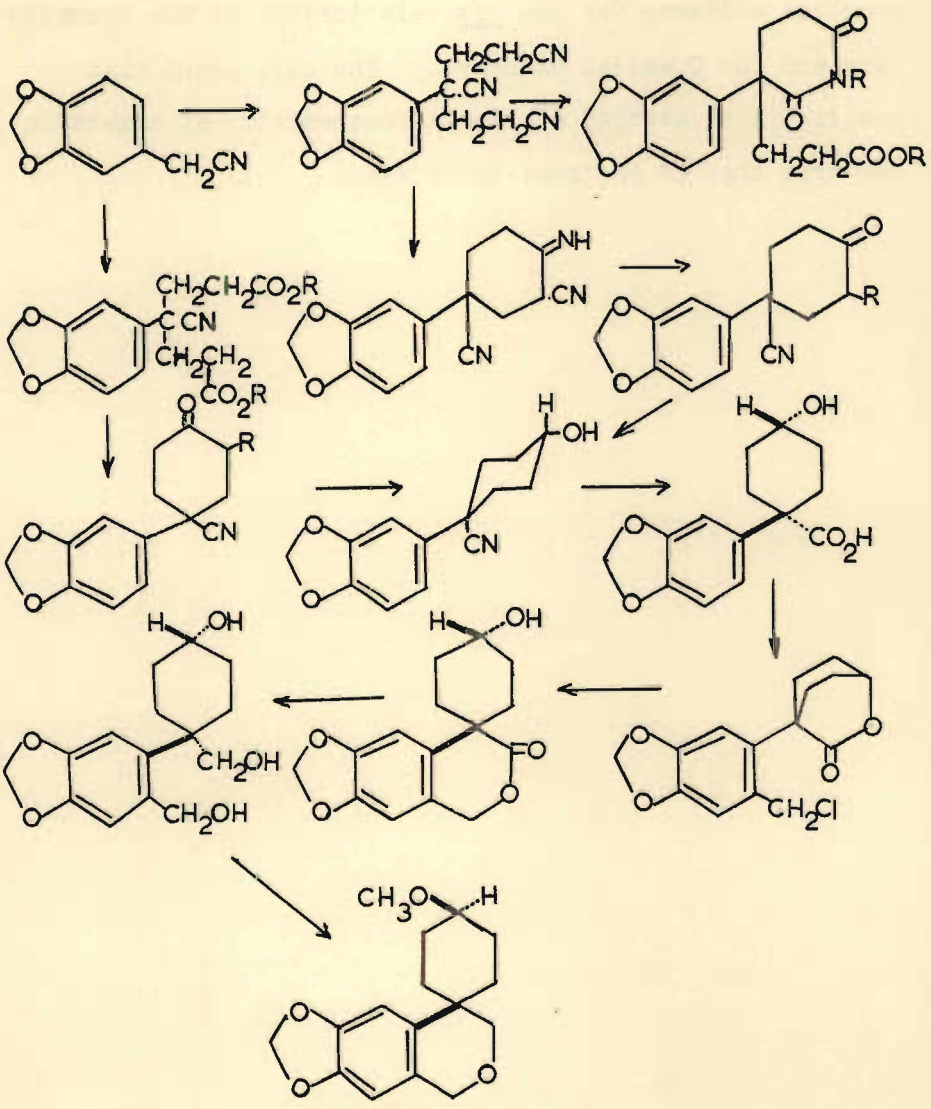


XVI

A stereospecific synthesis [31] of the Emde degradation product (XVIII) has provided confirmation regarding certain aspects of the stereochemistry of tazettine

proposed earlier.[29] The synthesis (see Chart I/9) provides evidence for the cis relationship of the aromatic ring and the C_3-axial methoxyl. The only point that remains to be settled on the stereochemistry of tazettine concerns that of the hemi-ketal ring.

CHART I/9



STEREOSPECIFIC SYNTHESIS OF THE EMDE BASE.

PART I.

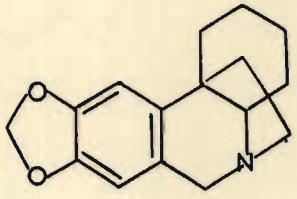
SECTION IV.

A REVIEW OF THE CHEMISTRY OF ALKALOIDS CONTAINING THE 5:10b-ETHANOPHENANTHRIDINE SKELETON.

The alkaloids of this group have all been characterised since 1956 [32] and are based essentially on the basic ring system, 5:10b-ethanophenanthridine. The alkaloids are elaborated from both enantiomorphs of this basis system and stem from variations in aromatic and aliphatic substitution.

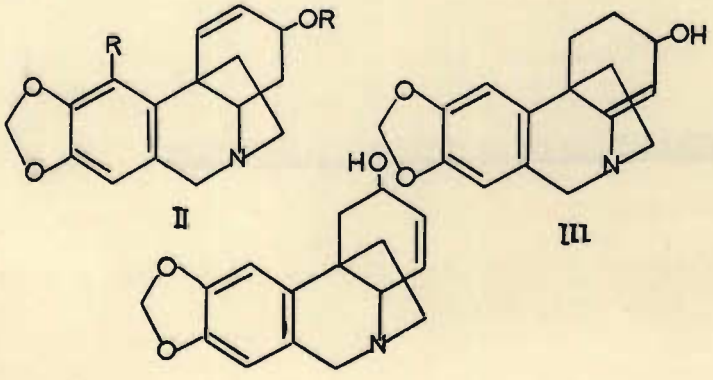
The ring system was demonstrated first for the alkaloid crinine in 1956.[32] Crinine was shown to be isomeric with caranine and to contain the same functional groups, viz., one methylenedioxyphenyl group, one hydroxyl and one aliphatic double bond. The double bond was neither conjugated with the aromatic ring nor contiguous with the hydroxyl function. Reagents, such as selenium dioxide and mercuric acetate, which are now known to oxidise alkaloids possessing the pyrrolo[de] phenanthridine ring system, were without effect on crinine. Furthermore the alkaloid was not dehydrogenated by palladium-on-charcoal at 200°. Wildman suggested that the most probable explanation for the stability of crinine to these reagents was that it possessed a spiro ring system since ring C in alkaloids of the lycorine type become aromatic under the mildest conditions. Proof

of this suggestion was obtained by degrading crinine, (Chart I/10), to the basic skeleton, (-)-crinane, which was shown to be 5:10b-octahydrophenanthridine (I) by synthesis of the racemic base (see Chart I/11).



I

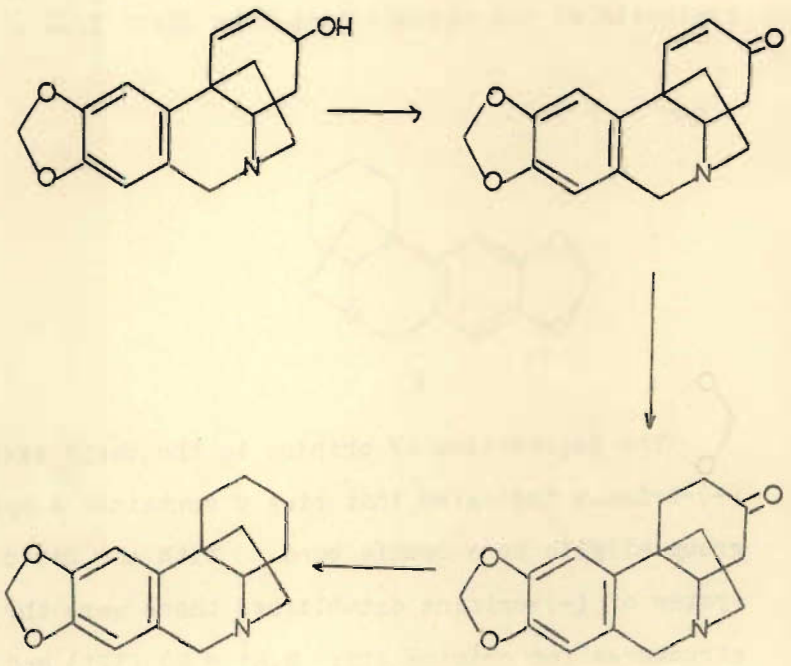
The degradation of crinine to the basic skeleton (-)-crinane indicated that ring C contained a hydroxyl group allylic to a double bond. With the basic ring system of (-)-crinane established there were three possible structures for crinine (II; R,R^1 = H) (III) and (IV).



II III

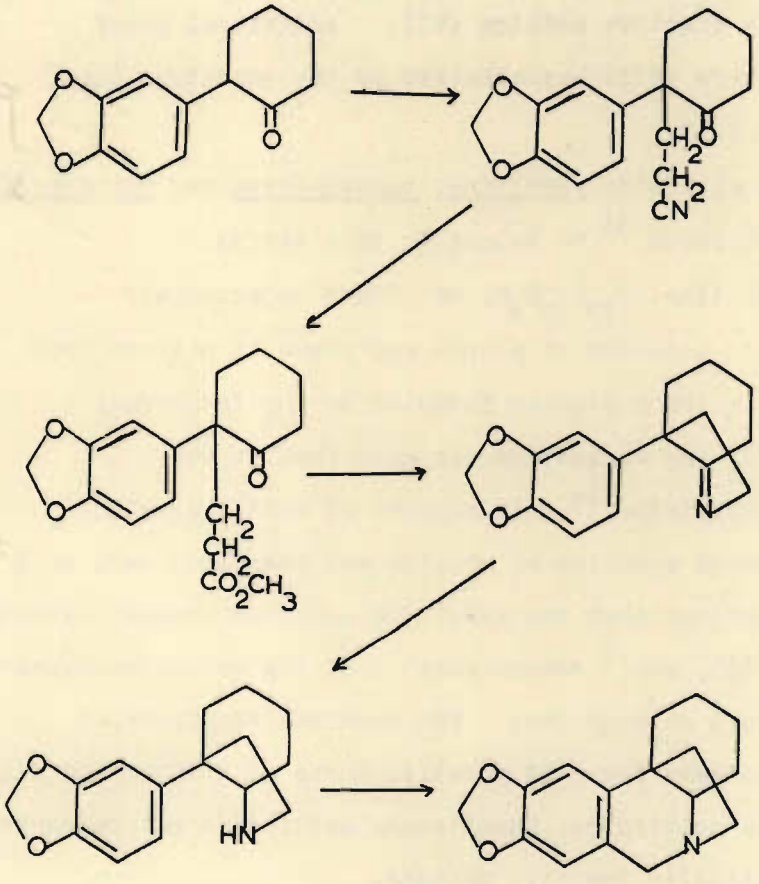
IV

CHART I/10



DEGRADATION OF CRININE TO (-)-CRINANE.

CHART I/11



SYNTHESIS OF (±)-CRINANE

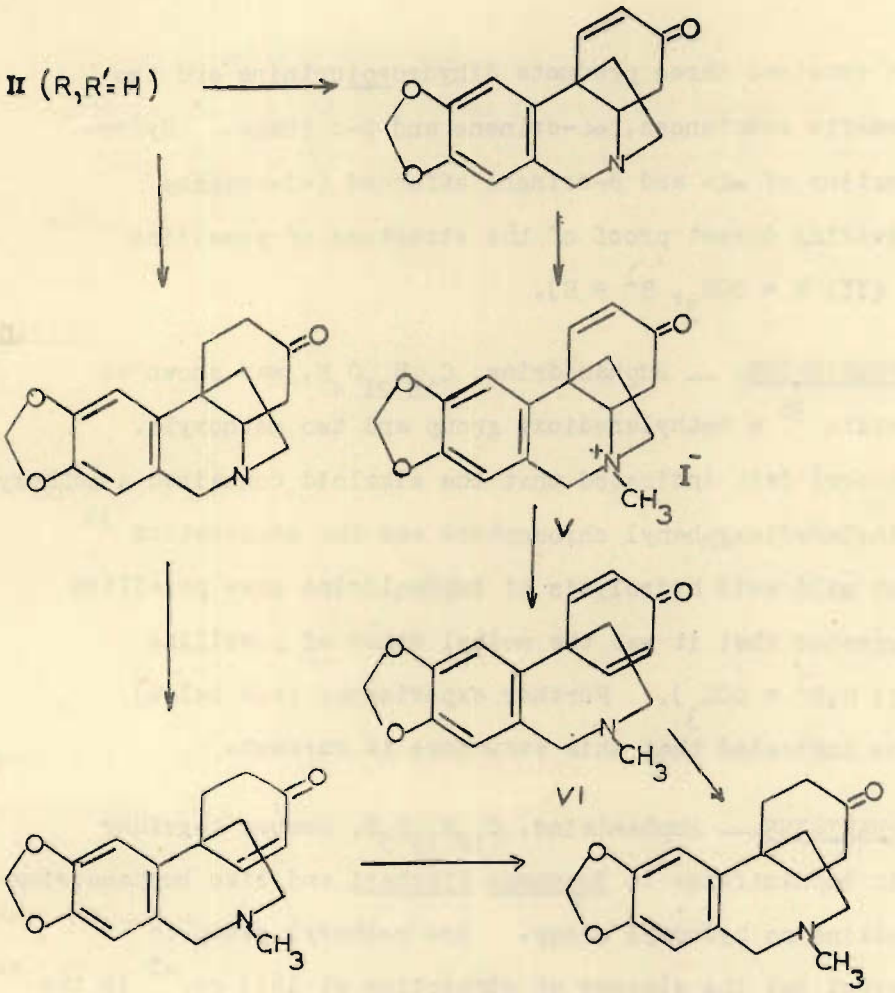
Structures (III) and (IV) were eliminated when it was found that crinenone methiodide (V) afforded an optically inactive methine (VI). Additional proof of structure (III) was obtained by the reactions shown in Chart I/12.

The alkaloids powelline, buphanadrine and buphanisine were soon shown [33] to belong to this series.

Powelline, $C_{17}H_{19}O_4N$, was found to accompany crinine in a number of plants and since it differs from crinine in the molecular formular by the functional group OCH_3 the suggestion was made that it was ar-methoxycrinine.[34] In support of this suggestion the infrared spectrum of crinine and powelline were very similar except that the powelline spectrum showed a strong band at 1613 $cm.^{-1}$ characteristic of the methoxymethylene-dioxyphenyl chromophore. The chemical reactions of powelline were found to parallel those of crinine and like crinenone methiodide, powellenone methiodide was converted to an optically inactive methine.

The reagent, sodium and amyl alcohol, was reported by Clayson [35] to demethoxylate hydrocotarnine and give hydrohydrastine in good yield. Applying this reaction to powelline Wildman did not obtain crinine as expected

CHART I/12



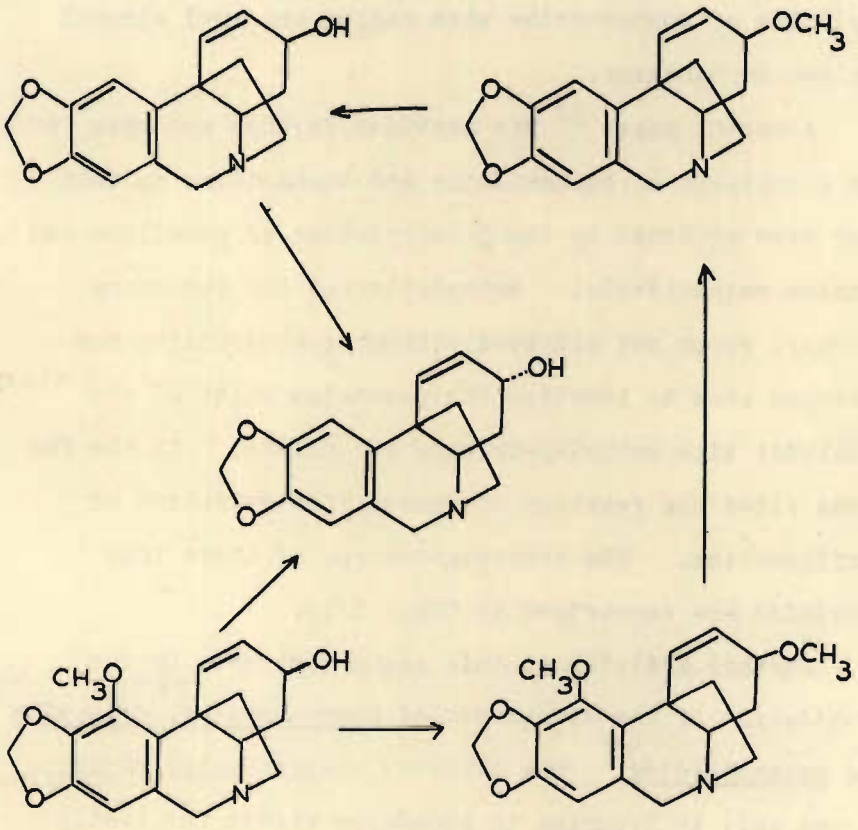
REACTIONS OF CRININE

but obtained three products dihydroepicrinine and two isomeric substances, ∝-crinene and β-crinene. Hydrogenation of ∝- and β-crinene afforded (-)-crinane providing direct proof of the structure of powelline as (II; R = OCH_3, R^1 = H).

BUPHANIDRINE. — Buphanidrine, $C_{18}H_{21}O_4N$, was shown to contain [36] a methylenedioxy group and two methoxyls. Spectral data indicated that the alkaloid contained a methoxymethylenedioxyphenyl chromophore and the observation [33] that mild acid hydrolysis of buphanidrine gave powelline suggested that it was the methyl ether of powelline (II; R,R^1 = OCH_3). Further experiments (see below) have indicated that this structure is correct.

BUPHANISINE.— Buphanisine, $C_{17}H_{19}O_5N$, occurs together with buphanidrine in Boophone fischeri and like buphanadrine contains no hydroxyl group. One methoxyl group is present but the absence of absorption at 1613 $cm.^{-1}$ in the infrared spectrum indicates that it is not aromatic. The structure became apparent when it was found [33] that buphanisine gave crinine on acid hydrolysis and it was formulated as O-methyl crinine (II; R = H,R^1 = OCH_3).

CHART I/13



INTERRELATIONSHIP OF CRININE, POWELLINE, BUPHANADRINE AND BUPHANISINE.

Support for this structure was obtained when the ar-demethoxylation of buphanadrine with sodium and amyl alcohol yielded buphanisine.

A recent paper [37] has provided further evidence for the structures of buphanidrine and buphanisine in that they were obtained by the O-methylation of powelline and crinine respectively. Methylation of the secondary hydroxyl group was achieved without quarternizing the nitrogen atom by treating the potassium salts of the alkaloids with methyl-p-toluene sulphonate. In the few cases cited the reaction proceeded with retention of configuration. The interconversions of these four alkaloids are summarised in Chart I/13.

Further addition to this group were made by the elucidation of the structures of haemanthamine [38,39], crinamine [39] and haemanthidine [40]. The alkaloid haemanthamine ranks second only to lycorine in abundance within the family of the Amaryllidaceae. Crinamine is a rare alkaloid and is usually present in only minor quantities in the few plants from which it has been isolated. Both crinamine and haemanthamine possess the molecular formular $C_{17}H_{19}O_4N$ and contain one methoxyl, one hydroxyl, one

one methylenedioxy group and an ethylenic double bond. Spectral data indicate that the double bond is not conjugated with the aromatic ring and the methoxyl is not present as a methoxymethylenedioxyphenyl group in either alkaloid.

Manganese dioxide and selenium dioxide were without effect on haemanthamine, suggesting it was neither an allylic alcohol nor derived from the pyrrolo[de]phenanthridine skeleton.

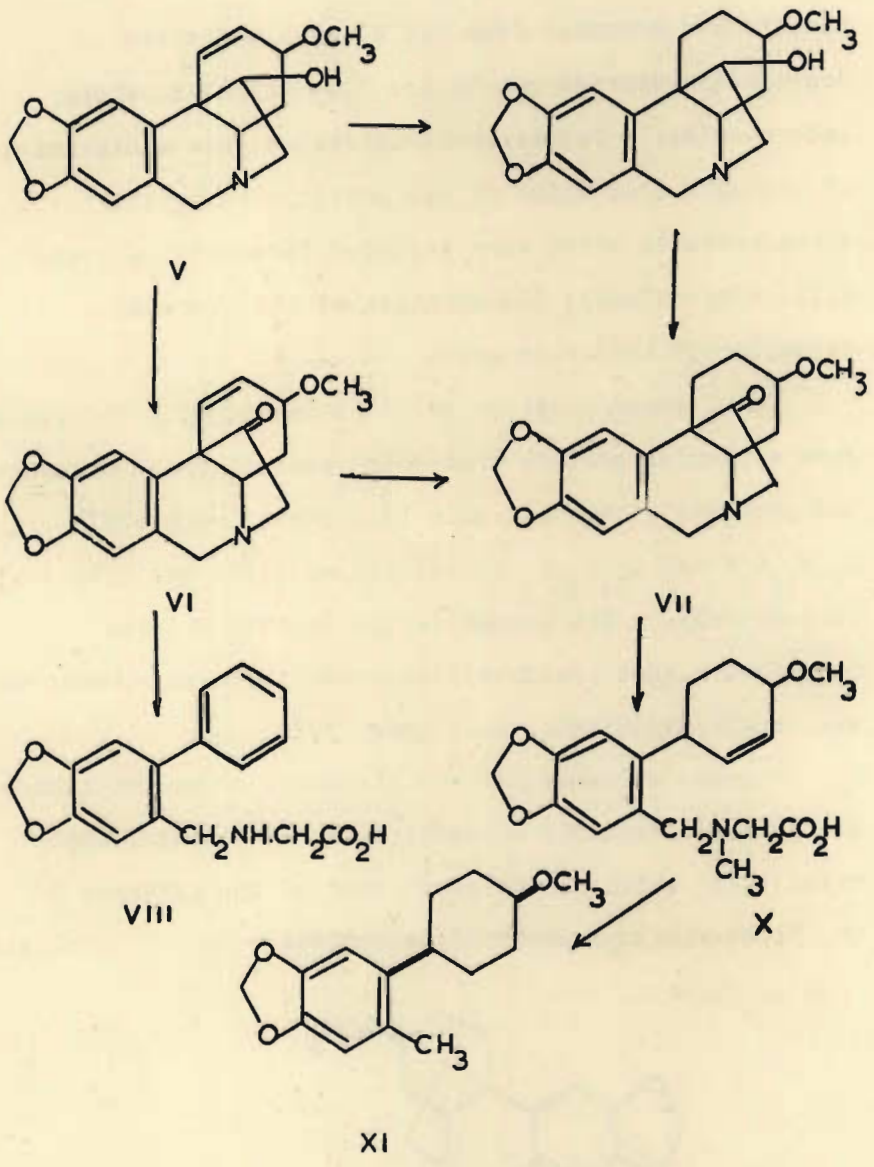
Furthermore O-acetyldihydrohaemanthamine was stable to oxidation with potassium permanganate solution.

Similar conditions oxidise acetyldihydro derivatives of the lycorine type to lactams. These results provided strong evidence for the presence of a spiro ring system in haemanthamine.

Oxidation of haemanthamine (V) with the chromium trioxide-pyridine reagent gave oxohaemanthamine (VI) which from its infrared spectrum obviously contained a ketone function in a five membered ring. Spectral data indicated that the carbonyl was not conjugated with either the double bond or the aromatic ring.

Oxodihydrohaemanthamine (VII) was prepared both by oxidation of dihydrohaemanthamine or reduction of the double bond in oxohaemanthamine. The alkaline degradation products of oxohaemanthamine and oxodihydrohaemanthamine gave valuable information regarding the structure (V) for haemanthamine. Oxohaemanthamine with potassium t-butoxide in t-butanol gave the amino acid (VIII) the structure of which was confirmed by synthesis. Under similar conditions oxodihydrohaemanthamine did not react but the methiodide when warmed with alkali gave a product (X) which after hydrogenation-hydrogenolysis was identical to a substance (XI) obtained by the reduction of the Hofmann degradation product of dihydrotazettine. The cis relationship of the methoxyl group and the aromatic nucleus in haemanthamine is evident from this last reaction since it has been established in tazettine by synthesis.

The degradative evidence presented above is sufficient to assign haemanthamine the structure (V).

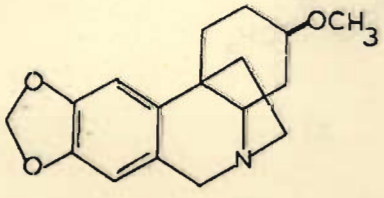


THE DEGRADATION OF HAEMANTHAMINE

Additional evidence for the structure of haemanthamine was advanced from the elegant oxidative degradation carried out by Dr. W.G. Wright[41] in these laboratories. Permanganate oxidation gave a mixture of products from which it was possible to isolate three products which were assigned formulars (XII and XIII) respectively, illustrative of the stepwise oxidation to hydrastic acid.

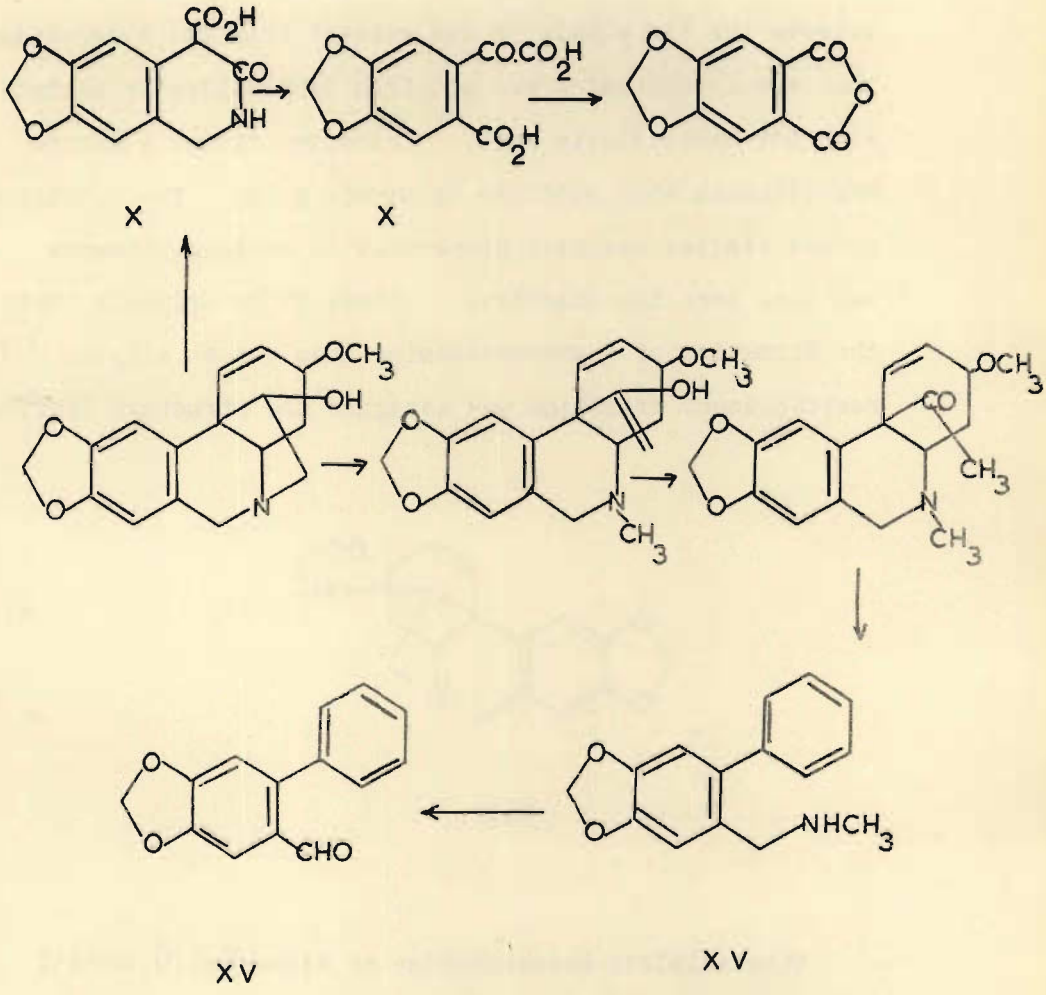
The Hofmann reaction which proceeded with difficulty gave a complex mixture from which amongst other unexpected products it was possible to isolate two products $C_{15}H_{15}O_2N$ and $C_{14}H_{10}O_3$ formulated as (XIV) and (XV) respectively. The aromatisation in this Hofmann degradation, not previously observed in these structures, was neatly explained. (See Chart I/14).

Further evidence for the structure of haemanthamine was obtained from its conversion to (+)-dihydrobuphanisine (XVI) which constitutes proof of the presence of the 5:10b-ethanophenanthridine nucleus.



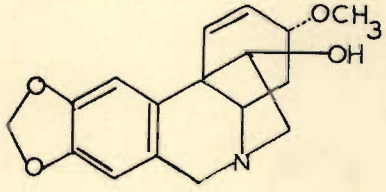
XVI

CHART 1/14



THE OXIDATION AND HOFMANN DEGRADATION OF HAEMANTHAMINE.

Crinamine is isomeric with haemanthamine and contains the same functional groups. The close relationship between the two alkaloids was evident from the observation that apohaemanthamine was obtained by treating crinamine with hot hydrochloric acid. Crinamine formed a ketone oxocrinamine when oxidised by chromic acid. The substance showed similar spectral properties to oxohaemanthamine but they were not identical. Since it is unlikely that the formation of apohaemanthamine involves an allylic rearrangement crinamine was assigned the structure (XVII).



XVII

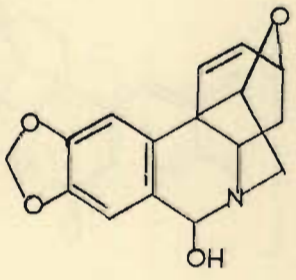
(The alkaloid haemanthidine is discussed in detail in view of its relationship to one of the new alkaloids isolated and characterised by the author).

Haemanthidine, $C_{17}H_{19}O_5N$, was shown to contain one

methoxyl group one ethylenic double bond and a methylenedioxy group. Acetylation of the alkaloid gave a compound which was formulated by Boit[42] as an O:N diacetate. Methylation of haemanthidine with methyl iodide or formaldehyde and formic acid followed by basification of the methiodide gave tazettine.

This result lead both Wildman[43] and Boit[42] to postulate that haemanthidine was de-N-methyl tazettine.

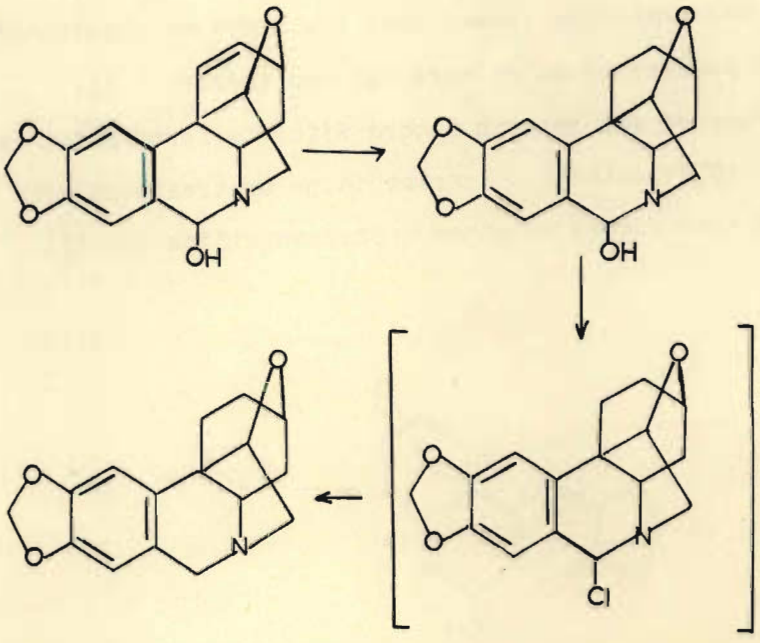
A further examination[40] of the acetylation product of haemanthidine showed that the infrared spectrum did not possess an amide carbonyl and therefore its structure was more in accord with its formulation as an O:O diacetate. Haemanthidine on treatment with hot acid formed an ether apohaemanthidine (XVIII).



XVIII

This compound contained one hydroxyl group, a double bond but no methoxyl. This reaction paralleled the formation of apohaemanthamine from haemanthamine and crinamine and suggested that some structural relationship would be found in common to these bases. Confirmation of this was obtained from the conversion of apohaemanthidine to dihydroapohaemanthamine as shown in Chart I/15.

CHART I/15

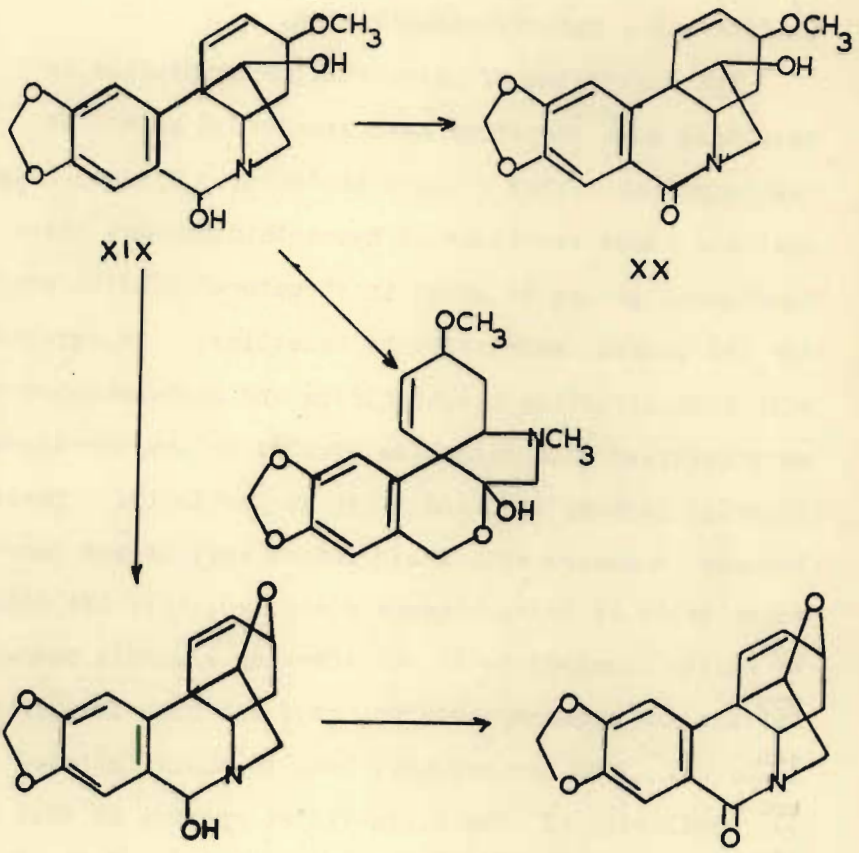


CONVERSION OF APOHAEMANTHIDINE TO APOHAEMANTHAMINE

The latter reaction established that haemanthidine contained the crinane nucleus and suggested that haemanthidine was a hydroxyhaemanthamine.

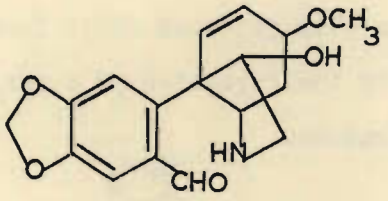
The conversion of haemanthidine methiodide to tazettine must therefore have involved a molecular rearrangement of the crinane skeleton. Wildman suggested that the above reactions of haemanthidine must place its functional groups as shown in structure (XIX) to account for its unusual conversion to tazettine. In agreement with this structure haemanthidine and apohaemanthidine were oxidised with manganese dioxide to the corresponding bicyclic lactams (XX) and (XXI) respectively. These lactams 'n accord with their unique environment behave essentially as amino-ketones since a), they are reduced by sodium borohydride to the starting alcohols haemanthidine and apohaemanthidine, b), the high frequency (1700 $cm.^{-1}$) of the carbonyl band in their infrared spectra, c), similarity of the ultra-violet spectra to that of 6,7-methylenedioxy-1-tetralone.

Wildman[44] has pointed out that haemanthidine might be expected to exist in equilibrium with the open chain and hemi-acetal forms (XXII; R = H and XXIII; R = H_3 respectively).



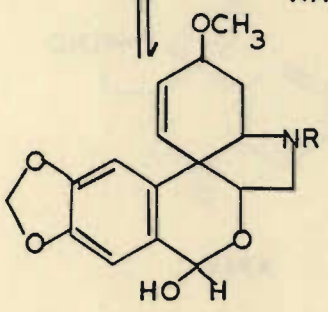
REACTIONS OF HAEMANTHIDINE

XIX ⇌



XXII

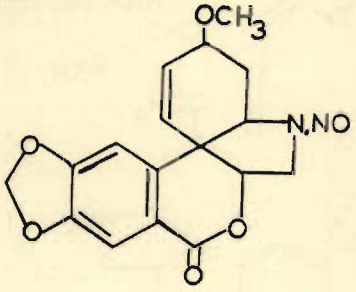
⇌



XXIII

The absence of a carbonyl maxima in the infrared and normal methylenedioxyphenyl ultra-violet spectrum would seem to exclude these structures. However, they are almost certainly present as reaction intermediates under certain conditions since haemanthidine reacts with nitrous acid to form the N-nitroso derivative of the hemi-acetal form (XXIII: R = NO). In support of this structure it was oxidised to the lactone (XXIV) by manganese dioxide. In acid solution it existed in

equilibrium with it open chain form (XXII; R = NO) as evidenced by the formation of a conjugated 2:4 dinitrophenyl hydrazone.



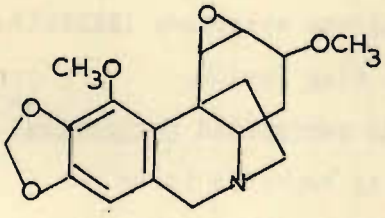
XXIV

The N-nitroso derivative like haemanthidine underwent alkaline oxidation-reduction to form N-nitrosonortazettine. This result indicates that the oxidation-reduction reactions of haemanthidine(and 3-epihaemanthidine) require neither a basic nitrogen atom nor the crinane ring system. From these results* it was suggested that the rearrangement was intramolecular and may proceed by hydride transfer.

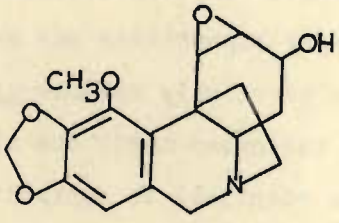
*This data was not available when Part II of this thesis was written. It does however support the postulates made regarding the intermediates in the proposed mechanism for the rearrangement of 3-epihaemanthidine to epitazettine.

The alkaloid undulatine [45] has been shown to possess a structure (XXV) containing a 1,2-epoxide ring. Most recently two more alkaloids crinamidine [44] (XXVI) and flexinine [44] (XXVII) have also been shown to contain a 1,2-epoxide bridge. Previously Boit et al., [46] had suggested that crinamidine was 4-hydroxy powelline on the basis of a), the resemblence of the infrared spectra of crinamidine and powelline, b), the formation of mono- and diacetyl crinamidine. With the structures of undulatine fairly rigidly established by Warnhoff [45] et al., the structure of crinamidine became evident since on O-methylation it afforded undulatine.

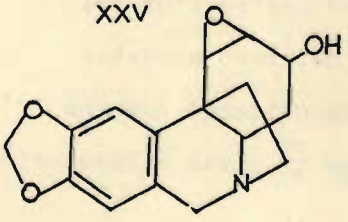
ar-demethoxylation of crinamidine gave a product identical with flexinine which on this basis was given the structure (XXVII).



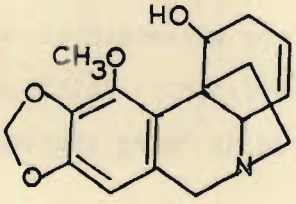
XXV



XXVI



XXVII

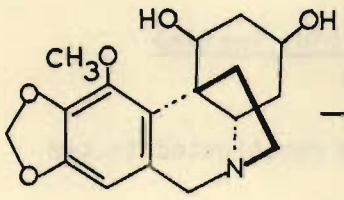


XXVIII

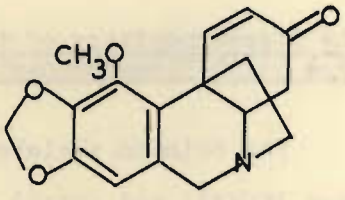
Buphanamine[44], $C_{17}H_{19}O_4N$, has been shown to belong to this group by its conversion to powelline and its reactions are best represented on the basis of structure (XXVIII).

Buphanitine, was first isolated by Tutin[47] in 1911 and the discrepancies regarding its derivatives was resolved in these laboratories by Dr. Goosen. An investigation of its chemistry was made and a structure (XXIX) was proposed. Oxidation of buphanitine gave buphanitenone (XXX), which was not identical with powellenone but which on Hofmann degradation gave powellenone methine (XXXI). Furthermore the removal of the ring C oxygen functions and reduction of the double bond gave buphanitane, not identical with powellane (XXXII). Accordingly buphanitane was assigned the structure (XXXIII) with the previously unknown cis B:C ring fusion.

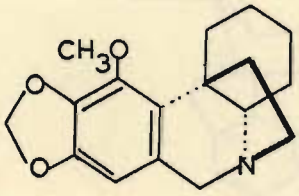
On the other hand, the alkaloid designated nerbowdine[44], which is identical to buphanitine, is reported to be obtained by partial synthesis from, and degradation to, powelline; but experimental data has not been presented for this conversion. This conflicting evidence remains unresolved and is being studied further in these laboratories.



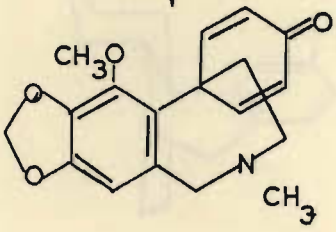
XXIX



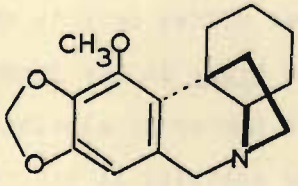
XXX



XXXII.



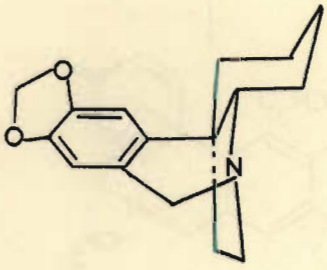
XXXI



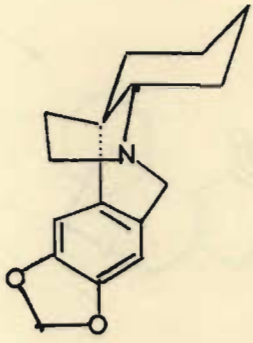
XXXII

The Stereochemistry of the Alkaloids Derived from 5:10b-Ethanophenanthridine.

The crinane skeleton may be constructed in two ways (XXXIV) and (XXXV).



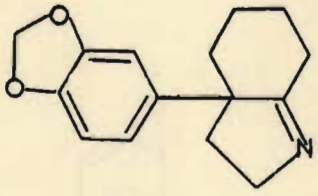
XXXIV



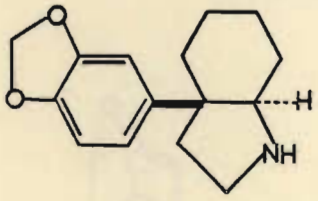
XXXV

These two structures differ only in the manner of B:C ring fusion which is *trans* in the former and *cis* in the latter. Since a number of alkaloids in this series possess analgesic activity it was suggested by Sugemoto and Kugita[48] that the crinane nucleus is represented by structure (XXXV) since this is similar to the configuration of morphine. The implication of this means that the catalytic reduction of the hexahydro indole (XXXVI), a key intermediate in the synthesis of

(+)-crinane, proceeds by the addition of hydrogen to the side opposite the phenyl group to yield the trans-octahydroindole (XXVII).



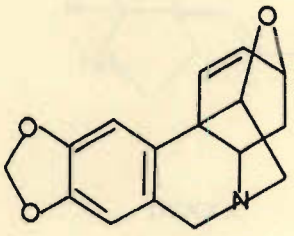
XXXVI



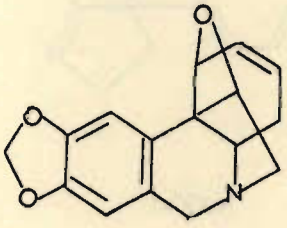
XXXVII

Thi suggestion was not compatible with observation[45] that haemanthamine on treatment with hydrochloric acid formed the demethoxy ether, apohaemanthamine (XXXVIII). Apohaemanthamine contains a C_3-C_{11} ether bridge which is sterically impossible on the basis of the structure containing the cis B:C ring fusion. The possibility of an allylic rearrangement during its formation to give structure (XXXIX) with a C_1-C_{11} ether bridge was also excluded since this structure, if possible, would be highly strained, and therefore prone to ring opening.

The fact that apohaemanthamine is stable to hot hydrochloric acid and to lithium aluminium hydride excludes this structure in favour of the strainless alternative.



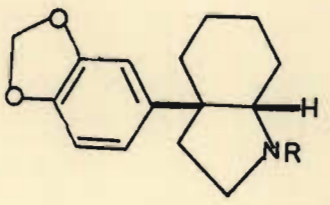
XXXVIII



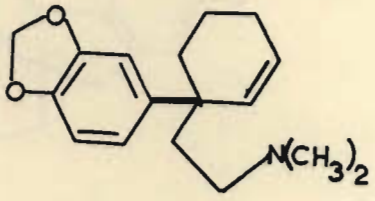
XXXIX

A recent investigation [49] of the stereochemistry of the octahydroindole obtained from the hexahydroindole (XXXVI) has been undertaken. Sodium borohydride reduction of the latter gave the same octahydroindole as obtained by catalytic reduction. Since this type of reduction would be expected to lead to the more stable <u>cis</u> fused isomer (XL; R = H) a further investigation was carried out. Hofmann degradation of the octahydroindole (XL; R = CH_3) gave the substituted cyclohexene (XLI). The structure

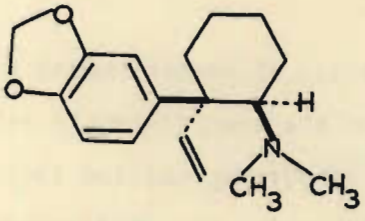
of this compound was proved by synthesis of its dihydro derivative. If the octahydroindole had possessed trans-structure (XXXVII)it would be expected to produce the substituted hexane (XLII) in a manner analogous to the simpler derivatives studied by Booth and King.[50]



XL



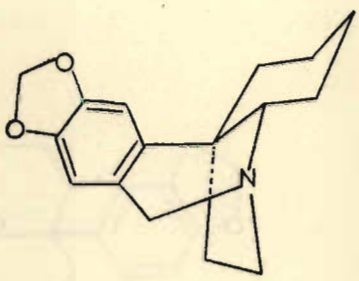
XLI



XLII

These results give independent confirmation of the trans B:C (cis C:D) ring fusion of the crinane nucleus.

The synthesis of (+)-crinane and the investigation of the stereochemistry of the octahydroindole (XIX; R = H) thus established the manner of ring fusion as represented in structure (XXXIV).



XXXIV

The degradation of haemanthamine to deoxydihydro-haemanthamine and the enantiomorphic relationship of this product to dihydrobuphanisine indicated that alkaloids in this series are elaborated from both enantiomorphs of the basic crinane nucleus. Chemical interrelationships have established that the alkaloids haemanthamine, crinamine, haemanthidine, 6-hydroxycrinamine

(3-epihaemanthidine) and haemultine are based on the same enantiomorphic skeleton, (+)-crinane. The isolation of vittatine[46] and (+)-epicrinine[51] and their enantiomorphic relationship to crinine and epicrinine respectively, also means they are based on the (+)-crinane skeleton. The degradation of crinine to (-)-crinane and its chemical relationship with powelline, buphanadrine, crinamidine, flexinine, undulatine, buphanamine, and buphanisine identify these alkaloids with the (-)-crinane nucleus.

The degradation of haemanthamine and tazettine to a common product had established the cis relationship of the aromatic nucleus and the methoxyl group in haemanthamine. In view of the enantiomorphic nature of deoxydihydrohaemanthamine and dihydrobuphanisine the methoxyl and the aromatic nucleus must also be cis in buphanisine.

All the known alkaloids in this series possessing a C_3-substituent, with three exceptions, have been shown to have this group cis (and diaxial) to the aromatic nucleus by chemical interconversions. The exceptions, crinamine, 3-epihaemanthidine and (+)-epicrinine have their C_3oxy substituent equatorial and trans to the aromatic ring.

The small group of alkaloids containing the 1,2-epoxide structure undulatine, crinamidine and flexinine have been shown[44] to possess the same stereochemistry with regard to the epoxide ring and the substituent at C_3. Further experiments involving reduction of the epoxide has indicated that the oxide ring is equatorial at C_1 and therefore trans to the aromatic ring and the C_3-substituent.

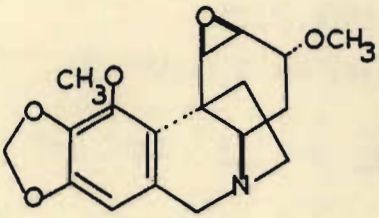
The absolute configuration of the (+)-and (-)-crinane skeleton followed from the observations made by the author[52] which are reported fully in Part III of this thesis. More recently Wildman[37] has come to the same conclusions regarding the absolute configurations of this group of alkaloids.

The structures and detailed stereochemistry of several alkaloids have appeared since the authors original proposals of absolute configuration in this series were made and they are shown in Chart I/16.

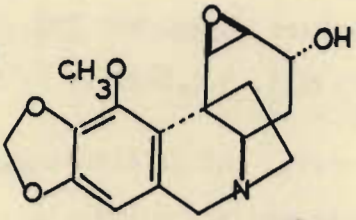
CHART I/16

THE STEREOCHEMISTRY OF SOME NEW ALKALOIDS.

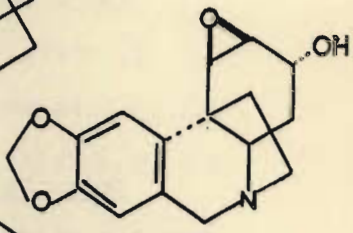
(-)-CRINANE



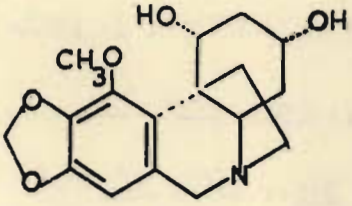
UNDULATINE



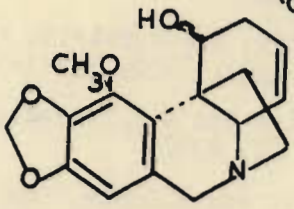
CRINAMIDINE



FLEXININE

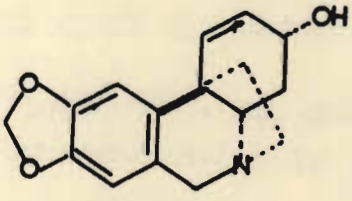


NERBOWDINE



BUPHANAMINE

(+)-CRINANE



(+)-EPICRININE

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PART II.

A
REVIEW OF ALKALOID
BIOSYNTHESIS.

The last ten years has witnessed considerable progress in our understanding of biosynthesis. In particular the role of the acetate unit in the biosynthesis of phenolic-enolic compounds has been firmly established by Birch [1] and other workers using labelled acetic acid. An extension of the simple acetate pathway to the terpenes and steroids was made by the brilliant work of Cornforth [2] and co-workers and now the acetate-mevalonate path constitutes an acknowledged route to certain terpenes and steroids.[3]

The acetate unit has been demonstrated to play an important role in the biosynthesis of certain classes of alkaloids by the work of Leete and Marion.[4] However in many cases it is not easy to see how a large number of alkaloids, and indeed many other natural products, can be accommodated in the usual acetate-polyacetate scheme.

The pioneering work of Robinson [5] in the field of alkaloid biogenesis with his initial success in synthesising tropenone under 'physiological' conditions gave a great impetus to the amino-acid precusor concept. The subsequent elucidation of the structures of many alkaloids, and their possible dissection in common amino-

acid units, has caused a general acceptance of the role played by amino-acids in alkaloid biosynthesis.

The types of reactions considered initially, before the nature of enzyme systems was fully understood, were those reactions taking place by ionic mechanisms in aqueous solutions preferably at room temperature and at approximately pH 7. Aldol condensations and Mannich type condensations are two of the most important reactions of this type which lead to the formation of the C-C and C-N bond.

In the proposals for any biosynthesis the reactions should conform to routes which are explicable in terms of the electronic theory of reaction mechanisms. We should however not fail to recognise the ability of an enzyme system to bring about stereospecific reactions and processes requiring a considerable amount of energy with consumate ease.

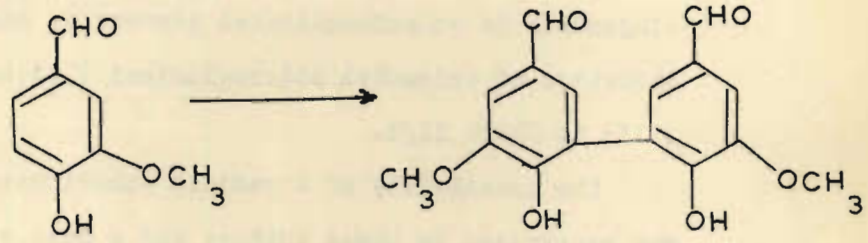
A most important contribution to biosynthetic theory was made recently by Barton and Cohen.[6] They have suggested in many cases phenol-phenol oxidative coupling is an important biosynthetic step. Although this idea had been presented earlier by Robinson for the biogenesis

of morphine and in a scheme proposed by Wenkert[7] in 1953 for morphine, the Erythrina and Amaryllidaceae alkaloids, Barton and Cohen have suggested its general application to a wide variety of natural products. Their ideas are presented in this section and particular attention is paid to its application in the Amaryllidaceae alkaloids.

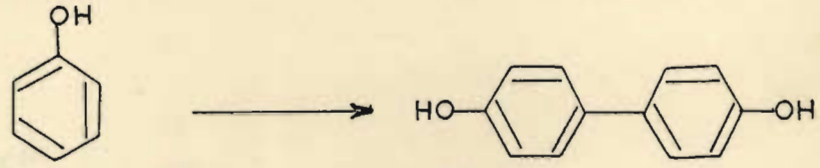
Certain reagents such as ferric chloride, potassium ferricyanide and lead tetra-acetate are known to oxidise phenols or phenolate anions to phenol radicles. These radicles are comparitively stable, relative to alkyl radicles, because of the delocalisation of the lone electron. Coupling of the phenol radicle can take place to form stable molecular products by several processes. Self-coupling is the most important process since it leads to stable dimers by either carbon-carbon, carbon-oxygen, and oxygen-oxygen linking. Obviously carbon-carbon coupling is the most important from biogenetic considerations and this may take place in three ways, ortho-ortho, ortho-para or para-para. Some laboratory examples of these oxidative couplings using one electron transfer oxidising agents are illustrated in Chart II/1. Carbon-oxygen coupling although of lesser importance in

CHART II/1

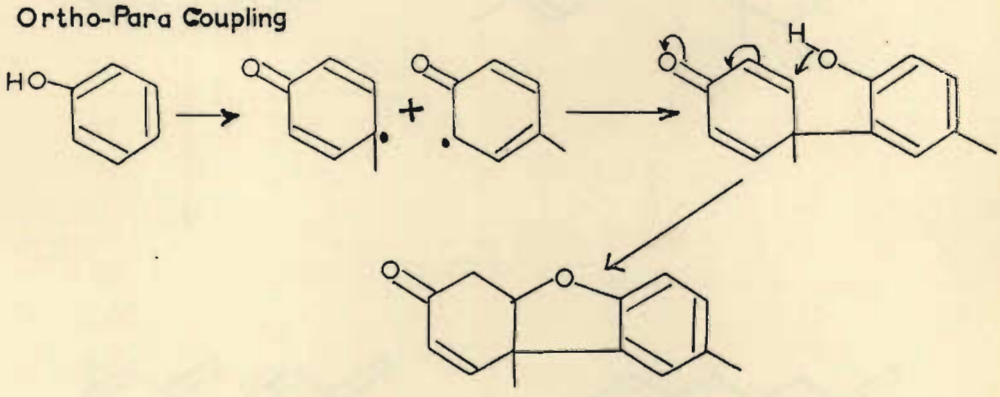
Ortho-Ortho Coupling



Para-Para Coupling



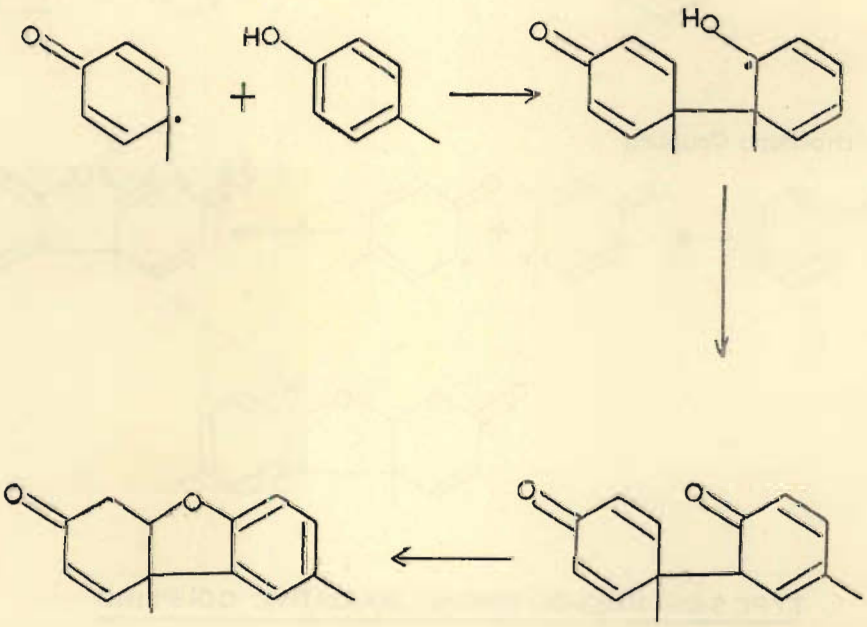
Ortho-Para Coupling



TYPES OF PHENOL-PHENOL OXIDATIVE COUPLING

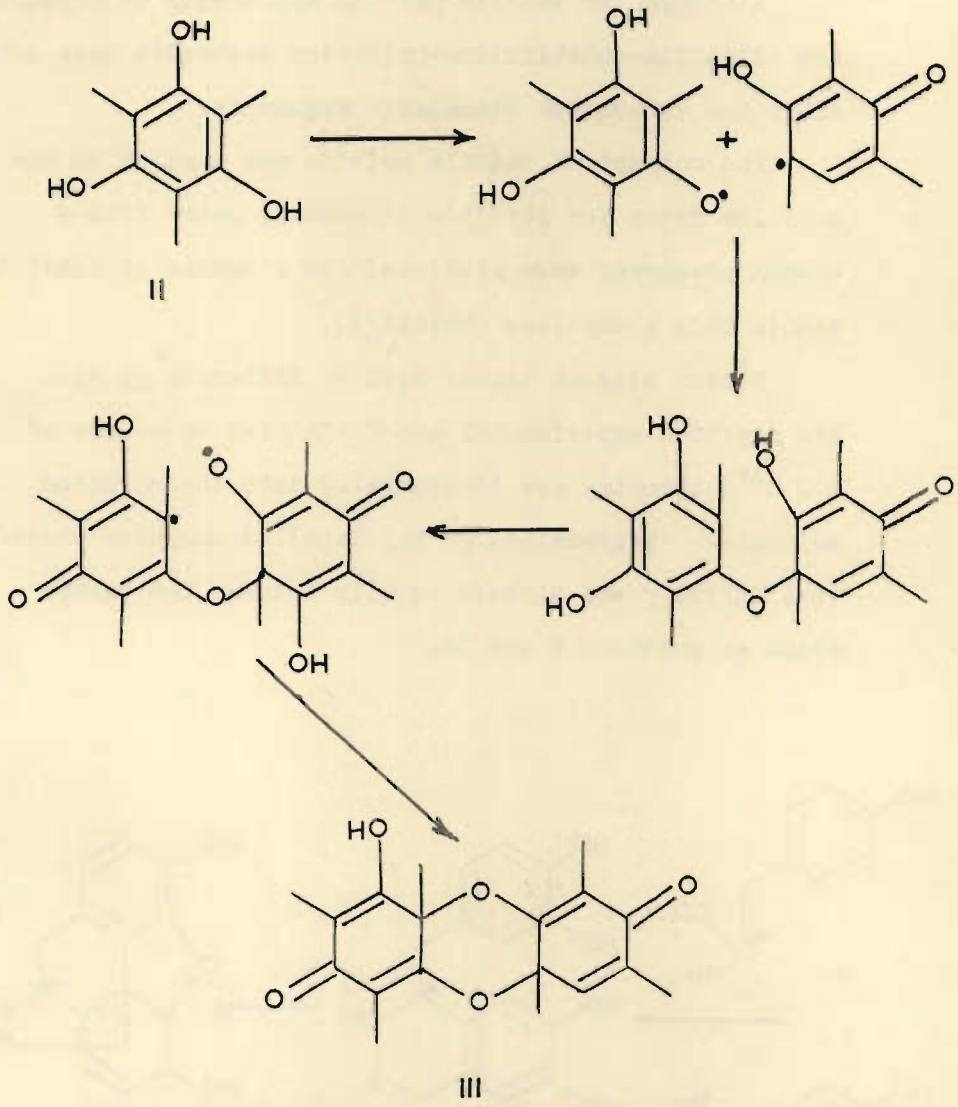
biogenesis is an authenticated process as shown by the oxidation of trimethyl phloroglucinol (II) to cederone (III) in Chart II/2.

The possibility of a radicle substitution mechanism was recognised by these authors and a case cited where this is a possible alternative to radicle pairing was in the formation of Pummer's ketone (I) as illustrated below.



I

CHART II/2



II

III

FORMATION OF CEDERONE: AN EXAMPLE OF CARBON OXYGEN COUPLING

Although the radicle pairing hypothesis is favoured, the oxidation-substitution-oxidation mechanism does not alter the subsequent biogenetic arguments.

The concept of radicle pairing was applied to the morphine group and possible biogenetic paths from a common precursor were predicted for a number of alkaloids within this group (see ChartII/3).

Recent elegant tracer work by Battersby[8] et al., has provided experimental proof that two molecules of [2 C^{14}] tyrosine are incorporated into the morphine molecule. Degradation of the labelled morphine showed that activity was divided equally between the carbon atoms at position 9 and 16.

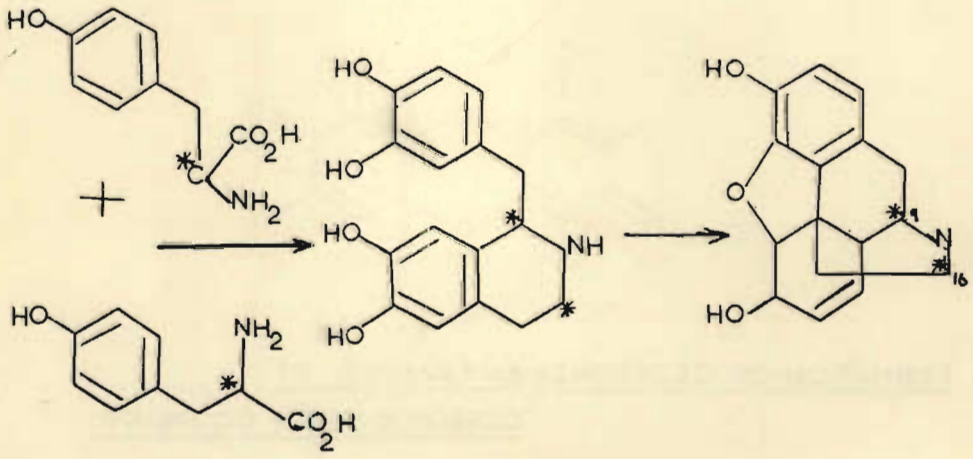
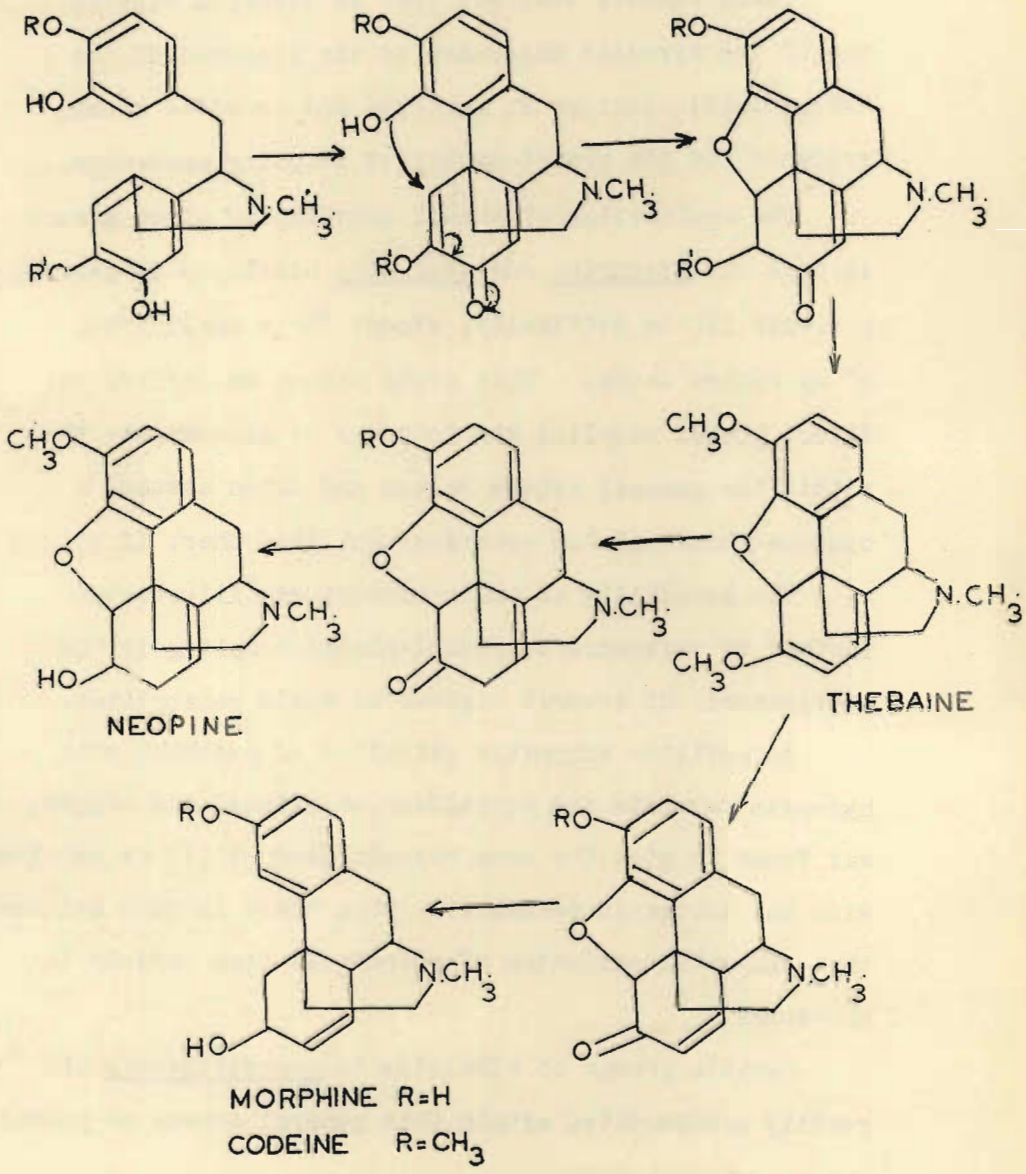


CHART II/3



BIOGENETIC PATHS TO THE MORPHINE ALKALOIDS

These results indicate that an aromatic ring of one of the tyrosine molecules is the precusor of the hydroaromatic nucleus in morphine and provides strong evidence for the phenol-oxidative coupling mechanism.

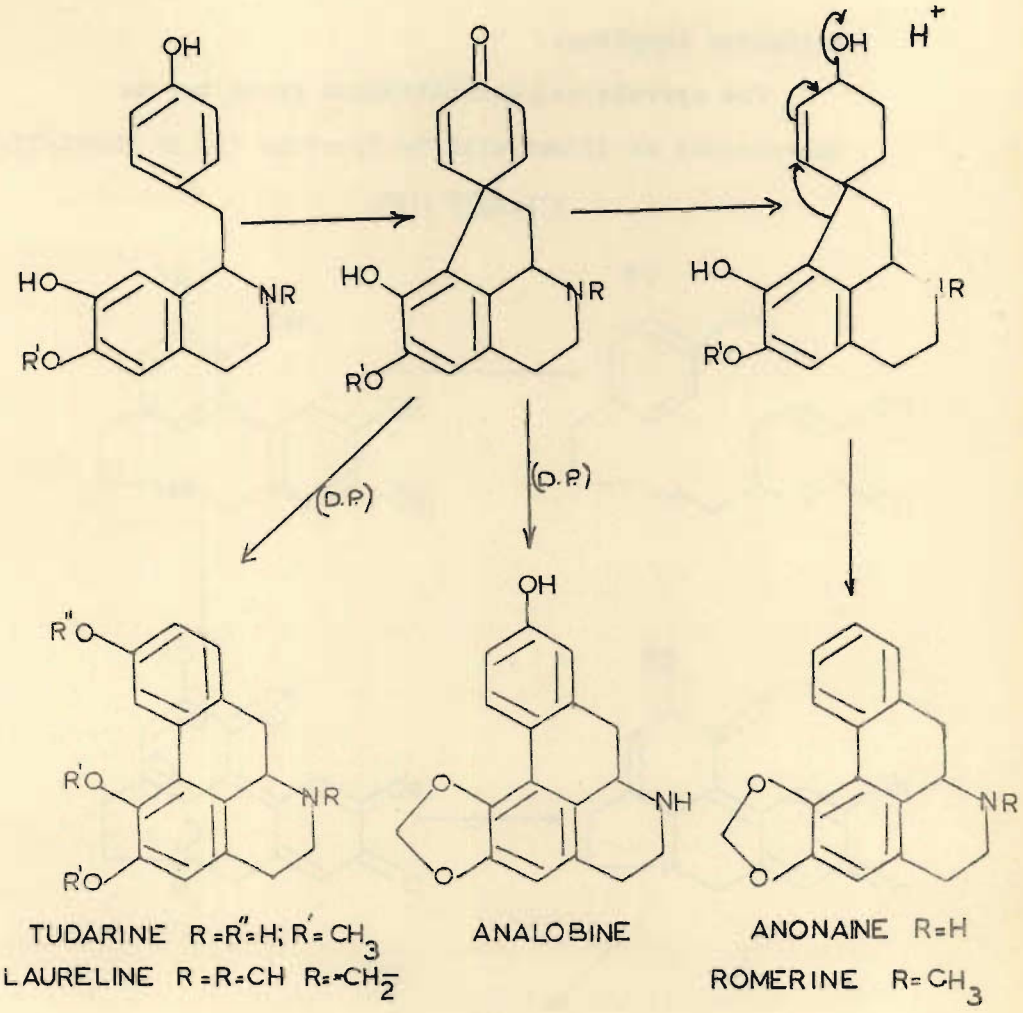
The application of phenol coupling to other groups such as the Erythrina and Aporphine alkaloids in general presents little difficulty, except for a small group of aporphine bases. This group cannot be derived by direct phenol coupling and in order to accommodate them within the general scheme Barton and Cohen assumed a dienone-phenol (D.P.) rearrangement (see Chart II/4).

The generality of their concept was illustrated further by reference to phenol-phenol coupling in the biosynthesis of several classes of mould metabolites.

In addition enzymatic oxidation of p-cresol with hydrogen peroxide and peroxidase,or oxidase and oxygen, was found to give the same racemic product (I) as obtained with the inorganic reagents. Thus there is much evidence that enzymatic oxidation of phenols is free radicle in character.

Certain groups of alkaloids in Amaryllidaceae are readily accommodated within this general scheme of phenol-

CHART III/4



TUDARINE R = R" = H; R' = CH_3

LAURELINE R = R = CH R = CH_2

ANALOBINE

ANONAINE R = H

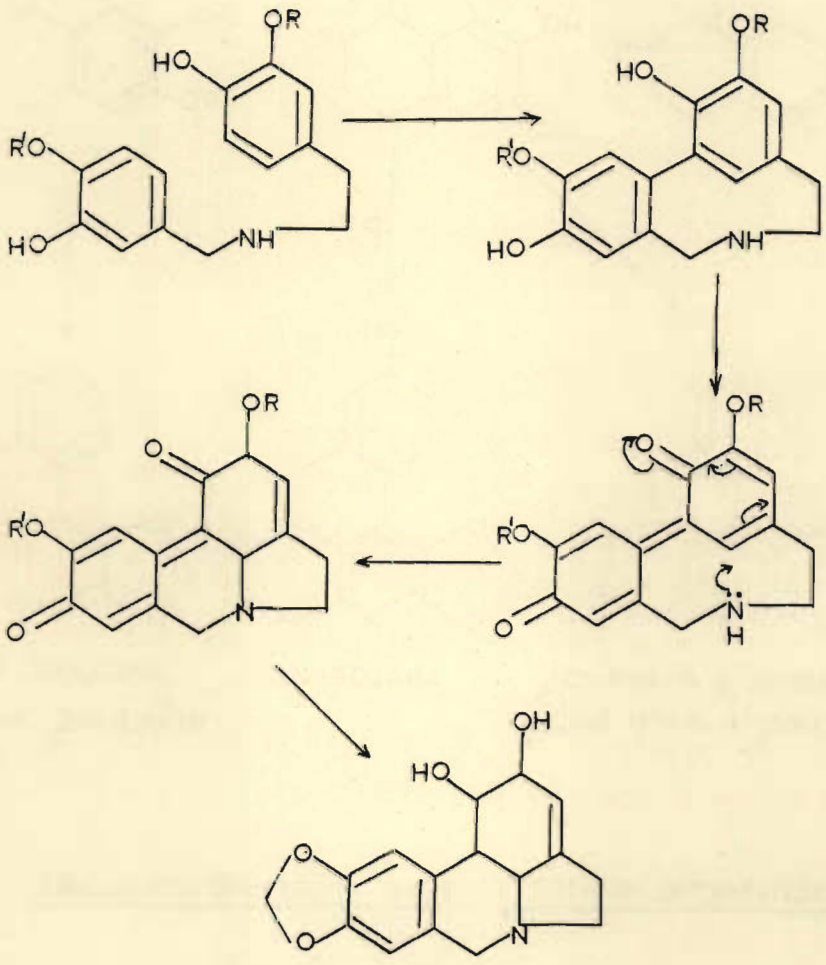
ROMERINE R = CH_3

BIOSYNTHETIC ROUTES TO SOME APORPHINE ALKALOIDS

oxidative coupling.

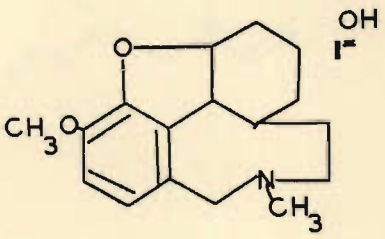
The pyrrolo[de]phenanthridine group may be constructed as illustrated by lycorine (V) in Chart II/5.

CHART II/5

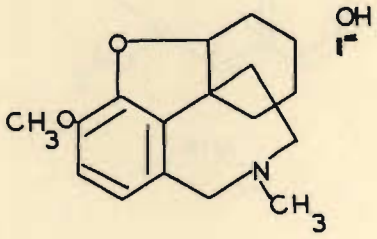


POSTULATED ROUTE TO LYCORINE

The alkaloid galathamine belonging to the dibenzfuran group had lead to two expressions (VI) and (VII) at the time.



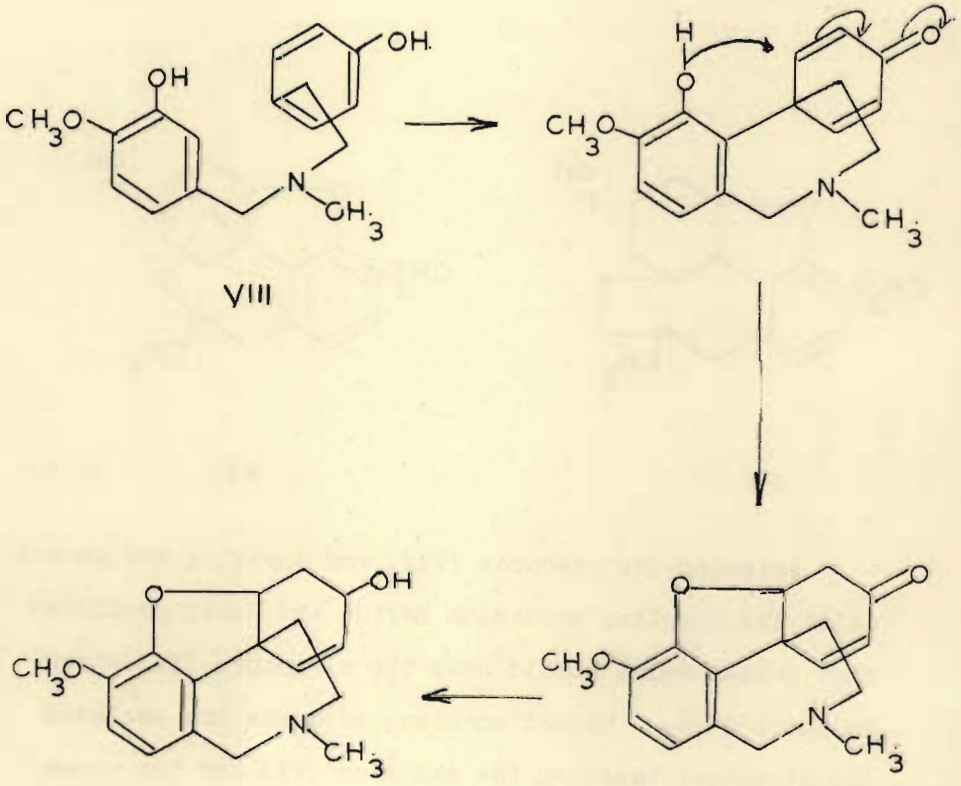
VI



VII

Assuming the precusor (VII) and applying the phenol oxidative coupling mechanism Barton and Cohen predicted that galanthamine should have the structure (IX) shown in Chart II/6. Recent chemical evidence has excluded the structure based on the skeleton (VI) and has shown that galanthamine is best represented by structure (IX) in complete accord with the prediction.

CHART II/6

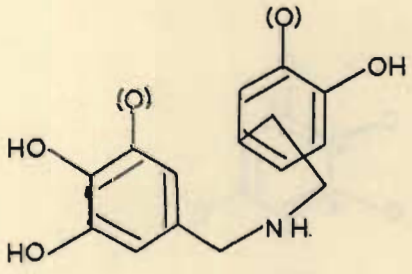


VIII

IX

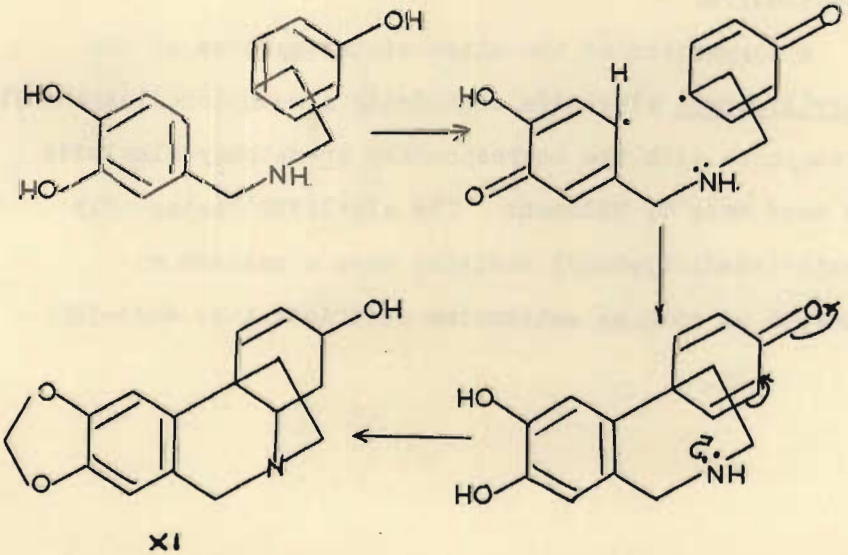
BIOGENETIC ROUTE TO GALANTHAMINE

The large group of alkaloids based on the 5:10b-ethanophenanthridine skeleton are also readily built up in theory from a common type of precursor (X) by phenol-oxidative coupling.



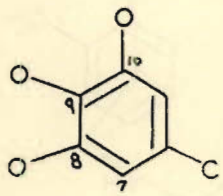
X

Crinine (XI), the parent alkaloid of this group may be constructed as follows.



XI

A most important consequence of the phenol-coupling mechanism is that in those alkaloids with three oxygen functions attached to the aromatic ring the position of these atoms should be as shown in the partial structure (XII).



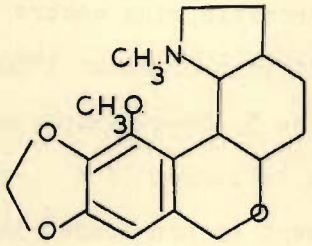
XII

Although no direct chemical proof of this substitution pattern has been published to date a certain amount of physical data is available in support of this type of substitution.

A comparison of the ultra-violet spectra of the Amaryllidaceae alkaloids containing a methylenedioxyphenyl chromophore with the corresponding ar-methoxy alkaloids has been made by Wildman. The alkaloids having only a methylenedioxyphenyl skeleton have a maximum at 292—297 mμ with an extinction coefficient of 4600-5200.

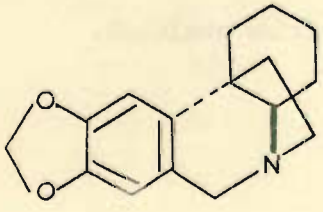
In the methoxymethylenedioxyphenyl alkaloids, absorption due to the substituted aromatic ring occurs at 286—288 mμ with an extinction coefficient of about 1700—1800. For the alkaloids based on the 5:10b-ethanophenanthridine skeleton there can be no influence of a C_1-substituent on the aromatic ring except by non-bonded interaction with a group bulkier than a hydrogen atom at the C_{10}-position. It is interesting to note that the extinction coefficients of the trioxy-aryl alkaloids with a C_1-substituent show a marked decrease to 1320—1500. Since the methylenedioxyphenyl alkaloids containing a C_1-substituent show no decrease in the ultra-violet extinction coefficients this decrease may be due to steric interaction with the aromatic chromophore which, as mentioned above, can only occur with a C_{10}-substituent.

The Amaryllidaceae alkaloids containing the skeleton (XIII), where the pyrrolidine ring D is attached so as to interfere considerably with a C_{11}-substituent on the aromatic ring, the extinction coefficients are lower than those based on the 5:10b-ethanophenanthridine skeleton.

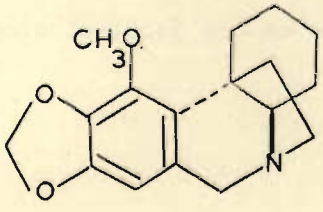


XIII

Some further evidence regarding the substitution pattern of the trioxy-aryl alkaloids is available from optical rotatory dispersion studies in the (-)-crinane and (+)-powellane series. There is a substantial amount of evidence for the (-)-crinane (XIV) and (+)-powellane (XV) skeletons having the same absolute configuration.



XIV



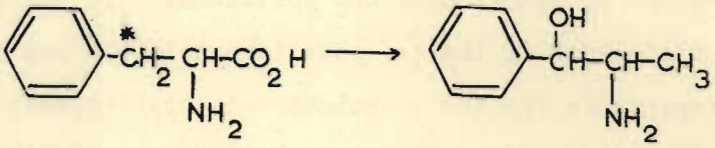
XV

However it has been found that whereas (-)-crinane exhibits a plain negative rotatory dispersion curve (+)-powellane showed, unexpectedly, a plain positive curve. A possible explanation of this has been advanced by Wildman on the basis of the position of the aromatic methoxyl group. As mentioned previously it is only when the methoxyl is placed in the C_{10}-position is it possible to get steric interference with the ring C hydrogen atom at C_1, the alternative position (C_7) presents no steric hinderance to either adjacent positions. It was suggested that distortion of the (+)-powellane molecule may therefore be responsible for the anomolous rotatory dispersion curve.

The biogenesis of those Amaryllidaceae alkaloids hydroxylated at C_{11} presents an interesting problem. Although postulated biosynthetic pathways are essentially structural rather than sequential, extensive tracer work in a number of cases has determined the order of events e.g. phenylalanine—tyrosine—tyramine—N-methyl-tyramine pathway to hordenine.[9]

Since a benzylic carbon atom is far easier to oxidise than that most unreactive structural unit, the isolated methylene group; therefore in spite of the danger in

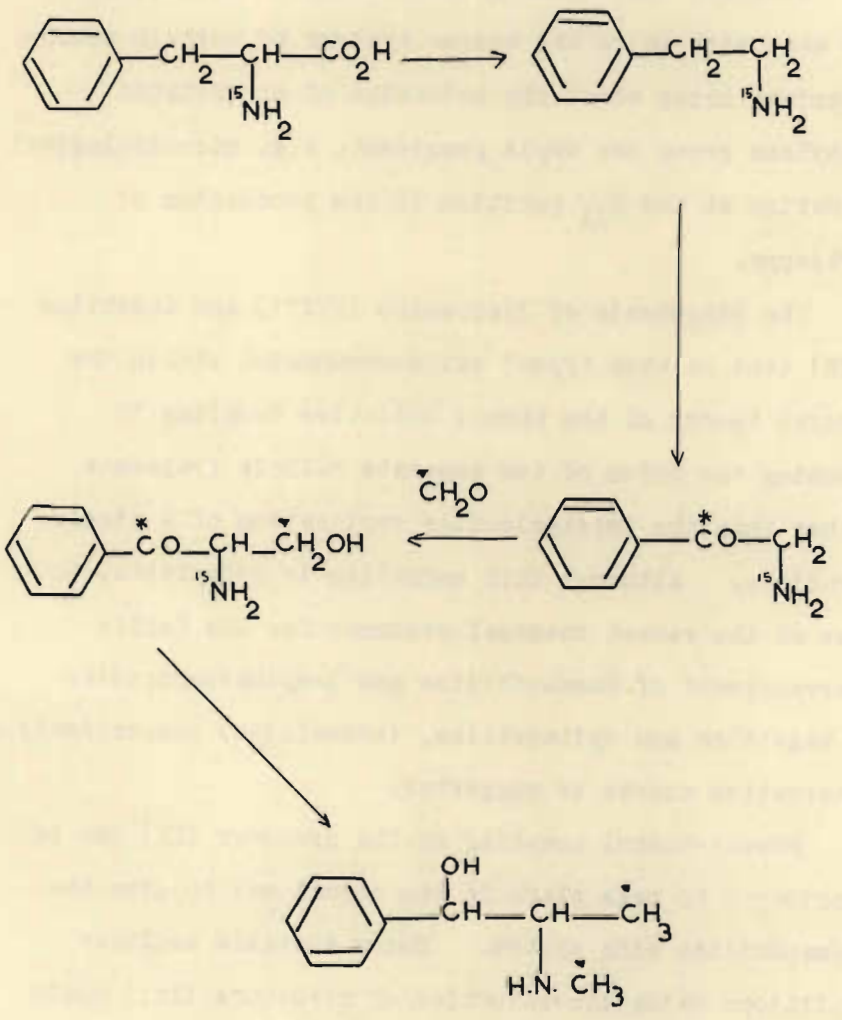
invoking laboratory analogies one would expect that oxidation occurs at this position before phenol coupling. Two such examples of benzylic oxidation in biosynthesis have been established by tracer studies from the incorporation of labelled phenylalanine into d-norpseudo ephidrine [10] (XVI) and the work of Shibita et al.,[11] on the biogenesis of ℓ-ephedrine (XVII).



XVI

The latter authors showed that both N^{15} phenylalanine and ω-amino-acetophenone (carbonyl C^{14}) were incorporated efficiently into the molecule. In conjunction with degradation experiments, in which the activity of the ℓ-ephedrine derived from labelled ω-amino-acetophenone was found to be restricted to the α-carbon atom; the biosynthesis was represented as shown in Chart II/7.

CHART II/7



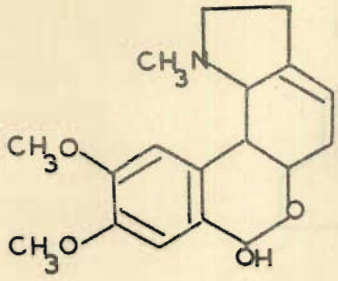
XVII

BIOGENESIS OF ℓ-EPHEDRINE

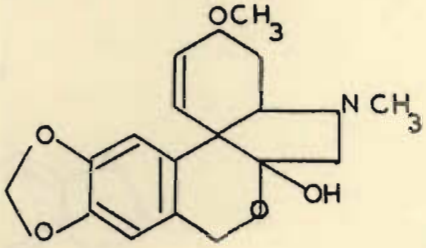
Although the author favours benzylic oxidation, the ease with which the enzyme systems of certain micro-organisms bring about the oxidation of an isolated methylene group has ample precident, e.g. microbiological oxidation at the C_{11} position in the production of cortisone.

The biogenesis of lycorenine (XVIII) and tazettine (XIX) (and related types) was accommodated within the general theory of the phenol oxidative coupling by assuming the union of two separate radicle fragments rather than the intramolecular cyclisation of a single diradicle. Although this mechanism is acceptable, in view of the recent chemical evidence for the facile rearrangement of haemanthidine and 3-epihaemanthidine to tazettine and epitazettine, (criwelline) respectively an alternative course is suggested.

Phenol-phenol coupling of the precusor (XX) can be considered to take place in the normal way to give the haemanthidine ring system. Under suitable cellular conditions oxidation-reduction of structure (XXI) could take place to give a nortazettine which is N-methylated in the usual way (see Chart II/8).



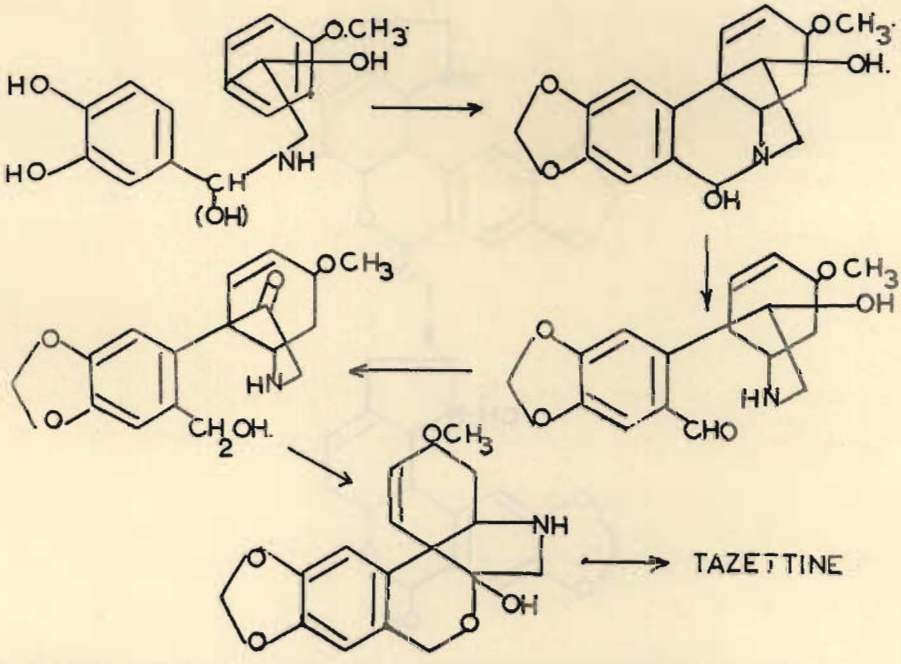
XVIII



XIX

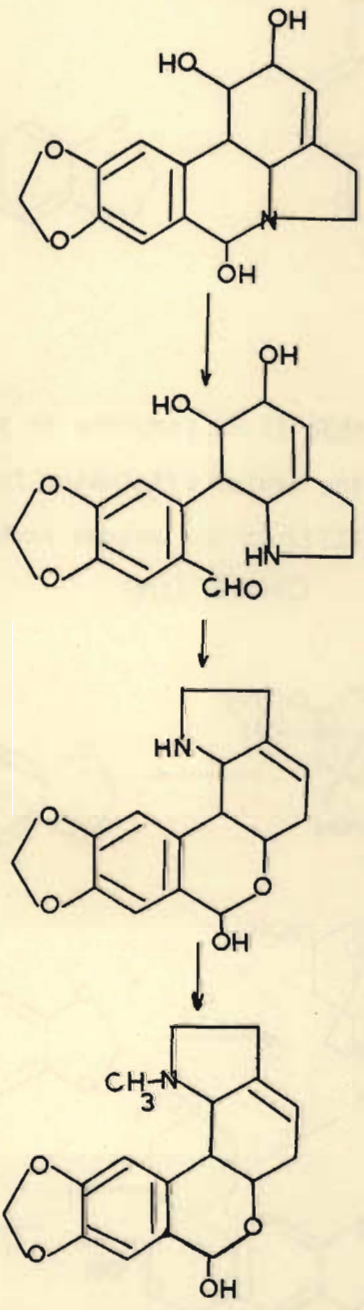
In view of this it is tempting to speculate that the lycorenine ring system originates from the lycorine type structure (XXII) in the manner shown in Chart II/9.

CHART II/8



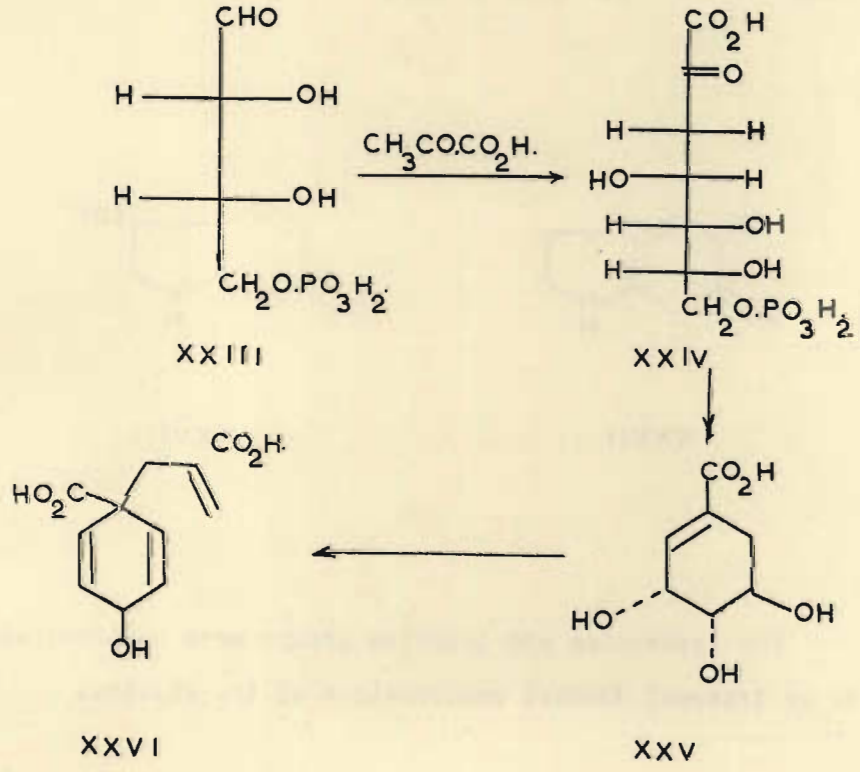
SUGGESTED BIOGENETIC ROUTE TO TAZETTINE

CHART II/9



POSSIBLE ROUTE TO THE LYCORENINE TYPE ALKALOIDS

Recently Wenkert[12] challenged the theory that alkaloids are the product of amino-acid metabolism and has suggested that they arise from carbohydrates. This idea was prompted by the discovery[13] that certain aromatic systems such as anthranillic and benzoic acids, phenylalanine and tyrosine are synthesised in bacterial cells from carbohydrates via D-erythrose-4-phosphate (XXIII), 2-keto-3-desoxy-7-phospho-D-glucoheptonic acid (XXIV), shikimic acid (XXV) and prephenic acid (XXVI).

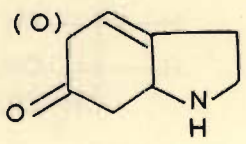


XXIII XXIV

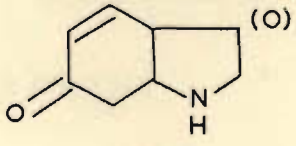
XXVI XXV

The universality of shikimate-prephenate biosynthetic pathways was suggested and biogenetic schemes for a large number of alkaloid groups were illustrated.

The biosynthesis of the Amaryllidaceae was considered to involve the union of a C_6-C_1 unit, derived from a shikimate precusor and a C_6-N-C_2 moiety originating from prephenate. All the alkaloids of this group with the exception of belladine were derived from the structures (XXVII) and (XXVIII), which are themselves readily synthesised from prephenic acid.



XXVII

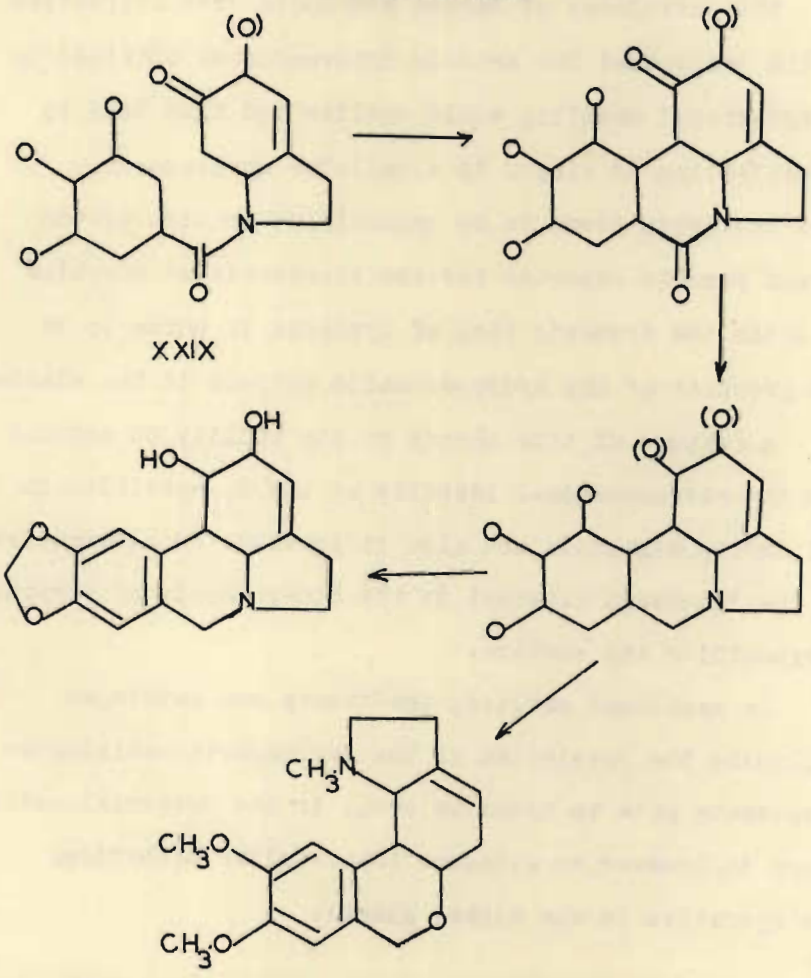


XXVIII

The lycorenine and lycorine groups were constructed by an internal Michael condensation of the shikimyl

derivatives of structure (XXIX), followed by hydration-dehydration, oxidation-reduction changes as shown in Chart II/10.

CHART II/10



WENKERT'S SCHEME FOR THE BIOGENESIS OF LYCORINE AND LYCORENINE

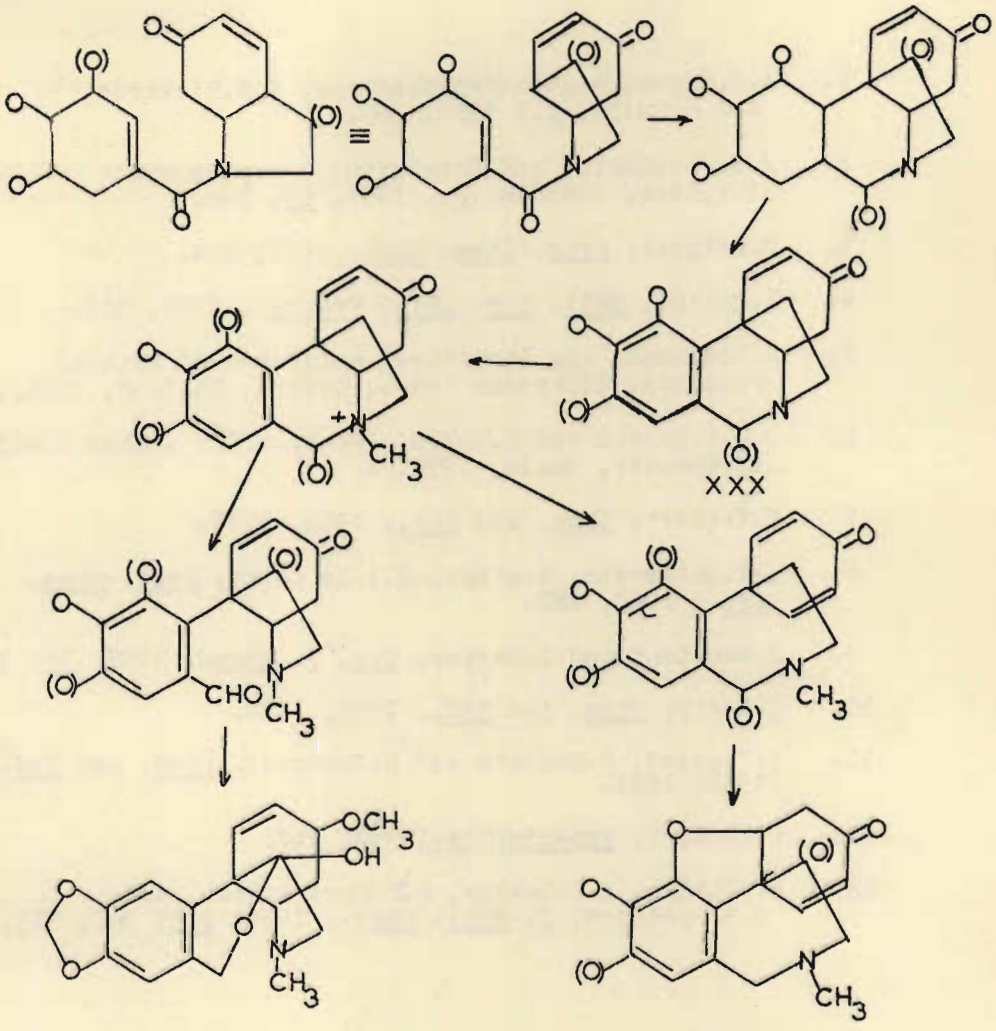
Similar schemes were presented for the biosynthesis of the crinine group (XXX), the dibenzfuran (XXXI) and tazettine (XXXII) types as shown in Chart II/11.

The postulates of Barton and Cohen were critisised on the basis that the ketonic intermediates obtained by phenyl-phenyl coupling would enolise and thus lead to aromatisation of ring C in a cellular environment. This critisism seems to be unjustified in view of the recent results reported for the biogenesis of morphine in which the aromatic ring of tyrosine is shown to be the precusor of the hydro-aromatic nucleus in the alkaloid.

A feature of this theory is its ability to account for the stereochemical identity of the C_{15}-position in all indole alkaloids and also it provides an alternative to the 'Woodward fission' in the biosynthesis of strychnine, corynanthine and emetine.

As mentioned earlier, the theory was developed following the revelation of the carbohydrate-shikimate-prephenate path to tyrosine etc., in the bacterial cell. There is, however, no evidence that similar mechanisms are operative in the higher plants.

CHART II/11



WENKERT'S PROPOSED SCHEME FOR AMARYLLIDACEAE ALKALOIDS

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PART III.

SECTION I.

THE ISOLATION AND CHEMISTRY OF A NEW ALKALOID, 3-EPIHAEMANTHIDINE FROM *HAEMANTHUS NATALENSIS* AND THE ABSOLUTE CONFIGURATION OF THE ALKALOIDS DERIVED FROM THE 5:10b-ETHANOPHENANTHRIDINE SKELETON.

EXTRACTION OF HAEMANTHUS NATALENSIS.—

The extraction of Haemanthus natalensis had been carried out previously in these laboratories[1] and in the United States by Wildman et al.[2] Both groups of workers succeeded in isolating the alkaloid Haemanthamine which is known to occur in many species of the Amaryllidaceae. The American workers were unable to resolve the remaining mixture of alkaloids despite the application of partition and absorption chromatography, and counter current distribution techniques.

A further attempt at resolving the alkaloids of this plant was made in these laboratories by Dr. Graham[3] using a buffer extraction method and although an amorphous product was obtained, reproducible analytical data and the preparation of derivatives indicated it was a pure substance.

The results of degradations were complex and few crystalline products obtained. In retrospect it is obvious that complete separation was not obtained and several degradations are now explicable assuming that the amorphous alkaloid was a mixture.

The author chose to reinvestigate a chromatographic method since a preliminary experiment with an amorphous specimen supplied by Dr. Graham had yielded crystalline

material when chromatographed on alumina.

Bulbs collected from the environs of Pietermaritzburg Natal were finally sliced and immediately dropped into 95% alcohol which stopped enzyme action and prevented decomposition. The sliced bulbs were allowed to stand for two weeks before decanting off the ethanol. The once extracted bulbs were re-extracted with boiling alcohol. The combined alcohol extracts were flash evaporated and steam distilled to remove any remaining alcohol. The residual solution, which was slightly acidic (pH 4.0) was filtered through kieselguhr (Celite 545) to remove the precipitated fats and after adjusting the filtrate to pH 2.0 it was extracted with ether. The alkaloidal bases were liberated by treating the aqueous phase with excess sodium carbonate and extracted into chloroform.

Removal of the chloroform left a clear amber gum which by careful chromatography over alumina was separated into haemanthamine, haemanthidine and a new alkaloid, 3-epihaemanthidine.

The crude alkaloid was dissolved in hot benzene containing the minimum amount of ethyl acetate (12% $^{v}/v$) to effect complete solution. Employment of a gradient

elution technique resulted in haemanthamine being eluted with benzene-ethyl acetate. Haemanthamine was identified by mixed melting point determination and comparison of its infrared spectrum with that of an authentic specimen.

3-Epihaemanthidine.—

Elution of the column with pure ethyl acetate and chloroform - containing a small percentage of alcohol gave a new alkaloid. A sample when crystallised from a dry acetone or acetone-light petroleum gave stout needles, m. p. 211° and an $[\alpha]_D$ +44° (in chloroform). The propensity for the alkaloid and its derivatives to solvate made the determination of its molecular formular difficult and a full examination of quite a number of derivatives was required before the $C_{17}H_{19}O_5N$ formular was established. Particular care was necessary when crystallising the alkaloid from acetone to make certain the solvent was quite dry, otherwise the alkaloid was obtained as the hemi-hydrate m. p. 135—140°. This hydrate was also obtained when the alkaloid was crystallised from 'wet' ethyl acetate. Crystallisation from ethyl alcohol gave needles m. p. 146° which analysed for the alcoholate $C_{17}H_{19}O_5N, C_2H_6O$. Each of the above forms, as expected showed differences in their infrared spectra when run in nujol.

The picrate crystallised from aqueous alcohol m. p. 146° and analysed for the hydrate $C_{17}H_{19}O_5N,C_6H_3O_7N_3,H_2O$. The methiodide when crystallised from water was also hydrated and after drying at 100°C at 0.01 mm., it analysed for $C_{17}H_{19}O_5N,CH_3I,\frac{1}{2}H_2O$. Further characterisation was effected by the preparation and analysis of the metho picrate which was not solvated in any way. (See Table I for analytical data).

The ultra-violet spectrum (See Fig.I) of the alkaloid showed two maxima of medium intensity at 242 mμ (ε,5620) and 293 mμ (ε,4680) which is very similar to that shown by lycorine and other alkaloids of the Amaryllidaceae possessing a methylenedioxy phenyl chromophore. The presence of a methylenedioxy group was demonstrated by a positive response to the Labat test and by the appearance of bands at 2725 $cm.^{-1}$, 1250 $cm.^{-1}$ and 931 $cm.^{-1}$ in the infrared spectrum of the alkaloid (Fig.2).

FIG 1

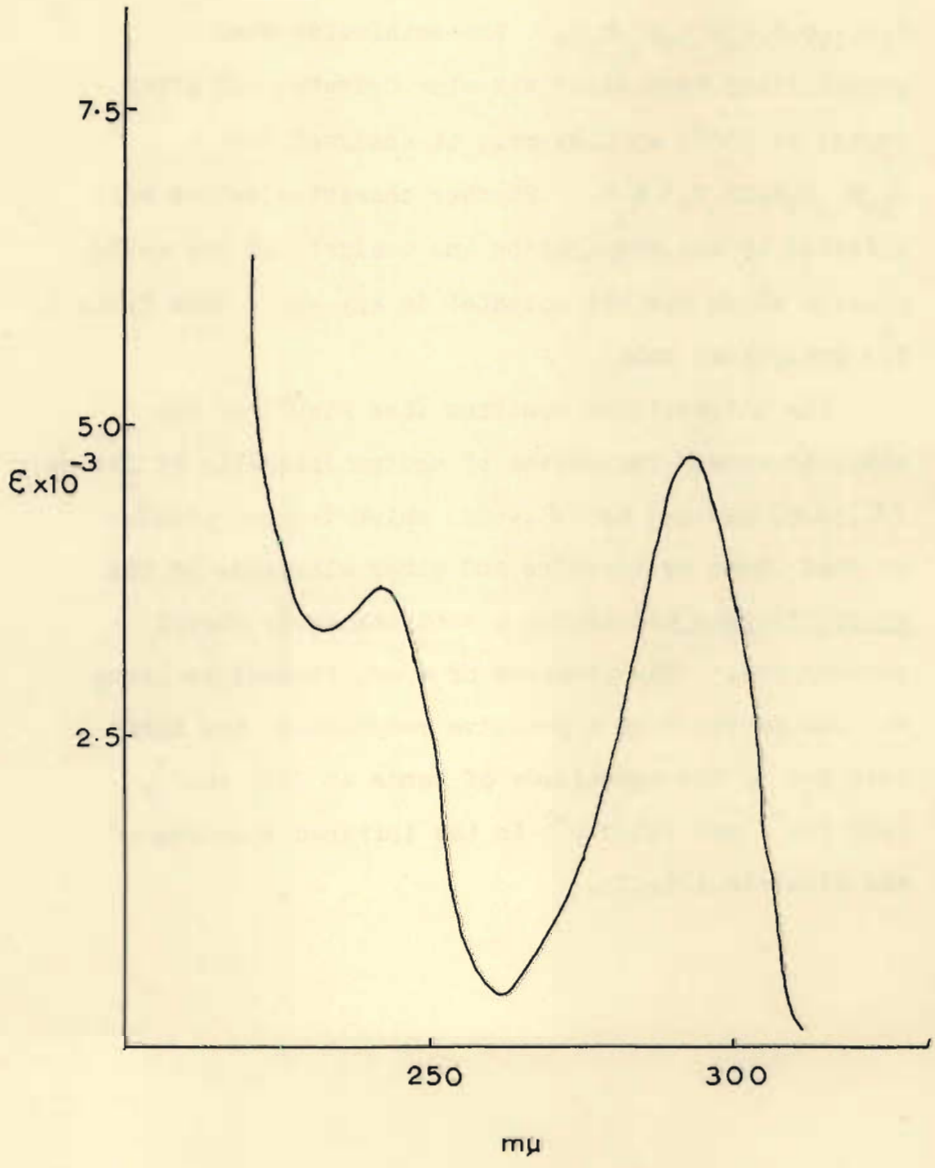
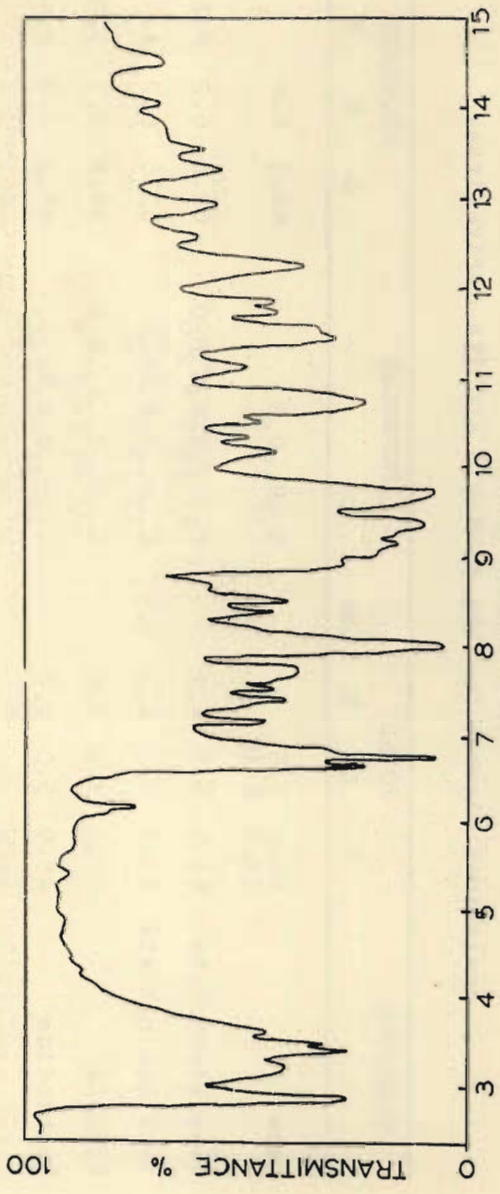


FIG. 2



3-EPIHAEMANTHIDINE

TABLE I.

Analytical figures for the alkaloid and its derivatives.

COMPOUND	FOUND				FORMULAR	REQUIRES			
	C	H	N	OMe		C	H	N	OMe
Base	64.0 64.2	5.8 6.15	- -	- -	$C_{17}H_{19}O_5N$	64.3	6.0		
Base Alcoholate	62.6	6.8	4.2		$C_{17}H_{19}O_5N, C_2H_6O$	62.8	6.2	4.2	
Base Hemihydrate	62.2	6.2	4.3	9.5	$C_{17}H_{19}O_5N, \frac{1}{2}H_2O$	62.6	6.2	4.3	9.3
Picrate	49.3	4.4	9.6		$C_{23}H_{21}O_{12}N_4, H_2O$	48.9	4.3	9.9	
Methiodide	45.9	5.0	2.8		$C_{18}H_{21}O_5N, \frac{1}{2}H_2O$	46.2	4.8	2.4	
Metho Picrate	51.1	4.0			$C_{18}H_{21}O_5N, C_6H_3O_7N_3$	51.4	4.3		

THE FUNCTIONAL GROUPS.

The oxygen atoms were associated with:

1). A methylenedioxy group (evidence presented above).

2). A Ziesel determination showed the presence of one methoxyl which in the absence of strong absorption at 1613 cm.$^{-1}$ (indicative of the methylmethylenedioxy phenyl chromophore) must be aliphatic. The absence of an N-methyl group was shown in this determination.

3). The presence of an hydroxyl group(s) was indicated by a sharp band at 3590 cm.$^{-1}$ in the infrared spectrum of the alkaloid when run in chloroform solution. Acetylation yielded an O:O diacetate as indicated by analysis and the strong ester carbonyl absorption at 1745 cm.$^{-1}$ in its infrared spectrum.

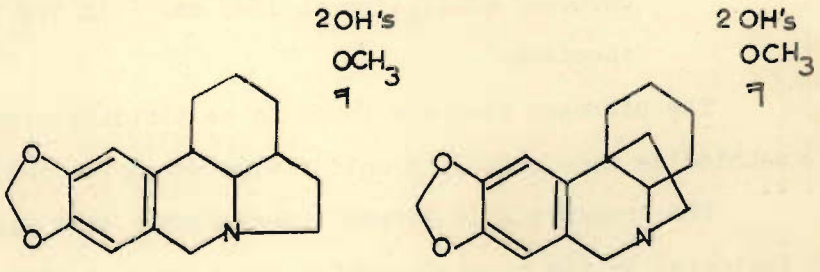
The nitrogen atom was found to be tertiary since the methiodide was soluble in cold sodium hydroxide solution.

The presence of a disubstituted double bond was indicated by the appearance of a sharp band at 1645 cm.$^{-1}$ in the infrared spectrum. However several attempts to reduce the alkaloid with hydrogen in the presence of Adams's

catalyst in acetic acid were unsuccessful.. Eventually reduction was effected by using a 1:1 ratio of catalyst to the compound and carrying out the reduction according to Linstead.[3a] The dihydro compound crystallised from ether m. p. 240—242° and analysed for $C_{17}H_{21}O_5N$. The formular may now be expanded to

$$C_6H_2(CH_2O_2)C_9H_{10}(OH)_2(OCH_3)(\equiv N)$$

and the presence of one ethylenic double bond requires that it is pentacylic (including the methylenedioxy ring). If it is assumed that the alkaloid possesses one of the known basic skeletons encountered in the Amaryllidaceae alkaloids, then since a lactone or cyclic ether functions are absent it may be represented by either of the partial structures.



ALKALINE REARRANGEMENT PRODUCT.

An experiment designed to test the stability of the alkaloid to alkaline hydrolysis with hot 10% aqueous potassium hydroxide proved most interesting. The product was isolated initially as a gum (although it was obtained crystalline at a much later date) and it was shown to be isomeric with the alkaloid by the preparation of a number of derivatives (see Table II). The hydrochloride crystallised from water or aqueous ethanol in silky needles m. p. 248—250° (dec.) $[\alpha]_D$ +198° (in water) and analysed for $C_{17}H_{19}O_5N,HCl.H_2O$. Further characterisation was afforded by the preparation of the picrate and the picrolonate.

The infrared spectra of the hydrochloride and the base revealed bands attributable to a methylenedioxy group. The ultra-violet spectrum was very similar to that of the alkaloid, showing two maxima at 241 mμ (ε,4570) and 290 mμ (ε,4370).

The rearrangement product was recovered unchanged after refluxing with selenium dioxide and also remained unaffected by refluxing 10% hydrochloric acid. Thus the presence of the pyrrolo [de] phenanthridine skeleton

TABLE II.

COMPOUND	FOUND					FORMULAR	REQUIRES				
	C	H	N	OMe	Cl		C	H	N	OMe	Cl
Base	61.2	5.7				$C_{17}H_{19}O_5N,H_2O$	60.9	6.3			
Hydrochloride	54.9 54.9 54.5	5.7 6.0 5.9			9.3 9.3	$C_{17}H_{19}O_5N,HCl$	54.9	6.0	3.8	8.4	9.6
Picrate	50.1	4.4	10.7			$C_{17}H_{19}O_5C_6H_3O_7N_3$	50.1	4.1	10.2		
Metho hydriodide	47.2 47.2	4.8 4.9		6.4		$C_{17}H_{18}O_5NCH_3,HI$	47.1	4.8		6.7	
Methyl Base	65.2	6.6	4.2	9.4		$C_{17}H_{18}O_5NCH_3$	65.2	6.4	4.2	9.3	
Picrolonate	55.7	4.6	10.1			$C_{17}H_{19}O_5N,C_{10}H_8O_5N_4$	55.9	4.7	12.0		

could be excluded and the substance was not an enol ether.

Acetylation with acetic anhydride and pyridine at room temperature gave a product which although analysing correctly for carbon and hydrogen, assuming the molecular formular $C_{17}H_{18}O_5N.COCH_3$, two determinations for acetyl by the normal procedure gave no acetic acid. The infrared spectrum (NUJOL) however provided convincing evidence for the presence of an acetyl group and from the low frequency of the carbonyl, 1618 $cm.^{-1}$, it must be attached to nitrogen. In addition a broad band at 3205 $cm.^{-1}$ suggested the presence of an hydroxyl group which in the solid state is bonded to the amide carbonyl.

Acetylation with the same reagents, but at an elevated temperature, gave a different compound. The infrared spectrum showed carbonyl absorption at 1758 $cm.^{-1}$ and 1641 $cm.^{-1}$ which was in accord with its formulation as an O:N diacetate, and although the carbon and hydrogen figures were consistent with this, the acetyl determination was again anomolous. (Found: C,62.7; H,5.85; Ac, 12.1, 12.2. $C_{17}H_{17}O_5N,2COCH_3$ requires C,62.8; H,5.8; Ac, 21.4).

It has been pointed out by previous authors[4] that where analytical data is ambiguous for compounds containing both N-acetyl and O-acetyl groups the infrared spectrum usually makes it possible to decide between the various structures. Another point regarding the infrared spectrum of this compound was the rather high frequency of the carbonyl absorption of the O-acetate. Alcoholic acetates usually absorb at lower frequencies in the range 1750 $cm.^{-1}$ to 1735 $cm.^{-1}$.

The formation of an O:N diacetate indicated:

(1) Treatment of the alkaloid with alkali had resulted in a molecular rearrangement,

(2) the rearrangement product possessed an N-H (or NH_2) group.

Treatment of the rearrangement product with methyl iodide in chloroform gave prisms m. p. 245—246° from ethanol. On dissolving this compound in alkali and extracting with ether or chloroform a base ($C_{17}H_{18}O_5NCH_3$) m. p. 214° was obtained.

Since both compounds gave the same picrate it is obvious that the compound m. p. 245—246° is the hydriodide of the N-methyl base m. p. 214°. This N-methyl

base was shown to contain a tertiary nitrogen atom since methylation with methyl iodide gave a quarternary salt. Thus the rearrangement product must be a secondary amine as suggested by its acetylation products.

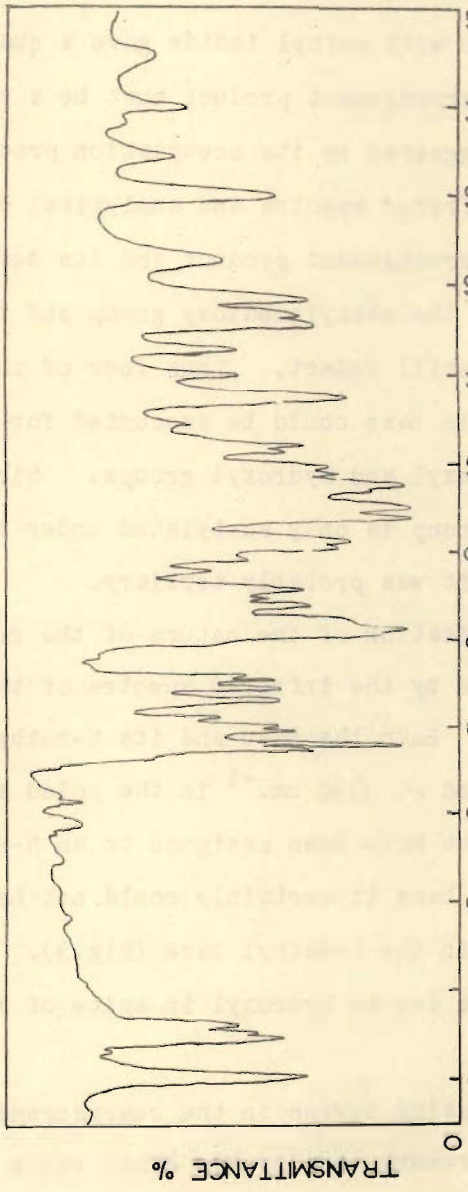
The infrared spectra and analytical data of the alkaline rearrangement product and its derivatives showed that the methylenedioxy group and the methoxyl group were still intact. Thus four of the five oxygen atoms in this base could be accounted for in a methylenedioxy, methoxyl and hydroxyl groups. Since the hydroxyl group is only acetylated under fairly rigorous conditions it was probably tertiary.

An indication of the nature of the remaining oxygen was provided by the infrared spectra of the above compounds. Both the base and its N-methyl derivative showed a band at 3330 $cm.^{-1}$ in the solid state which although might have been assigned to an N-H stretching mode in the base it certainly could not have this assignment in the N-methyl base (Fig.3). It must therefore be due to hydroxyl in spite of its very low frequency.

The missing oxygen in the rearrangement product was seemingly present as a cyclic ether since the foregoing

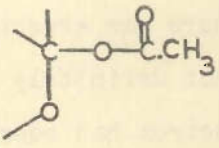
140

FIG. 3


EPITAZETTINE
WAVELENGTH (MICRONS)
TRANSMITTANCE %
0
3
4
5
6
7
8
9
10
11
12
13
14
15

evidence, and the absence of carbonyl absorption excludes other oxygen functions.

Furthermore the high frequency of the O-acetyl is explicable if an adjacent oxygen function is present as in the partial structure (I).



I

This partial structure might also account for the low frequency of the hydroxyl absorption in the base since some tertiary lactol hydroxyls are known[5] to absorb at even lower frequencies in the solid state, due to intermolecular hydrogen bonding.

Since this partial structure was present in acetyl tazettine (I) the possibility of a similar structure for the rearrangement product was considered.

The presence of the tazettine ring system was strongly suggested when the product obtained by oxidation of the N-methyl base with manganese dioxide gave a

compound analysing for $C_{18}H_{19}O_6N$. That an extra oxygen molecule should have been incorporated in the molecule is both significant and unusual with this reagent, which is normally only expected to oxidise an allylic hydroxyl to the $\alpha\beta$-unsaturated ketone. The similarity of the ultra-violet spectrum to that of the N-methyl base indicated that an $\alpha\beta$-unsaturated chromophore was absent in the molecule, although it was not definitely excluded until the ultra-violet spectrum had been examined both at varying concentrations and different pH.

Though the spectrum showed some variation with concentration and pH no evidence was obtained for the presence of an $\alpha\beta$-unsaturated ketone chromophore.

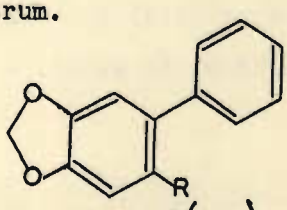
This oxidation found parallel in the reported manganese dioxide oxidation of tazettine to a lactone-amide, tazettamide.

The infrared spectrum of the manganese dioxide oxidation product of the N-methyl base showed carbonyl absorption at 1733 $cm.^{-1}$ and 1667 $cm.^{-1}$ in chloroform solution, which was in excellent agreement with absorptions reported for the corresponding compound

obtained from tazettine.

The evidence so far accumulated suggested that the N-methyl base of the rearrangement product was a stereo-isomer of tazzetine though different positions for the double bond and the methoxyl were not excluded at this juncture.

This premise was given further support when Dr. Wright of these laboratories found that the Hofmann degradation products of the N-methyl base were identical to those reported for tazettine. The Hofmann degradation of the quarternary salt obtained from the N-methyl base gave an oily methine. Treatment of the methine with dilute hydrochloric acid gave 6-phenylpiperonyl N:N dimethyl glycine (structure II), isolated as the picrate, and 6-phenylpiperonyl chloride (structure III) m. p. 59°. 6-phenylpiperonyl chloride was characterised by converting it to 6-phenylpiperonyl alcohol (structure IV) m. p. 101° which was identified by mixed melting point determination and from its infrared spectrum.



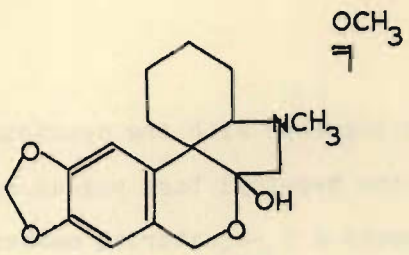
II, R= $CH_2O.CO.CH_2N(CH_3)_2$
III, R= Cl
IV, R= OH

The isolation of 6-phenylpiperonyl chloride provides evidence for the substitution pattern on the aromatic ring of the alkaloid and its rearrangement product i.e. that it is a 3:4-methylenedioxyphenyl derivative. The rearrangement product may be assigned the partial structure (V) with the double bond and methoxyl in ring C.

A comparison of the molecular rotations of the N-methyl base and the lactone-amide with those of certain tazettine derivatives proved useful.

Tazettine was reported [6] to be demethylated to a pair of epimeric allylic alcohols, tazettinol and iso-tazettinol. The latter compound predominated and was shown to possess the hydroxyl in the more stable configuration, since oxidation of either deoxytazettinol or deoxyiso-tazettinol to the $\alpha\beta$-unsaturated ketone followed by metal-hydride reduction gave predominently deoxyiso-tazettinol (see Part I section III).

This lead to the conclusion that the hydroxyl in iso-tazettinol is equatorial and that the C_3-O bond in tazettine and tazettinol is axial.



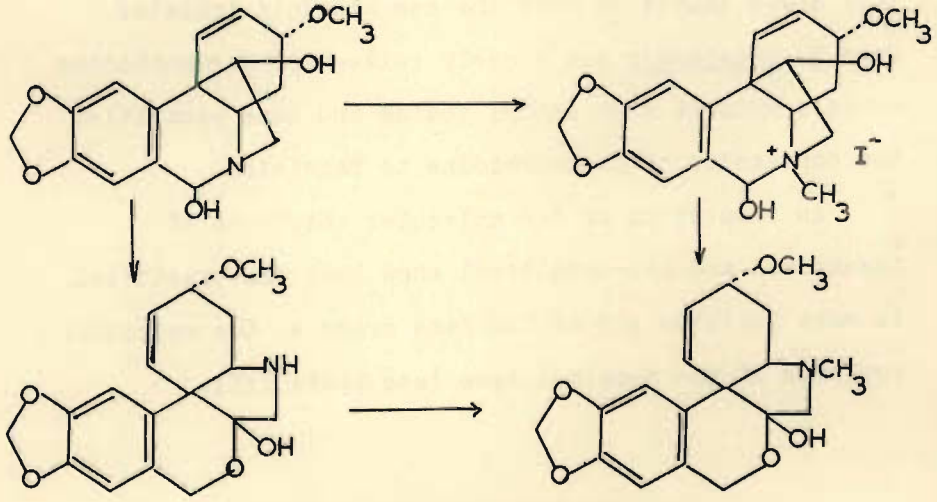
V

During the course of the early stages of this work Wildman reported[7] a revised structure for Haemanthidine and showed that its conversion to Tazettine was accompanied by a molecular rearrangement, (see Part I, section IV). The evidence obtained at this stage indicated that the new alkaloid isolated from H. natalensis was closely related to Haemanthidine since treatment with methyl iodide and base paralleled the conversion of Haemanthidine to Tazettine.

An inspection of the molecular rotations of tazettinol and iso-tazettinol show that iso-tazettinol is more positive and of the same order as the molecular rotation of the N-methyl base (see Table III).

These results together with the previous evidence suggested that if the N-methyl base was an epimer of tazettine it possessed a C_3-equatorial methoxyl group. If this supposition was correct the structure of the alkaloid as 3-epihaemanthidine is indicated. A possible mechanism for the reported conversion of haemanthidine to apohaemanthidine is represented as shown in Chart III/I. If this is correct then 3-epihaemanthidine on treatment with hydrochloric acid would be expected to form the same compound, since a common resonance stabilised carbonium ion intermediate (Chart III/I) would be involved.

CHART III/4



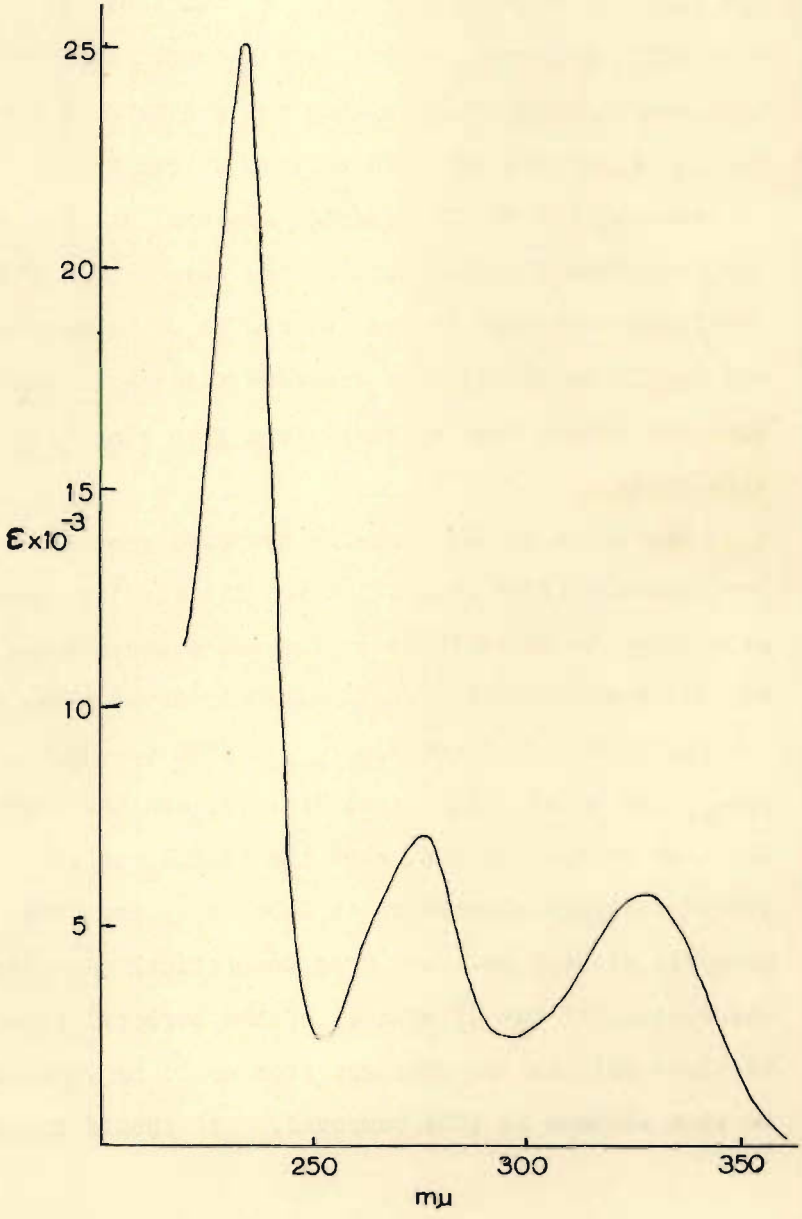
THE ALKALINE REARRANGEMENT OF

3-EPIHAEMANTHIDINE AND ITS METHIODIDE

That apohaemanthidine was obtained by treating the alkaloid with 6N hydrochloric acid provided conclusive evidence for the 3-epihaemanthidine structure. This reaction not only defined the position of the functional groups but also indicated that the stereochemistry of the benzylic hydroxyl in haemanthidine and 3-epihaemanthidine must be the same. The mild conditions employed in the conversion of haemanthidine and 3-epihaemanthidine to apohaemanthidine indicated that the bridge head hydroxyl is cis to ring C in both alkaloids.

Oxidation of the benzylic hydroxyl group in 3-epihaemanthidine gave a lactam with similar spectral properties to those reported for oxohaemanthidine.[7] The ultra-violet spectrum in ethanol showed three maxima $\lambda_{max.}$ 234 mμ (ε ,22,900), $\lambda_{max.}$ 275 mμ (ε ,6,170), $\lambda_{max.}$ 326 mμ (ε ,5,130) (see Fig. 4), and the infrared spectrum of the compound when run in CCl_4 solution showed carbonyl absorption at 1696 $cm.^{-1}$ and free hydroxyl at 3605 $cm.^{-1}$. From theoretical considerations the overlap of the π orbital of the carbonyl group and the lone pair on the nitrogen atom would be expected to be at a minimum in this compound. It should therefore

FIG. 4



possess the properties of an amino ketone rather than a lactam and the spectral evidence supports this view.

The preparation of the N-methyl base of the alkaline rearrangement product was achieved by a more direct route.

Treatment of the methiodide of 3-epihaemanthidine with cold dilute sodium hydroxide solution or sodium carbonate solution gave the N-methyl base of the rearrangement product. This reaction paralleled the reported conversion of haemanthidine to tazettine and gave further support to postulate that the N-methyl base was epitazettine.

The reactions shown in Chart III/2 would seem to represent a reasonable mechanism for the rearrangement of 3-epihaemanthidine. (IX).

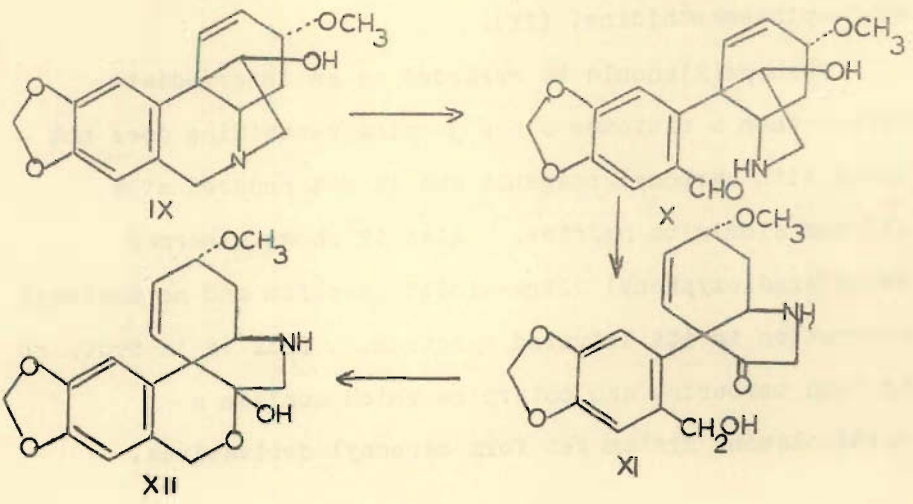
Perhaps(X)should be regarded as an intermediate rather than a tautomer since 3-epihaemanthidine does not react with carbonyl reagents and is not reduced with lithium aluminium hydride. Also it shows a normal methylenedioxyphenyl ultra-violet spectrum and no carbonyl absorption in its infrared spectrum. This is in contrast to both berberine and cotarnine which contain a carbinolamine system yet form carbonyl derivatives.

It is obvious that the carbinolamine system in these alkaloids must be more mobile than that found in 3-epihaemanthidine.

The intermediate (X) is then considered to undergo oxidation-reduction to the keto-alcohol (XI) which ring closes to the cyclic lactol (XII).

Thus if (X) is regarded as an intermediate in a base catalysed reaction then the corresponding intermediate for the methiodide should be formed more readily due to the increased inductive effect of the quarternary nitrogen (Chart III/3) i.e behaving as a normal carbinolamine.

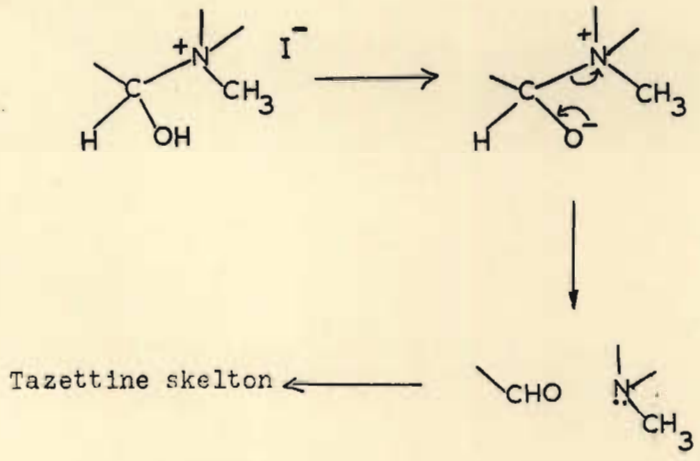
CHART III/2



The fact that the methiodide rearranges with cold sodium hydroxide solution or sodium carbonate solution whereas it is necessary to use hot sodium hydroxide to effect the rearrangement of the alkaloid would seem to support the postulated mechanism.

The rearrangement of both the alkaloid and the methiodide are summarised in Chart III/4

CHART III/3



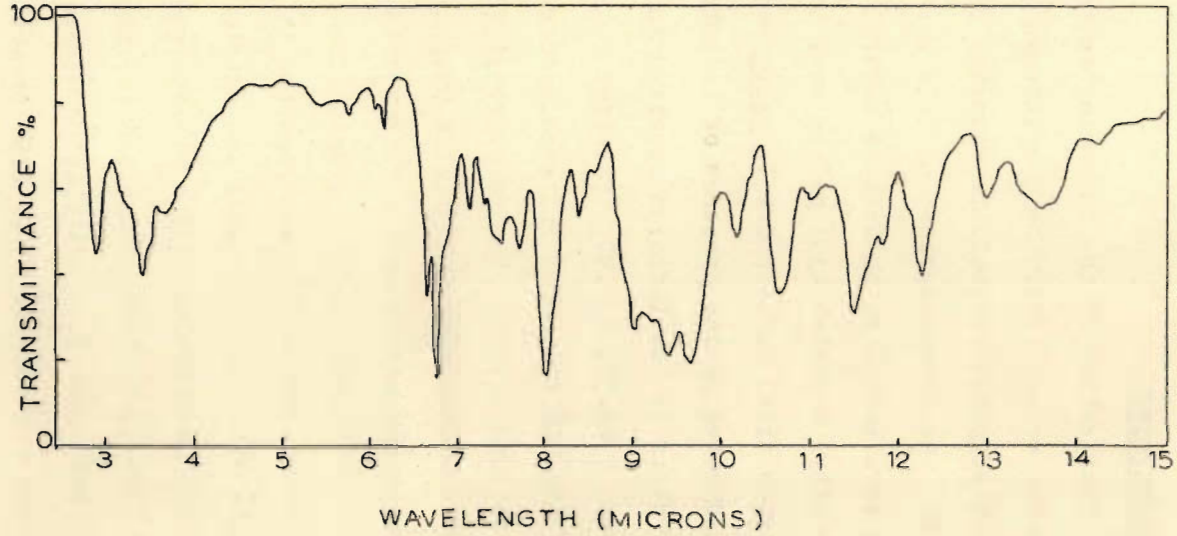
REARRANGEMENT OF THE METHIODIDE.

Since this work was completed and submitted for publication a note [8] reporting the isolation of 3-epihaemanthidine from *Crinum* species appeared. The structure of the alkaloid was given, and the alkaline rearrangement of its methiodide was reported to yield a compound which was found to be identical with *criwelline*, an alkaloid isolated by Boit.

HAEMANTHIDINE.

Combination of the chromatogram fractions emerging from the column after the 3-epihaemanthidine had been eluted afforded crystalline material of wide melting range. Re-chromatography of this material on alumina gave an alkaloid which despite several crystallisations from ethyl acetate still melted over the range 180—182°. A sample dried for 4 hours in vacuo was still solvated as indicated by the presence of an ester carbonyl absorption in the infrared spectrum and analysis (Found: C,63.50; H,5.97; Calc.for $C_{17}H_{19}O_5N,\frac{1}{2}CH_3CO_2C_2H_5$. C,63.15; H,6.4). The infrared spectrum showed (Fig.5) absorptions attributable to hydroxyl ($CHCl_3$) 3595 $cm.^{-1}$. It gave a deep wine colour with sulphuric acid and with concentrated nitric acid a yellow colour which when diluted with water gave a crystalline precipitate. The alkaloid haemanthidine was reported[9] to give the same results with sulphuric acid and nitric acid.

The preparation of the picrate gave a compound of m. p. 209—210° (dec.)(reported for haemanthidine picrate m. p. 206° (dec.)). Acetylation with acetic anhydride afforded a diacetate m. p. 219° (reported[9] for haemanthidine



HAEMANTHIDINE

154
FIG. 5

diacetate 183° clear at 200—205°).

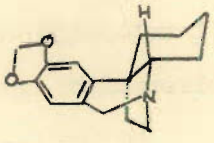
As the result of the above differences in the melting points of the alkaloid and derivatives to those reported for haemanthidine it was decided to investigate the action of dilute alkali on the methiodide of the base. After treating the methiodide with 10% potassium hydroxide solution and extracting with chloroform a gum was obtained which afforded prisms m. p. 202—204°C, after several crystallisations from acetone. (Reported[10] for tazettine m. p. 212—213° (vac.) 210—211°, 208—209°, 207—208°). It analysed correctly for $C_{18}H_{21}O_5N$ and the optical rotation and the ultra-violet spectrum were in good agreement with the reported values $[\alpha]_D$ +158° (reported $[\alpha]_D$ +160°) $\lambda_{max.}$ 242 mμ (ε,4790), $\lambda_{max.}$ 295 mμ (ε,4470),(reported $\lambda_{max.}$ 242 mμ (ε,4790), $\lambda_{max.}$ 295 mμ (ε,4470).

Further confirmation of its identity as tazettine was obtained by the isolation of isotazettinol from acid hydrolysis. The isotazettinol was converted to O-methylisotazettine methopicrate which was required for comparison with epitazettine methopicrate.

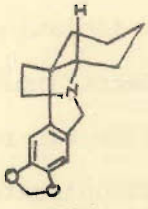
STEREOCHEMISTRY OF 3-EPIHAEMANTHIDINE.

1. The Skeleton.

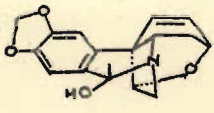
Only two stereochemical conformations (XIII) and (XIV) are possible for the alkaloids based on the 5:10b-ethanophenanthridine skeleton. Wildman[11] has pointed out that formation of apohaemanthidine and apohaemanthamine is only possible if haemanthidine and haemanthamine are based on the stereostructure (XIII) or its mirror image. It is sterically impossible for the structure containing the cis B:C ring fusion to give a C_3-C_{11} ether bridge, and hence the stereostructure of apohaemanthidine may be expressed as (XV). Therefore in view of its conversion to apohaemanthidine 3-epi-haemanthidine must be based on the same enantiomorphic skeleton as haemanthidine and haemanthamine (trans B:C ring fusion).



XIII



XIV



XV

2. The C_6-hydroxyl.

Since 3-epihaemanthidine yields apohaemanthidine under identical conditions to that used for the preparation of apohaemanthidine from haemanthidine the configuration of the benzylic hydroxyl must be the same in both alkaloids.

It has been reported[12] that sodium borohydride reduction of oxohaemanthidine and oxo-apohaemanthidine affords haemanthidine and apohaemanthidine respectively.

An inspection of molecular models revealed that the C_6 position is not sterically hindered and metal-hydride reduction of a keto group in this position would be expected to yield the thermodynamically more stable alcohol.[13] Thus on this basis the C_6-hydroxyl may be assigned the pseudo equatorial configuration in haemanthidine and 3-epihaemanthidine.

3. The C_{11} hydroxyl.

The mild conditions employed in the formation of apohaemanthidine imply that the C_{11}-hydroxyl must be *cis* to ring C. Recently[14] spectroscopic evidence has been presented to confirm this indication. If the C_{11}-hydroxyl is *cis* to ring C it is favourably situated

for hydrogen bonding with the π electrons of the 1-2 double bond and therefore reduction of the double bond would cause some shift in the hydroxyl stretching frequency. In the alternative position the hydroxyl may bond with the π electrons of the aromatic ring and reduction of the 1-2 double bond should not affect the hydroxyl frequency. Wildman[14] has shown that the hydroxyl stretching band in haemanthamine occurs at 3598 cm.$^{-1}$, which is 27 cm.$^{-1}$ lower than that in the dihydro derivative when both are observed in CCl_4 at high dilution, indicating that the overlap of the hydrogen of the hydroxyl is occurring with the ring C double bond.

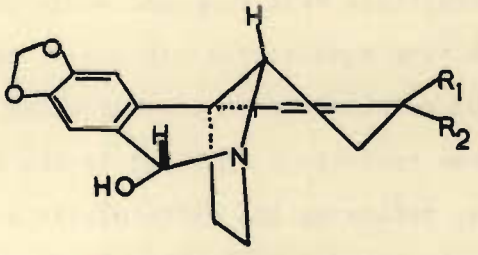
Since hydrogenolysis of the benzylic hydroxyl group in haemanthidine yields haemanthamine the configuration of the C_{11} hydroxyl must be the same in both alkaloids.

C_3-methoxyl.

A study of the dissociation constants of the pair of allylic alcohols tazettinol and isotazettinol, and degredation studies lead the Japanese workers[15] to conclude that the methoxyl in tazettine is axial and

cis to the aromatic ring. This was recently confirmed by the synthesis of a degradation product (see section on the stereochemistry of tazettine). The conditions employed for the conversion of haemanthidine methiodide to tazettine are extremely mild and there is no reason to doubt that these groups are also cis in haemanthidine; and on this basis haemanthidine may be assigned the complete stereo-structure (XVI) (or its mirror image).

If the assumption made above that the configuration of the C_{11}-hydroxyl is the same in both haemanthidine and 3-epihaemanthidine is correct, then 3-epihaemanthidine must possess an equatorial methoxyl which is trans to the aromatic ring. Therefore 3-epihaemanthidine is represented as (XVII) (or its mirror image).

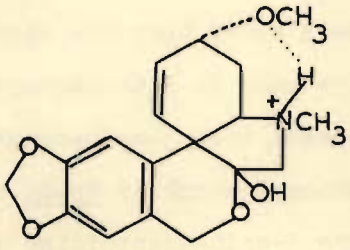


XVI; R_1=OCH_3, R_2=H

XVII; R_1=H, R_2=OCH_3

Stereochemistry of epitazettine.

The above conclusion regarding stereochemistry of 3-epihaemanthidine therefore defines most of the stereochemistry of the product obtained by rearranging the methiodide. The methoxyl is equatorial and trans to the aromatic ring and therefore fulfills the prediction made earlier from an analysis of rotational data of the tazettinols.



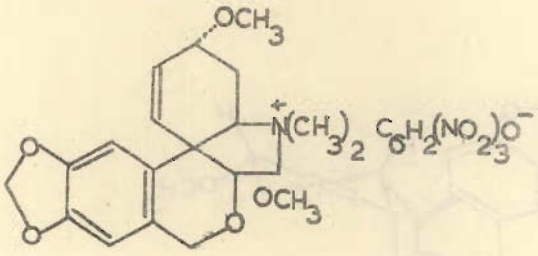
XVIII

On demthylation this compound would be expected to yield the same equilibrium mixture of tazettincl and isotazettinol as obtained on demethylating tazettine.

The same conditions employed in the demethylation of tazettine, refluxing 10% hydrochloric acid, were without effect on the N-methyl base. More drastic conditions employing hydriodic acid in glacial acetic

acid gave intracticable products.

A possible explanation of the stability of epitazettine to hot hydrochloric acid is that the proton of the conjugate acid from epitazettine (XVIII) is hydrogen bonded with the oxygen of the methoxyl group and thereby stabilising it. This explanation has been advanced by Ikeda et al.[15], to explain the greater basicity of deoxy-isotazettinol than of deoxytazettinol.

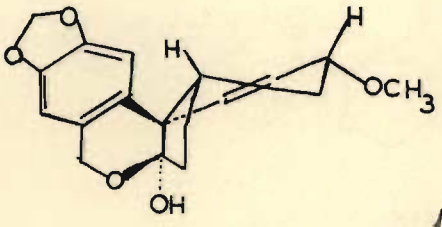


XIX

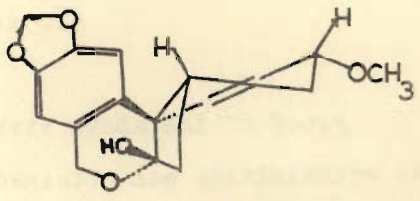
Proof of the above structure of the N-methyl base as epitazettine was obtained by methylating with dimethyl sulphate and isolating the quarternary salt as its picrate (XIX).

This proved to be identical with the product obtained from treating isotazettinol with dimethyl sulphate.

The stereochemistry of the hemi-ketal ring in epitazettine and tazettine are shown to be the same by this reaction and the structure of epitazettine may be presented as (XX) or (XXI).



XX



XXI

These structures only differ in the mode of ring B:D fusion and a decision between them is not readily made.

The Absolute Configuration of 3-Epihaemanthidine and Alkaloids Containing the 5:10b-Ethanophenanthridine (trans B:C) Ring System.

Wildman [11] has shown that the alkaloids containing the 5:10b-ethanophenanthridine ring system are elaborated from both enantiomorphs of this basic nucleus.

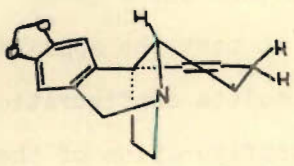
The alkaloids, crinine, powelline, buphanisine, buphanidrine, buphanamine and undulatine were related to the (-)-crinane nucleus. Vittatine, haemanthamine, haemanthidine, haemultine and crinamine were shown to contain the enantiomorphic (+)-crinane nucleus. With this information and the fact that all the above alkaloids except buphanamine contained a common allyl ether or allyl alcohol system (1:2 double bond and a 3-oxy substituent) it was of interest to test the application of Mills's rule for assigning absolute configuration to this series. In addition the configuration of the 3-oxy substituent was known to be pseudo axial in all the above alkaloids except crinamine.

3-Epihaemanthidine has been shown in the previous section to have its methoxyl in the pseudo-equatorial

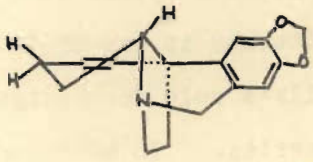
configuration, also it must be derived from the (+)-crinane nucleus since it is converted to apohaemanthidine.

The investigations into the chemistry of powelline and crinine had led to the preparation of the 3-epimers and there was a sufficient number of compounds to test the application of the Mills's rule to this series.

(+)-Crinene* and (-)-crinene may be represented by the structures (XXII) or (XXIII) [or vice versa].



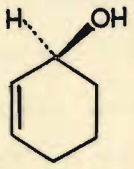
XXII



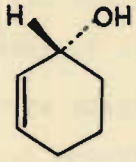
XXIII

* Wildman has found that in course of demethoxylating powelline two isomeric compounds were obtained which on catalytic reduction gave (-)-crinane. These could only differ in the position of the double bond in ring C and were designated α-and β-crinenes. For the sake of the discussion in this section the name crinene will be used for the structure possessing 1:2 unsaturation.

Mills[16] has shown that for allylic alcohols and certain of their derivatives in terpenes and steriods structure (XXIV) is more laevorotatory than its stereoisomer (XXV).



XXIV



XXV

The application of this rule for structure (XXII) would mean that for alkaloids based on this enantiomorphic skeleton those possessing a pseudo axial oxygen function at position 3 (XXII, R_1, = OH,OCH_3, R_2 = H) would be more laevorotatory than their epimers. For those alkaloids based on the enantiomorphic skeleton (XXII) an oxygen function in the pseudo axial configuration (XXIII, R_1 = OH,OCH_3, R_2 = H) would on the basis of Mills's rule be less laevorotatory than their epimers.

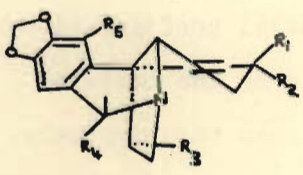
An inspection of the molecular rotations (see Table IV) of the alkaloids derived from the (+)-crinane nucleus revealed that those possessing the pseudo axial 3-oxy

substituent were more laevorotatory than their epimers.

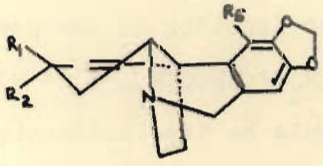
The alkaloids known at present to be derived from the (-)-crinane skeleton (except buphanamine) all possess a C_3-pseudo axial substituent and a comparison of the rotations of their synthetic epimers, where possible, revealed that a C_3-pseudo axial substituent was less laevorotatory than its C_3-epimer (see table (V)).

These data indicate that the absolute configuration of the (+)-crinene structure must be as represented as structure (XXII) and (-)-crinene its mirror image (XXIII).

Thus (+)-crinane is (4aR,10bR)-1,2,3,4,4a,5,6,10b-octahydro-8,9-methylenedioxy-5,10b-ethanophenanthridine.



XXVI



XXVII

TABLE IV.

MOLECULAR ROTATIONS OF ALKALOIDS
DERIVED FROM THE (+)-CRINANE NUCLEUS.

C_3 pseudo axial substituent.		C_3 pseudo equatorial substituent.	
Haemanthamine	M_D = + 59	Crinamine	M_D = + 496
Haemanthidine	M_D = - 129	3-epihaemanthidine	M_D = + 156
6-oxohaemanthidine	M_D = - 130	6-oxo-epihaemanthidine	M_D = + 91

TABLE V.

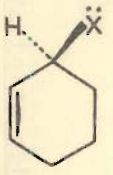
MOLECULAR ROTATIONS OF ALKALOIDS
DERIVED FROM THE (-)-CRINANE NUCLEUS.

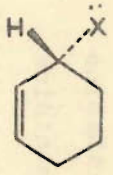
C_3 pseudo axial substituent.		C_3 pseudo equatorial substituent.	
Crinine	M_D = - 51	epicrinine	M_D = - 385
Powelline	M_D = 0.0	epipowelline	M_D = - 310

The absolute configurations* of the alkaloids based on the 5,10b-ethanophenanthridine nucleus are given in Table VI.

This work has now received further support from the observation[17] that crinanone shows a negative rotatory dispersion curve, which is predicted from the octant rule[18] on the basis of the above assignment of absolute configuration.

The use of epimeric methyl ethers for comparison of molecular rotations in applying Mills's rule is also justified in view of the recent work of Brewster[19] who considers that structure (XXVIII), where $\ddot{X}$ is more polarisable than hydrogen it will be more laevorotatory than its epimer (XXIX).





* Since the publication of these results[20] Wildman has come to the same conclusion regarding the absolute configuration of alkaloids based on the 5,10b-ethanophenathridine skeleton (see section on stereochemistry of the alkaloids derived from 5,10b-ethanophenanthridine).

TABLE VI.

Absolute Configuration of Amaryllidaceae Alakloids derived from 5,10b-Ethanophenanthridine.

Alkaloid.	Formula	R_1	R_2	R_3	R_4	R_5
(+)-Crinene	XXVI	H	H	H	H	-
Haemanthidine	XXVI	OMe	H	OH	OH	-
epiHaemanthidine	XXVI	H	OMe	OH	OH	-
Haemanthamine	XXVI	OMe	H	OH	H	-
Crinamine	XXVI	H	OMe	OH	H	-
Vittatine	XXVI	OMe	H	H	H	-
Haemultine	XXVI	H	H	OH	H	-
(-)-Crinene	XXVII	H	H	-	-	H
Crinine	XXVII	OH	H	-	-	H
epiCrinine	XXVII	H	OH	-	-	H
Buphanasine	XXVII	OMe	H	-	-	H
Powellane	XXVII	H	H	-	-	OMe
Powelline	XXVII	OH	H	-	-	OMe
epiPowelline	XXVII	H	OH	-	-	OMe
Buphanidrine	XXVII	OMe	H	-	-	OMe

SECTION II.

THE ALKALOIDS OF NERINE KRIGEII.

The alkaloids of Nerine krigeii were first investigated by Wildman[21] in 1955. In addition to lycorine, two new alkaloids, neronine and krigeine, were isolated and evidence was presented for their structures (see Part I, section II). The author has reinvestigated the alkaloids of this plant and in addition to the above three alkaloids a new alkaloid, for which the name krigenamine is proposed, has been isolated. The method of extraction employed by the author suggests that the lactone alkaloid, neronine does not occur in the plant but is produced as an artefact in the isolation procedure.

EXTRACTION.— Bulbs of Nerine krigeii, collected in May from Maraisburg, Transvaal, were sliced and dropped into hot ethanol. The alcohol was filtered off and the residual solid material dried and re-extracted with alcohol. The combined extracts were concentrated in a climbing film evaporator and the concentrate was steam distilled to remove the volatiles and to precipitate the fats.

In view of the ready disproportionation of lycorenine and krigeine on alumina it seemed desireable to establish unequivocally the presence of a lactone alkaloids in the crude plant extract. Fortunately means were readily

available to make a decision on this matter in that the known lactone alkaloids of the Amaryllidaceae show an unusual property in the chloroform solubility of their hydrochlorides.

The concentrate was acidified with hydrochloric acid and then extracted with chloroform. The chloroform extracts gave no precipitate with silicotungstic acid or Mayer's reagent indicating the absence of alkaloids possessing chloroform soluble hydrochlorides in the plant.

The subsequent isolation of the lactone alkaloids, neronine and oxokrigenamine, thus arose as the result of disproportionation on alumina.

The alkaloidal bases were obtained in the normal way by basifying the aqueous solution and extracting with chloroform containing 5% ethanol.

Concentration of the chloroform extract caused the precipitation of crude lycorine which after purification was identified by its infrared spectrum. Removal of all the chloroform left the remaining alkaloids as a dark brown viscous gum.

Chromatography of the crude alkaloid extract over alumina yielded the known alkaloids neronine and krigeine and two new bases. One of these bases, for which the name krigenamine is proposed, had been isolated by the author in a previous extraction of Nerine krigeii. The other base was readily characterised as a lactone from its spectral properties and like neronine it must have occurred as the result of disproportionation on alumina during chromatography. This lactone base was subsequently identified as oxokrigenamine. Elution of the column with chloroform gave a large amount of non-crystalline alkaloid(s) in the early fractions. This was followed by fractions which contained mixtures of oxokrigenamine and neronine which were only separated satisfactorily by re-chromatography. Subsequent fractions gave neronine followed by neronine-krigenamine mixtures. In those fractions containing mixtures of neronine and krigenamine advantage was taken of the chloroform solubility of neronine hydrochloride in separating it from krigenamine. On changing the solvent to chloroform 1-5% ethanol small amounts of krigeine were eluted.

KRIGENAMINE.—— Trituration of the crude gummy krigenamine with ethyl acetate gave a white solid which could be crystallised from acetone, ethanol, or ethyl acetate. It afforded silky needles after several crystallisations from ethyl acetate, m. p. 210—211° $[\alpha]_D$ +210° (in chloroform).

The ultra-violet spectrum of the alkaloid showed a maximum centred around 280 mu (ε, 1,385) (Fig.6).

The close similarity of the infrared spectra of krigenamine (Fig.7) and that of the known alkaloid, krigeine (Fig.8) suggested that they must possess features in common. The presence of a methoxymethylenedioxyphenyl chromophore was indicated by a strong absorption band at 1616 $cm.^{-1}$. Also the low intensity of the aromatic chromophore in the ultra-violet spectrum of the alkaloid was compatable with the presence of this grouping. A positive response to the Labat test confirmed the presence of a methylenedioxy group.

Analysis of krigenamine indicated the molecular formula $C_{18}H_{21}O_5N$. Furthermore the presence of one methoxyl group and one N-methyl group was established by standard analytical procedures. Although the

FIG.6

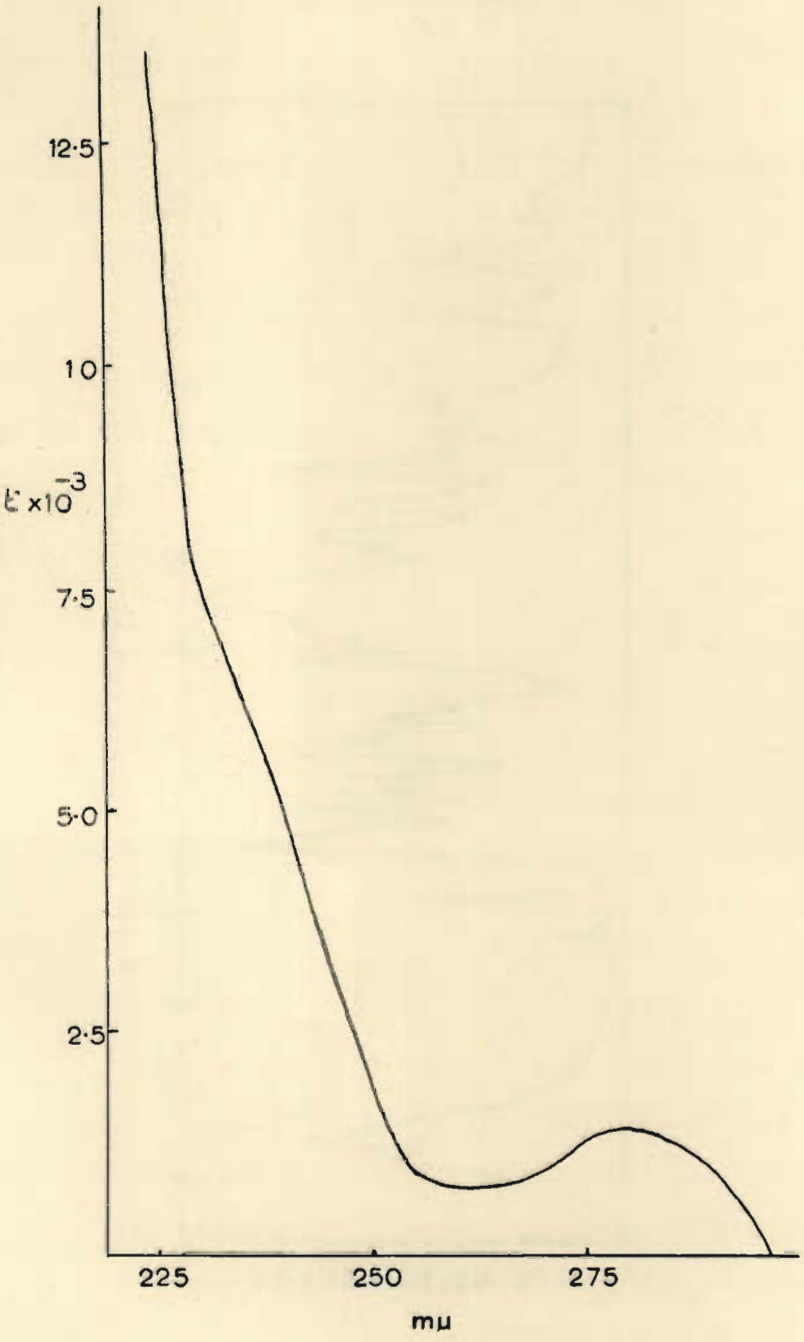
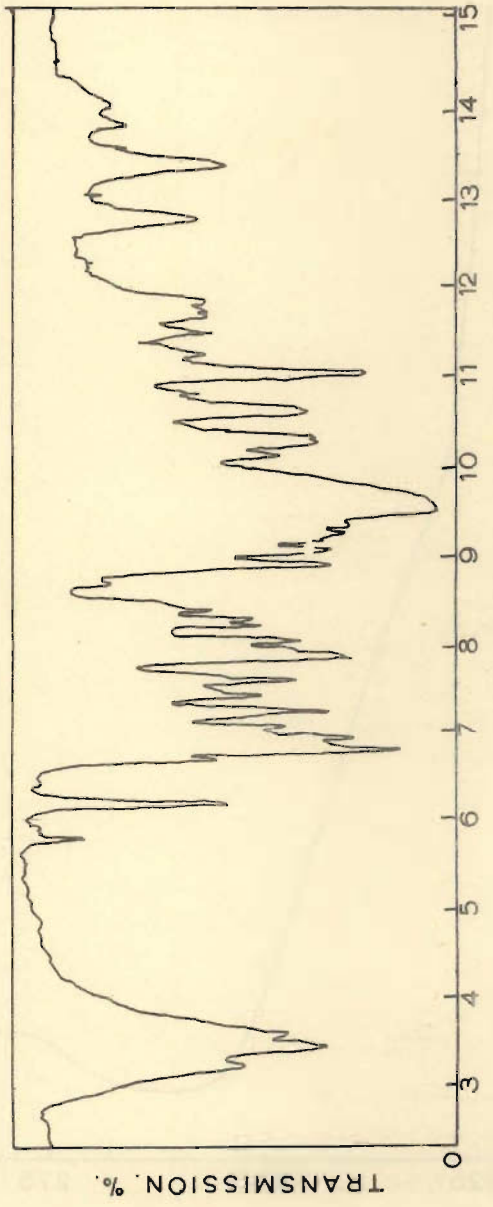
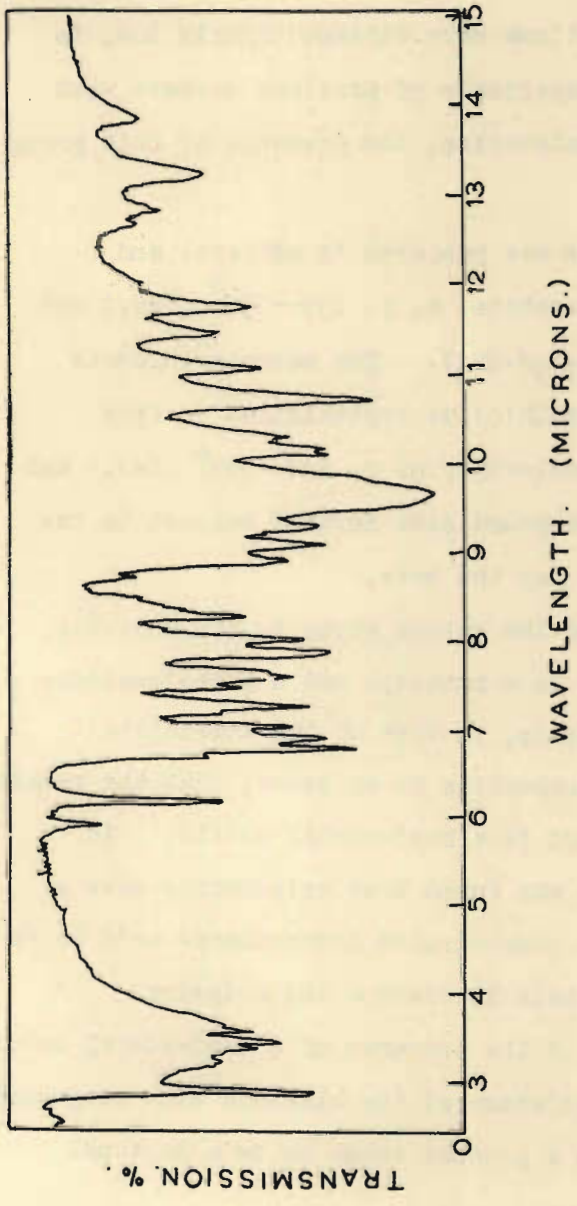


FIG. 7



KRIGENAMINE.

FIG. 8



KRIGEINE.

N-methyl determinations were dissapointingly low, in view of the same experience of previous workers with lycorenine and homolycorine, the presence of this group was indicated.

The methiodide was prepared in methanol and crystallised from acetone, m. p. 235—237° (dec.) and analysed for $C_{18}H_{21}O_5N.CH_3I$. The methoperchlorate prepared from the methiodide crystallised in fine needles from methanol-ether m. p. 248—249° (dec.) and analysis of this compound gave further support to the $C_{18}H_{21}O_5N$ formular for the base.

Since three of the oxygen atoms in the molecule were accounted for in a methoxyl and a methylenedioxy group, it seemed likely, in view of the alkaloid's similar spectral properties to krigeine, that the remaining two were present in a hemi-acetal moiety. In support of this it was found that krigenamine gave a yellow colour with concentrated hydrochloric acid as do the known hemi-acetals lycorenine and krigeine.

Confirmation of the presence of a hemi-acetal moiety was obtained by oxidation of the alkaloid with manganese dioxide which gave a product shown to be a lactone.

The oxidation was studied spectrophotometrically and during the course of three hours the absorption which had appeared at 1717 $cm.^{-1}$ reached a maximum. Although the compound from this oxidation was not isolated in crystalline form, its infrared spectrum was identical to the spectrum of crystalline oxokrigenamine isolated by chromatography of the crude alkaloid extract.

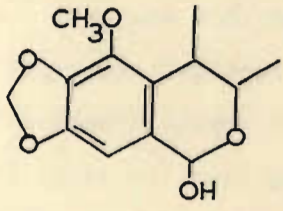
Oxokrigenamine crystallised from 'wet' ethyl acetate as massive prisms m. p. 70—75°. Further recrystallisations from the solvent did not improve the melting point and after drying at room temperature for 3 hours it analysed for the hydrate. However after drying for 3 days under high vacuum it was found that the melting point had changed and the sample had now an m. p. 146° $[\alpha]_D$ = +117°. Further characterisation of this compound was afforded by the preparation of the methiodide which crystallised from ethanol as rhombs m. p. 254—255° (dec.).

Analytical data on oxokrigenamine was in excellent agreement with the formular $C_{18}H_{19}O_5N$. The ultra-violet spectrum showed an intense maxima at 228 mμ (ε, 22,900)

and 285 mμ (ε , 6,026), and an inflection at 310 mμ (ε , 2,570).

This was very similar to the spectrum reported for neronine and indicated the compound was an aromatic δ-lactone. In common with the other known δ-lactones of the Amaryllidaceae the hydrochloride was soluble in chloroform.

The evidence accumulated at this stage indicated krigenamine could be represented by the partial structure I.*



I

*The methoxyl group is placed in this position in accordance with biogenetic and spectral reasons presented in Part II.

On the assumption that krigenamine possessed a lycorenine type nucleus then the preceeding analytical data indicates the presence of one ethylenic double bond. However attempts to reduce krigenamine and oxokrigenamine with hydrogen in the presence of a palladium-charcoal catalyst were unsuccessful; the bases being recovered unchanged. Catalytic hydrogenation of krigenamine in the presence of platinum resulted in the absorption of two moles of hydrogen. The reduction product crystallised from ether giving needles m. p. 145—155°, which was not improved by further re-crystallisations from this solvent. Chromatography in chloroform or ether resulted the rapid elution of the reduction product and crystallisation from ether again gave crystals melting over the range 145—155°. Since the catalytic hydrogenation of lycorenine is known to proceed by the simultaneous hydrogenolysis of the benzylic hydroxyl group and reduction of the double bond, to yield two isomeric decxydihydro compounds, a similar course was considered possible for the reduction of krigenamine. In support of this it was found that the crude hydrogenation product showed no absorption

attributable to a hydroxyl group in its infrared spectrum.

The failure to obtain a sharp melting point of the krigenamine reduction was therefore probably due to it being a mixture of the isomeric deoxydihydro compounds. In view of this a further attempt at resolving this mixture was made. This was partially successful in that one pure isomer was obtained after chromatography over alumina in benzene-petrol. Crystallisation of the later fractions from the column gave needles m. p. 160—162° $[\alpha]_D$ = +48° which analysed correctly for the deoxydihydro compound. Rotational data presented in Table I suggests that this compound has a trans C:D ring juncture and is accordingly named β-deoxydihydrokrigenamine (V). Further evidence for the structure of this compound was obtained by its preparation by another route. (vide infra).

Reduction of either krigenamine or oxokrigenamine (II) with lithium aluminium hydride gave a diol which crystallised from acetone in needles m. p. 171—172° $[\alpha]_D$ = -36°. (Found: C, 63.60; H, 6.75. calculated for $C_{18}H_{23}O_5N$. C, 63.85; H, 6.95). The ultra-violet spectrum showed a shoulder at 240 mμ (ε , 5,220) and a broad maximum at 280—285 mμ (ε , 1,400). On the basis

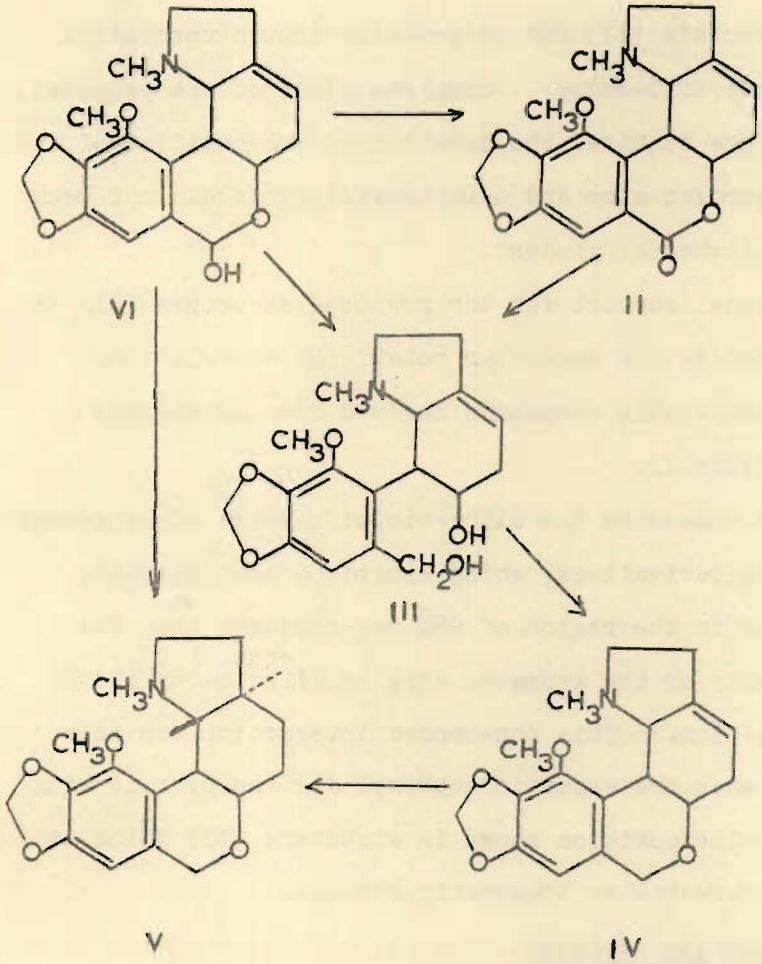
of the lycorenine type skeleton for krigenamine this may be represented by structure (III) in Chart III/5.

The diol was readily converted to the anhydro base, deoxykrigenamine (IV) by 5% sulphuric acid at 100°C. This compound was not obtained crystalline and it was characterised through its crystalline picrate and methiodide.

Deoxykrigenamine in the presence of a platinum catalyst absorbed one mole of hydrogen and gave an oily product which was resolved by chromatography into two fractions. The first of these was an oil and has not been characterised. However the second component eluted from the column crystallised from ether m. p. 160—162°. A mixed melting point determination of this compound and the crystalline product obtained from the reduction of krigenamine showed no depression. Furthermore the infrared spectra of these samples were identical. Thus the structure of the krigenamine hydrogenation product as a deoxydihydrokrigenamine is established by its method of preparation from deoxy-krigenamine.

The preceeding data is strong evidence in favour

CHART III/5



REACTIONS OF KRIGENAMINE

of structure (VI) for krigenamine though conclusive evidence is lacking. Complete proof of the proposed structure requires the location of the position of the nitrogen atom and unfortunately this has not been accomplished at present.

Some support for the proposed structure (VI) is obtained by the molecular rotational correlations with comparable compounds derived from lycorenine (see Table I).

Furthermore the ultra-violet spectra of krigenamine and its derivatives, which exhibit a low intensity maximum in the region of 280 mμ, suggests that the planarity of the aromatic ring is affected by steric interaction. This non-bonded interaction can only occur when the aromatic methoxyl and the pyrrole ring are in the position shown in structure (VI) which is also preferred on biogenetic grounds.

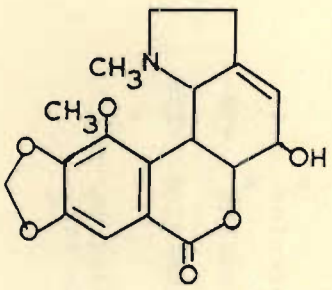
KRIGEINE AND NERONINE.

Krigeine and neronine were isolated from Nerine krigeii and characterised by Wildman[21] in 1955. Evidence was presented in favour of structure (VII) for neronine and is reviewed in Part I of this thesis. The structure of krigeine (VIII) was assigned on the basis of its oxidation to oxoneronine with manganese dioxide.

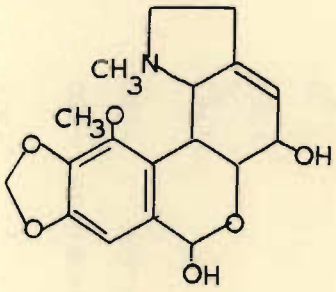
TABLE I.

	M_D		M_D	ΔM_D
Lycorenine	+549	Homolycorine	+282	+267
Krigenamine	+695	Oxokrigenamine	+385	+310
Homolycorine	+282	Tetrahydrohomolycorine*	-310	+592
Oxokrigenamine	+385	Tetrahydro-oxokrigenamine	-120	+505
Tetrahydrohomolycorine*	-310	Deoxylycorenine	+275	-585
Tetrahydro-oxokrigenamine	-120	Deoxykrigenamine	+387	-407
Deoxylycorenine*	+275	(-Deoxydihydrolycorenine*	-43	+318
		(β-Deoxydihydrolycorenine*	+58	+217
Deoxykrigenamine	+387	β-Deoxydihydrokrigenamine	+152	+235

All values refer to rotations recorded in chloroform except those with an asterisk in which ethanol was the solvent.



VII

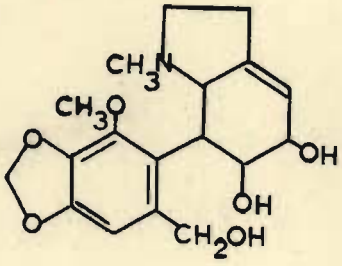


VIII

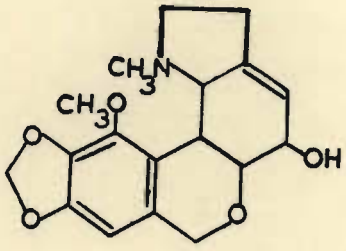
These two bases have been isolated by the author in this current study and additional evidence is presented in support of the structures proposed by Wildman.

Lithium aluminium hydride reduction of krigeine afforded tetrahydroneronine (IX) which indicates that the hydroxyl group in krigeine and neronine at the C_5-position have the same configuration.

In agreement with structure (IX) proposed for tetrahydroneronine it underwent facile dehydration to deoxykrigeine (X) with 5% sulphuric acid. Crystallisation of the product for ethyl acetate gave pure deoxykrigeine as prisms, m. p. 171—172° $[\alpha]_D$ = +196°. (Found: C, 64.93; H, 6.24. calculated for $C_{18}H_{21}O_5N$. C, 65.24; H, 6.39.).



IX



X

SECTION III.

THE ALKALOIDS OF HAEMANTHUS MAKENII.

The rare alkaloid coccinine has been isolated from Haemanthus makenii, a species of Amaryllidaceae for which no previous chemical study has been reported.

EXTRACTION.

Bulbs of Haemanthus makenii collected in May from the environs of Pietermaritzburg were sliced and extracted with boiling 95% ethanol. The once extracted bulbs were dried and re-extracted with a further quantity of alcohol.

The combined alcohol extracts were flash evaporated and steam distilled to remove the remaining alcohol and volatile compounds. The cooled solution, which was slightly acidic, contained a large amount of suspended fats which were removed by filtering through keiselguhr. The neutral and acidic material was extracted into ether, and the aqueous phase was basified with sodium carbonate. Repeated extraction of the aqueous phase was carried out with aliquots of chloroform until a negative reaction to Meyer's reagent was obtained. Removal of the solvent from the chloroform extract gave the crude alkaloidal material in the form of a viscous brown gum. The yield

of crude alkaloid was 0.6% on the dry weight of the bulbs.

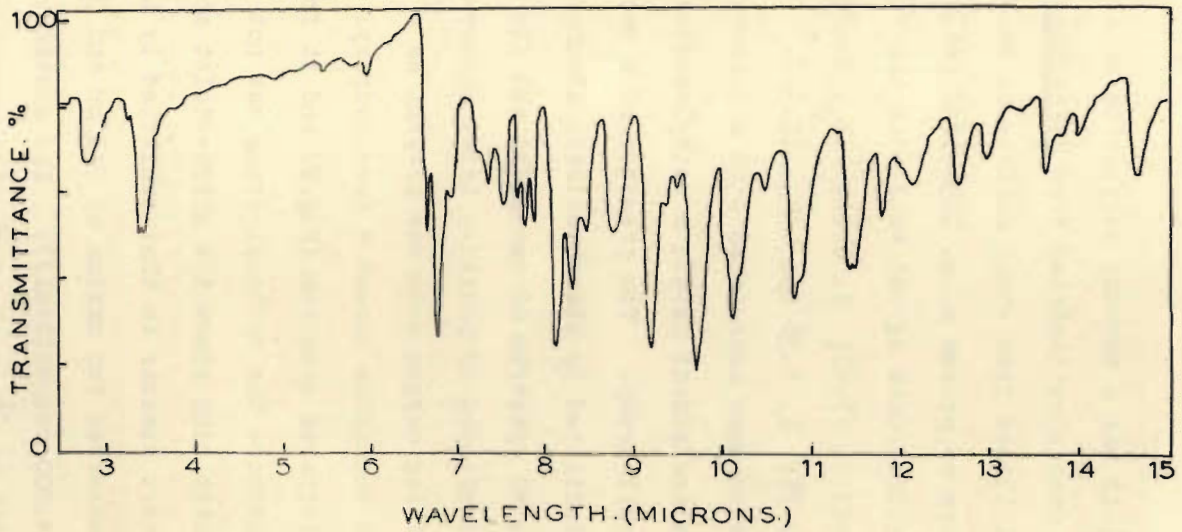
Chromatography of the crude alkaloid extract on alumina provided a clear separation of two alkaloids. The early fractions contain an alkaloid which crystallised readily from ether containing a small amount of ethanol. This alkaloid was subsequently shown to be coccinine which had previously been isolated by Wildman[22] from Haemanthus coccineus and several other Haemanthus species. The second alkaloid eluted from the column was presumed to be montanine since its melting point and the melting point of the perchlorate derivative agreed well with the figures reported for this alkaloid.

COCCININE.

Coccinine, $C_{17}H_{19}O_4N$, was first reported by Wildman[22] et al., in 1955. Several derivatives were prepared and the oxygen atoms were associated with a methylenedioxy group, one hydroxyl and one methoxyl function. Furthermore the alkaloid was reported to absorb one mole of hydrogen when reduced with platinum in acetic acid, and the nitrogen was shown to be tertiary. In view of the absence of N-methyl and C-methyl groups it was suggested that the

alkaloid was a methoxy derivative related to lycorine.

Coccinine isolated from Haemanthus makenii crystallised from ethyl acetate or ether-ethyl acetate mixture as prisms m. p. 163—164° $[\alpha]_D$ = -185°. Analytical data agreed well with the formular $C_{17}H_{19}O_4N$. (Found: C, 67.85; H, 6.24; OCH_3, 10.72. requires C, 67.76; H, 6.36 one OCH_3, 10.30). The oxygen functions were associated with a methoxyl, (from the above analytical data), a methylenedioxy group and one hydroxyl group. The presence of a methylenedioxy group was indicated by characteristic absorption bands in the infrared spectrum of coccinine and its derivatives and was confirmed by positive Labat colour-test. The remaining oxygen atom was present as a hydroxyl group since coccinine showed a free hydroxyl absorption band in its infrared spectrum (Fig.9) and it formed an O-acetate. Furthermore the methoxyl group was not attached to the aromatic ring since the ultra-violet spectrum of coccinine was very similar to that exhibited by hydrastine in that it possessed two maxima at 242 mμ and 293 mμ (ε, 4,370 and 4,900 respectively). The absence of strong absorption at 1616 $cm.^{-1}$ in the infrared spectra of coccinine and its



COCCININE.

194
FIG.9

derivatives also excluded the presence of the methoxy-methylenedioxyphenyl chromophore in these compounds.

The methiodide was prepared in methanol and crystallisation from ethyl acetate-ethanol gave fine needles m. p. 219—220° which was the same melting point as that reported for coccinine methiodide crystallised from water. However samples of coccinine perchlorate differed considerably in melting point from the value reported. Wildman reports a melting point of 254—255° (dec.) for a sample crystallised from methanol but samples of coccinine perchlorate prepared by the author melted much higher at 279—280° (dec.) when crystallised from a number of solvents including methanol. Analytical data on the perchlorate was in excellent agreement with the $C_{17}H_{19}O_4N$ formular for the base. (Found: C, 50.8; H, 5.23. calculated for $C_{17}H_{19}N.HClO_4$, C, 50.71; H, 5.02). The analytical results for coccinine and its derivatives are summarised in Table I.

The reduction of coccinine over platinum catalyst in acid medium was reported to give an oil for which no derivatives or analytical data were presented. Thus

TABLE I.

Analytical figures for coccinine and its derivatives.

COMPOUND	FOUND C	FOUND H	FORMULAR	REQUIRES C	REQUIRES H
Coccinine	67.85	6.24	$C_{17}H_{19}O_4N.$	67.76	6.36
Methiodide	48.40	5.10	$C_{18}H_{22}O_4NI.$	48.70	5.02
Perchlorate	50.80	5.23	$C_{17}H_{20}O_8N.Cl.$	50.71	5.02
Acetate	66.50	6.00	$C_{19}H_{21}O_5N.$	66.50	6.20

a re-investigation of the reduction of coccinine was desireable.

In an experiment using a palladium catalyst no uptake of hydrogen was observed and the alkaloid was recovered unchanged. However using a platinum catalyst in an acid medium, coccinine was rapidly reduced taking up 1.4 mole of hydrogen. A second experiment under the same conditions showed an uptake of 1.5 mole of hydrogen. These results were at variance with the hydrogenation results reported by the previous workers in which only 1 mole of hydrogen was absorbed. The crude reduction product was obtained as an oil but this could be separated into three components by chromatography over alumina.

The first component eluted from the column crystallised from ethyl acetate as prisms m. p. 179—180°. This compound analysed for the dihydro compound and was designated α-dihydrococcinine. It gave an oily O-acetate (ν C=O at 1738 cm.$^{-1}$ and OAC 1250 cm.$^{-1}$) which was characterised as its crystalline perchlorate. The methiodide was also non-crystalline but satisfactory analytical data was obtained. The ultra-violet spectrum

of α-dihydrococcinine showed normal methylenedioxyphenyl absorption but the low wave length maximum in coccinine had been reduced to an inflection.

The second hydrogenation product eluted from the column has not been obtained crystalline and was characterised through its crystalline methiodide. Since the analytical data indicated that it was isomeric with α-dihydrococcinine it has been designated β-dihydrococcinine.

A third compound was obtained from the hydrogenation in approximately 20% yield. It was rather unstable in solution showing a pronounced tendency to become yellow on attempted crystallisation. A clue to the structure of this compound was obtained from the infrared spectrum of its acetylation product which showed carbonyl absorption at 1738 $cm.^{-1}$ and 1642 $cm.^{-1}$. The latter absorption is obviously due to an amide carbonyl and indicated the acetylation product was an O,N-diacetate. Subsequent analytical data supported this assignment (Found: C, 64.18; H, 6.59. calculated for $C_{17}H_{21}O_4N.2COCH_3$ C, 64.76; H, 6.99). Thus in view of the absence of an NH group in coccinine this compound must

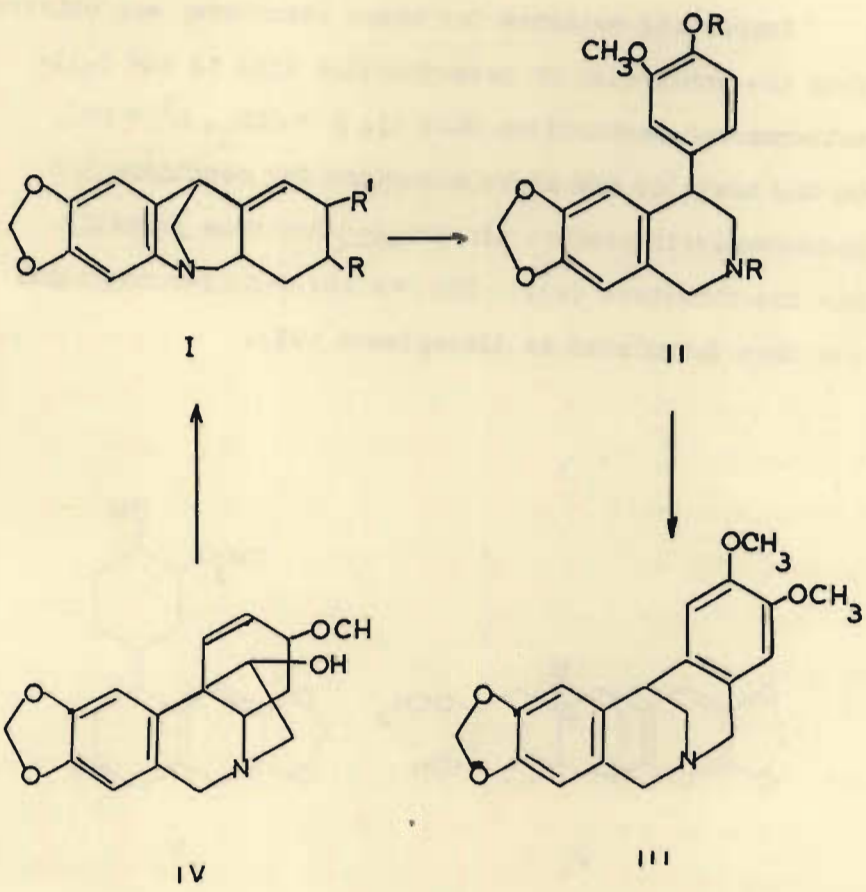
have originated from reductive clevage of a C-N bond. Consequently it has been called dihydrosecococcinine. The isolation of the above products from the hydrogenation of coccinine established the presence of a double bond, which is at least trisubstituted, and the isolation of the seco compound suggested that the double bond was allylic to the nitrogen atom. Lycorine and other alkaloids based on the pyrrolo[de]phenanthridine skeleton with a 3,3a-double bond all possess the double bond allylic to the nitrogen and yet show nc tendency to hydrogenolyse. Furthermore the presence of the lycorine type skeleton in coccinine was excluded since the alkaloid was recovered unchanged from reluxing with selenium dioxide in alcohol. In view of this stability to selenium dioxide it seemed that the alkaloid was based on thc 5:10b-ethanophenanthridine skeleton. However the infrared spectrum in the range 1500—600 $cm.^{-1}$ was very different to alkaloids of the crinine type and it seemed likely that coccinine was based on a skeleton not previously encountered in the alkaloids of this group.

In order to gain some information about the hydroxyl group some oxidation studies were carried out. Manganese

dioxide was without effect on coccinine, the alkaloid being recovered in 75% yield from two experiments. This indicated the absence of an allyl or benzyl alcohol system in the alkaloid. Several attempts to oxidise the alcoholic hydroxyl with the chromium trioxide-pyridine reagent under different experimental conditions failed to yield any ketonic material, the alkaloid being recovered in high yield in each case. In view of its resistance to oxidation it might have been concluded that the hydroxyl was tertiary but this was unlikely in view of the ease with which coccinine is acetylated.

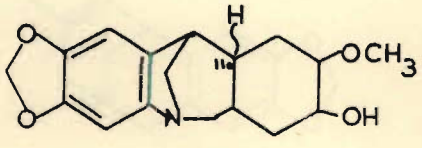
At this stage of the work the structure of coccinine was proposed by Wildman[23] and co-workers.

Few details of this work are known since only an abstract of the paper is available. Structure (I; R = OH, R^1 = OCH_3) was proposed for coccinine and montanine since Oppenauer oxidation of these alkaloids gave the optical active secondary amino-phenol (II; R = H) which gave an O,N dimethyl derivative (II, R = CH_3) with diazomethane. Treatment of the amino-phenol (II; R = CH_3) with formaldehyde and formic acid followed by diazomethane, gave a product (III), the structure of which was proved by synthesis and resolution.

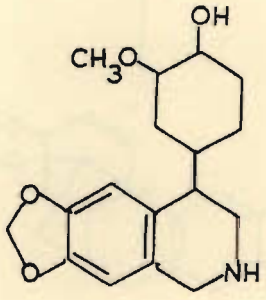


REACTIONS OF COCCININE

Supporting evidence for these structures was obtained from the conversion of haemanthamine (IV) to the 5,11-methanomorphenanthridine base (I; R = OCH_3, R^1 = OH). On the basis of the above structure for coccinine the hydrogenolysis product dihydroseco<u>seco</u>coccinine probably has the structure (V). The ∝- and β-dihydrocompounds are then formulated as 11a-epimers (VI).



VI



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PART IV.

EXPERIMENTAL

All melting points are uncorrected.

SECTION I.

THE ALKALOIDS OF HAEMANTHUS NATALENSIS.

The Extraction of Haemanthus Natalensis.

Bulbs of Haemanthus natalensis collected in March from the environs of Pietermaritzburg were sliced and soaked in 95% alcohol (20 litres) for two weeks. The ethanol extract was concentrated to a small bulk (1 litre) and the concentrate treated by passing steam until precipitation of the fats was complete.

Filtration of the fats through kieselguhr (Celite 545) gave a clear yellow filtrate. The filtrate was acidified to pH 2.0 and extracted with chloroform (3 x 200 ml.). A silico-tungstic acid test on the chloroform extract gave no precipitate and the extract was discarded. The aqueous phase was basified with sodium carbonate and the alkaline solution extracted repeatedly with chloroform (200 ml. x 25) until no more material was being extracted. The combined chloroform extracts (5 litres) were dried over anhydrous sodium sulphate and after filtration, the chloroform was removed by distillation. The residue (42 g.) was a clear amber gum and represented a 1% yield based on the dry weight of the bulbs. The gum was dissolved in benzene containing 12% ethyl acetate (1200 ml.) and chromatographed on alumina (1 kg.) previously activated by heating at 100°

for 4 hours. The column was eluted with benzene containing increased amounts of ethyl acetate, followed by pure ethyl acetate and chloroform and chloroform-ethanol mixtures.

The ethyl acetate eluates (800 ml.) afforded haemanthamine (natalensine) (11.7 g.) which crystallised from acetone as prisms m. p. 202—203°, (reported [1] m. p. 202—203°). It was identified by comparison of its infrared spectrum with that of an authentic sample and by mixed melting point determination. Confirmation of its identity was obtained by the preparation of the picrate which gave from water, needles m. p. 220—222°, (reported [1] m. p. 220—222°). The chloroform eluates (2 litres) and chloroform 1 - 2% ethanol (500 ml.) yielded a new alkaloid 3-epihaemanthidine (9.0 g.).

Chloroform containing 5 - 10% ethanol (1 litre) and chloroform containing 20 - 50% ethanol eluted crystalline material (7.2 g.) of wide melting range.

This was dissolved in chloroform (100 ml.) and rechromatographed on alumina (120 g.). Elution with chloroform and chloroform containing ethanol (200 ml.) gave a brown gum (84 mg.) which was not investigated.

The chloroform eluates containing 2 - 4% ethanol afforded crude haemanthidine (4.1 g.).

A further quantity of gum (2.4 g.) was eluted from the column with chloroform containing 10 - 30% ethanol. Attempts to crystallise this material were unsuccessful and it was not investigated further.

Yields of alkaloid based on the dry weight of bulbs;

Haemanthamine	0.3%
3-Epihaemanthidine	0.23%
Haemanthidine	0.10%

3-Epihaemanthidine.—

Crude 3-epihaemanthidine in dry acetone, treated with light petroleum ether until the solution was turbid, gave prisms, m. p. 205—209°. Three crystallisations from acetone-light petroleum raised the melting point to 211°, $[\alpha]_D$ = +44° (c, 1.0 in chloroform). The ultra-violet spectrum in ethanol showed two maxima at 242 mμ and 293 mμ, (ε, 5620 and 4680 respectively).

Analysis: Sample dried 6 hours at 100°/0.02 mm.

Found:	C, 64.0, 64.2;	H, 5.8, 6.15.
$C_{17}H_{19}O_5N$ requires	C, 64.3;	H, 6.0.

The infrared spectrum (in nujol) was recorded.

$\nu_{max.}$ cm.$^{-1}$	Assignments.
3064 (w)	C-H vib. of disubstituted double bond.
3021 (w)	C-H vib. or aromatic ring.
2725 (w)	C-H vib. of methylenedioxy ring.
1645	disubstituted double bond.
1618) 1499)	aromatic ring vibration.
1250) 1036)	methoxy and methylenedioxy groups.
931	methylenedioxy group.

3-Epihaemanthidine when crystallised from aqueous acetone or 'wet' ethyl acetate afforded large prisms of the hemi-hydrate m. p. 135—140°.

Analysis: Sample dried for 4 hours at 20°/0.02 mm.

Found:	C, 62.2;	OMe, 9.5;	H, 6.2;	N, 4.3.
$C_{17}H_{19}O_5N.\frac{1}{2}H_2O$ requires	C, 62.6;	OMe, 9.3;	H, 6.2;	N, 4.3.

3-Epihaemanthidine Picrate.—

A few drops of saturated aqueous picric acid was added to a solution of 3-epihaemanthidine in ethanol. A crystalline precipitate formed immediately which after several recrystallisations from ethanol afforded silky yellow needles of 3-epihaemanthidine picrate, m. p. 146°.

Analysis:

Found:		C, 49.3; H, 4.4; N, 9.6.
$C_{17}H_{19}O_5N.C_6H_5O_7N_3.H_2O$	requires	C, 48.9; H, 4.3; N, 9.9.

3-Epihaemanthidine Methiodide.—

To 3-epihaemanthidine (25 mg.) in methanol (2 ml.) was added 2 drops of methyl iodide and the solution was allowed to stand at room temperature for 24 hours. The solvents were removed in vacuo and the residual gum (28 mg.) was crystallised from a very small volume of aqueous acetone, giving needles m. p. 174—175°, $[\alpha]_D$ +57° (c, 1 in ethanol).

Analysis:

Found:		C, 45.90; H, 5.02; N, 2.83.
$C_{18}H_{22}O_5NI.\frac{1}{2}H_2O$	requires	C, 46.16; H, 4.73; N, 2.37.

3-Epihaemanthidine Methopicrate.—(Kindly prepared by Dr. W.G.Wright of these laboratories).

The methiodide in ethanol was treated with a few drops of aqueous picric acid and the turbidity produced was removed by warming. The solution was cooled and the solid product which was recrystallised from ethanol to give crystals, m. p. 246°.

Analysis: Dried for 4 hours at 100°/0.02 mm.

Found: C, 51.1; H, 4.0.

$C_{24}H_{24}O_{12}N_4$ requires C, 51.4; H, 4.3.

Anhydrodemethylhaemanthidine (apohaemanthidine).—

3-Epihaemanthidine (100 mg.) was heated with 6N hydrochloric acid (25 ml.) for 2 hours at 80-90°. The solution was cooled and basified with sodium carbonate and then extraction with chloroform (3 x 25 ml.) yielded a gum (90 mg.). Chromatography of the gum on alumina in benzene and elution with benzene and benzene-methanol mixtures afforded crude anhydrodemethylhaemanthidine. Several crystallisations of this material gave pure anhydrodemethylhaemanthidine as stout needles m. p. 195—196° (dec.). $[\alpha]_D^{23}$ +125° (c, 0.73 in chloroform). The ultra-violet spectrum in ethanol had a shoulder at 240 mμ and a maximum at 293 mμ (ε, 3,890 and 5,620 respectively).

(Reported [2] for this compound m. p. 195—196°, $[\alpha]_D$ +123°, $\lambda_{sh.}$ 240 mμ $\lambda_{max.}$ 294 mμ (ε, 3720 and 5010 respectively)),

Analysis: Sample dried 100° for 4 hours at 0.02 mm.

Found:	C, 67.0; H, 5.8; OMe, 0.0.
$C_{16}H_{15}O_5N$ requires	C, 67.35; H, 5.3; OMe, 0.0.

Manganese Dioxide Oxidation of 3-Epihaemanthidine.

3-Epihaemanthidine (200 mg.) in dry chloroform (50 ml.) was shaken for 30 minutes with manganese dioxide (2 g.) prepared according to Attenburrow [3] et al. The solution was refluxed for 2 minutes and the manganese dioxide filtered off and washed with ethanol. Removal of the solvent from the filtrate gave a gum (130 mg.) which, twice crystallised from ethyl acetate gave prisms of 6-oxo-3-epihaemanthidine m. p. 195—196° $[\alpha]_D^{23}$ +29° (c, 0.9 in $CHCl_3$). The ultra-violet spectrum in ethanol showed three well defined maxima $\lambda_{max.}$ 234 mμ, $\lambda_{max.}$ 275 mμ and $\lambda_{max.}$ 326 mμ (ε, 22,900, 6,170 and 5,130 respectively). The infrared spectrum in nujol:

$\nu_{max.}$ cm.$^{-1}$	Assignments
3300 (broad)	bonded hydroxyl
1677 (s,broad)	bonded six ring lactone (conjugated)
1616 (s)	conjugated aromatic ring.

Infrared spectrum in carbon tetrachloride:

$\nu_{max.}$ cm.$^{-1}$	Assignments
3605	free hydroxyl.
1696 (s)	six ring lactone (conjugated).

Analysis: Dried at 100° for 4 hours at 0.02 mm.

Found: C, 64.2; H, 5.5.

$C_{17}H_{17}O_5N$ requires C, 64.8; H, 5.4.

Dihydro-3-epihaemanthidine.

a). 3-Epihaemanthidine (100 mg.) in ethanol (50 ml.) was shaken with hydrogen in the presence of 10% palladium on charcaol catalyst. No absorption of hydrogen took place during the course of 3 hours. The catalyst was filtered off and the solvent in the filtrate was removed in vacuo leaving a gum (92 mg.). Crystallisation of the gum acetone gave prisms m. p. 209—210° (79 mgs.). A mixed melting point determination with starting material showed no depression.

b). 3-Epihaemanthidine (65 mg.) in glacial acetic acid (10 ml.) was shaken with hydrogen in the presence of platinum oxide (100 mg.). Hydrogen (1.1 mole was absorbed

during the course of 30 minutes. The platinum was removed by filtration and ethanol added to the filtrate. Removal of the solvent in vacuo left a residue of colourless glass (59 mg.) which solidified on trituration with ether. The solid twice crystallised from ether gave needles (28 mg.) of dihydro-3-epihaemanthidine m. p. 240—242°.

Analysis: Dried for 4 hours at 100°C at 0.02 mm.

Found: C, 63.60; H, 6.47.

$C_{17}H_{21}O_5N$ requires C, 63.93; H, 6.63.

Acetylation of 3-Epihaemanthidine.

3-Epihaemanthidine (100 mg.) in dry pyridine (2 ml.) and acetic anhydride (1 ml.) added. After allowing to stand at room temperature for 72 hours the solvents were removed in vacuo leaving a brown gum (106 mg.). Attempts to crystallise the gum were unsuccessful and it was sublimed at 145—150°/0.01 mm. to give a white amorphous solid. The infrared spectrum in chloroform solution had strong bands at 1745 $cm.^{-1}$ and 1250 $cm.^{-1}$ indicative of an O-acetate; no hydroxyl absorption was present. Analytical data permitted its formulation as

an 0:0 diacetate.

Analysis:

Found:	C, 62.85;	H, 5.8.
$C_{17}H_{17}O_5N,2COCH_3$ requires	C, 62.78;	H, 5.77.

The Alkaline Rearrangement of 3-Epihaemanthidine.

3-Epihaemanthidine (1 g.) was heated on a water bath for 1 hour with 10% potassium hydroxide solution (25 mls.). After cooling, the solution was extracted with chloroform (5 x 50 ml.). The aqueous phase was acidified with 2N hydrochloric acid, rebasified with sodium bicarbonate solution, and extracted with chloroform (3 x 50 ml.). The chloroform extracts were combined, dried over anhydrous sodium sulphate, and the chloroform removed by distillation; a brown gum (830 mg.) remained. The gum was dissolved in 2N hydrochloric acid (50 ml.) and the solution decolourised by heating with charcoal. The charcoal was filtered off and concentration of the filtrate afforded white silky needles de-N-methylepitazettine hydrochloride m. p. 240—245°. Recrystallisation from 2N hydrochloric acid raised the melting point to 248—250° $[\alpha]_D^{23}$ +198° (c, 1.1 in H_2O). The ultra-violet spectrum showed two

maxima. $\lambda_{max.}$ 241 mμ and $\lambda_{max.}$ 290 mμ (ε, 4570 and 4370 respectively).

The infrared spectrum was recorded as a nujol mull.

$\nu_{max.}$ cm.$^{-1}$	Assignments
3400	alcoholic OH and/or OH of hydrate.
2695) 2610)	hydrochloride.
1624 (w.broad)	double bond and water of crystallisation.
1237	methoxyl and/or methylenedioxy.
1167	methoxyl.
939	methylenedioxy.

Analysis:

Found: C, 54.9, 54.9;
H, 5.7, 6.0;
Cl, 9.3.

$C_{17}H_{19}O_5N.HCl.H_2O$ requires C, 54.9;
H, 6.0;
Cl, 9.3.

de-N-Methylepitazettine.

The samples of de-N-methylepitazettine obtained by basifying the hydrochloride were usually amorphous and reactions were carried out on these specimens.

An amorphous sample had an $[\alpha]_D^{23}$ +227° (c, 1 in $CHCl_3$) and the ultra-violet spectrum in ethanol had two maxima. $\lambda_{max.}$ 240 mμ and $\lambda_{max.}$ 290 mμ (ε, 5,620 and 4,570 respectively).

The infrared spectrum was recorded in a nujol mull.

$\nu_{max.}$ cm.$^{-1}$	Assignments
3330	bonded tertiary lactol hydroxyl.
1650 (w)	disubstituted double bond.
1235	methoxyl and methylenedioxy groups.
1173	methoxyl.
931	methylenedioxy.

At a late stage in the work it was found that trituration of the amorphous base with acetone furnished needles m. p. 195°.

Epitazettine Hydriodide.

de-N-Methylepitazettine (230 mg.) regenerated from the hydrochloride was dissolved in chloroform (5 ml.) and methyl iodide (1 ml.) added. After allowing to stand at

room temperature for 2 hours the solvents were removed and the residual gum crystallised from ethyl acetate-ethyl alcohol giving prisms of epitazettine hydriodide (223 mg.) m. p. 240—243°. One crystallisation from ethanol raised the melting point to 245—246°.

Analysis:

Found:	C, 47.2; 47.2;
	H, 4.8, 4.85;
	OMe, 6.4; N-Me, 3.1.
$C_{18}H_{22}O_5NI$ requires	C, 47.1;
	H, 4.8;
	OMe, 6.7; N-Me, 6.3.

de-N-Methylepitazettine Picrate.

de-N-Methylepitazettine (100 mg.) in water (3 ml.), containing 2 drops of dilute acetic acid, was treated with excess aqueous picric acid solution until precipitation was complete. The precipitate was filtered and washed with water; two recrystallisations from ethanol furnished needles of de-N-methylepitazettine picrate, m. p. 242—244°.

Analysis:

Found:	C, 50.1;	H, 3.74;
	N, 10.7.	
$C_{17}H_{19}O_5N, C_6H_3O_7N_3$ requires	C, 50.1;	H, 4.05;
	N, 10.17.	

de-N-Methylepitazettine Picrolonate.

de-N-Methylepitazettine hydrochloride (50 mg.) dissolved in water (3 ml.) and excess aqueous picrolonic acid solution was added dropwise until precipitation was complete. The precipitate after two recrystallisations from ethanol gave bright yellow plates of de-N-methylepitazettine picrolonate m. p. 230—235°. The melting point was not improved by further crystallisation from ethanol.

Analysis: Sample dried for 4 hours at 100°/0.02 mm.

Found:	C, 55.7;	H, 4.6;
	N, 10.1.	
$C_{17}H_{19}O_5N, C_{10}H_{10}O_5N_4$ requires	C, 55.9;	H, 4.7;
	N, 12.0.	

Attempted Oxidation of de-N-Methylepitazettine with Selenium Dioxide.

To de-N-methylepitazettine (27 mg.) in ethanol (25 ml.) selenium dioxide (25 mg.) was added and the solution refluxed for 2 hours. No precipitation of selenium occurred during this time and the solution was concentrated, N hydrochloric acid (2 ml.) added, and the solution filtered. The filtrate after concentration and setting aside at room temperature for several hours gave five white needles, (16 mg.) m. p. 240—242°. The infrared and ultra-violet spectra were identical to the spectra of de-N-methyl-epitazettine hydrochloride.

Attempted Acid Hydrolysis of de-N-Methylepitazettine.

de-N-Methylepitazettine hydrochloride (197 mg.) was refluxed with 2N hydrochloric acid (25 ml.) for 2 hours. The solution on concentrating and cooling gave fine silky needles (180 mg.), m. p. 249—250° (dec.). The infrared spectrum was identical to the spectrum of the starting material.

O:N Diacetate of de-N-Methylepitazettine.

de-N-Methylepitazettine (200 mg.) was dissolved in pyridine (1 ml.) and acetic anhydride (1 ml.) added.

The solution was set aside at room temperature for 48 hours and then the solution was concentrated to half its original volume. After cooling it was poured into ice cold sodium carbonate solution and the precipitate formed was filtered off and crystallised from ethanol. Several crystallisations from ethanol gave prisms (97 mg.) of the O:N-diacetate, m. p. 210—212°. The ultra-violet spectrum in ethanol had $\lambda_{max.}$ 239 mμ and $\lambda_{max.}$ 292 mμ (ε, 4680 and 4570) respectively.

The infrared spectrum was recorded as a nujol mull.

$\nu_{max.}$ cm.$^{-1}$	Assignments
3035 (w)	aromatic C-H.
2775 (w)	C-H of methoxyl group.
1758 (vs)	carbonyl of O-acetate with an adjacent oxygen function.
1641 (vs)	carbonyl of N-acetate.
1487	methylenedioxy.
1235 (vs,broad)	C-O of O-acetate + methoxyl and methylenedioxy groups.
1176	methoxyl.
936	methylenedioxy.

Analysis:

Found: C, 62.7; H, 5.85; Ac, 12.1, 12.2.

$C_{21}H_{23}O_7N$ requires C, 62.8; H, 5.8; Ac, 24.45.

The N-Acetate of de-N-Methylepitazettine.

de-N-Methylepitazettine (100 mg.) in pyridine (1 ml.) cooled to 0° in an acid bath. Acetic anhydride (1 ml.) was added dropwise and the mixture allowed to stand at 0° for 1 hour. It was then poured into ice cold sodium carbonate solution and the aqueous solution extracted with chloroform (3 x 25 ml.). After drying the chloroform furnished a clear gum (83 mg.).

The gum crystallised from ether containing ethanol (1 drop) as prisms of the N-acetate, m. p. 223—224°. The infrared spectrum of the sample in nujol was recorded.

$\nu_{max.}$ cm.$^{-1}$	Assignments
3205 (s; broad)	bonded hydroxyl.
1618 (s; broad)	bonded carbonyl of N-acetate.
930	methylenedioxy group

Analysis:

Found:	C, 63.71;	H, 6.01;	Ac, 0.0.
$C_{19}H_{21}O_6N$ requires	C, 63.51;	H, 5.88;	Ac, 11.97.

Epitazettine.

a). 3-Epihaemanthidine methiodide (50 mg.) in water (5 ml.) was treated with sodium carbonate solution (5 ml.)

and after shaking for 5 minutes the solution was extracted with chloroform. The chloroform extract gave crude epitazettine (30 mg.) which crystallised from acetone as prisms of pure epitazettine, m. p. 210—11°. There was no depression of m. p. when mixed with a sample prepared by method (b).

b). de-N-Methylepitazettine (100 mg.) was dissolved in chloroform (5 ml.) and methyl iodide (1 ml.) added. After allowing to stand at room temperature for 20 minutes the solvent was distilled off and the residue crystallised from ethanol giving prisms of epitazettine hydriodide m. p. 245—246°. The epitazettine hydriodide was dissolved in water and treated with sodium carbonate solution.

Extraction of this solution with chloroform (3 x 25 ml.) afforded epitazettine (60 mg.) m. p. 210—11° after two crystallisations from ethanol. $[\alpha]_D^{23}$ +288° (c, 1.0 in chloroform).

Analysis:

Found:	C, 65.22;	
	H, 6.60;	N-Me, 3.94, 7.1;
	N, 4.19;	OMe, 9.35, 8.43.
$C_{18}H_{21}O_5N$ requires	C, 65.26;	
	H, 6.39;	N-Me, 8.74;
	N, 4.22;	OMe, 9.34.

The infrared spectrum was recorded in nujol.

$\nu_{max.}$ cm.$^{-1}$	Assignments
3325 (m.s.)	bonded tertiary lactol hydroxyl.
2780 (m.)	C-H of methoxy group.
1650 (w)	double bond.
1509	aromatic ring.
1487	methylenedioxy.
1237	methoxyl and/or methylenedioxy groups.
1171	methoxyl group.
936	methylenedioxy group.

Epitazettine Picrate.

a). Aqueous picric acid solution was added to a solution of epitazettine hydriodide in water and the precipitate when crystallised from ethanol afforded needles of epitazettine picrate, m. p. 233° (dec.).

b). Epitazettine in ethanol was treated with aqueous picric acid and the precipitate was crystallised from ethanol giving needles of epitazettine picrate, m. p. 233°.

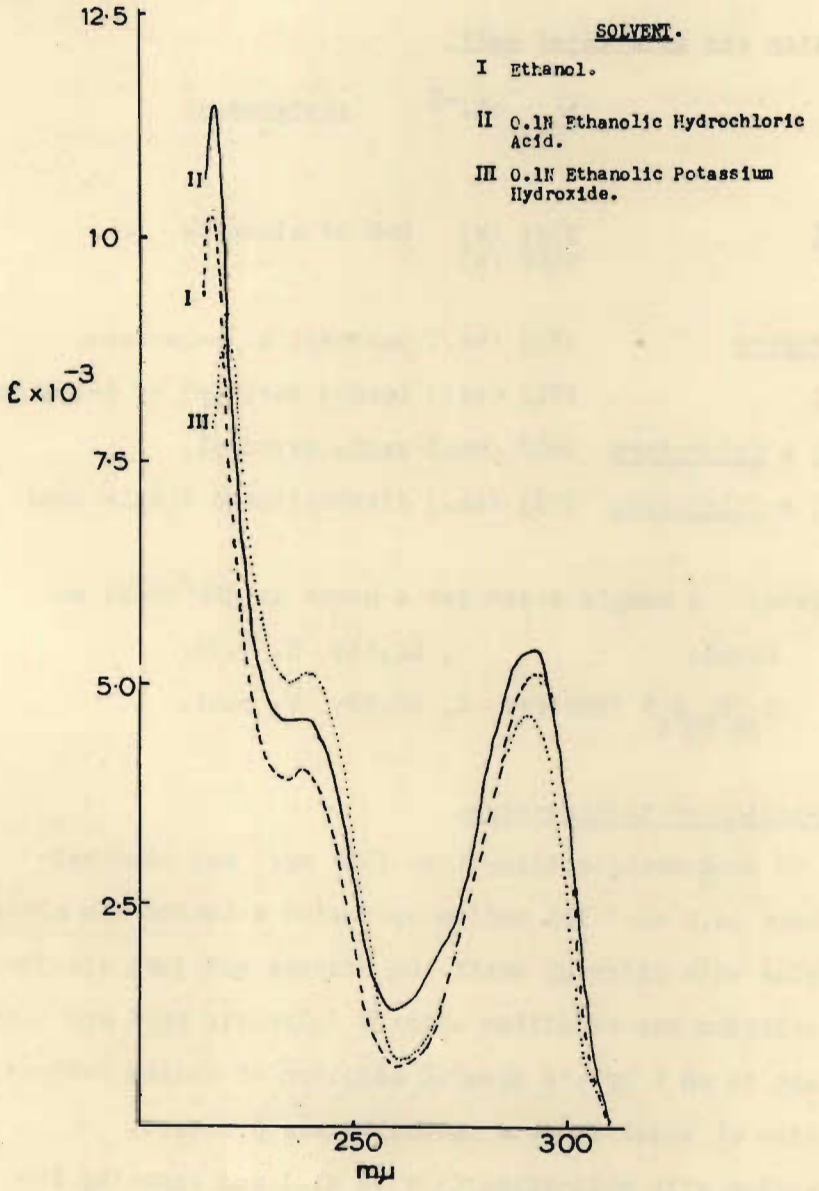
Analysis:

Found:	C, 51.2;
	H, 4.4.
$C_{24}H_{24}O_{12}N_4$ requires	C, 51.4;
	H, 4.3.

Manganese Dioxide Oxidation of Epitazettine.

Epitazettine (350 mg.), dry chloroform (100 ml.), and manganese dioxide (3 g.), prepared according to Attenburrow et al.[3], were shaken for 42 hours. The manganese dioxide was filtered off and washed with a little chloroform containing ethanol. Removal of the solvent from the filtrate left a gum (240 mg.). The gum could be crystallised from acetone or ethanol. Crystallisation from acetone afforded epitazettamide as plates, m. p. 253—254°. $[\alpha]_D$ +187° (c, 0.75 in chloroform). The ultra-violet spectrum in ethanol was very similar to that of epitazettine with two maxima at $\lambda_{max.}$ 238 mμ (ε, 3890) and $\lambda_{max.}$ 292 mμ (ε, 4898). A study of the effect of concentration and pH on the ultra-violet spectrum was made and no deviation from Beer's law was found in ethanol but the 'end' absorption increased appreciably both at high and low pH. (see Fig.10). The infrared spectrum was run in chloroform

FIG.10


12·5
10
7·5
5·0
2·5
ε x10⁻³
I
II
III
250
300
mμ
SOLVENT.
I Ethanol.
II 0.1N Ethanolic Hydrochloric Acid.
III 0.1N Ethanolic Potassium Hydroxide.

solution and as a nujol mull.

	$\nu_{max.}$ cm.$^{-1}$	Assignments
Nujol	2721 (w) 2667 (w)	C-H of aldehyde.
Chloroform	1733 (vs.)	carbonyl of δ-lactone.
Nujol	1712 (vs.)	bonded carbonyl of δ-lactone.
Nujol & Chloroform	1667 (vs.)	amide carbonyl.
Nujol & Chloroform	1653 (sh.)	disubstituted double bond.

Analysis: A sample dried for 4 hours at 100°/0.01 mm.

Found: C, 62.61; H, 5.56.

$C_{18}H_{19}O_6N$ requires C, 62.57; H, 5.61.

Methylation of Epitazettine.

To de-N-methylepitazettine (150 mg.) and dimethylsulphate (4.0 ml.) 10% sodium hydroxide solution was added dropwise with stirring until the mixture was just alkaline. The solution was acidified with 2N sulphuric acid and then brought to pH 6 by the careful addition of sodium carbonate solution at which stage a turbidity was produced. Extraction with chloroform (3 x 50 ml.) and removing the

solvent afforded a clear gum (130 mg.). The picrate was prepared by adding aqueous picric acid solution to a portion of the gum (30 mg.) dissolved in ethanol. Crystallisation from ethanol gave yellow plates of O-methylepitazettine methopicrate, m. p. 210—212° (reported [4] for O-methylisotazettine methopicrate, m. p. 204—205°). However, it showed no depression on mixed melting point with O-methylisotazettine methopicrate, m. p. 210—212° prepared from isotazettinol.

Analysis:

Found:		C, 53.06; H, 5.09; OMe, 10.5.
$C_{20}H_{26}O_5N, C_6H_2O_7N_3$	requires	C, 53.10; H, 4.60; OMe, 10.5.

Attempted Demethylation of Epitazettine.

Epitazettine (150 mg.) refluxed with glacial acetic acid (10 ml.) containing hydriodic acid (S.G., 1.7; 1.5 ml.) for 1 hour with water (50 ml.) and the excess iodine destroyed with sodium thiosulphate solution. The solution was basified with potassium carbonate solution and extracted with chloroform (4 x 25 ml.). Removal of the

chloroform gave a gum (130 mg.) which defied all attempts at purification. Attempts to prepare derivatives gave intractable products.

Haemanthidine.

Crude haemanthidine was crystallised several times from ethyl acetate to give prisms of haemanthidine, m. p. 180—182° (reported[5] 189°). Further crystallisations from ethyl acetate did not increase the melting point and from the melting behaviour of the crystals they appeared to be solvated.

Analysis: Dried for 4 hours at 100°/0.01 mm.

Found: C, 63.50; H, 5.97.

$C_{17}H_{19}O_5N \cdot \frac{1}{2} CH_3CO_2C_2H_5$ requires C, 63.15; H, 6.4.

The infrared spectrum of the above analysis sample had an ester carbonyl absorption at 1738 $cm.^{-1}$ which was only removed on prolonged drying.

Colour Tests.

1. Treatment of haemanthidine (1 mg.) with concentrated sulphuric acid gave a deep wine coloured solution.

2. Haemanthidine (5 mg.) on treating with concentrated nitric acid produced a deep yellow colour. The addition

of water (10 ml.) caused the colour to disappear and a crystalline precipitate formed.

Haemanthidine Picrate.

Ethanolic picric acid solution was added to haemanthidine in ethanol. After the addition of water and allowing to stand a crystalline precipitate was obtained. Crystallisation of the precipitate from ethanol afforded prisms of haemanthidine picrate, m. p. 209—210° (dec.) (reported [5] 208°, dec.).

Analysis:

Found:	C, 50.06; H, 4.35.
$C_{17}H_{19}O_5N, C_6H_3O_7N_3$ requires	C, 50.1; H, 4.02.

Haemanthidine Methopicrate.

Haemanthidine (20 mg.) in ethanol (2 ml.) was refluxed with methyl iodide (0.5 ml.) for 30 minutes. Removal of the solvent left a pale yellow gum which was dissolved in ethanol (0.5 ml.) and aqueous picric acid solution added. Large rhombic crystals separated out on standing and recrystallisation from aqueous alcohol gave haemanthidine methopicrate, prisms, m. p. 216—218° (dec.).

Analysis: Sample dried for 4 hours at 100°/0.01 mm.

Found: C, 51.3; H, 4.34.

$C_{18}H_{21}O_5N, C_6H_2O_7N_3$ requires C, 51.32; H, 4.49.

Acetylation of Haemanthidine.

Haemanthidine (60 mg.) was dissolved in pyridine (0.5 ml.) and acetic anhydride (0.3 ml.) added. The solution was allowed to stand at room temperature for 15 hours and then refluxed for 10 minutes. Removal of the solvents *in vacuo* left a colourless gum. Crystallisation from ether-hexane gave prisms m. p, 192—195°. Two further crystallisations from ether yielded prisms of pure haemanthidine 0:0 diacetate, m. p. 219°.

Analysis:

Found: C, 62.62; H, 6.44; Ac, 24.40.

$C_{17}H_{17}O_5N, 2COCH_3$ requires C, 62.78; H, 5.77; Ac, 21.4.

The Rearrangement of Haemanthidine to Tazettine.

Haemanthidine (330 mg.) in ethanol (5 ml.) was refluxed with methyl iodide (2 ml.) for 30 minutes.

Removal of the solvents in vacuo left a methiodide as a pale yellow gum. 10% sodium hydroxide solution (15 ml.) was added to the gum and after agitating and allowing to stand for several minutes the solution was extracted with chloroform (3 x 20 ml.). The chloroform extract was dried over anhydrous potassium carbonate and after filtration and removal of the solvent a solid residue was obtained. Treatment of the residue with ethanol gave crystals of crude tazettine. Recrystallisation from ethyl acetate gave prisms of tazettine, m. p. 200—202° further crystallisation from ethanol raised the melting point to 209—210°, $[\alpha]_D$ +158° (c, 1.0 in chloroform). The ultra-violet spectrum in ethanol showed two maxima $\lambda_{max.}$ 242 (ε, 4,900) $\lambda_{max.}$ 295 (ε, 4,470) (reported for tazettine m. p. 210—11°, 207—208° $[\alpha]_D$ +160.4).

Analysis:

Found:	C, 65.16;	H, 6.3.
$C_{18}H_{23}O_5N$ requires	C, 65.26;	H, 6.39.

Acid Hydrolysis of Tazettine and Methylation of Isotazettinol.

Tazettine (310 mg.) was refluxed for 1 hour with 10% hydrochloric acid (10 ml.) and then heated at 100° for

3 hours. The solution was basified with sodium carbonate and extracted with chloroform (4 x 25 ml.). After drying and removing the chloroform a gum (255 mg.) remained. Chromatography of the gum over alumina (15 g.) using benzene-ethyl acetate and chloroform-ethanol mixtures gave isotazettinol (134 mg.) and unchanged tazettine (85 mg.).

Isotazettinol and tazettine gave a large depression on a mixed melting point determination.

Isotazettinol (120 mg.) methylated with dimethyl sulphate in the same manner as described above for the methylation of epitazettine. Conversion to the methopicrate and recrystallisation from ethanol gave plates of O-methylisotazettine methopicrate, m. p. 210—212°. (Literature m. p. 204—205°). A mixed melting point determination showed no depression.

SECTION II.

THE ALKALOIDS OF NERINE KRIGEII.

Extraction of Nerine Kregii.

The bulbs were sliced and dropped into boiling 95% ethanol (5 litres) and allowed to simmer for 6 hours. After filtering, the bulbs were crushed, dried, and re-extracted with hot ethanol (3 litres) for a further 4 hours. The combined ethanol extracts were flash evaporated to a convenient volume (2 litres). The remaining traces of ethanol and volatiles were removed by steam distillation after adjusting to pH 2.0 with hydrochloric acid. Small amounts of fats precipitated during the steam distillation and these were removed by filtration through kieselguhr (Celite 545). The filtrate was washed with ether (5 x 300 ml.) to remove nonbasic material and since no precipitate was formed with Mayer's reagent the washings were discarded. The aqueous solution was then extracted with chloroform (5 x 300 ml.). An aliquot (100 ml.) of the chloroform extract was dried over anhydrous sodium sulphate and the solvent removed leaving only small trace of residue. This residue was dissolved in N hydrochloric acid (2 ml.) and divided

into two equal portions. The addition of Mayer's reagent (0.5 ml.) to one portion gave no precipitate and a similar result was obtained on adding silicotungstic acid to the other portion. This result indicated the absence of alkaloids with chloroform soluble hydrochlorides in the crude plant extract. The aqueous phase was then basified by the addition of solid sodium bicarbonate and extracted exhaustively with chloroform containing 5% ethanol (20 x 200 ml.). On concentrating the chloroform extract to about 1 litre a brown solid (7.2 g.) precipitated and was filtered off. A portion (0.2 g.) of this solid was dissolved in hot ethanol and the solution decolourised with charcoal. After cooling and filtering small prisms separated out from the solution m. p. 266—268° (dec.).

The infrared spectrum of this substance was identical with the spectrum of lycorine.

The chloroform filtrate was concentrated to a dark brown viscous resin (79 g.). This was dissolved in hot chloroform (800 ml.) and after cooling it was chromatographed on activated alumina (1.5 kg.).

Elution with chloroform gave a considerable quantity of gum (A) (18 g.) in the early fractions. The next few fractions contained neronine (4.0 g.) which crystallised from 'wet' ethyl acetate as prisms m. p. 128—130°, resolidifying and melting at 196—197°. Continued elution with chloroform gave fractions (B) (14 g.) containing mixtures of neronine and other alkaloids. Elution with 5% ethanolic chloroform gave fractions containing small amounts of krigeine (1.1 g.). Crystallisation of the crude krigeine from acetone gave prisms m. p. 207—210° (dec.).

Rechromatography of the Gum A.

The gum (A) (18 g.) was rechromatographed on alumina (500 g.) using benzene-chloroform 1:1. This solvent (700 ml.) eluted a viscous yellow gum (8.1 g.) which showed absorptions in its infrared spectrum characteristic of a methoxymethylenedioxyphenyl chromophore. This substance could not be induced to crystallise and was set aside for a later study. Elution with chloroform (450 ml.) gave crude oxokrigenamine as a pale yellow gum (2.7 g.) which crystallised from 'wet' ethyl acetate as massive prisms m. p. 70—75°. Continued elution with chloroform (1 litre) gave crude neronine (4.5 g.).

The remaining elutes contained small quantities of brown gums which were not investigated.

Separation of the Constituents of Fraction B.

Fraction B (14 g.) was dissolved in chloroform (600 ml.) and washed with N hydrochloric acid (3 x 50 ml.). The chloroform layer, washed with water, basified with ammonia, and dried over anhydrous sodium carbonate. Removal of the chloroform afforded a further quantity of crude neronine (ca. 2.8 g.).

The hydrochloric acid washings were, basified with ammonia and extracted with chloroform (5 x 100 ml.); after drying the solution over anhydrous sodium carbonate, removal of the chloroform left a clear yellow gum (11 g.). The gum dissolved in chloroform (250 ml.) was chromatographed on aluminium oxide (500 g.). The early fractions contained an alkaloid (6 g.) exhibiting absorption in its infrared spectrum characteristic of a methoxymethylenedioxyphenyl chromophore and a hemi-acetal ring. Cursory efforts to crystallise this alkaloid were unsuccessful and it was reserved for further study.

The following fractions afforded crude krigenamine

(3.1 g.) which crystallised from ethyl acetate in needles m. p. 209—211°.

The remaining fractions for this column were not investigated.

KRIGENAMINE.

Crude krigenamine (0.2 g.) extracted with three portions of hot ethyl acetate (20 ml.), the combined extracts concentrated and on standing the solution deposited silky needles of pure krigenamine m. p. 210—211° $[\alpha]_D$ = +210 (c, 0.43 in chloroform). The ultra-violet spectrum recorded in ethanol showed a broad maximum of low intensity at 280 mμ (ε , 1,385). The infrared spectrum was recorded as a nujol mull.

$\nu_{max.}$ cm.$^{-1}$	Assignments
1616 (s)	methoxymethylenedioxyphenyl group.
1054 (s.broad)	hemi-acetal.
929 (s)	methylenedioxy group.

Analysis:

Found: C, 65.00; H, 6.49; OCH_3, 8.7; NCH_3, 6.03.

Calculated for $C_{18}H_{21}O_5N$ C, 65.24; H, 6.39; OCH_3, 9.36; NCH_3, 8.77.

Krigenamine methiodide.

Krigenamine (50 mg.) in methanol (5 ml.) and methyl iodide (0.5 ml.) added. The solution was allowed to stand at room temperature for 5 hours and then the solvents were removed in vacuo. The residual glass crystallised on trituration with acetone and recrystallised from the same solvent giving prisms of pure krigenamine methiodide m. p. 235—237° (dec.) $[\alpha]_D$ = +150° (c, 1.2 in water).

Analysis:

Found:	C, 48.19; H, 5.58.
Calculated for $C_{19}H_{24}O_5NI$	C, 48.21; H, 5.11.

Krigenamine methoperchlorate.

Krigenamine methiodide in methanol (0.5 ml.) treated with ether until solution became turbid. To this solution methanol (1 drop) was added followed by perchloric acid (1 drop). The solution on scratching gave a crystalline solid which after two crystallisations

from methanol-ether gave pure krigenamine methoperchlorate m. p. 248—249° (dec.).

Analysis:

Found:	C, 51.50;	H, 5.57.
$C_{19}H_{24}O_5N.HClO_4$ requires	C, 50.96;	H, 5.40.

Oxokrigenamine hydrate.

Crude oxokrigenamine dissolved in 'wet' ethyl acetate and the solution concentrated. After standing for several hours the solution deposited massive prisms of oxokrigenamine hydrate m. p. 70—75°. Two recrystallisations from ethyl acetate did not improve the melting point. The infrared spectrum of oxokrigenamine hydrate in chloroform showed strong absorption at 1717 $cm.^{-1}$ (aryl conjugated lactone) and 1616 $cm.^{-1}$ (methoxymethylenedioxyphenyl).

Analysis: Dried at room temperature at 0.02 mm. for 3 hrs.

Found:	C, 62.36; H, 6.0; N, 4.13; OCH_3, 8.79; NCH_3, 6.36.
$C_{18}H_{19}O_5N,H_2O$ requires	C, 62.24; H, 6.10; N, 4.03; OCH_3, 8.92; NCH_3, 8.35.

Oxokrigenamine.

A sample of oxokrigenamine hydrate m. p. 70—75° was dried at 20° at 0.02 mm. for 72 hours. Dehydration was complete after this time and the oxokrigenamine had a melting point of 146°. $[\alpha]_D$ = +117° (c, 1.0 in chloroform). The ultra-violet spectrum showed an intense maximum at 228 mμ (ε, 22,900) maximum at 285 mμ (ε, 6,026) and an inflection at 310 mμ (ε, 2,570).

Analysis:

Found: C, 65.26; H, 6.0.

$C_{18}H_{19}O_5N$ requires C, 65.64; H, 5.82.

Oxokrigenamine methiodide.

Methyl iodide (0.5 ml.) added to oxokrigenamine hydrate (50 mg.) in ethanol (5 ml.). Small prisms of oxokrigenamine methiodide separated out after two hours. Recrystallisation from ethanol gave pure oxokrigenamine methiodide m. p. 254—255° (dec.).

Analysis:

Found: C, 48.34; H, 5.30.

$C_{19}H_{26}O_5N.I$ requires C, 48.01; H, 5.50.

Lithium Aluminium Hydride Reduction of Krigenamine and Oxokrigenamine.

a). Krigenamine (200 mg.) in tetrahydrofuran (30 ml.) refluxed for 24 hours with lithium aluminium hydride (200 mg.). The excess reagent was destroyed by adding water, the precipitated aluminium hydroxide filtered off and the filtrate extracted with chloroform (3 x 20 ml.). After drying the chloroform extract over anhydrous potassium carbonate the solvent was removed to leave a glass. Trituration with acetone gave a white solid which crystallised from acetone to give pure tetrahydro-oxo-krigenamine as stout needles (165 mg.) m. p. 171—172° $[\alpha]_D = -36^\circ$ (c, 1.01 in chloroform). The ultra-violet spectrum in ethanol showed an inflection at 240 mμ (ε,5,220) and a broad maximum at 282 mμ (ε , 1,400).

b). Oxokrigenamine (hydrate) (200 mg.) in tetrahydrofuran was refluxed with lithium aluminium hydride (200 mg.) for 15 hours. The product worked up as described above yielded a gum (185 mg.). Recrystallisation of the crude product from acetone afforded stout needles m.p. 171—172° which on admixture with the product obtained from the lithium aluminium hydride reduction

of krigenamine showed no depression.

Analysis:

Found:	C, 64.60;	H, 6.75.
$C_{18}H_{23}O_5N$ requires	C, 64.85;	H, 6.95.

Deoxykrigenamine.

Tetrahydro-oxokrigenamine (170 mg.) heated at 90° for 2 hours with 5% sulphuric acid (25 ml.). The solution cooled, extracted with chloroform (3 x 10 ml.), basified with ammonia, and re-extracted with chloroform (3 x 15 ml.). The latter chloroform extract was dried over anhydrous sodium sulphate and the solvents removed in vacuo to leave a clear glass. Attempts to crystallise this were unsuccessful.

Ethanolic picric acid added to the glass and the amorphous picrate, which precipitated immediately, was dissolved by warming. The solution on cooling gave pure deoxykrigenamine picrate needles m. p. 205—206° $[\alpha]_D$ = +123° (c, 0.82 in chloroform).

Analysis:

Found:	C, 53.26;	H, 4.68.
$C_{24}H_{24}O_{11}N$ requires	C, 52.94;	H, 4.44.

Catalytic Reduction of Krigenamine.

Krigenamine (290 mg.) in glacial acetic acid (35 ml.) was shaken in an atmosphere of hydrogen, in the presence of platinum oxide (80 mg.). The rapid absorption of hydrogen took place and two moles were taken up during the course of 80 minutes. The catalyst was filtered off and the acetic acid removed *in vacuo*. The residual gum was dissolved in chloroform (40 ml.) and washed with sodium carbonate solution. After drying the chloroform solution over anhydrous sodium carbonate the solvent was removed by distillation *in vacuo* leaving a gum (260 mg.). Attempts to crystallise this gum were unsuccessful and it was chromatographed over alumina in chloroform-ether (1:3). All the material was eluted rapidly in two small fractions (60 ml.) with this solvent. The second fraction crystallised from a small volume of ether (*ca.* 0.5 ml.) in prisms m. p. 148—156°. Further crystallisation from ether did not improve the melting point. The material from the column was recombined, dissolved in benzene-petrol (1:1) and recromatographed over alumina. Elution with benzene-petrol (1:1) (100 ml.) gave only traces of gum. Pure benzene (50 ml.) eluted a gum (30 mg.) which was very soluble in all organic solvents and could not be

crystallised. Continued elution with benzene (75 ml.) afforded a further quantity of gum which crystallised from ether to yield β-deoxydihydrokrigenamine as long prisms 160—162° $[\alpha]_D$ = +48° (c, 1.02 in chloroform).

Analysis:

Found:	C, 67.95; H, 7.15.
$C_{18}H_{23}O_4N$ requires	C, 68.12; H, 7.31.

Catalytic Reduction of Deoxykrigenamine.

Deoxykrigenamine (87 mg.) and platinum oxide (25 mg.) in glacial acetic acid were shaken in the presence of hydrogen. The rapid absorption of hydrogen took place and 1 mole was taken up in the course of 60 minutes. After removing the acetic acid in vacuo the residue was dissolved in chloroform (30 ml.) and washed with sodium carbonate solution. The chloroform solution dried and the solvent removed to leave a residue (80 mg.); which was dissolved in benzene-petrol (1:1). Chromatography over alumina yielded two distinct fractions. The first was a gum and could not be crystallised; the second fraction however crystallised from ether as long needles m. p. 160—162°. The infrared spectrum of this

compound was identical to the spectrum of β-deoxydihydro-krigenamine prepared by catalytic reduction of krigenamine. Furthermore a mixed melting point determination of these compounds showed no depression.

KRIGEINE.

Crude krigeine crystallised twice from aqueous acetone to furnish pure krigeine m. p. 209—210° (dec.) $[\alpha]_D$ = +245° (c, 0.27 in ethanol). The ultra-violet spectrum in ethanol showed an inflection at 240 mμ and a broad maximum centred at 285 mμ (ϵ, 990)

(Reported [6] for krigeine m. p. 209—210° $[\alpha]_D$ = +234°).

Analysis:

Found: C, 62.40; H, 6.23.

$C_{18}H_{21}O_6N$ requires C, 62.24; H, 6.10.

Tetrahydroneronine.

Krigeine (200 mg.) in dry ether (120 ml.) was refluxed for 23 hours with lithium aluminium hydride (300 mg.). The reaction mixture poured onto crushed ice, N, sodium hydroxide (25 ml.) added and the ether layer separated. The aqueous phase re-extracted first with ether (3 x 25 ml.)

and then with chloroform (3 x 75 ml.). After drying the extracts the solvents were removed leaving a gum (160 mg.). The gum crystallised from 'wet' ethyl acetate giving tetrahydroneronine hydrate as prisms, m. p. 177—178° $[\alpha]_D$ = -7.2° (c, 0.9 in chloroform). The ultra-violet spectrum measured in ethanol showed a shoulder at 240 mμ (ε, 5,370) and a broad maximum at 285 mμ (ε, 1,950). (Reported for tetrahydroneronine m. p. 178—179°).

Analysis:

Found: C, 58.57; H, 6.53.

$C_{18}H_{21}O_6N.H_2O$ requires C, 58.84; H, 6.86.

Deoxyneronine.

Tetrahydroneronine (287 mg.) heated with 5% sulphuric acid (15 ml.) for 2 hours. The solution was, cooled, extracted with chloroform, and the aqueous phase basified with ammonia. Extraction of the basic solution with chloroform (3 x 20 ml.) yielded the crude product as a gum (260 mg.) which crystallised from ethyl acetate as prisms. Two recrystallisations from ethyl acetate furnished pure

deoxyneronine, prisms, m. p. 171—172° $[\alpha]_D$ = +196°.

Analysis:

Found:	C, 64.93;	H, 6.39;
	OCH_3, 9.39.	
$C_{18}H_{21}O_5N$ requires	C, 65.24;	H, 6.38;
	OCH_3, 9.81.	

SECTION III.

THE ALKALOIDS OF HAEMANTHUS MAKENII.

Extraction of Haemanthus Makenii.

Extraction of the sliced bulbs of Haemanthus makenii with hot 95% ethanol was carried out in the usual way and the extracts concentrated in a climbing film evaporator. The concentrate (1 litre) contained a large amount of fats and these were removed by filtering through kieselguhr (celite 545). The filtrate was acidified to pH 2.0 with hydrochloric acid and then steam distilled. Filtration through kieselguhr was again necessary to remove precipitated fats. The clear filtrate extracted with ether (5 x 200 ml.) and the ether extract dicarded. Solid sodium carbonate was added to the extract until it was alkaline and the bases were removed by extracting exhaustively with chloroform. Removal of the chloroform left the crude alkaloid extract as a pale brown resin (9.4 g.) which represented a 2-4% yield based on the dry weight of the bulbs. A portion (4.7 g.) of the extract was taken and treated with hot benzene (150 ml.), filtered from a small amount (120 mg.) of insoluble matter, and then chromatographed on alumina (120 g.).

Crude coccinine (2.9 g.) was eluted with benzene-ethyl acetate (2:1) (700 ml.). Elution with chloroform (500 ml.) gave a second alkaloid (1.1 g.) which crystallised from water as needles m. p. 88—89° and gave a perchlorate m. p. 250—252°. The values are in agreement with the melting points reported [7] for montanine and montanine perchlorate respectively. No further investigation of this alkaloid was attempted by the author.

COCCININE.

Crude coccinine isolated from the column on trituration with ether gave a solid which on crystallisation from this solvent gave pure coccinine, prisms m. p. 163—164° $[\alpha]_D$ = -185° (c, 1.0 in chloroform). The ultra-violet spectrum had two maxima at 242 mμ and 293 mμ (ε, 4,370 and 4,900 respectively). The infrared showed absorptions attributable to alcoholic hydroxyl (3,598 $cm.^{-1}$).

(Reported [7] for coccinine m. p. 162—163° $[\alpha]_D$ = -189°).

Analysis:

Found:	C, 67.85; H, 6.24; N, 4.91; OCH_3, 10.72; NCH_3, 0.00. Neutral equivalent 304.9.
$C_{17}H_{19}O_4N$ requires	C, 67.76; H, 6.36; N, 4.65; OCH_3, 10.30. Neutral equivalent 301.3.

Coccinine Methiodide.

To coccinine (50 mg.) in methanol (2 ml.) methyl iodide (2 drops) added and the solution refluxed for 10 minutes. The solvents were removed in vacuo to leave a glass (53 mg.) which crystallised from ethyl acetate as micro-needles m. p. 219—220° (dec.).

(Reported[7] for coccinine methiodide m. p. 219—220° (from water)).

Analysis:

Found:	C, 48.40; H, 5.10; OCH_3, 8.44; NCH_3, 4.20.
$C_{18}H_{22}O_4N.I$ requires	C, 48.70; H, 5.02; OCH_3, 6.93; NCH_3, 6.48.

Coccinine methopicrate.

Alcoholic picric acid (1 ml.) added to coccinine methiodide (50 mg.) in ethanol (1 ml.). The yellow precipitate, which formed immediately, was filtered off and crystallised from ethanol. Two recrystallisations afforded pure coccinine methopicrate, needles, m. p. 318—319° (dec.). No analytical data could be obtained for the compound since it exploded on combustion.

Coccinine Perchlorate.

Perchloric acid (1 drop) added to coccinine (20 mg.) in ether. A white precipitate formed immediately and was redissolved by the addition of methanol (2 drops). The solution on standing deposited needles of coccinine perchlorate m. p. 279—280° (dec.). The melting point was unchanged after crystallising from methanol.

(Reported [7] for coccinine perchlorate m. p. 254—255° (dec.)).

Analysis:

Found:	C, 50.80;	H, 5.23.
$C_{17}H_{19}O_4N$ requires	C, 50.71;	H, 5.02.

Coccinine Acetate.

Coccinine (50 mg.) in dry pyridine (1 ml.) treated with acetic anhydride (2 drops) and allowed to stand at room temperature for 48 hours. The solvents were removed *in vacuo* and the residual gum pumped under high vacuum to remove traces of pyridine. The crude product (53 mg.) was dissolved in acetone and light petroleum added until a slight turbidity formed. After standing several days at room temperature large prisms were deposited from the solution. Recrystallisation from the same solvent gave pure coccinine acetate m. p. 148—150°. The infrared spectrum showed strong bands at 1738 $cm.^{-1}$ and 1250 $cm.^{-1}$ in accordance with its formulation as an O-acetate.

Analysis:

Found:		C, 66.50; H, 6.0; Ac, 14.15.
$C_{17}H_{18}O_4N.COCH_3$	requires	C, 66.50; H, 6.20. Ac, 12.53.

Coccinine Acetylperchlorate.

Coccinine acetate (15 mg.) in ether (1 ml.) containing methanol (1 drop) treated with perchloric acid (1 drop). A crystalline precipitate separated after a few minutes and on recrystallisation from methanol-ether pure coccinine acetylperchlorate m. p. 258—260° (dec.) was obtained.

Analysis:

Found: C, 51.60; H, 5.28.

$C_{19}H_{21}O_6N.HClO_4$ requires C, 52.24; H, 4.82.

Attempted oxidations of Coccinine.

a). Coccinine (95 mg.), in pyridine (2 ml.), was added to an ice cold solution of chromium trioxide (100 mg.) in pyridine (1.5 ml.) and the solution allowed to stand at room temperature for 48 hours. After treating with water the solution was filtered, and the filtrate, basified with sodium hydrogen carbonate, extracted with chloroform (4 x 20 ml.) and the chloroform extracts dried over anhydrous sodium sulphate. Removal of the chloroform afforded a gum (90 mg.) which on treatment with ether gave prisms m. p. 162—164°. The infrared spectrum of this substance contained no carbonyl

absorption and was identical to the spectrum of coccinine.

b). The above experiment was repeated on the same scale and instead of standing at room temperature the reaction mixture was warmed on a water bath for 2 minutes. Working up the product as described above gave unchanged starting material m. p. 158—162°.

c). Coccinine (25 mg.) in ethanol (10 ml.) refluxed for 2 hours with selenium dioxide (25 mg.). No precipitation of selenium occurred and the solvent was removed in vacuo. Coccinine was recovered unchanged from the chloroform extraction of this residue.

d). Coccinine (100 mg.) in chloroform (50 ml.) stirred for 3 hours with manganese dioxide (1 g.). The manganese dioxide was filtered off, washed with ethanol, and the solvents removed. The residue, a gum (80 mg.) could not be induced to crystallise. The ultra-violet spectrum of the product showed two maxima at 242 mμ and 293 mμ. A sample was sublimed at 140—160°/0.1 mm. giving a glass which after dissolving in ether and adding

perchloric acid gave prisms m. p. 278—280°. The infrared spectrum of this sample was identical with the spectrum of coccinine perchlorate.

Hydrogenation of Coccinine.

Coccinine (500 mg.) in N/10 hydrochloric acid was shaken with hydrogen in the presence of platinum oxide (120 mg.). Absorption of hydrogen (1.5 moles) took place during the course of 2 hours. After filtering off the catalyst the filtrate was basified with ammonia, extracted with chloroform (4 x 25 ml.), and the chloroform extract dried over anhydrous sodium sulphate. Removal of the chloroform left a gum (416 mg.) which was dissolved in chloroform-ether 1:1 (30 ml.) and chromatographed over alumina (25 g.). The early fractions contained a small amount of gum (60 mg.) which could not be crystallised. Chloroform (50 ml.) eluted α-dihydrococcinine (120 mg.) which crystallised from ethyl acetate in prisms m. p. 178—181°. Continued elution with chloroform (50 ml.) gave crude β-dihydrococcinine as a gum (100 g.). Chloroform-methanol elutes gave a pale yellow resin (90 mg.)

characterised as dihydrosecococcinine.

α-Dihydrococcinine.

Crude α-dihydrococcinine on recrystallising from ethyl acetate gave pure α-dihydrococcinine prisms, m. p. 180—181°. The ultra-violet spectrum in ethanol showed an inflection at 242 mμ and a maximum at 292 mμ (ε, 4,890).

Analysis:

Found: C, 66.83; H, 6.98.

$C_{17}H_{21}O_4N$ requires C, 67.31; H, 6.98.

α-Dihydrococcinine perchlorate.

One drop of 70% perchloric acid added to α-dihydrococcinine (20 mg.) in methanol (1 ml.) and ether added to turbidity. On setting aside the solution deposited prisms (18 mg.) which recrystallised from methanol-ether furnishing pure α-dihydrococcinine perchlorate m. p. 241—243°.

Analysis:

Found: C, 48.20, 48.36; H, 6.36, 6.06.

$C_{17}H_{21}O_4N.HClO_4.H_2O$

requires C, 48.40, ; H, 5.30,

∝-Dihydrococcinine Methiodide.

∝-Dihydrococcinine (25 mg.) in methanol (3 ml.) refluxed with methyl iodide (0.2 ml.) for 30 minutes. The solvents removed in vacuo and the product, as a gum, could not be obtained crystalline. It was dissolved in water and evaporated to dryness $[\alpha]_D$ = +14° (c, 1.0 in ethanol).

Analysis:

Found: C, 46.30; H, 6.14.

$C_{17}H_{21}O_4N.CH_3I.H_2O$ requires C, 46.66; H, 5.66.

β-Dihydrococcinine Methiodide.

To β-dihydrococcinine (30 mg.) in acetone (1 ml.) methyl iodide (ca. 0.2 ml.) added and the solution allowed to stand at room temperature for 3 hours. The solvent removed in vacuo and the residual gum crystallised from water giving β-dihydrococcinine methiodide, needles m. p. 255—257° (dec.). $[\alpha]_D$ = +17.5°.

Analysis:

Found: C, 47.60; H, 5.50.

$C_{18}H_{24}O_4N.I.\ H_2O$ requires C, 47.60; H, 6.0.

O,N-Diacetyldihydrosecococcinine.

Dihydrosecococcinine (50 mg.) in pyridine (1 ml.) and acetic anhydride (0.5 ml.) refluxed for 15 minutes. Solvents removed in vacuo and the residual gum sublimed at 140—150°/0.1 mm. The sublimate showed strong carbonyl absorption at 1738 $cm.^{-1}$ and 1642 $cm.^{-1}$ in accordance with its formulation as an O:N diacetate.

Analysis:

Found: C, 64.18; H, 6.59.

$C_{17}H_{21}O_4N.2COCH_3$ requires C, 64.76; H, 6.99.

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