THE CHEMISTRY OF CERTAIN AMARYLLIDACEAE ALKALOIDS

A Thesis

Submitted to

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by

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Doctor of Philosophy

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TO GOPAL

AND

TO MY PARENTS

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I would like to express my sincere thanks to Professor G.W.Kirby for his guidance throughout the course of this work.

I must also thank the technical staff at Loughborough University of Technology for their invaluable assistance.

I am thankful to The Whitehall Foundation, New York, for the financial support by awarding me 'The George M.Moffatt Scholarship', which made it possible to carry out this work.

Finally, I would like to express my deep gratitude to my parents for providing me with initial opportunities for attempting this degree and to my husband, Gopal, for his patient encouragement.

Abstract

The chemistry of alkaloids of the galanthamine group is reviewed. Oxidation of galanthamine with chromic acid or manganese dioxide, to give narwedine and a minor product, is described. On the basis of chemical and spectral data this minor product is shown to be keto-aldehyde containing a newly formed pyrrolidine ring.

The structure of the phenolic alkaloid, chlidanthine, of the galanthamine group has been determined by NO-dimethylation to give (-)-galanthamine methyl ether methiodide which was in turn prepared from (-)-galanthamine via (-)-epigalanthamine. The relative stereochemistry of the pair of epimeric, allylic alcohols derived from Pummerer's ketone has been determined. The conversion of these alcohols into allylic chlorides with thionyl chloride and with trisdimethylaminophosphine and carbon tetrachloride has been studied in detail.

The biosynthesis of chlidanthine has been studied. Tritium labelled (±)-narwedine, (±)-galanthamine and (±)-epigalanthamine were fed to Chlidanthus fragrans plants. Biosynthetic conversion of tritiated galanthamine and narwedine into chlidanthine was observed. Additionally, narwedine was efficiently converted into galanthamine thus confirming the long suspected precursor-product relationship of these alkaloids. The observed lack of incorporation of (±)-epigalanthamine into chlidanthine served to confirm the relative configuration of the latter alkaloid.

CONTENTS

CHAPTER 1

CHEMISTRY OF ALKALOIDS

OF THE GALANTHAMINE GROUP

The alkaloids of Amaryllidaceae family have been of great interest to organic chemists for a number of years. The plants of this family produce a large variety of alkaloids which are structurally related and probably derived biosynthetically from similar precursors. Numerous alkaloids have been isolated from various Narcissus, Crinum, Haemanthus, Galanthus, Lycoris and Nerine species. Full accounts of all these alkaloids have been published.[1,2,3]

The alkaloids of this family can be divided into groups based on their carbon skeleton types. Alkaloids containing,

a) a dibenzofuran skeleton, for example, galanthamine (1)

b) a pyrrolophenanthridine skeleton, for example, galanthine (2)

c) 5-10b-ethanophenanthridine skeleton, for example, crinine (3)

d) an N-benzylphenethylamine skeleton, for example, belladine (4)

e) a (2)-benzopyrano(3,4-g)indole skeleton, for example, lycorenine (5)

f) a (2)-benzopyrano(3,4-c)indole skeleton, for example, pretazettine. (6)

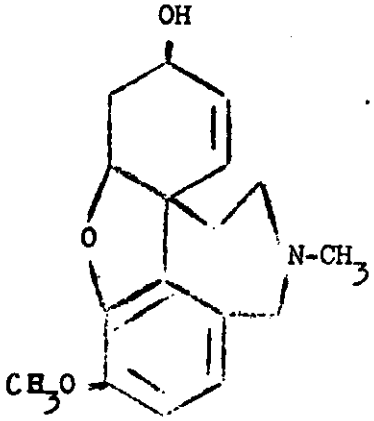
g) a 5,11-methanomorphanthridine skeleton, for example, coccinine (7)

and finally,

h) the alkaloid, nivalidine (8) was identified as 6-O-methylapogalanthamine.

Last four classes of alkaloids can be derived from first three classes, which occur the most frequently in nature, by the processes of cleavage, rearrangement and recyclisation. Thus lycorenine (5) can be derived by oxidative cleavage of

galanthine (2) and recyclisation. Similarly pretazettine (6) can be derived by oxidative cleavage of the crinene type skeleton followed by internal oxidation, reduction and recyclisation. The alkaloids coccinine (7) and nivalidine (8) can be derived by rearrangement of carbon skeleton.



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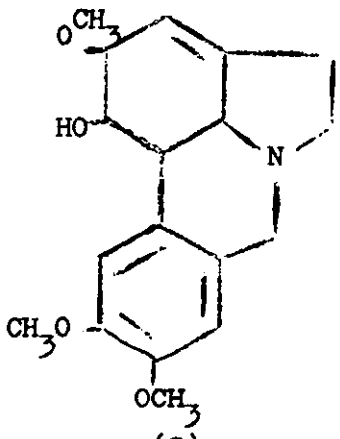

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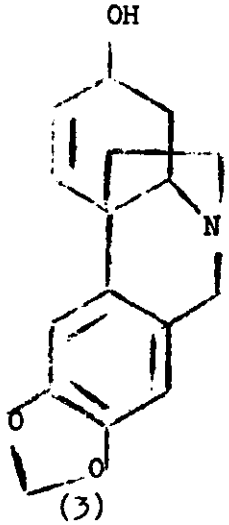

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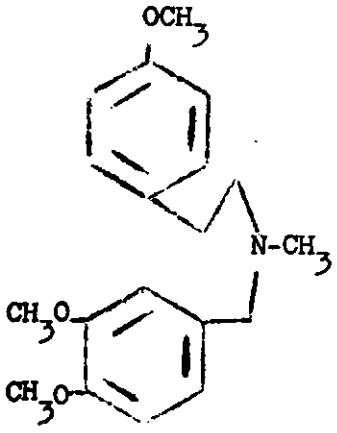

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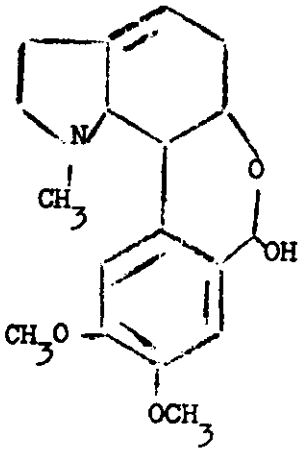

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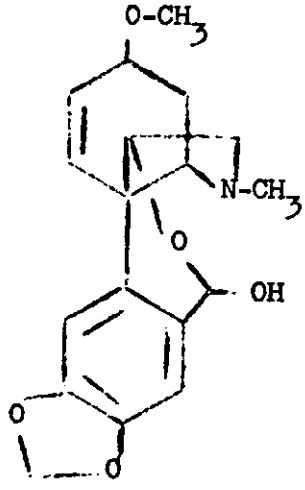

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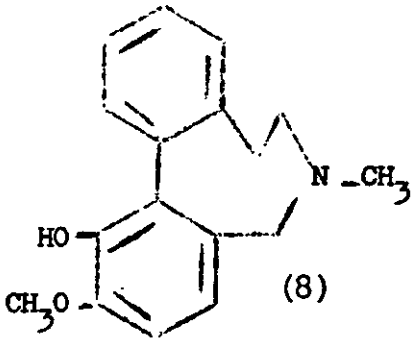
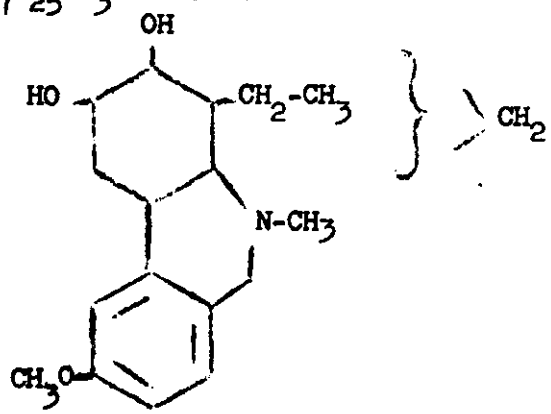


(8)

The alkaloid galanthamine, of the dibenzofuran group, was first isolated by Proskurnina and Yakovleva[4] as a constituent of Galanthus woronowi. Uyeo and Kobayashi[5] isolated the same alkaloid from Lycoris radiata and named it lycoremine. Galanthamine, $C_{17}H_{21}NO_3$, is a tertiary base, containing one nonphenolic hydroxyl group, one methoxyl group and one N-methyl group. It contains one double bond, since on catalytic hydrogenation it forms dihydrogalanthamine. Dihydrogalanthamine was found to be identical with alkaloid lycoramine,[5] which was first isolated by Kondo and his associates[6,7] as a constituent of Lycoris radiata. They assigned the molecular formula $C_{17}H_{25}NO_3$ and proposed the partial formula (9) for lycoramine.[7]



(9)

But the revised formula $C_{17}H_{23}NO_3$ proposed by Uyeo and Kobayashi[5] for lycoramine was not compatible with the formula $C_{17}H_{25}NO_3$ proposed by Kondo and Ishiwata.[7]

Also experimental data of Kondo and Ishiwata[7] showing the presence of two hydroxyl groups was inconsistent with the results obtained by Uyeo and Koizumi.[8] Thus to prove the relationship between galanthamine and lycoramine, the chemistry of lycoramine was studied very carefully by many workers.

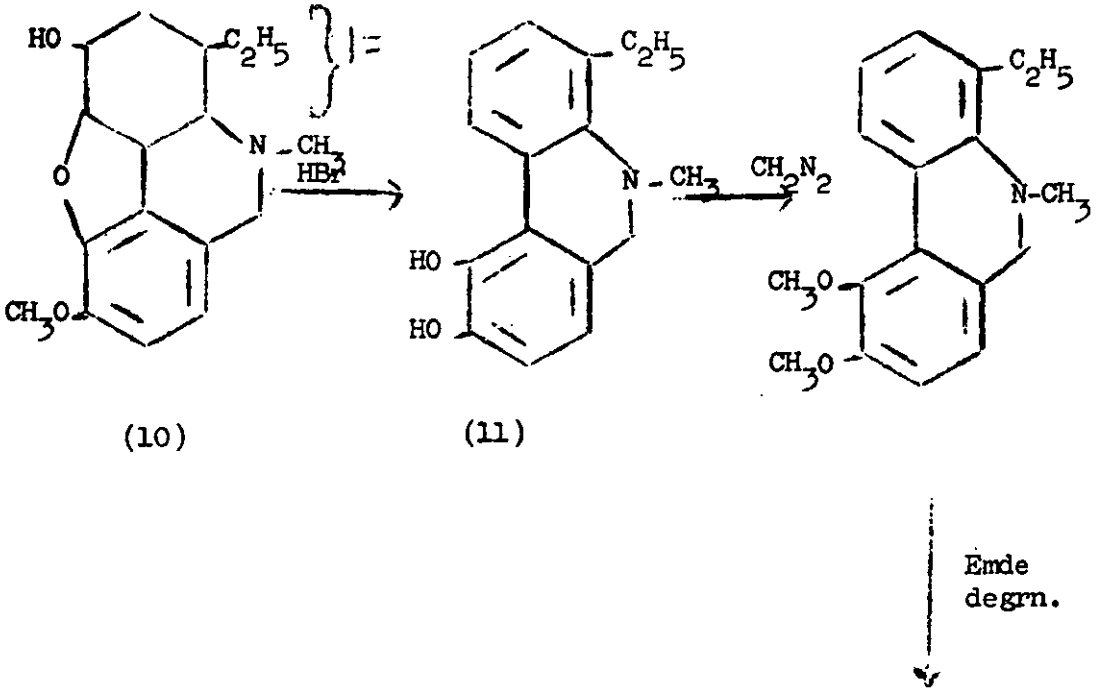
Uyeo and Koizumi[8] reinvestigated the chemistry of lycoramine and galanthamine and corrected a number of errors in the work of

earlier workers. They found that the two bases differed markedly towards the action of concentrated mineral acids. When lycoramine was treated with acetic acid and hydrobromic acid, it was demethylated and the secondary alcoholic hydroxyl group was replaced by bromine. The product was a monohydric phenol that could be reduced and methylated to give deoxylycoramine. The third oxygen was unaffected. Therefore it was suggested[8] that it must be a part of cyclic ether system.

On the other hand, when galanthamine was treated with mineral acids, it gave apogalanthamine monomethyl ether and apogalanthamine, $C_{16}H_{17}NO_2$ in which both oxygen atoms were present as phenolic hydroxyl groups. The composition indicated the presence of second aromatic nucleus which was not present in parent alkaloid but presumably was formed by the dehydration of hydroxyl group and the elimination of the ether system. Thus the ether linkage in galanthamine could be easily cleaved by acid in contrast to the ether linkage in lycoramine.

To find more structural information about galanthamine, degradation of methylated apogalanthamine was very helpful. Proskurnina and Yakovleva[9] methylated apogalanthamine with diazomethane and obtained two products - one a methylated apogalanthamine and second an uninvestigated base. Permanganate oxidation of Emde base of methylated apogalanthamine gave galanthamic acid $C_{16}H_{14}O_6$. Accepting the views of the Japanese workers[6,8] for the structure of lycoramine, Proskurnina and Yakovleva assigned the structure (10) for galanthamine, structure (12) for galanthamic acid and structure (11) for apogalanthamine.

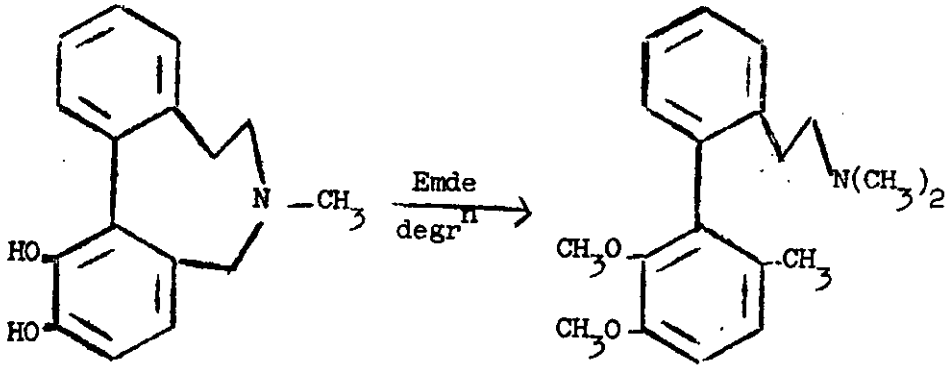
They proposed the following sequence of reactions for the degradation of galanthamine to galanthamic acid.



(10) (11) (12)

But the work done by the Russians was repeated by Japanese workers,[10,11] as the results obtained for lycoramine were incompatible. It was found that hydrobromic acid converted galanthamine into apogalanthamine, but hydrochloric acid gave O-methylapogalanthamine which was identical with the uninvestigated base obtained by Proskurnina and Yakovleva,[9]

during methylation of apogalanthamine with diazomethane. Methylation of O-methylapogalanthamine with methyl iodide gave OO-dimethylapogalanthamine methiodide. Emde degradation of this methiodide followed by oxidation with potassium permanganate gave two acids. Kobayashi and his associates[10] considered these two acids to be (15) and (16). They thought that the methyl group in acid (15) must have been formed during Emde degradation, as apogalanthamine does not contain any C-CH_3 groups. On the basis of this, the Emde base was assigned the structure (14) and apogalanthamine the structure (13).



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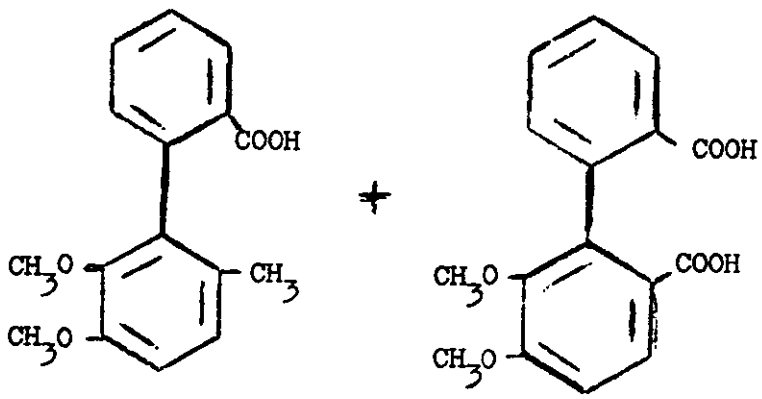
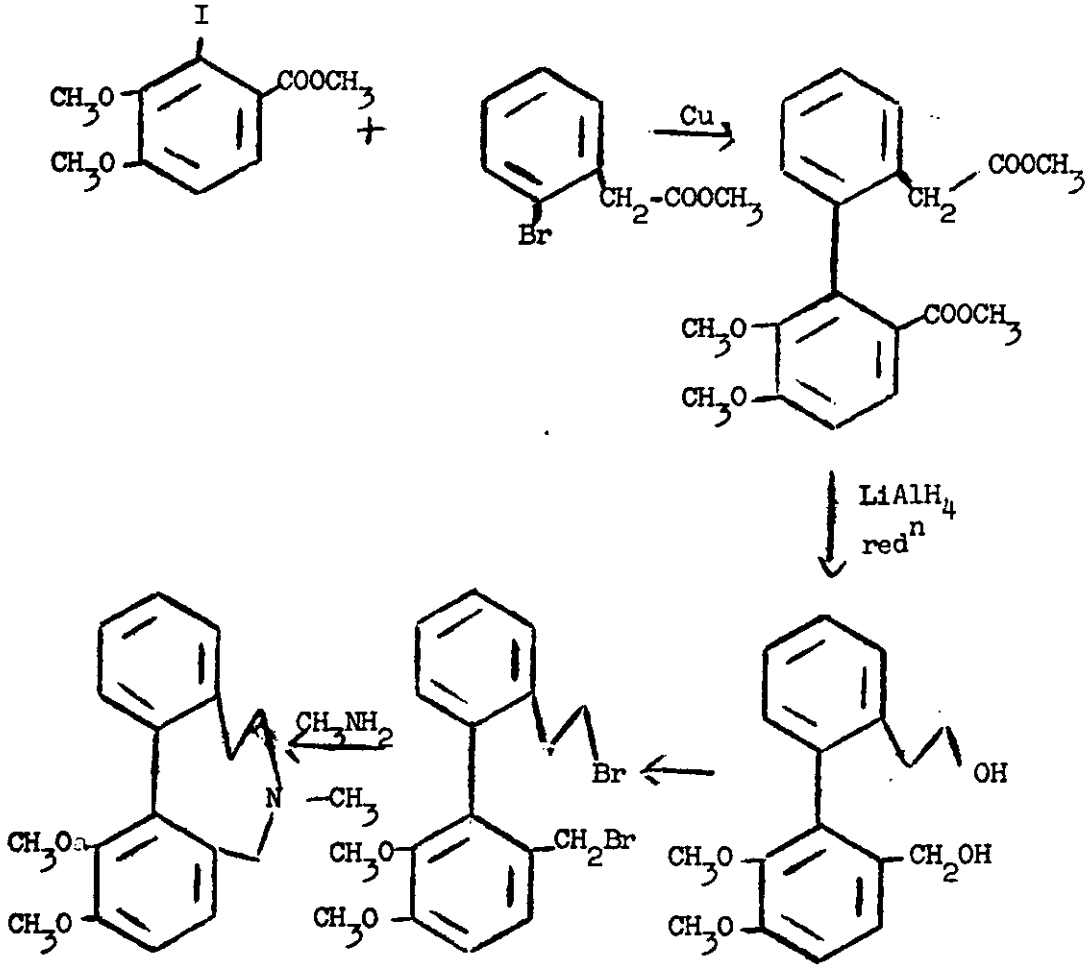
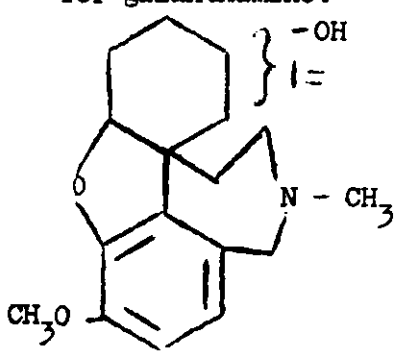


(15) (16)

This was further confirmed by the total synthesis of OO-dimethylapogalanthamine.[11] The synthetic route was as follows:



On the basis of these experimental data, Kobayashi and his associates[10] suggested two partial formulae (17) and (18) for galanthamine.



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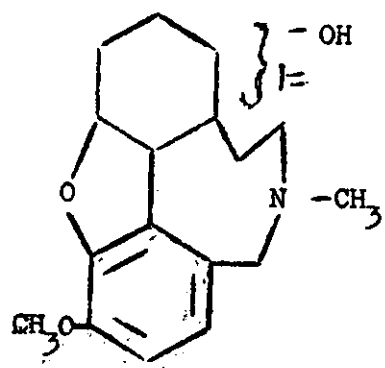
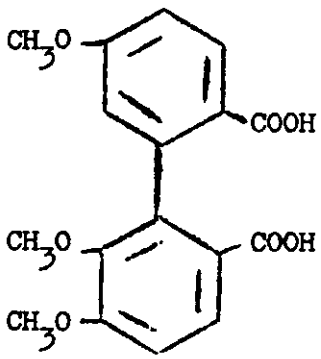


(18)

The hydroxyl group in galanthamine was found to be allylic since the oxidation of galanthamine to galanthaminone (narwedine) takes place readily.[12,13] Galanthaminone was found to be an α,β-unsaturated ketone. A series of degradation experiments was carried out on galanthaminone analogous to the degradations carried out on galanthamine. Thus treatment with mineral acid afforded hydroxyapogalanthamine. Methylation and Emde degradation of hydroxyapogalanthamine afforded a dicarboxylic acid (19), from which it was clear that hydroxyapogalanthamine could be represented by (20).



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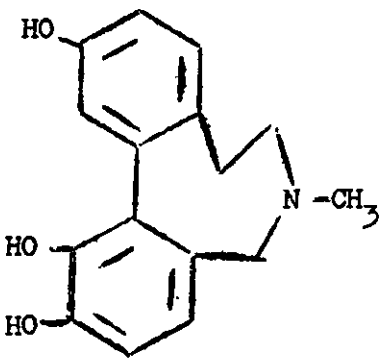
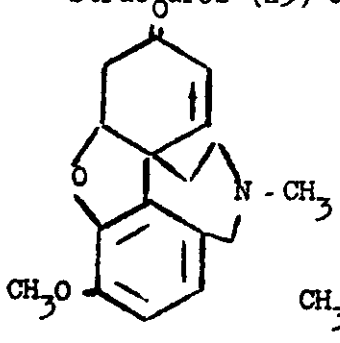


(20)

These results provided the evidence for the structure of galanthaminone and galanthamine. Thus galanthamine was represented by structures (21) or (22) and galanthamine by structures (23) or (24).



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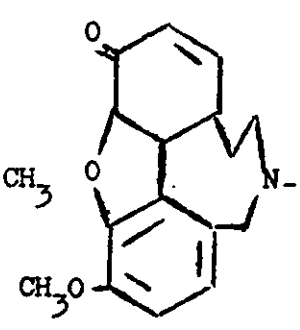

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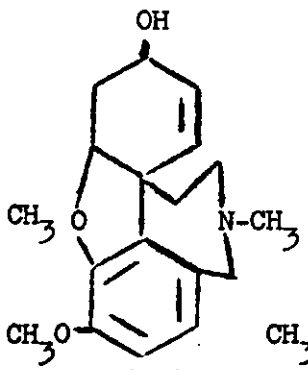

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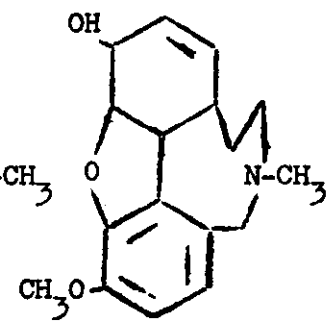
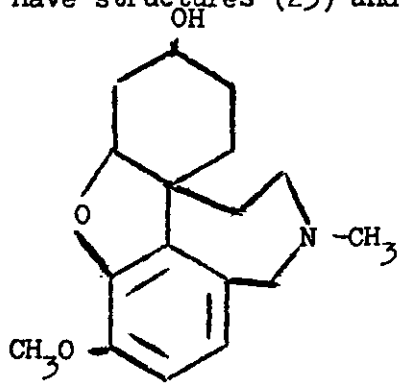


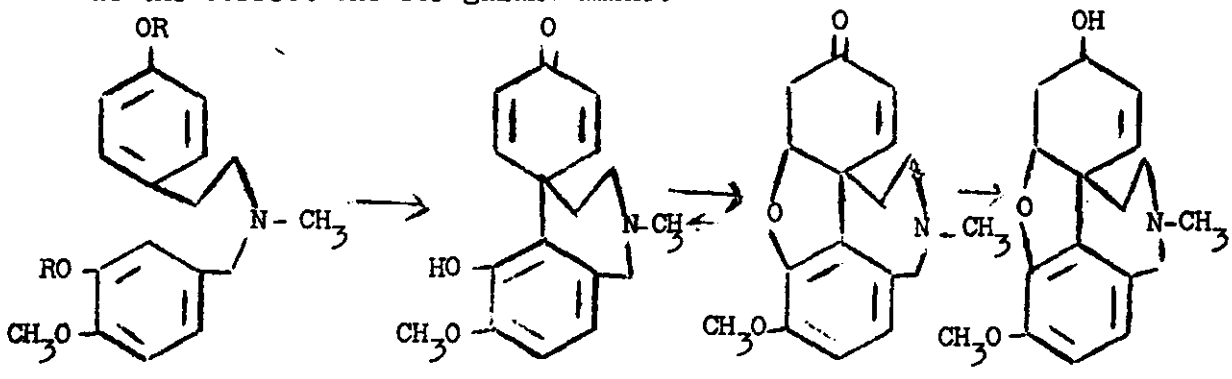
(24)

If the conversion of galanthamine and galanthaminone to their respective apoderivatives takes place without rearrangement then structure (24) is suitable for galanthamine. But this formula was discarded since the formation of a piperonylidene derivative by galanthaminone indicated the presence of a $-CH_2-CO-$ system in it. Therefore galanthamine, was represented by structure (21) and accordingly galanthamine and lycoramine were assumed to have structures (23) and (25).

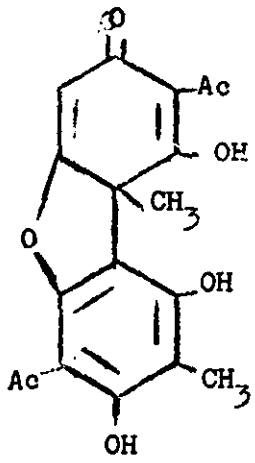


(25)

With the biogenetic theory as a background, Barton and Cohen[14] suggested the biosynthetic route for galanthamine indicated by the formulae (26) to (29). These reactions have analogy in the synthesis of usnic acid[15](30) and of Pummerer's ketone[16](31). Thus it was possible for them to propose the constitution (29) as the correct one for galanthamine.



(26) (27) (28) (29)



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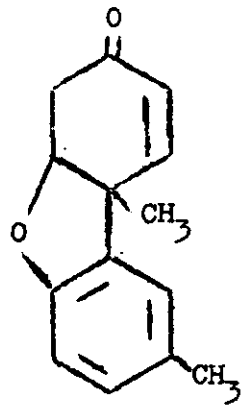


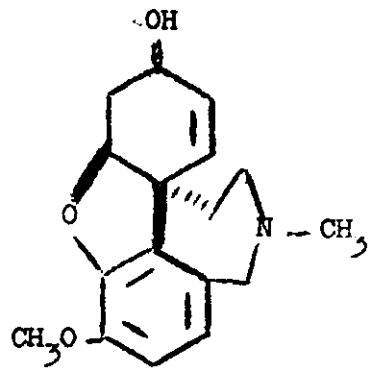
(31)

Isolation of the alkaloids belladine[17](4) and narwedine[18](28) supported this proposal for the biosynthesis and constitution of galanthamine entirely satisfactory.

To confirm the structure (29) for galanthamine, Barton and Kirby[19] undertook the total synthesis of (-)-galanthamine, following the proposed[14] biogenetic route, using the radioactive diphenol [(26),R=H], as the precursor. The consitution of this diphenol was confirmed by treatment with diazomethane, which gave belladine (4). A prior synthesis of belladine has been recorded.[20] Oxidation of the diphenol [(26), R=H] with various oxidising agents gave narwedine in very low isolated yields. The formation of narwedine was first detected by tracer experiments. Racemic narwedine served a very important role in the synthesis and resolution of galanthamine.

In the course of their studies, Barton and Kirby[19] obtained two stereoisomeric alcohols, (±)-galanthamine and (±)-epigalanthamine from the reduction of (±)-narwedine by

lithium aluminium hydride. These two alcohols differed markedly in their physical properties. (±)-Galanthamine was eluted more easily from an alumina column and it was more soluble in nonpolar solvents than was (±)-epigalanthamine. The hydroxyl group in (±)-galanthamine showed strong intramolecular hydrogen bonding with the oxide bridge, which is a well-known phenomenon.[21] Thus these two groups were placed cis to each other. (±)-Epigalanthamine had a hydroxyl group which was not hydrogen bonded. These facts, along with the observation that (-)-narwedine racemises but does not epimerise under basic conditions led them to the idea that in narwedine (and hence in galanthamine and epigalanthamine) the oxide ring has the more stable cis-fused configuration. The racemisation of narwedine occurs in polar solvent and is catalysed by the presence of base. The furan ring opens up with the loss of one of the methylene protons. The alkaloid narwedine itself is sufficiently basic to achieve this ring opening. The resulting dienone (28) can then reclose to regenerate (±)-narwedine, by the addition of phenolic hydroxyl group to either double bond of the dienone system. By interpreting the difference in rotations of (-)-galanthamine and (-)-epigalanthamine according to Mill's generalisation, they assigned the absolute configuration (32) to galanthamine, Accordingly epigalanthamine was represented by the absolute configuration (33). The stereochemistry of galanthamine was further supported by X-ray methods.[22]



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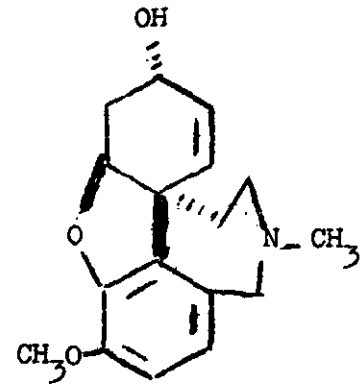
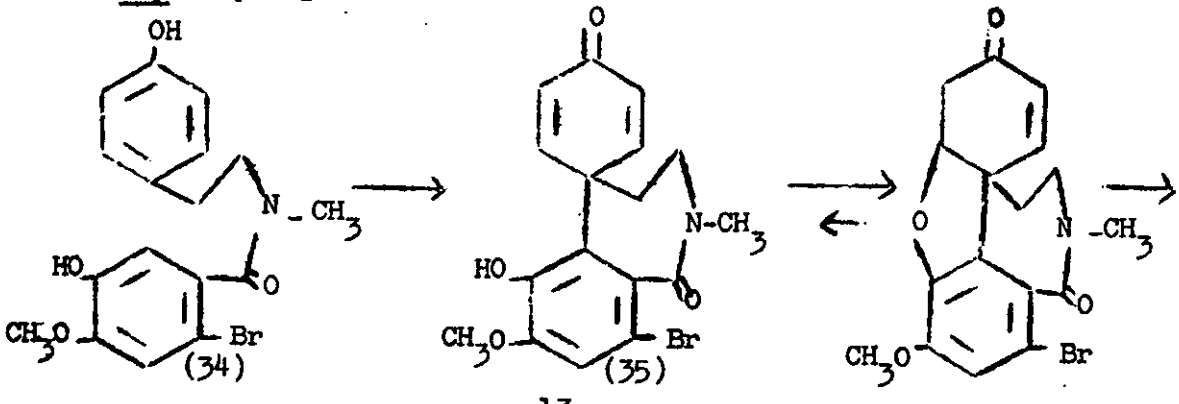


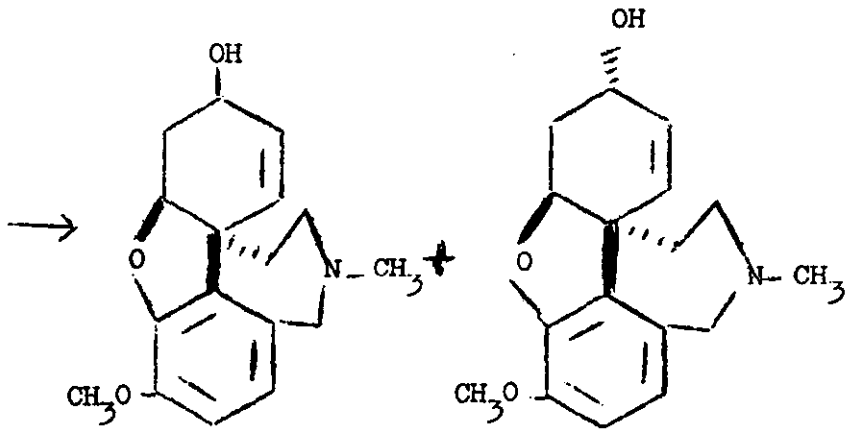
(33)

In order to achieve the total synthesis of natural (-)-galanthamine Barton and Kirby[19] tried to resolve either (±)-narwedine or (±)-galanthamine. But the resolution was unsuccessful by standard methods. However partially racemic (-)-narwedine [$[\alpha]_D = -88^\circ$] and (+)-narwedine [$[\alpha]_D = +36^\circ$] were obtained from the crystallisation of the reaction product from manganese dioxide oxidation of (-)-galanthamine with acetone and ethanol, respectively. Both products had i.r. spectra identical with those of (±)-narwedine and both were found to racemise in ethanol to give (±)-narwedine at the same rate. The spontaneous generation of (+)-narwedine during crystallisation from ethanol was found to be caused by the presence of small amounts of unreacted (-)-galanthamine. For the preparative work (±)-narwedine and (-)-galanthamine were used in a 2:1 ratio and the mixture was crystallised from ethanol and triethylamine. (±)-narwedine [$[\alpha]_D = +306^\circ$] isolated, was further purified to [$[\alpha]_D = +405^\circ$] by crystallisation from benzene. (-)-Narwedine was obtained in the similar way by using (+)-galanthamine and (+)-epigalanthamine as resolving agents. They came to the conclusion that this phenomenon was not just due to a seeding effect, since addition of (+) or (-)-narwedine

crystals to a saturated solution of (±)-narwedine in ethanol did not cause any significant resolution. Resolution was also independent of the functional groups present in resolving agent: O-acetyl and dihydro derivatives as well as N-methosalts of (-)-galanthamine were equally effective. At present this phenomenon can be best explained by adsorption phenomenon. The adsorption of traces of (-)-galanthamine on the surface of developing narwedine crystals might encourage the crystallisation of (+)-narwedine or inhibit the crystallisation of (-)-narwedine. The resolved (-)-narwedine was then reduced with lithium aluminium hydride to give a mixture of two alcohols, (-)-galanthamine and (-)-epigalanthamine, which could be easily separated by chromatography on alumina.

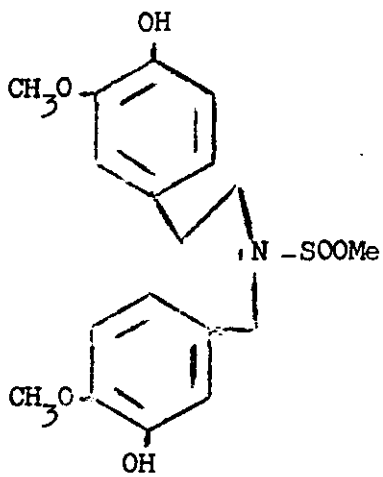
Recently Kametani[23] and his co-workers have published a modified total synthesis of (±)-galanthamine through phenol oxidation. Oxidation of 2-bromo-5-hydroxy-N-(4-hydroxyphenethyl)-4-methoxy-N-methylbenzamide(34) gave a narwedine type of dienone (35) in good yield. Reduction of (35) gave (±)-galanthamine and (±)-epigalanthamine in good yields. Introduction of a bromine atom para to the hydroxyl group apparently inhibited p-p coupling between the rings and thereby favoured o-p coupling.





(32) (33)

It has been also reported[24] that the methyl sulphonyl group was used to protect nitrogen during oxidative condensation of Ph-CH_2-NH-$(CH_2)_2$Ph and the ring substituted derivatives. The ring closure reaction led to various Amaryllidaceae alkaloids and confirmed the biosynthetic route suggested for these alkaloids. The synthesis of galanthamine (1) and nivalidine (8) was achieved by oxidative condensation and rearrangement of (36) and (37).



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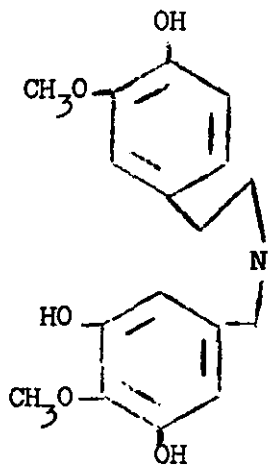

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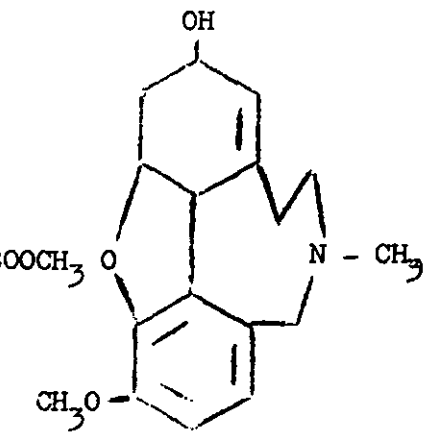
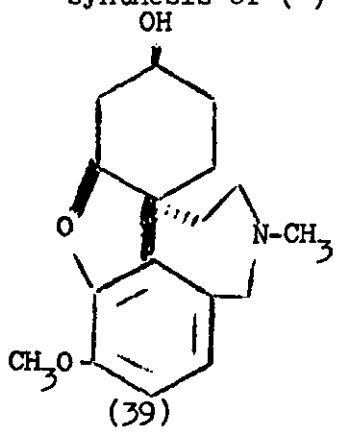


(38)

Galanthamine epimerises to (-)-epigalanthamine when treated with hot dilute mineral acid.[25,26] The incorrect structure (38) was assigned first to this isomerised product.[27] Since it gave O-methylapogalanthamine when further refluxed with concentrated mineral acid, the structure (38) seemed improbable. Hence the work was repeated by Kirby and Tiwari.[25] They found that oxidation of the isomerised product gave (±)-narwedine. They concluded that the isomerised product was (±)-epigalanthamine. During this study the n.m.r. spectrum of (±)-narwedine was studied in great detail. In the n.m.r. spectrum of narwedine the olefinic protons gave an AB quartet. But the doublet due to β proton was further split into a double doublet with a smaller coupling constant of 2 Hz. This was shown to be due to long range coupling with 4a proton by decoupling experiments. These results were further verified by deuteration studies on narwedine and Pummerer's ketone (31).

Alkaloid lycoramine, $C_{17}H_{23}NO_3$, was studied by many workers during the study of alkaloid galanthamine. It was found to be dihydrogalanthamine and has the absolute configuration (39) analogous to that of galanthamine.[19] Recently the total synthesis of (±)-lycoramine has been reported.[28,29]



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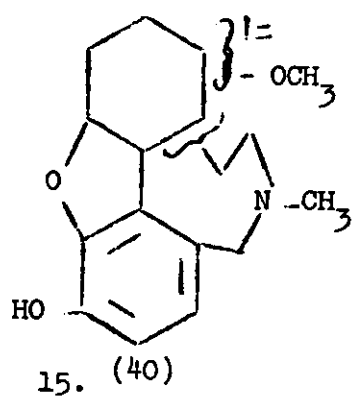

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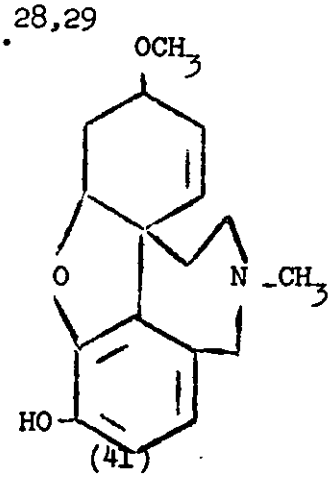
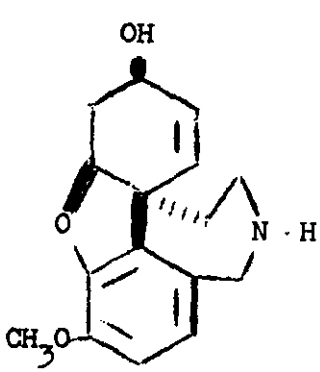


(41)

Chlidanthine is another alkaloid from the dibenzofuran group. The alkaloid, $C_{17}H_{21}NO_3$, was isolated from Chlidanthus fragrans by Boit.[30] The base contains one methoxyl group and one N-methyl group. Its method of isolation suggested in addition the presence of a phenolic hydroxyl group. Further studies showed that the alkaloid contains one double bond.[31] Upon treatment with hydrobromic acid chlidanthine was converted into apogalanthamine.[32] From these degradation data chlidanthine was represented by the partial formula (40), suggesting that it contains a galanthamine type of ring system. Recently, though no specific spectral data have been given, a report has been published that n.m.r. spectrum of chlidanthine is similar to that of galanthamine and thus supporting the assigned structure (41) for chlidanthine.[33]

Narcissamine, an alkaloid of the same group, was first isolated by Boit and Ehmke[34] from several garden varieties of daffodils. The alkaloid has the molecular formula $C_{16}H_{19-21}NO_3$ and was characterised as a secondary base containing one methoxyl and one double bond, but no N-methyl group. Fales and his co-workers[35] concluded that narcissamine is des-N-methylgalanthamine (42), molecular formula $C_{16}H_{19}NO_3$. It was also shown that galanthamine methiodide was identical with N-methylnarcissamine methiodide obtained by methylation of narcissamine,



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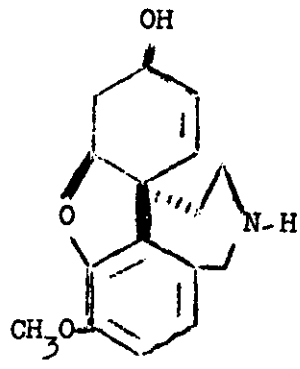

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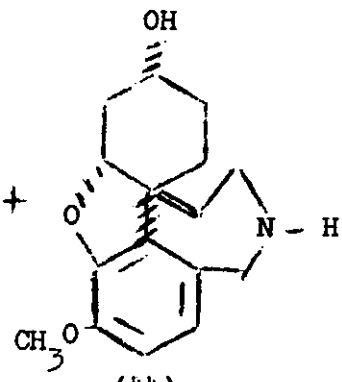
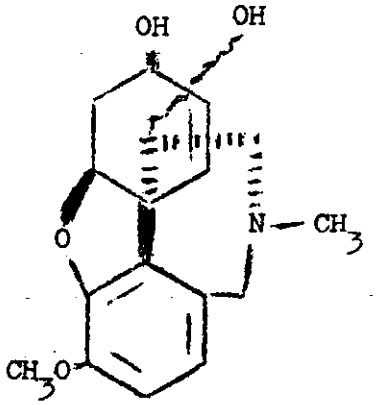


(44)

But a recent investigation on this alkaloid has shown[36] that narcissamine is in fact a quasi-racemic mixture containing equimolar amounts of (-)-N-demethylgalanthamine (43) and (+)N-desmethyl-dihydrogalanthamine (44). This conclusion was based on the fact that narcissamine showed a low rotation when compared with (-)-galanthamine. Also on hydrogenation, only 0.5 equivalent of hydrogen was absorbed by the alkaloid. The reduction product was pure, but completely racemic. The n.m.r. spectrum of narcissamine showed only one olefinic proton by integration.

The alkaloid habranthine was isolated[37] from Habranthus brachyandrous. It has the molecular formula $C_{17}H_{21}NO_4$ and contains the galanthamine type of ring system. This was proved by the fact that, on treatment with thionyl chloride followed by lithium aluminium hydride, it gave deoxylycoramine.[8] The structure (45) was deduced largely by comparison of the i.r., n.m.r. and mass spectra of habranthine with those of galanthamine and related alkaloids. The stereochemistry was further supported by the close resemblance of the O.R.D. and C.D. curves of habranthine with those of galanthamine. This is the first



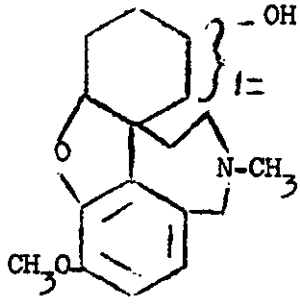
(45)

alkaloid of the galanthamine group to have a hydroxyl group at position 12, although a number of alkaloids of the crinine family have been shown to have hydroxyl groups at the analogous position.

CHAPTER 2

AN OXIDATION PRODUCT OF GALANTHAMINE

Until 1957 the structure of galanthamine was represented by the partial formulae (17) or (18), as suggested by Kobayashi and his associates.[10] With biogenetic theory as a background, Barton and Cohen[14] suggested the structure (32) (excluding stereochemistry) for galanthamine.



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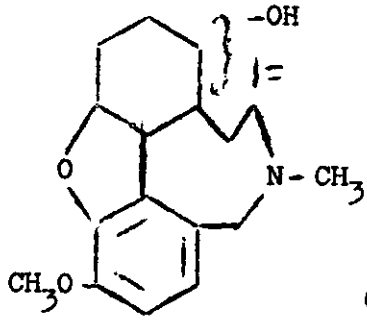

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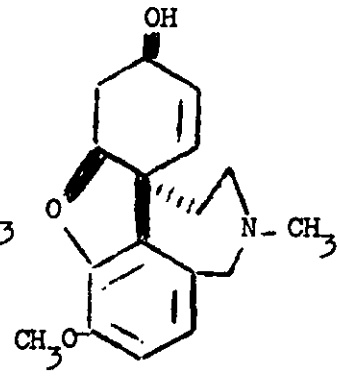
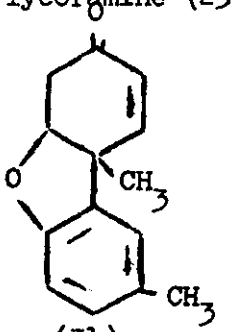


(32)

Barton and Kirby[19] undertook the synthesis of galanthamine to prove the constitution. During the course of their studies, they oxidised (-)-galanthamine with manganese dioxide[38,18] or chromium trioxide to obtain narwedine (21). They also obtained from the chromium trioxide oxidation an unidentified crystalline compound as a minor product along with narwedine. We have found that the same by-product is also produced during the manganese dioxide oxidation. To prove the structure of this unidentified oxidation product, spectroscopic studies have now been made and oxidation experiments carried out on Pummerer's ketone (31), lycoramine (25), and narwedine (21).



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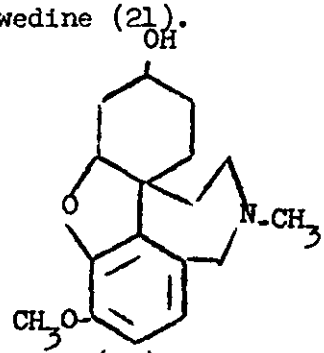

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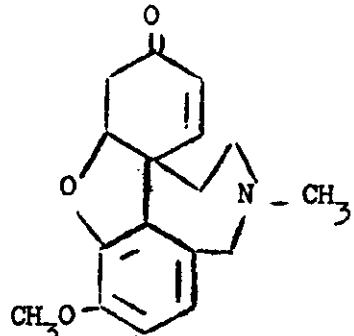


(21)

The unidentified oxidation product of (-)-galanthamine was prepared as previously reported. It was separated from narwedine (21) by chromatography. Crystallisation of the compound from ether or methanol, gave needles with a wide range of melting point 153° to 160°, which was increased to 190°-200° after a second crystallisation from methanol. The mass spectrum with accurate mass measurement gave the molecular formula as $C_{17}H_{19}NO_4$ i.e. formally a hydroxylation product of narwedine. However in the i.r. spectrum hydroxyl absorption was absent. But in carbonyl region it showed bands at 1720 and 1690 $cm.^{-1}$, indicating the presence of two carbonyl groups, (narwedine showed only a 1720 $cm.^{-1}$ band in the i.r. spectrum). Reduction with sodium borohydride in methanol gave material lacking carbonyl absorption. This indicated the absence of an amide group in the compound. The u.v. spectrum showed bands at λ max. 238, 279, 322 nm. (ϵ 11600, 10400, 6500 respectively). This suggested the presence of extended conjugation in the compound. The band at 322 nm. was ascribed to the conjugation between an aromatic ring and a carbonyl group.[39] Mishima et al.[40] prepared compound (46) and reported a u.v. spectrum nearly the same as ours; λ max. 231, 279, 327 nm. (ϵ 11700, 12400, 7700 respectively).

At this point, amounts of the by-product were too small to run an n.m.r. spectrum. While large amounts of the by-product were being collected, some oxidation experiments were carried out on Pummerer's ketone (31), to discover if oxidation of narwedine involved attack on or adjacent to nitrogen. Pummerer's ketone (31) resembles narwedine (21) closely in structure but lacks

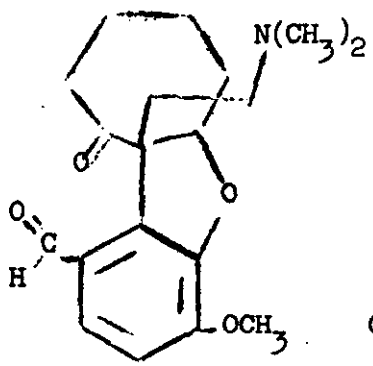
the nitrogen ring system. When Pummerer's ketone was treated with manganese dioxide, starting material was recovered. The reaction was followed by t.l.c. on alumina GF_{254}. No change was observed. This indicated that electrophilic attack by the reagent must be on the nitrogen ring in narwedine, most probably on the benzylic carbon atom. In order to confirm this and to see if the double bond is involved in oxidation, the oxidation of lycoramine (25) was carried out in similar manner.

Lycoramine (25) is dihydrogalanthamine. When it was oxidised with activated manganese dioxide,[38] two products were obtained. However the products were not fully characterised. One of them was separated in a reasonably pure state by column chromatography on alumina grade III in benzene; however it did not crystallise. It showed in the i.r. spectrum bands at 1725 and 1670 $cm.^{-1}$ These bands were assigned to saturated ketone and amide functions. The u.v. spectrum was similar to the u.v. spectrum of the by-product of galanthamine. It showed bands at λ max. 236, 278, 322 nm. To confirm the presence of an amide group, the compound was reduced with sodium borohydride. In the i.r. spectrum of the reduction product, the 1725 $cm.^{-1}$ band was absent, but the 1670 $cm.^{-1}$ band remained. This confirmed the presence of an amide group in the oxidation product of lycoramine.

From the results of oxidations on Pummerer's ketone and lycoramine, a conclusion was drawn that oxidation in narwedine must have occurred on the nitrogen ring, most probably on the benzylic carbon atom but not to give an amide.

When sufficient of the unidentified by-product obtained during the oxidation of galanthamine was collected, the n.m.r.

spectrum was run in deuteriochloroform. It showed a singlet at τ 0.0, suggesting the presence of an aldehyde function. Two aromatic protons appeared as doublets at τ 2.53 and τ 3.07 (J=8.7 Hz), which showed the presence of one deshielded aromatic proton (τ 2.53). These observations suggested the presence of an aldehyde function attached to the aromatic ring. Thus on the basis of these spectral data, it was possible to assign the structure (47) to this oxidation product.



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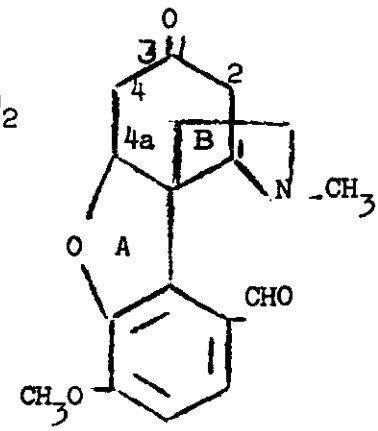

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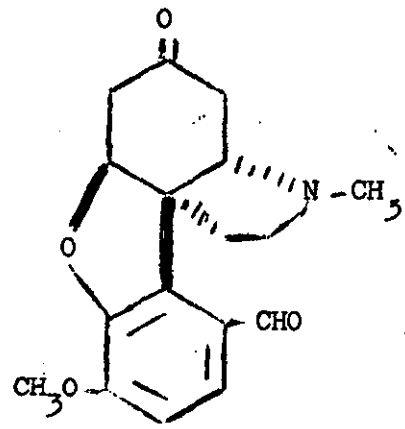


(48)

The stereochemistry of the compound (47) can in principle be represented by structures in which the five-membered A and B rings are cis-cis, cis-trans, and trans-cis fused to the cyclohexanone ring. Trans-trans fusion is sterically not possible.

The correct stereochemical assignment of the keto-aldehyde followed from the appearance of the methine proton (4a) signal in n.m.r. spectrum. It appeared at τ 5.23 as a distorted triplet (line separations 2.8 and 3.1Hz). The lack of large coupling with the neighbouring methylene group showed that the

methine proton was equatorial and the oxygen substituent axial. The aryl group must therefore have been equatorial and the ethanamine bridge linked from the axial position at C-4b to the equatorial position at C-1. Both 5-membered rings were therefore cis-fused to the cyclohexanone ring. The ketoaldehyde (47) was optically active and must have the same absolute configutation (48) as galanthamine (32).

The melting point and optical rotation of the keto-aldehyde increased with repeated crystallisation from methanol without any corresponding change in the spectroscopic or chromatographic properties. Narwedine is known to racemise partially during its preparation from galanthamine and it is probable that the by-product (47) was also obtained partially racemic. Confirmation of this was obtained later (see Chapter 4). The by-product (47) gave an optical rotation - 168° in ethanol, which was increased to -263° after two crystallisations from methanol.

The keto-aldehyde (47) is formally a hydroxylation product of narwedine (21). Its formation is consistent with,

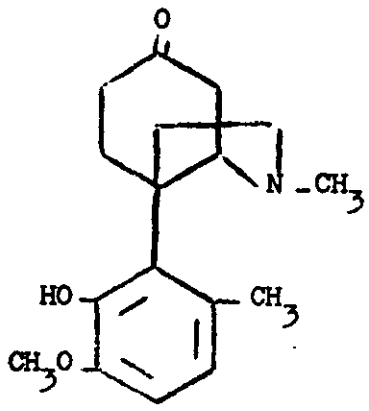
(1) hydroxylation at the benzylic carbon atom,

(b) opening of the resulting carbinolamine before further oxidation to a lactam can occur and,

(c) addition of the liberated amino group to the enone system.

Presumably benzylic hydroxylation of both galanthamine and narwedine can occur leading ultimately to the same product (47). Oxidation of (±)-narwedine with manganese dioxide gave material having u.v. and i.r. (solution) spectra identical with those of the keto-aldehyde (47).

An attempt was made to convert the keto-aldehyde (47) into a compound (49) having the ring system of mesembrine (50). Comparison of the ORD/CD curves of these two compounds would then have provided confirmation[41] of the absolute configuration of mesembrine. Catalytic hydrogenation of keto-aldehyde was studied carefully.



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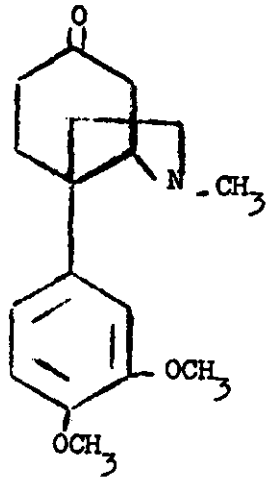

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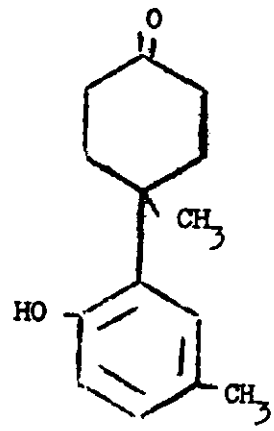


(51)

Barton and his co-workers[15] prepared the ketophenol (51) by catalytic hydrogenation of Pummerer's ketone over 10% palladised charcoal in the presence of sodium ethoxide, when the oxide bridge in Pummerer's ketone opens easily. The hydrogenation of keto-aldehyde was carried out similarly with the expectation that the aldehyde group would be reduced to a methyl group and the oxide bridge would open to give a phenolic hydroxyl group to yield ketophenol (49).

In order to see whether the aromatic aldehyde group could be reduced to a methyl group, veratraldehyde was hydrogenated over 10% palladised charcoal. The hydrogenation product of veratraldehyde showed a u.v. band at λ max. 285 nm. Disappearance of the band at 305 nm., which was present in u.v. spectrum of veratraldehyde,

indicated that the aldehyde group in veratraldehyde was reduced. The i.r. spectrum showed the absence of the 1680 $cm.^{-1}$ band, characteristic of an aromatic aldehyde group. The n.m.r. spectrum in deuteriochloroform showed a sharp singlet of three protons at τ 7.7, indicating the presence of a new methyl group.

When the ketoaldehyde (47) was hydrogenated over 10% palladised charcoal in the presence of sodium ethoxide, a mixture of three compounds was obtained. The major product obtained after chromatography showed on t.l.c., on alumina GF_{254} plates, a single spot with R_F 0.4, different from keto-aldehyde. The u.v. spectrum showed bands at λ max. 230, 285 nm. Absence of the 322 nm. band indicated that the aldehyde group in keto-aldehyde was reduced. The i.r. spectrum showed bands at 3560 and 1720 $cm.^{-1}$ indicating the presence of hydroxyl and saturated ketone functions. However the n.m.r. spectrum of the compound did not show a singlet of three protons due to methyl group. We could not characterise the hydrogenation product from the spectral data.

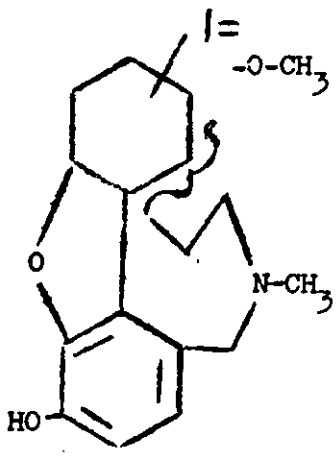
The hydrogenation of the keto-aldehyde (47) was then tried under different experimental conditions. When the keto-aldehyde was hydrogenated over 10% palladised charcoal in the absence of sodium ethoxide, one mole of hydrogen was rapidly consumed. The reaction mixture showed one spot on t.l.c., different from that of the keto-aldehyde. The u.v. spectrum of the compound showed a band at λ max 285 nm. and the band at 322 nm. due to the aromatic aldehyde group in the keto-aldehyde had disappeared. Thus reduction of aldehyde group had taken place. When the hydrogenation was further carried out in the presence of sodium ethoxide, the

hydrogenated product showed one spot on t.l.c. with the same R_F value as the keto-aldehyde. But the u.v. spectrum of the compound was different from that of the keto-aldehyde. It showed bands at λ max 232, 260, 290 and 305 nm. The i.r. spectrum showed bands at 3540 and 1720 $cm.^{-1}$, indicating the presence of a hydroxyl group and saturated ketone function. However the n.m.r. spectrum of the compound did not show a singlet of three protons due to methyl group. We could not characterise this hydrogenation product from the spectral data.

CHAPTER 3

STRUCTURE AND STEREOCHEMISTRY OF CHLIDANTHINE

The alkaloid chlidanthine, of the galanthamine group, was investigated by Boit and his co-workers.[30,31,32] By 1957, chlidanthine was represented by partial structure (52) which suggested a close relationship to galanthamine (53). Recently a resemblance has been noted between the n.m.r. spectra of chlidanthine and galanthamine and the structure (54) was assigned to the alkaloid, though no details were given.



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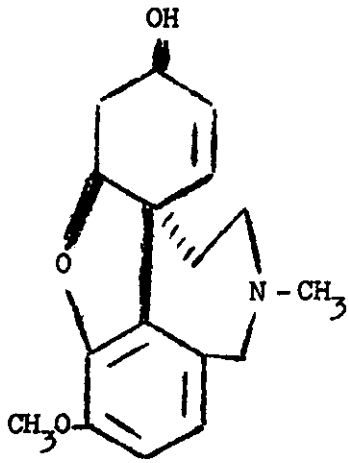

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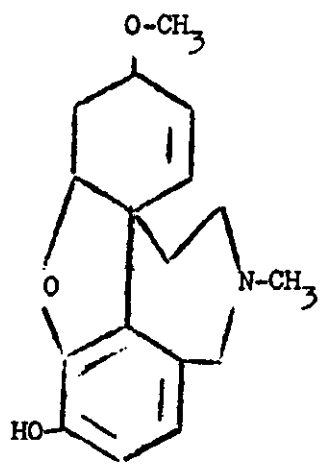
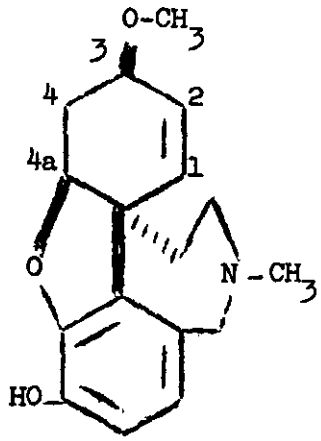


(54)

We propose the structure and stereochemistry (55) fpr chlidanthine. Accurate mass measurements on chlidanthine and O-acetylchlidanthine confirmed Boit's molecular formula, $C_{17}H_{21}NO_3$, for the alkaloid. In the mass spectrum of chlidanthine the molecular ion m/e 287, formed the base peak (100%). A peak at m/e 202 (37%), $C_{12}H_{12}NO_2$, may be tentatively assigned to the ion (56) since the spectra of galanthamine (53),

epigalanthamine (57) and narwedine (58) showed strong peaks (respectively 36, 65 and 19% of the corresponding base peaks) at m/e 216 and not at m/e 202.



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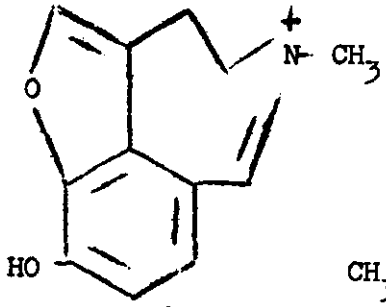

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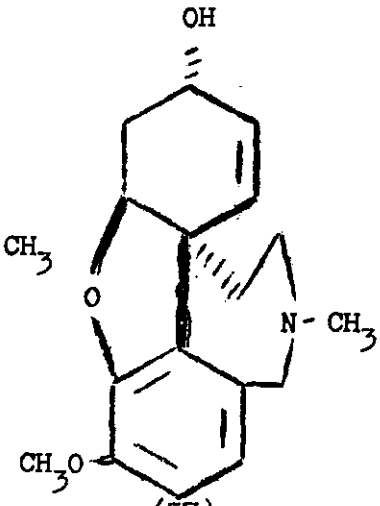
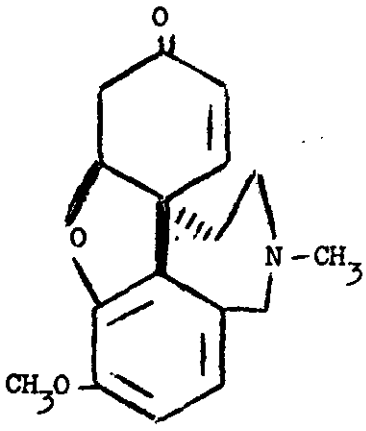


(57)



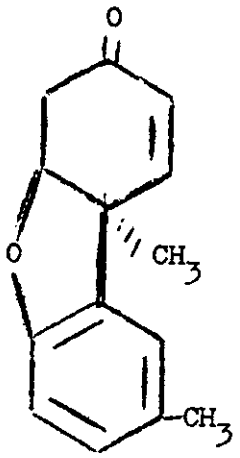
(58)

To study the n.m.r. spectrum of chlidanthine, it was acetylated in order to increase its solubility in deuteriochloroform. The n.m.r. spectrum of O-acetylchlidanthine in deuteriochloroform confirmed the gross structural features of the molecule. The aromatic protons appeared at τ 3.17 and 3.42 (J=8.2 Hz). The olefinic proton at position 1 appeared at τ 3.80 as a doublet

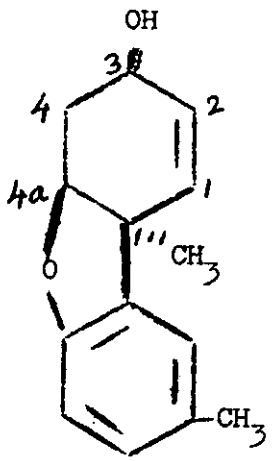
(J=11 Hz) and the olefinic proton at position 2 appeared at τ 4.02 as a quartet (J=11.0 and 3.4 Hz). The methine proton at position 4a gave a distorted triplet (J=ca. 3 Hz) at τ 5.43. The Ar-CH_2 protons gave a quartet (J=15.7 Hz) at τ 5.86 and 6.32. Alkyl-O-methyl and N-methyl groups gave singlets at τ 6.65 and 7.62. The acetyl group gave a singlet at τ 7.76. The superficial resemblance of this spectrum to that of galanthamine was not taken as reliable evidence for relative configuration of chlidanthine. In galanthamine the conformation of the cyclohexene ring is defined by hydrogen bonding between the hydroxyl group and the ether linkage[19,22] whereas in the corresponding ether or its C-3 epimer flattening of the cyclohexene ring might well take place.

In order to study the structure and stereochemistry of chlidanthine, Pummerer's ketone (59) was chosen as a model compound. Since chlidanthine was available only in small amounts, it was not possible to investigate many reactions on the alkaloid itself.

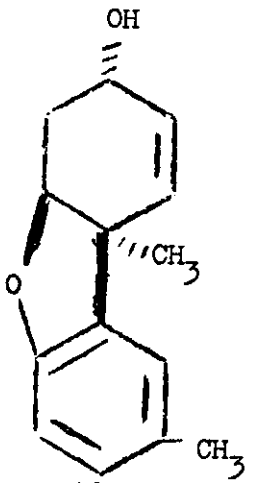
Pummerer's ketone (59) required for the purpose was prepared by the methods of Pummerer[42] and of Bacon.[43] It was reduced with lithium aluminium hydride to give two stereoisomeric alcohols (60) and (61), which were easily separated by chromatography. Elution of the mixture from alumina gave first an oily alcohol (60) and second, a crystalline alcohol (61). The crystalline alcohol had been obtained previously[16] from the Meerwin-Ponndorf reduction of Pummerer's ketone. The i.r. spectra of the two



(59)



(60)

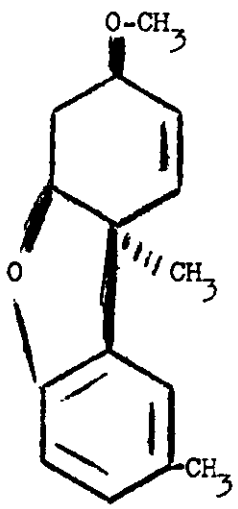


(61)

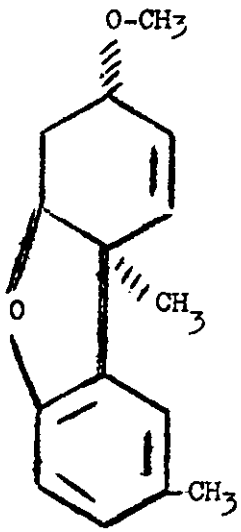
alcohols (60) and (61) in dilute carbon tetrachloride solution showed hydroxyl bands at $\nu_{max.}$ 3560 cm.$^{-1}$ and 3610 cm.$^{-1}$ respectively, which indicated the presence of intramolecular hydrogen bonding in alcohol (60) and not in alcohol (61). This observation defined the relative configuration of the alcohols and emphasised a structural analogy[19] with galanthamine (53) and epigalanthamine (57) respectively. Support for an intramolecular hydrogen bond in alcohol (60) came from the n.m.r. spectrum in deuteriochloroform. The proton at C-3 gave a multiplet, τ 5.86, of at least 8 lines (separation of extreme lines, 23.1 Hz). Addition of deuterium oxide caused collapse of this signal into a broad quartet (separation, 12.9 Hz), indicating a CH-OH coupling constant of 10.2 Hz. This was supported by a doublet at τ 7.6, for the hydroxylic proton, although the high field component appeared only as a shoulder on the Ar-CH_3 singlet. However, in hexadeuteriodimethylsulphoxide, the hydroxylic proton gave a doublet, τ 5.33 (J=5.8 Hz), the coupling now being characteristic[44] of a freely rotating

hydroxyl group. The larger value observed in deuteriochloroform is explicable[45] if rotation of the hydroxyl group is prevented by intramolecular hydrogen bonding.

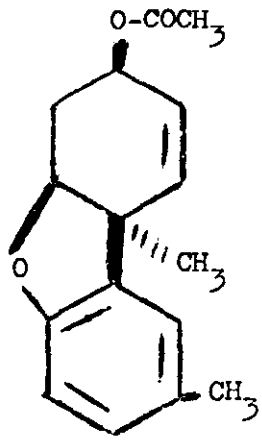
In order to prepare compounds structurally similar to chlidanthine, the two alcohols (60) and (61) were methylated in good yield with freshly prepared silver oxide in neat methyl iodide. Acetylation of the alcohols was carried out with acetic anhydride in the presence of pyridine. When the n.m.r. spectra of the alcohols (60) and (61) and their derivatives (62), (63), (64) and (65) were compared with that of O-acetylchlidanthine some common features were observed in the n.m.r. spectra of the oily alcohol (60) and O-acetylchlidanthine.



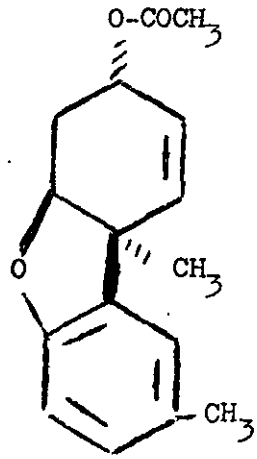
(62)



(63)



(64)



(65)

In the n.m.r. spectrum of the oily alcohol (60) the olefinic protons at positions 1 and 2 gave an AB quartet at τ 4.45 and 4.1 ($J_{1,2}$ = 10 Hz) with further splitting of the C-2 proton bands by the neighbouring methine proton at position 3 into a quartet (J=4.5 Hz), indicating that the hydroxyl group was quasi-axial.[46] The methine proton at position 4a gave a broadened triplet (J=ca.3 Hz) at τ 5.4 which could arise from splitting by the methylene protons at position 4. In the n.m.r. spectrum of O-acetylchlidanthine, the olefinic protons at position 1 and 2 and the methine proton at position 4a gave signals similar to those observed for the oily alcohol (60) in deuteriochloroform. The olefinic proton at position 1 gave a doublet at τ 3.80 (J= 11.0 Hz) and the olefinic proton at position 2 gave a quartet at τ 4.02 (J= 11.0 and 3.4 Hz). This indicated that the methoxyl group in chlidanthine must be quasi-axial[46] as is the hydroxyl group in the oily alcohol (60). The methine proton at position 4a gave a distorted triplet at τ 5.43 (J=ca. 3 Hz). These similarities led us to the conclusion that chlidanthine has a stereochemical configuration (55)

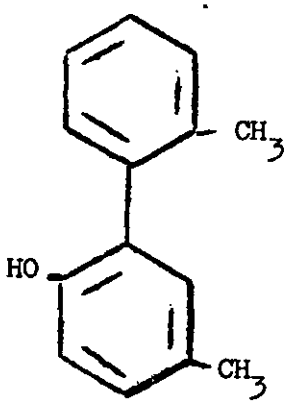
analogous to that of the oily alcohol (60) and in turn to galanthamine (53) with only difference in the positions of hydroxy and methoxy groups.

In the n.m.r. spectra of the crystalline alcohol (61), the methyl ether (63), and the acetate (65), the methylene protons at position C-4 gave multiplets of fourteen lines, τ ca. 7.1 - 8.5. The line spacings and chemical shifts were closely similar for all three compounds (Table 1), thus providing a useful criterion for stereochemistry at C-3 in this series. However the n.m.r. spectrum of the oily alcohol (60) differed markedly from the spectra of the methyl ether (62) and the acetate (64). The spectra of the two derivatives could not be analysed by first order methods but it seems likely that the large difference observed might arise from conformational "flipping" of the cyclohexene ring. Hydrolysis of the acetate (64) with alkali was carried out in order to see whether the acetate had a structure corresponding to that of the parent alcohol (60). The parent alcohol (60) was obtained in good yield.

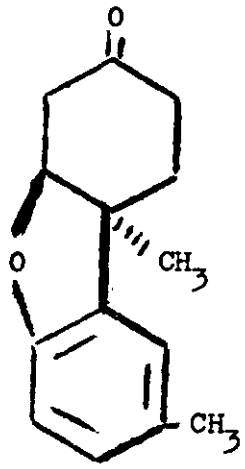
To see whether it is possible to convert chlidanthine into epigalanthamine (57), epimerisation of the methyl ether (62) was carried out by refluxing it with dilute hydrochloric acid. The expected epimeric alcohol (61) was obtained, but was accompanied by ca. 50% the rearrangement product (66). The n.m.r. spectrum of this compound in deuteriochloroform showed a complex multiplet pattern due to seven aromatic protons, τ 2.7 to 3.2. The aromatic hydroxyl proton gave a singlet at τ 5.33, which

disappeared after addition of deuterium oxide. Two sharp singlets due to two methyl groups appeared at τ 7.7 and 7.85. The low yield of the epimeric alcohol (61) was however discouraging and it was decided not worthwhile to expend our limited supply of chlidanthine on this experiment.

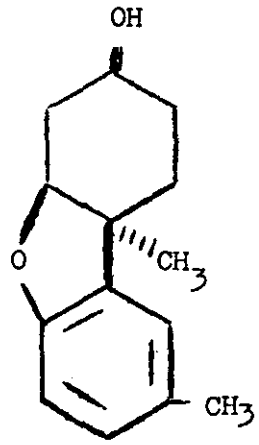
Pummerer's ketone (59) was hydrogenated in ethanol over 10% palladised charcoal.[15] Dihydro-Pummerer's ketone (67) on lithium aluminium hydride reduction gave two stereoisomeric alcohols (68) and (69). The two alcohols were readily separated by chromatography. The alcohol (68), eluted first from the column, was an oil. It slowly crystallised on standing. The second alcohol (69), eluted later from the column, was crystalline in nature. This alcohol has been previously obtained.[16] The i.r. spectra of the two alcohols (68) and (69) in dilute carbon tetrachloride solution showed hydroxyl bands at $\nu_{max.}$ 3600 cm.$^{-1}$ and 3650 cm.$^{-1}$ respectively, thus establishing the relative configuration and proving the structure of previously known epimer (69).



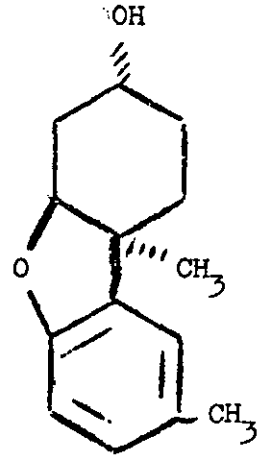
(66)



(67)

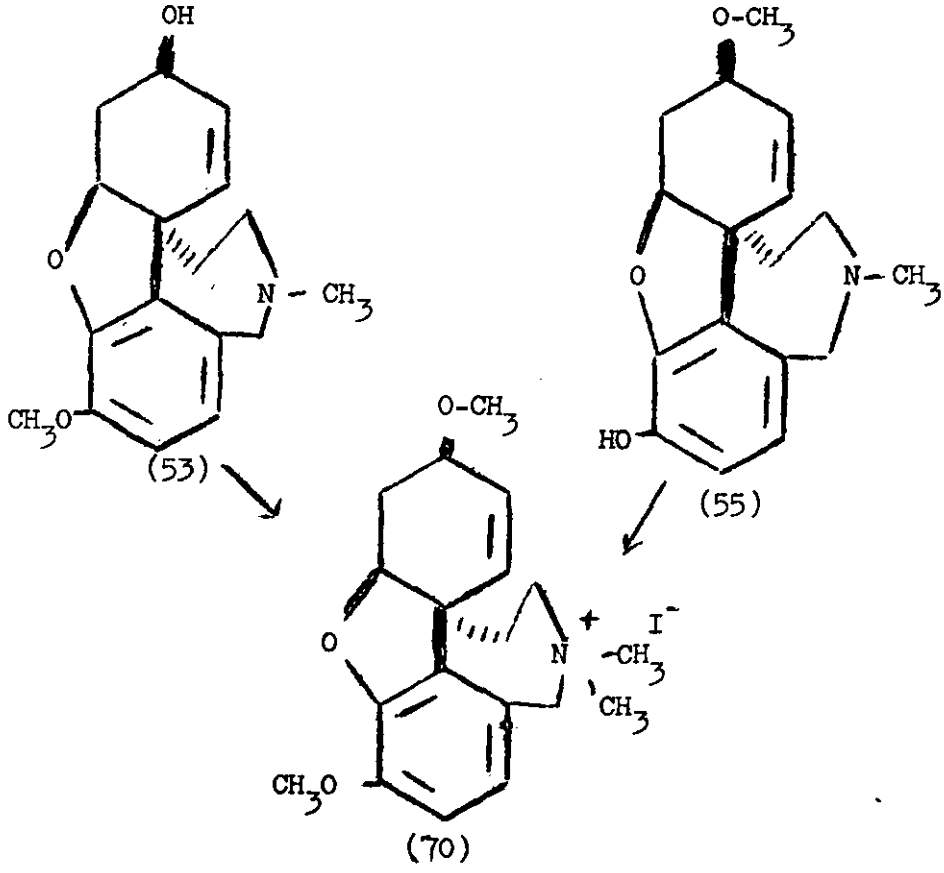


(68)

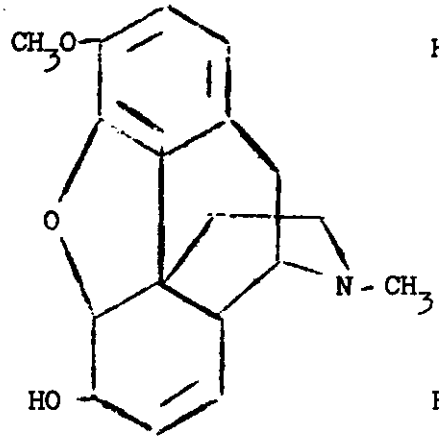


(69)

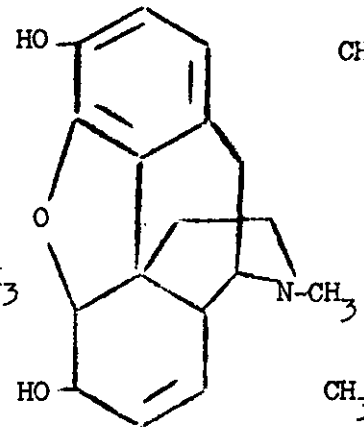
To establish the stereochemical relationship between chlidanthine (55) and galanthamine (53), we decided to compare directly the methyl ether methiodides of chlidanthine and galanthamine. Thus the derivative from both the alkaloids was expected to have the identical physical constants.



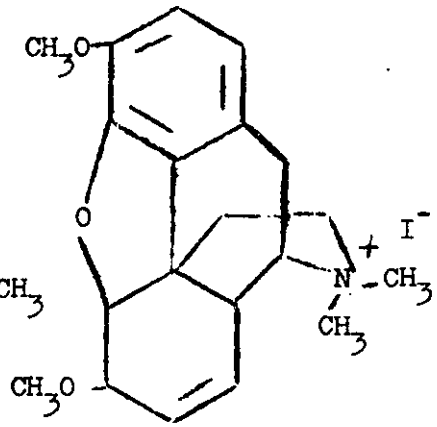
To conserve supplies of starting materials, the reactions were tried on model compounds to standardise the experimental conditions. Codeine (71), morphine (72) and the epimeric alcohols (60) and (61) were chosen for the purpose.



(71)

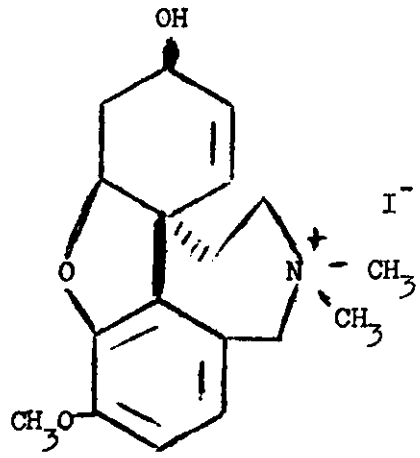


(72)



(73)

The allylic hydroxyl group in codeine (71) was successfully methylated[47] by dimethyl sulphate in presence of alkali. On the addition of saturated potassium iodide solution, the methiodide (73) crystallised out in almost 90% yield. But attempts to methylate galanthamine in a similar manner were frustrated by the insolubility of the methiodide (74), which formed very rapidly and the reluctance of the hydrogen-bonded hydroxyl group to methylate.



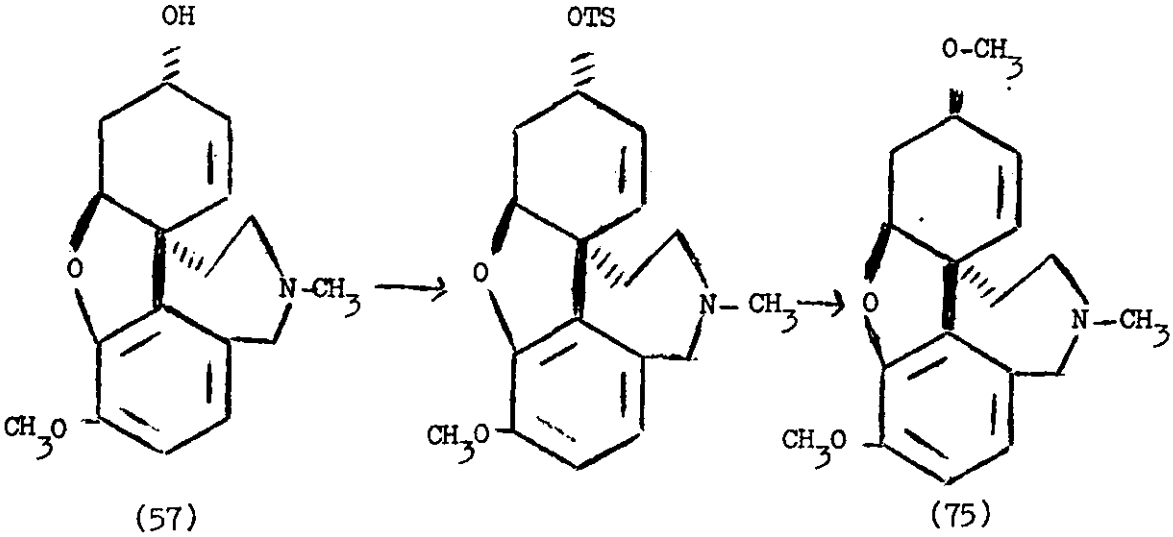
(74)

The methylation of codeine (71) was tried in the presence of strong bases in dimethylformamide or dimethylsulphoxide. The reaction was carried out using sodium hydride or barium oxide or silver oxide, but no well-defined products were isolated.

The alcohols (60) and (61) were successfully methylated in good yield with freshly prepared silver oxide in neat methyl iodide (see above). However attempts to methylate galanthamine under similar experimental conditions failed because of the insolubility of the galanthamine methiodide (74).

To overcome these difficulties in methylating galanthamine, we decided to replace the hydroxyl group stereospecifically. It is known[48] that in the conversion of an optically active alcohol into its tosyl derivative the reaction takes place at the oxygen of the hydroxyl group rather than at the asymmetric carbon atom; hence the configuration about the carbon atom remains unchanged. But the displacement of $^{\ominus}$OTs by $^{\ominus}$OMe or $^{\ominus}$OEt results in the inversion of the configuration (i.e. Walden inversion). Thus it could have been possible to obtain the

methyl ether of galanthamine (75) indirectly from epigalanthamine (57) following the route shown below.



(57) (75)

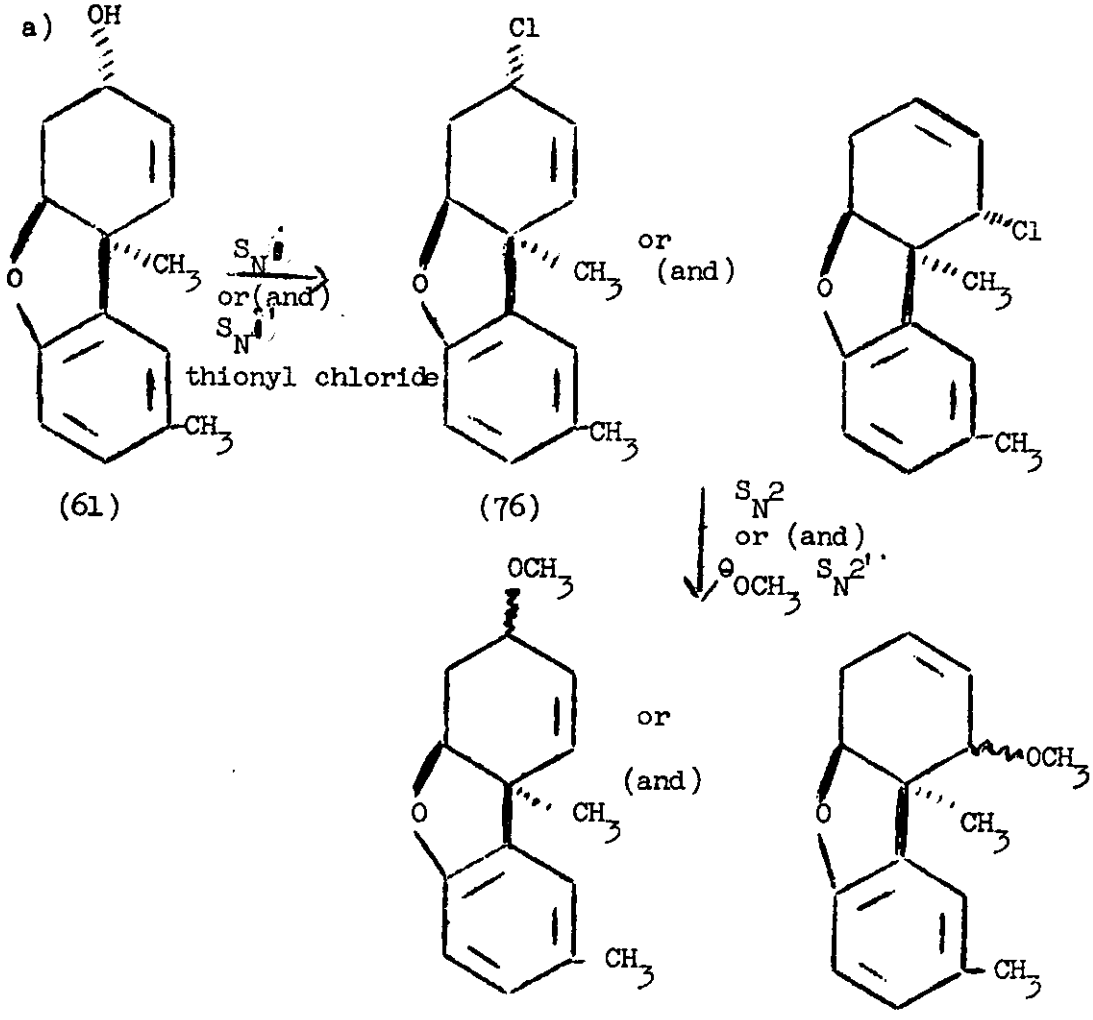
The tosylation experiment was first tried on the model alcohol (61). The alcohol was treated with tosyl chloride at 0° in pyridine for 18 to 20 hours. The reaction mixture was decomposed with crushed ice and the product extracted with chloroform. Examination of oily product showed that the alcohol had reacted completely. However the n.m.r. spectrum indicated the absence of any tosyl derivative. It seemed probable that the product was an allylic chloride or a mixture of allylic chlorides. We therefore decided to prepare various allylic chlorides directly.

One of the more usual methods of converting alcohols into alkyl chlorides is treatment with thionyl chloride, $SOCl_2$. The reaction proceeds through an alkyl chlorosulphite intermediate, which decomposes to the alkyl chloride and sulphur dioxide.[49]

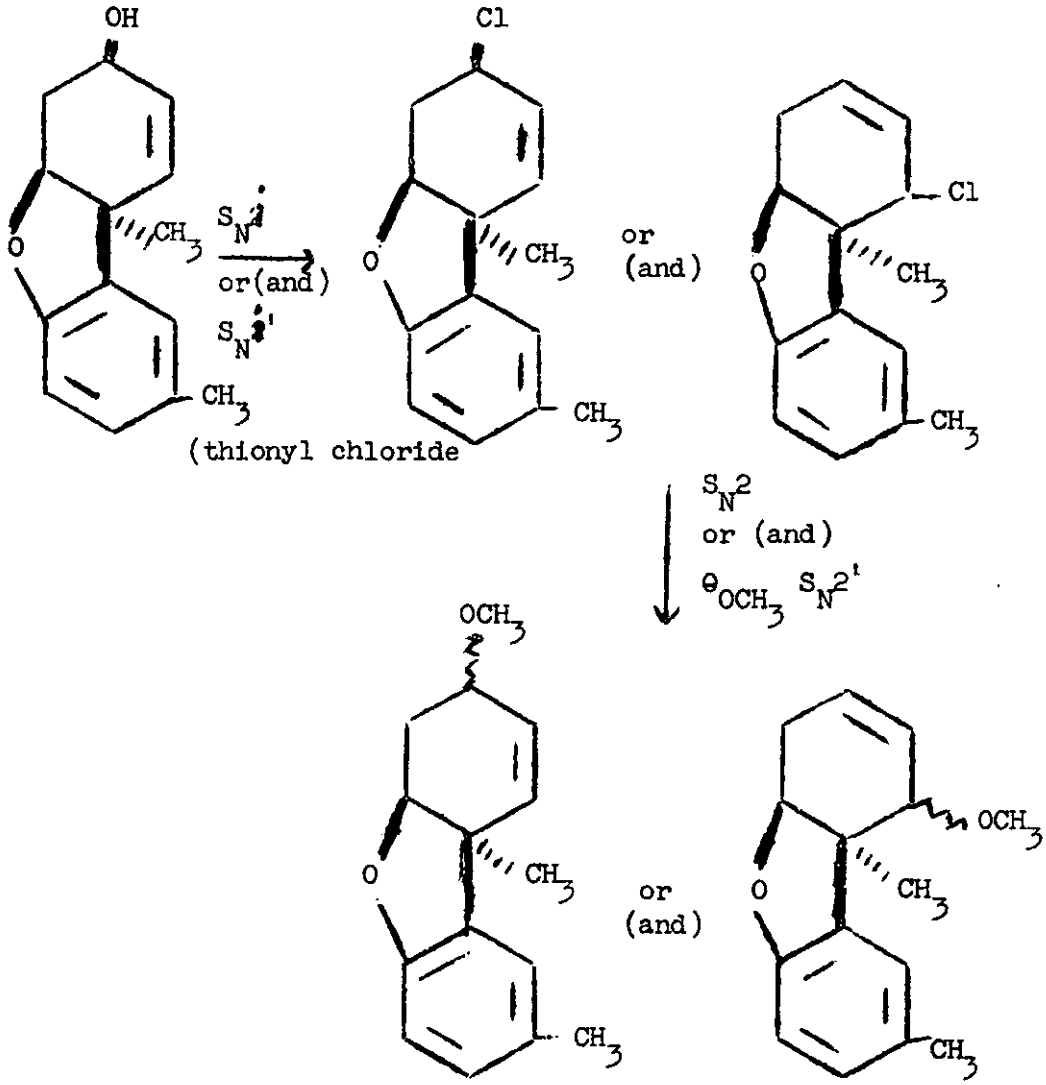
$$R\text{-}OH + SOCl_2 \longrightarrow R\text{-}O\text{-}\overset{\overset{O}{\|}}{S}\text{-}Cl \longrightarrow RCl + SO_2$$

With optically active alcohols, this substitution is almost unique in that it sometimes (but not always) results in the retention of the configuration, S_Ni mechanism), about the α-carbon atom, even though no neighbouring groups are involved. For allylic systems, another mode of reaction is also possible - internal substitution with allylic rearrangement (S_Ni' mechanism). Thus the reactions of thionyl chloride with both alcohols (60) and (61) and the subsequent reactions of the products with methoxide ion were investigated. The expected possible routes were as shown below.

a)

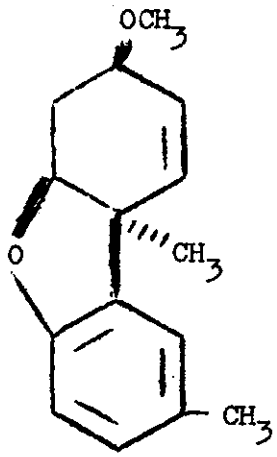


b)

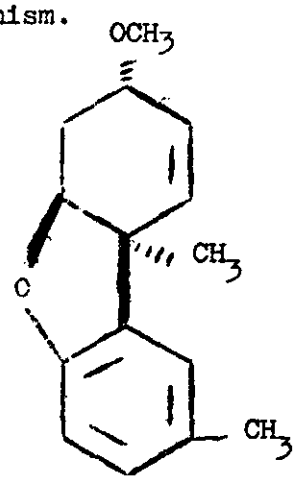


When the model alcohol (61) was treated with thionyl chloride in solvents like benzene or chloroform, the reaction proceeded smoothly at room temperature and was complete in half an hour. The reaction was followed on t.l.c. on alumina GF_{254} plates. The product had R_f 0.8 in the solvent system 10% ethyl acetate-benzene. It also showed only one spot on silica gel plates.

The derivative was easily purified by column chromatography on alumina. The oily product appeared, from its n.m.r. spectrum, to consist solely of the allylic chloride (76), produced with retention of configuration at C-3. The spectrum showed thirteen of the C-4 methylene signals (the fourteenth was obscured by a methyl signal at τ 7.74) characteristic of this configuration (see above and Table 1). Thus the reaction followed totally the S_Ni mechanism. Treatment of the allylic chloride with sodium methoxide in methanol gave the methyl ether (62). The reaction was followed on t.l.c. on silica gel plates. Examination of the crude reaction mixture by n.m.r. spectroscopy showed the presence of a minor (ca. 10%) constituent, probably the epimer (63). The mixture was separated by chromatography. The major methyl ether (62) had n.m.r. and i.r. spectra identical with those of the ether prepared directly from the alcohol (60) by methylation with methyl iodide. The displacement of halogen had therefore occurred, as expected, largely with inversion at C-3. The reaction followed mainly the S_N2 mechanism.

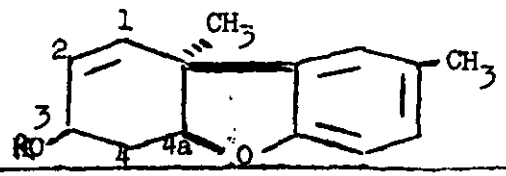


(62)



(63)

TABLE 1



N.m.r. spectra (in $CDCl_3$)

Compound	Proton 1	Proton 2	Protons at position 4		Proton 4a	Aromatic protons	Aromatic CH_3	Alkyl CH_3	OR
R=H Signal Pattern	Doublet	Doublet	axial Doublet-quartet doublet.	equatorial Two triplets	Triplet	Complex multiplet.	Singlet		
τ	4.5	4.23	8.24	7.39	5.4	2.9 to 3.4 7.74		8.58	8.02 disappeared on deuteration.
J	10Hz	10Hz	2.9,13.7, 9.8Hz	4.5, 13.7 Hz	4.3Hz				
R=CH_3 Signal Pattern	Doublet	Doublet .	Doublet-quartet doublet	Two triplets	Triplet	Complex multiplet	Singlet	Singlet	Singlet
τ	4.3	4.15	8.25	7.39	5.35	2.9 to 3.4 7.74		8.58	6.6
J	10Hz	10Hz	2.9,13.7, 9.6	5.1,13.7 Hz	3Hz				
R=$COCH_3$ Signal Pattern	Singlet due to two protons		Doublet, quartet doublet	Two triplets	Triplet	Complex multiplet	Singlet	Singlet	Singlet
τ	4.35		8.12	7.4	5.4	2.9 to 3.4 7.74		8.58	7.92
J			2.8,14.2, 9.5 Hz	4.6, 14.2 Hz	4.2Hz				
OR=Cl Signal Pattern	Doublet	Doublet	Doublet quartet quartet	Two triplets	Triplet	Complex multiplet	Singlet		
τ	4.35	4.1	7.86	7.35	5.35	2.9 to 3.4 7.74		8.58	
J	10Hz	10Hz	3.3,14.2 9.3Hz	5.1,13.9Hz					

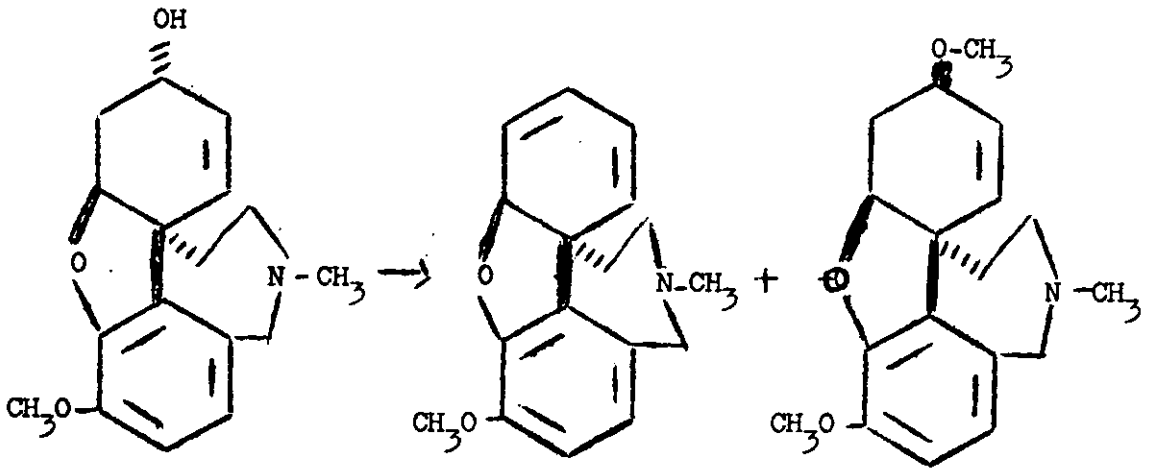
However, before carrying out reactions of this type with alkaloids, it was essential to see whether the presence of base induces any change in the mode of reaction, as the alkaloids themselves are basic in nature. Often, if the reaction is carried out in the presence of base, for example, pyridine, the hydrogen chloride liberated during formation of the chlorosulphite intermediate is converted to Cl^-; this then readily attacks the intermediate to expel $^-O\text{-}SO\text{-}Cl$ with inversion.[50] The reaction then becomes of a normal S_N2 type, proceeding with inversion. However, when the alcohol (61) was treated with thionyl chloride in the presence of pyridine, the usual allylic chloride (76) was obtained in quantitative yield. Thus the presence of base made no difference to the mode of reaction. The same result was obtained in chloroform as well as in benzene.

In contrast when the alcohol (60) was treated with thionyl chloride in dry chloroform, the reaction, which was complete in half an hour (t.l.c. observation), gave a mixture of products. The crude oily product appeared to consist of only one compound by examination on alumina t.l.c. plates, but on silica gel plates, it was found to be a mixture of two compounds with a very small difference in R_f values. The two components were not separable either by column or preparative t.l.c. The presence of two components was confirmed by n.m.r. spectral observations. In the n.m.r. spectrum two singlets due to methyl groups appeared at τ 8.6 and 8.69 with a ratio of intensities ca. 3:2. The major component of the mixture was recognised as the derivative (76),

but the other component was not positively identified. Again the same results were obtained in chloroform or benzene in the presence or absence of pyridine. The formation of the derivative (76) as a major product with the inversion of configuration indicated that the reaction followed the S_N2 mechanism in preference to S_Ni mechanism. Treatment of the reaction mixture with sodium methoxide in methanol gave a mixture of the ethers (62) and (63). The methyl ether (62) was obtained as the major component. The two ethers were separated by preparative t.l.c. on silica gel. They had n.m.r. spectra identical to those of the ethers obtained by direct methylation of the alcohols (60) and (61) with methyl iodide. The unexpected formation of the compound (76) might be explained if attack at C-3 cis to the aryl and oxide ring substituents is slow relative to the competing S_N2 attack trans to these substituents.

These preliminary studies provided us with a route for the indirect methylation of galanthamine. Epigalanthamine (57), the C-3 epimer of galanthamine (53), required for the purpose was prepared easily by epimerisation of galanthamine with hot hydrochloric acid.[25] Epigalanthamine (57) was treated with thionyl chloride in dry chloroform at room temperature. The total product was obtained as the hydrochloride. It was not possible to establish the stereochemical configuration of the product by n.m.r. spectroscopy, owing to high insolubility of the hydrochloride in deuteriochloroform. As the stability of the product was not known, it was not advisable to regenerate

the free base. The total product was, therefore, allowed to react with sodium methoxide in methanol. Chromatographic separation of the reaction mixture gave two compounds. The product eluted first from the column crystallised easily and was assigned, on the basis of u.v., n.m.r., and mass spectra, the constitution (77). The u.v. spectrum showed a band at $\lambda_{max.}$ 261 nm. (ε 4035) which indicated that the compound was a diene. In the n.m.r. spectrum two aromatic protons gave a singlet at τ 3.33. Four vinyl protons gave a multiplet at τ 3.8. The methine proton at position 4a gave a doublet at τ 5.12. The methylene protons near the benzene ring appeared at τ 5.78 and 6.28 as a quartet. The aromatic methoxy and N-methyl groups gave three proton singlets at τ 6.12 and 7.60 respectively. The mass spectrum showed a molecular ion at m/e 269.



)57) (77) (75)

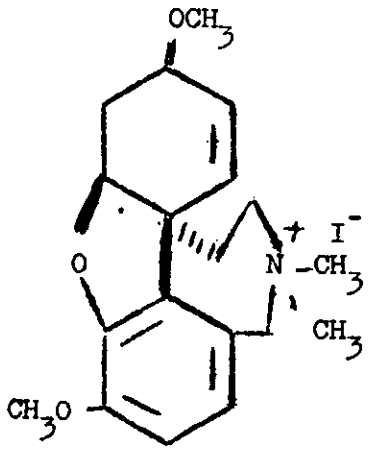
The second compound was obtained as an oil, which appeared (t.l.c.; n.m.r.) homogeneous. It was judged by its n.m.r. spectrum and method of preparation to be galanthamine methyl

ether (75). In the n.m.r. spectrum, the two aromatic protons gave a singlet at τ 3.41. The two olefinic protons at positions 1 and 2 gave an AB quartet (H_1, τ 3.77, J=10 Hz and H_2, τ 4.05, J= 10 H_2 and ca. 4 Hz). The doublet due to proton at position 1 was further split up into a quartet, indicating that the methoxyl group was quasi-axial.[46] The methine proton signal at position 4a appeared as a triplet at τ 5.45 (J=ca. 4Hz). The aromatic methoxyl and N-methyl groups gave sharp three proton singlets at τ 6.2 and 7.65. The singlet due to three protons at τ 6.6 indicated the presence of methoxy group in cyclohexene ring. The n.m.r. spectrum thus indicated the correct stereochemical configuration of galanthamine methyl ether.

When the n.m.r. spectra of galanthamine methyl ether (75) and O-acetylchlidanthine were compared, many common features were observed. The olefinic protons at positions 1 and 2 and the methine proton at position 4a gave similar signals.

The oily methyl ether of galanthamine (75) reacted with methyl iodide in methanol to give, in good yield, a highly crystalline methiodide (70). The compound had m.p. 274° and gave a specific rotation, $[\alpha]_D$-90°.

Morphine (72) was chosen as a model compound to standardise the experimental conditions for methylation of chlidanthine. It was successfully methylated[47] by dimethyl sulphate in the presence of alkali. After the addition of the saturated potassium iodide, the methiodide (73) crystallised out and was obtained in 90% yield. In a similar manner chlidanthine (55) was successfully methylated . The methiodide (70) had m.p. 274° and specific rotation, $[\alpha]_D$- 87°.



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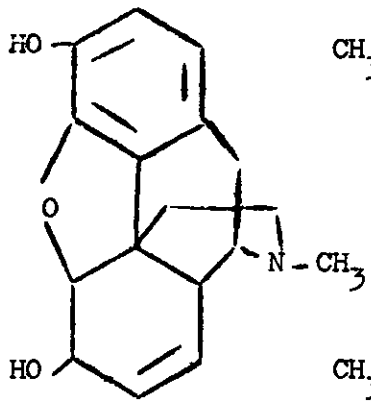

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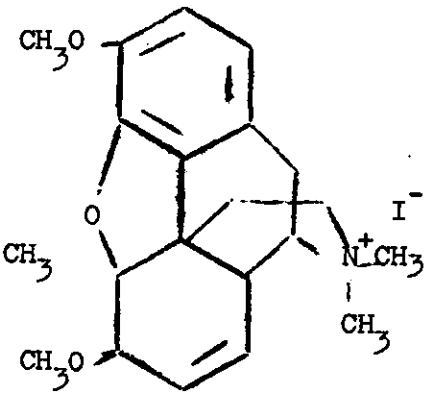


(73)

The methiodides obtained from the alkaloids galanthamine and chlidanthine had identical i.r. spectra (KBr disc). There was no depression of the m.p. of the mixture. Therefore chlidanthine has the relative and absolute configuration (55).

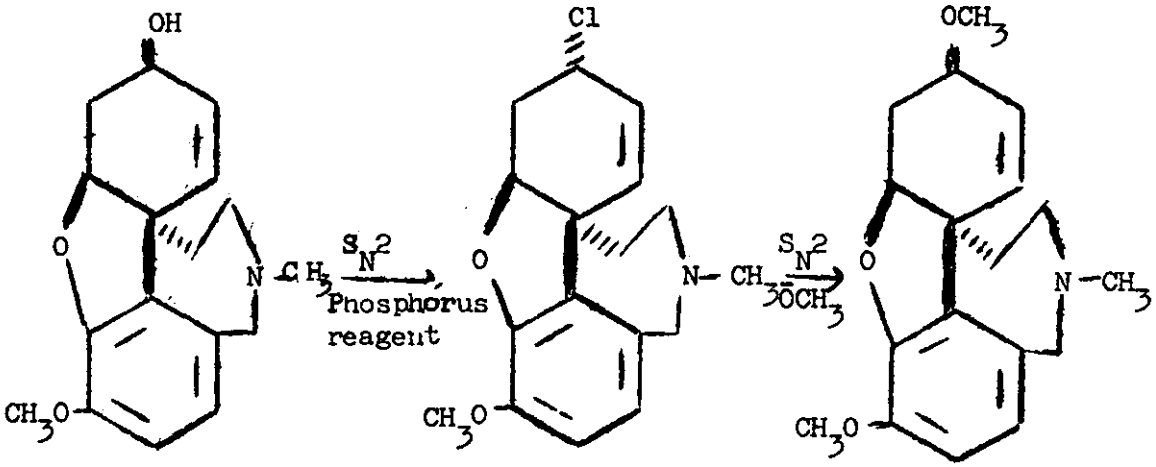
Recently it has been shown [51, 52, 53] that the formation of primary and secondary alkyl halides takes place in good yield by the interaction of tertiary phosphines with carbon tetrahalides in the presence of the corresponding alcohols. The reaction is of quite general scope and has the advantages of proceeding under mild conditions to give neutral by-products. Trisdimethyl and carbon tetrachloride are known[54] to convert alcohols into the corresponding chloro-compounds with inversion of configuration. The following mechanism was suggested

$$(Me_2N)_3P: + Cl\text{-}CCl_3 \xrightarrow{\text{fast}} (Me_2N)_3\overset{+}{P}\text{-}Cl\ \bar{C}Cl_3$$

$$(Me_2N)_3\overset{+}{P}\text{-}Cl\ \bar{C}Cl_3 + R\text{-}OH \xrightarrow{\text{fast}} (Me_2N)_3\overset{+}{P}\text{-}O\text{-}R\ Cl^- + CHCl_3$$

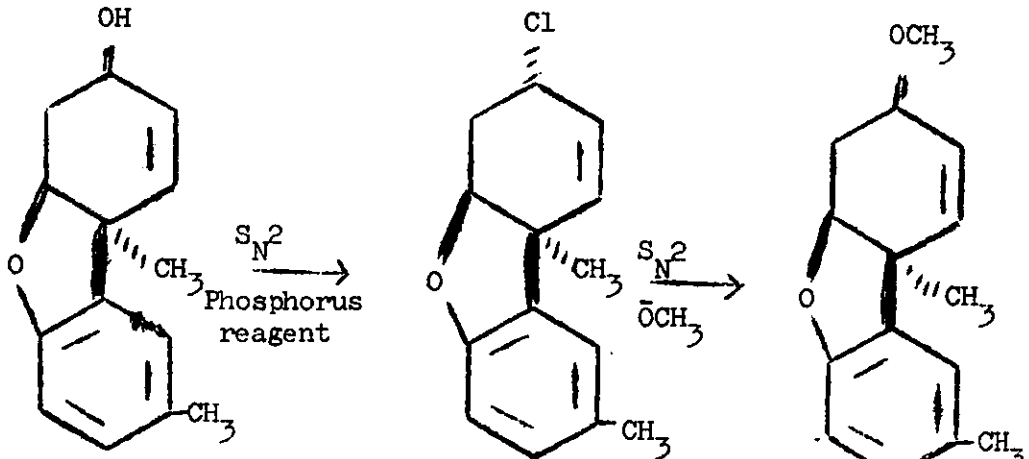
$$(Me_2N)_3\overset{+}{P}\text{-}O\text{-}R\ Cl^- \xrightarrow{\text{slow}} (Me_2N)_3P{=}O + R\text{-}Cl$$

We decided to examine the effect of this reagent on our model alcohols (60) and (61). The methyl ether (75) could in principle be obtained from galanthamine (53) following the route shown below and the necessity of epimerising galanthamine to epigalanthamine could then be avoided.



(53) (75)

The alcohol (60) was accordingly treated with phosphorus reagent in a mixture of carbon tetrachloride and chloroform at -20° and the reaction mixture allowed to warm up to room temperature. The resulting product was purified by chromatography and identified as allylic chloride (76) by n.m.r. spectra comparison with the material obtained from the thionyl chloride reaction on the alcohol (61). As expected, the reaction had followed the S_N2 mechanism with inversion of configuration. Treatment of the product with sodium methoxide in methanol gave the methyl ether (62) with a second inversion.



(60) (76) (62)

When the alcohol (61) was treated with the phosphorus reagent, a mixture of chloro-compounds was produced. One component of the mixture was recognised (n.m.r.) as the derivative (76), but the other components could not be identified. However, when the total mixture was treated with sodium methoxide in methanol, a mixture of the two methyl ethers (62) and (63) was obtained. They were separated by preparative t.l.c.: the ether (62) was the major product. Again, it appears that attack cis to the aryl and oxide bridge substituents is difficult and clean inversion is not observed. However it is known[54] that cleavage of the intermediate phosphorus derivative is slow and might well be sensitive to steric interference. The product (76) might arise by S_Ni decomposition of a covalent phosphorus intermediate or by a pair of consecutive S_N2' reactions or by S_N1 process.

This reaction was then carried out with galanthamine, but

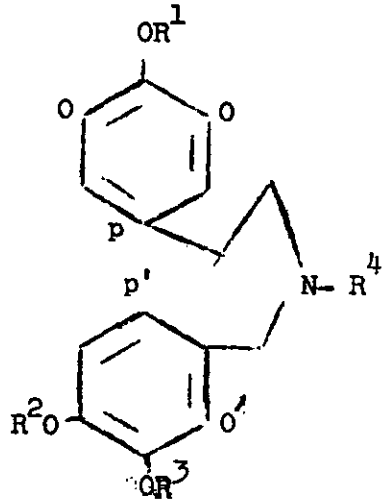
without success. From t.l.c., the crude product obtained appeared to be a mixture of several compounds, which were difficult to separate by chromatography.

CHAPTER 4

BIOSYNTHESIS OF CHLIDANTHINE

The alkaloids of the Amaryllidaceae family are very closely related biogenetically, though they differ widely in structure. All these alkaloids have in common a hydroaromatic C_6-C_2 unit. Two different biogenetic theories have been proposed to explain the biogenesis of the Amaryllidaceae alkaloids.

The biogenetic theory proposed by Barton and Cohen[14] is now the accepted one. They proposed that many naturally occurring compounds might be formed from phenols. Oxidation of the phenols would produce phenol radicals, which are relatively stable due to the spread of the odd electron over the ortho and para positions of the aromatic ring. The alkaloids are formed by oxidative coupling of two phenolic rings of the precursor of general nature (78) where R=H or some suitable group, e.g. alkyl or a part of the enzyme surface. The alkaloids are formed by ortho-para, para-ortho and para-para intramolecular carbon-carbon coupling reactions.



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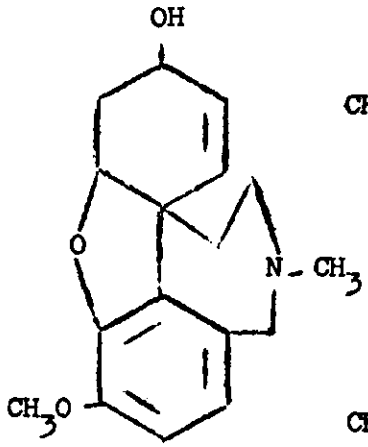

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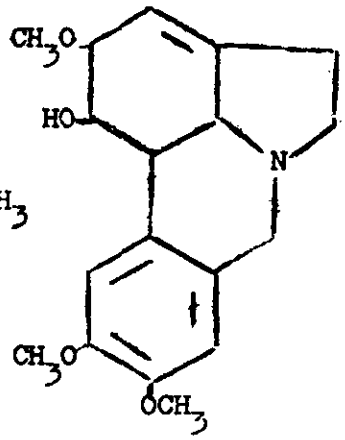

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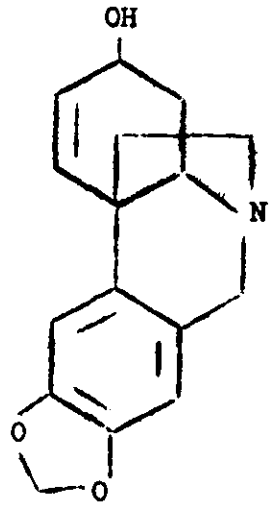
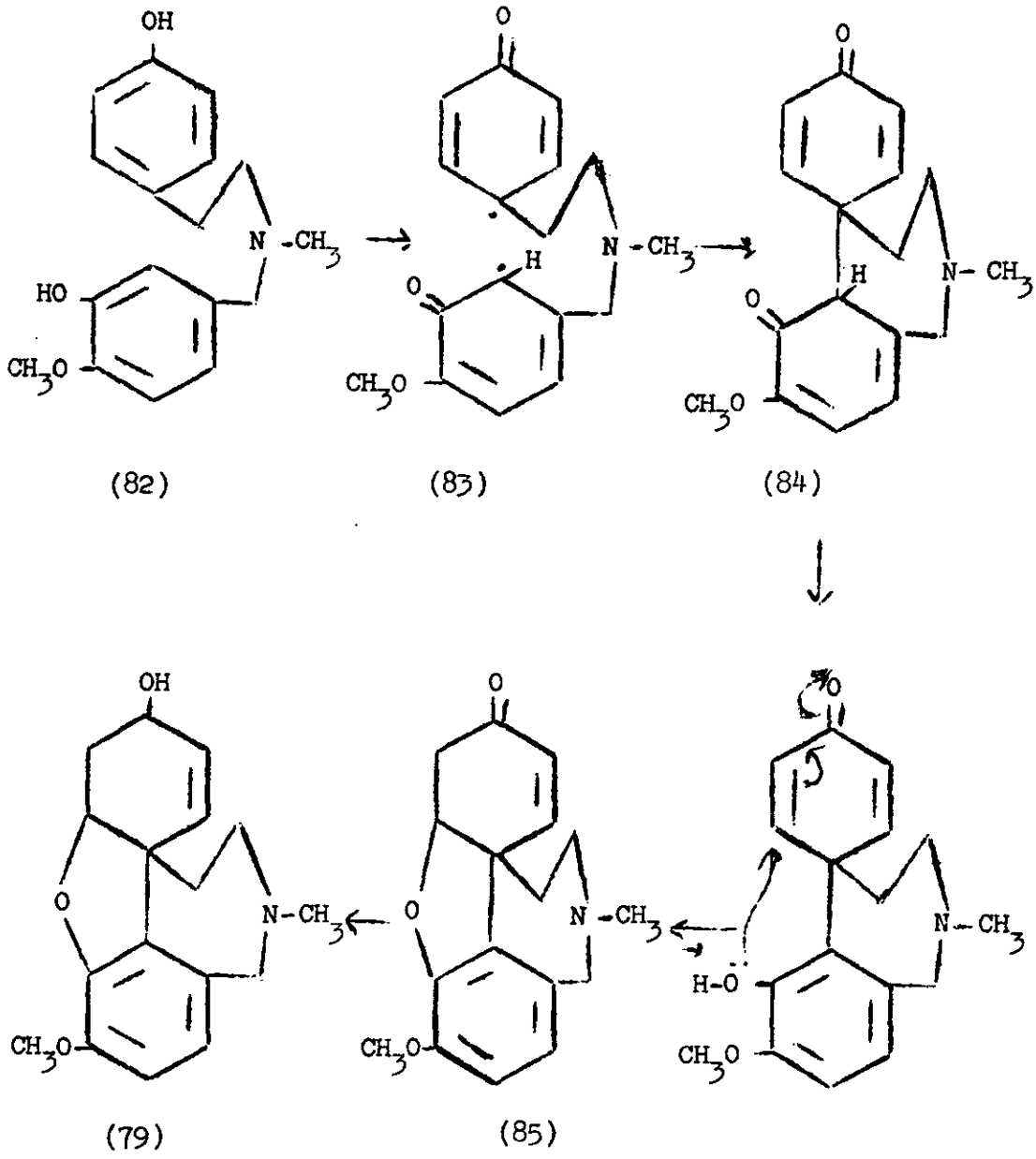
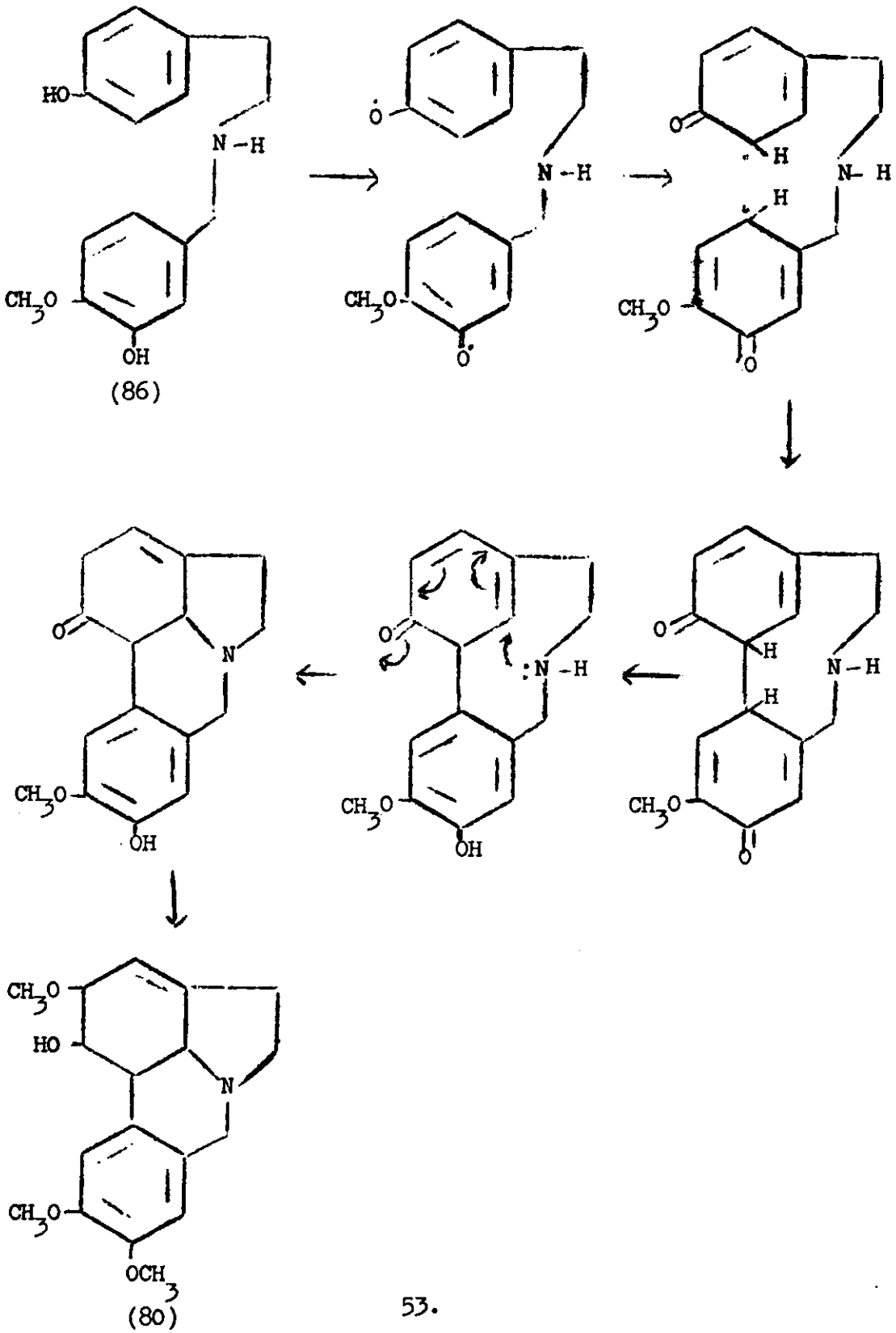


(81)

Thus the alkaloids, galanthamine (79), galanthine (80) and crinine (81), which represent three main groups of Amaryllidaceae alkaloids, can be derived by o'-p, p'-o, and p'-p coupling reactions. The alkaloid galanthamine (79)can hypothetically be derivable from the phenol (82) by ortho-para oxidative coupling. Oxidation of phenol (82) would give the diradical (83), which on radical pairing would give the dienone system (84). Aromatisation of the bottom ring in the conventional manner followed by a Michael type addition of the phenolic oxygen to the dienone would form narwedine (85). The reduction of this would then give galanthamine (79).

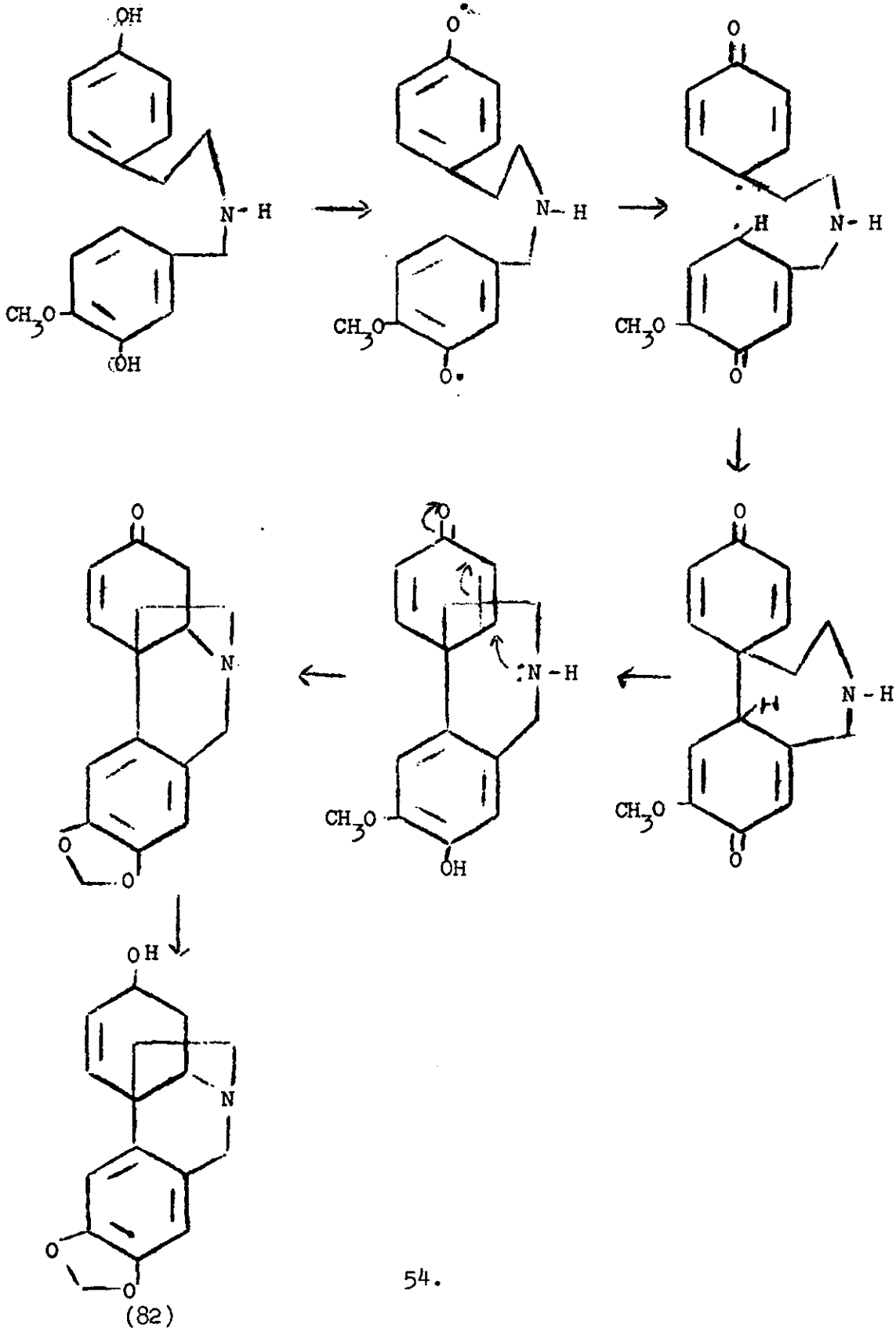


A similar sequence of reactions by <u>para-ortho</u> coupling of the precursor (86) would produce galanthine (80) and <u>para-para</u> coupling would produce crinine (81).



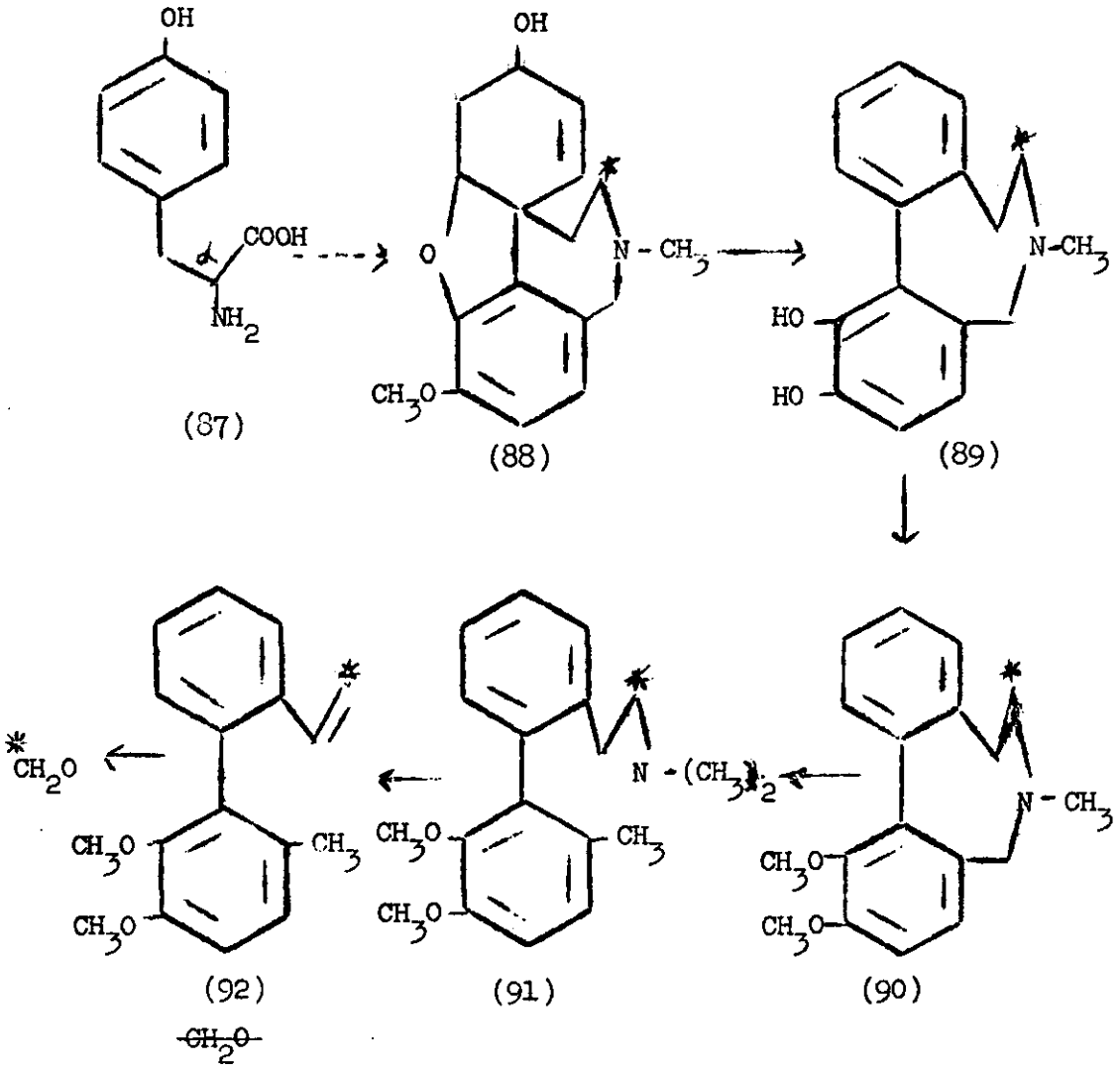
(86)

(80)



(82)

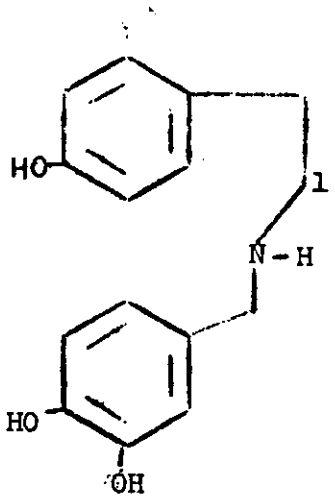
Wenkert[55] suggested a fundamentally different route, from prephenic acid and shikimic acid, which would build up the tetra-cyclic alkaloidal system by strain-free cyclisations and which would not involve aromatic precursors at any stage. Successful feeding experiments with aromatic precursors now clearly disproved Wenkert's proposals. The chemical feasibility of Barton and Cohen's scheme was confirmed by Barton and Kirby,[19,56] (see chapter 1, page 10). Also it has now been well established by several groups of workers,[56 to 63] that tyrosine (87) provides the C_6-C_2 unit for the major Amaryllidaceae alkaloids. Biosynthesis of galanthamine was carefully studied by Barton and his co-workers. In early experiments, Barton and Kirby[56] injected [α-^{14}C]tyrosine (87) into the snowdrop Galanthus elwesii. Galanthamine isolated from these plants was radioactive and incorporation (0.14%) was observed. However, they did not carry out further degradations to prove the labelling pattern in galanthamine. Later, Barton and his co-workers[57] obtained radioactive galanthamine (88)/(0.012% incorporation) when [α-^{14}C]tyrosine (87) was injected into 'King Alfred' daffodils. The degradation of radioactive galanthamine (88) was carried out by a method based on the work of Kobayashi and Uyeo[5] to establish the labelling pattern in the alkaloid. It gave the results outlined below.



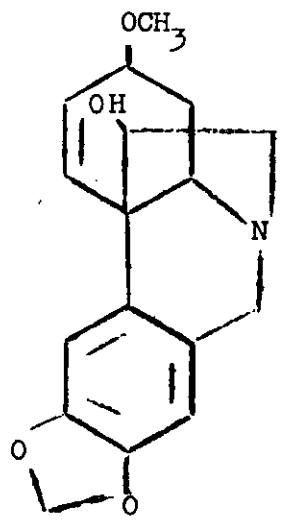
When radioactive galanthamine (88) was refluxed with hydrobromic acid, it gave apogalanthamine (89), which was methylated to give the dimethyl ether of apogalanthamine (90). Emde degradation of the corresponding methochloride gave the base (91), which was further degraded by Hofmann elimination to give the vinyl diphenyl (92), which contained all the activity of the original alkaloid. Ozonolysis of (92) gave formaldehyde, isolated as its dimedone derivative, containing all the activity. It was thus shown that [α-^{14}C]tyrosine was

incorporated into galanthamine and it served as a precursor for the C_6-C_2 unit.

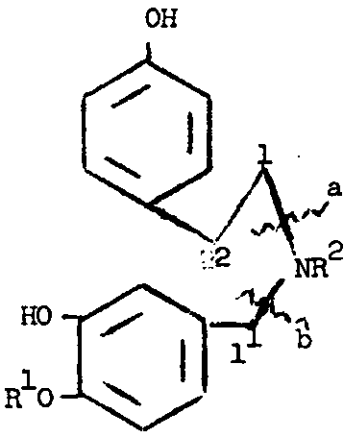
According to Barton and Cohen's proposition[14] the key step in the biogenesis of the Amaryllidaceae alkaloids is the oxidative coupling of the aromatic intermediates such as the diphenol, norbelladine (93). In order to prove this, Barton and his co-workers[57,64] fed [1-^{14}C]norbelladine (93) to 'King Alfred' daffodils and observed incorporation into galanthamine (79), galanthine (80) and haemanthamine (94) with incorporations of 0.014, 0.003 and 0.25% respectively. Degradation of the radioactive galanthamine by the method described earlier proved the position of the label. They also prepared likely precursor diphenols (95) singly labelled with ^{14}C either in their N-methyl group where applicable or at position 1 and fed them to mature 'King Alfred' daffodils.



(93)



(94)



(95a); $R^1=R^2=CH_3$

(95b); $R^1=H$, $R^2=CH_3$

(95c); $R^1=CH_3$, $R^2=H$

(95d); $R^1=R^2=H$

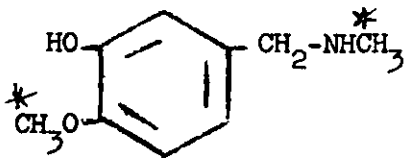
They observed that of the four biogenetically likely precursor diphenols, three (95a), (95b), and (95d) were incorporated into galanthamine with an efficiency similar to that observed with tyrosine. However, the O-methylnorbelladine (95c) did not serve as a precursor for galanthamine and no incorporation was observed even in later experiments. Thus it was possible for them to suggest a definite order of methylation in the biogenesis of galanthamine. The possible biosynthetic route suggested was: norbelladine (95d)— N-methylnorbelladine (95b)— NO-dimethylnorbelladine (95a)— galanthamine (79).

The results obtained did not establish that precursors, e.g. NO-dimethylnorbelladine (95a), are not cleaved at bonds a or b or a and b, before conversion into the 'true' precursors of the alkaloids. To clarify this point Barton and his coworkers[57] carried out feeding experiments with multiply labelled

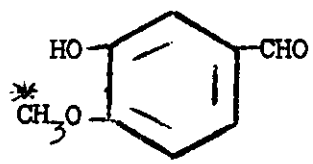
precursors. The diphenol (95a) was labelled in the N- and O-methyl groups and fed to 'King Alfred' daffodils in the usual way. The derived radioactive galanthamine was demethylated to give the separate activities for the N- and O-methyl groups. The ratio of activities was found to be the same as in the precursor. This result was confirmed by carrying out oxidation of radioactive galanthamine with manganese dioxide to give narwedine, which was also demethylated. Thus the possibility of cleavage of bond 'b' was excluded. In a similar way the triply labelled precursor (95a) with labels in the O-methyl, N-methyl groups and at position 1, was used to exclude cleavage of bonds a and b and to show that partial O- and N-dimethylation did not take place.

Though it has now been established that tyrosine provides the C_6-C_2 unit for the major Amaryllidaceae alkaloids, less is known about the origin of the C_6-C_1 unit. To study this problem Barton and his co-workers[57] fed doubly labelled N-methylbenzylamine (96) to 'King Alfred' daffodils. Radioactive galanthamine was isolated (0.019% incorporation), all the activity being located in the O-methyl group. This suggested that degradation of the amine to either 3-hydroxy-4-methoxy-benzylamine or, more probably, isovanilline (97) was responsible for incorporation. However, feeding experiments with O-methyl-labelled isovanillin were frustrated by lack of absorption of this substance by the plant. But Suhadolnik, Fischer and Zulalian[60] observed earlier incorporation of tritiated protocatechualdehyde into lycorine, although the distribution of radioactivity in the alkaloid remained to be determined.

The work done by Barton and his co-workers[57] established two separate routes leading from aromatic amino acids to the alkaloids. The first, involving step-wise methylation of norbelladine, has already been described. The second proceeds from an aromatic unit bearing a methoxyl group, e.g. isovanillin (97). For galanthamine biosynthesis, it must not involve the intermediate formation of the O-methylnorbelladine (95c). One possible intermediate could be aldimine (98). Methylation, decarboxylation, and reduction of this aldimine could then give NO-dimethylnorbelladine (95a) and hence galanthamine.



(96)



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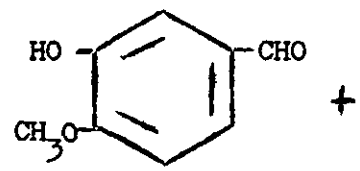

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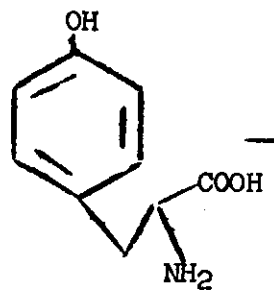

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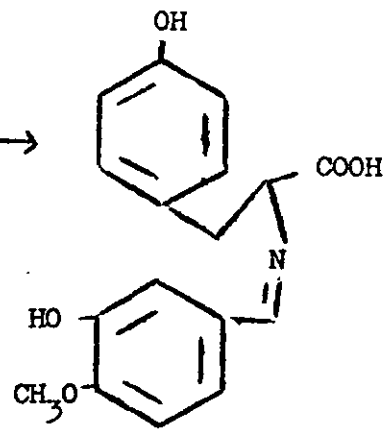
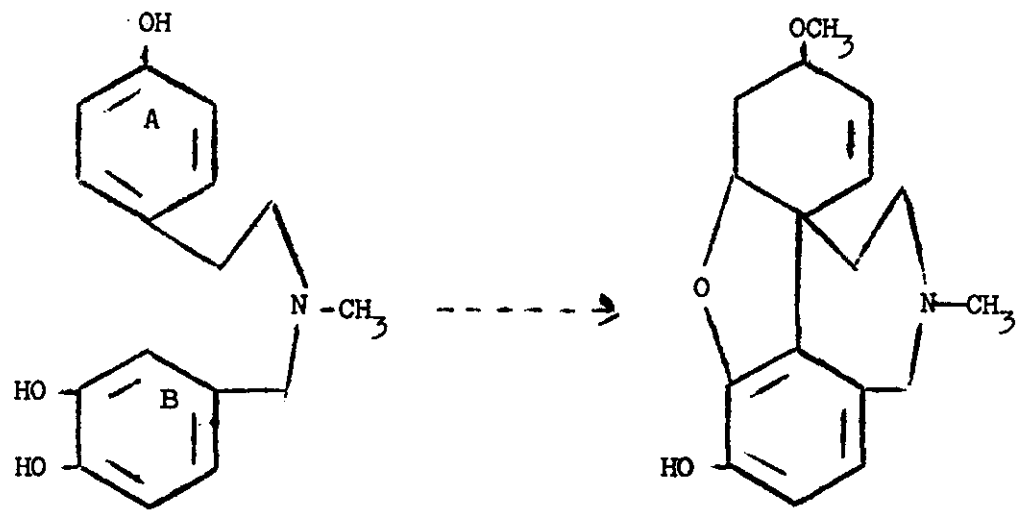


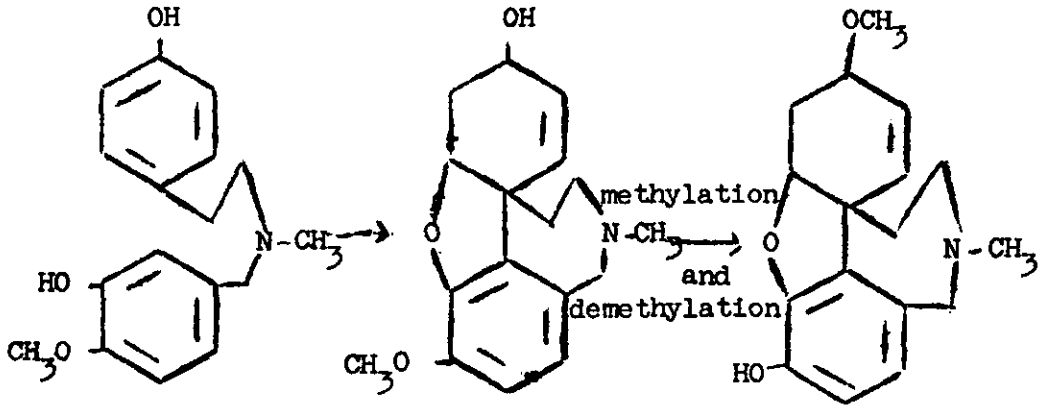
(98)

The alkaloid chlidanthine (100) has a structure and stereochemistry analogous to that of galanthamine, differing only in the positions of methoxyl groups (see Chapter 3). We decided to investigate the biosynthesis of chlidanthine. It could involve cyclisation of a derivative of the type (99) with two hydroxyl groups on ring B, thus by-passing the route to galanthamine. Alternatively, chlidanthine could be derived from galanthamine by methylation and demethylation (not necessarily in this order). Thus the possible two routes are as shown below.

1.

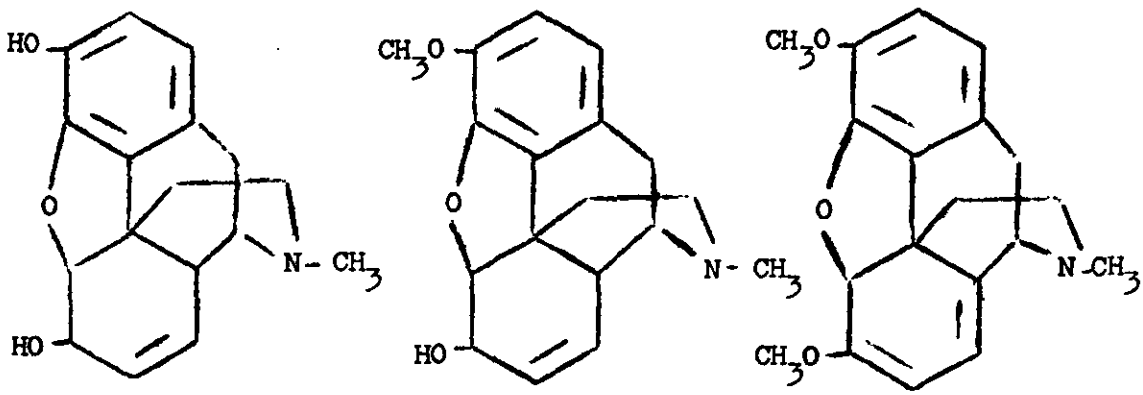


(99) (100)



(95a) (79) (100)

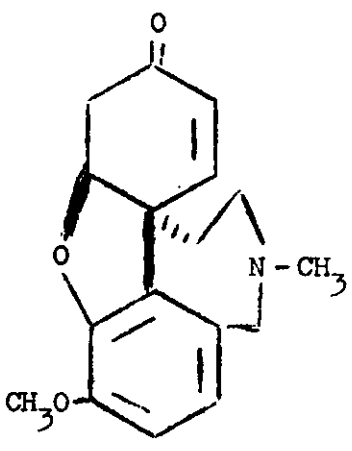
Late stage demethylation was observed in the conversion of codeine (102) into morphine (101).[65,66] Battersby and Harper[65] fed [α-^{14}C]tyrosine to mature poppy plants. The plants were worked up after set times. They observed rapid incorporation of the activity into thebaine (103) followed by a rise in the activity in codeine (102) and a steady fall in the activities of codeine (102) and thebaine (103) relative to that of morphine (101). They interpreted their results by the assumption of a biosynthetic pathway running from tyrosine⟶ thebaine (103)⟶ codeine (102)⟶ morphine (101).



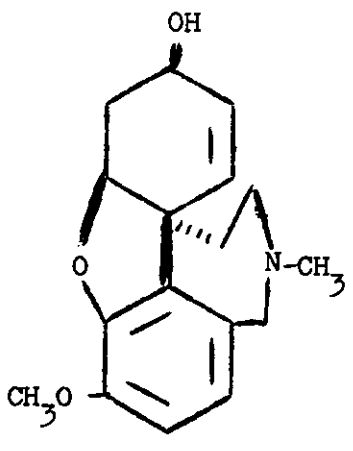
(101) (102) (103)

This work was further supported by Rapaport and Stermitz.[66] They carried out feeding experiments with Papaver somniferum using radioactive thebaine (103), codeine (102), and morphine (101), in which it was found that thebaine was converted into codeine, codeine was converted into morphine and no other conversions took place within this group. They also observed that in Papaver orientale, radioactive thebaine was incorporated into oripavine. This established thebaine as the precursor of other hydrophenanthrene alkaloids and O-demethylation as an important biosynthetic pathway.

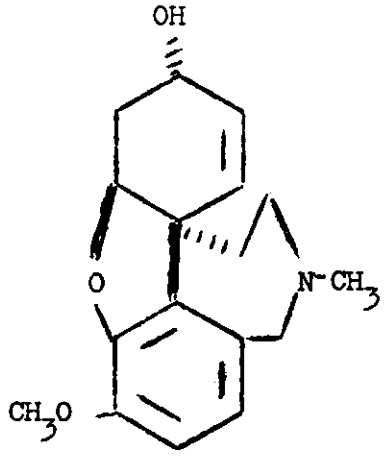
The second route, therefore, appeared to us an attractive possibility in the biosynthesis of chlidanthine. To test the possibility of second route and to find confirmation of the stereochemistry of chlidanthine, we decided to carry out biosynthetic experiments with Chlidanthus fragrans plants. Narwedine (104), galanthamine (105), or epigalanthamine (106) were the likely precursors.



(104)



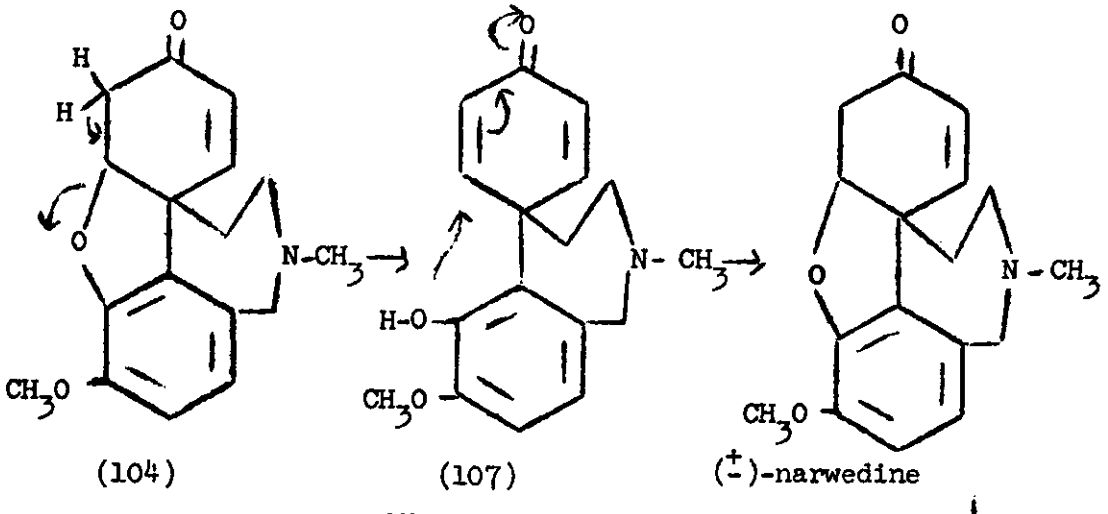
(105)

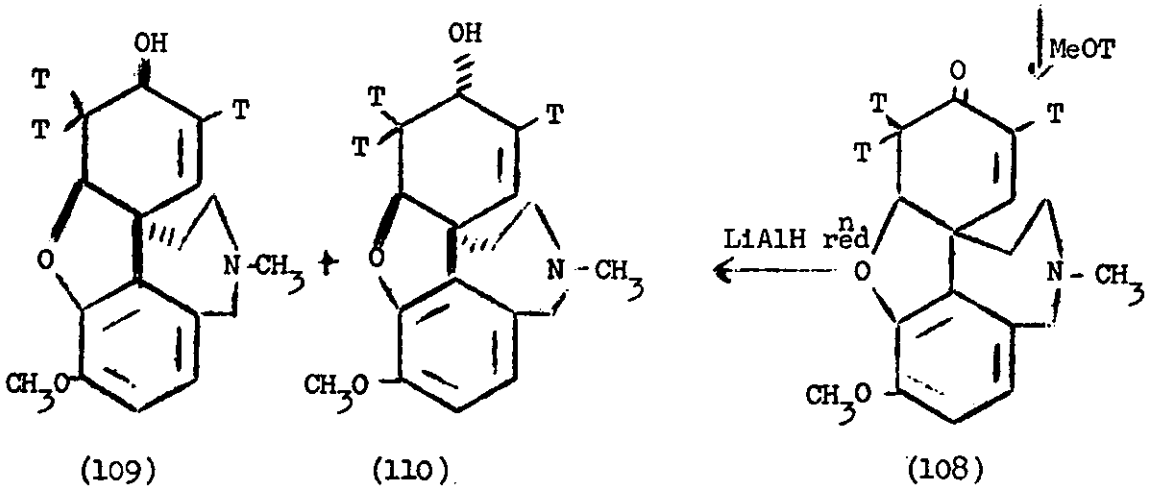


(106)

Biosynthetic Experiments:

Narwedine (104) is known[19] to racemise in hydroxylic solvents. In polar solvents, the oxide ring opens up with the loss of one of the methylene protons. The alkaloid itself is sufficiently basic to achieve this ring opening. The resulting dienone (107) can then reclose to regenerate (±)-narwedine, by the addition of the phenolic oxygen to either of the double bond. As a result of this methylene protons at position 4 and proton at position 2 are exchangeable in hydroxylic solvents. In accordance with this (±)-[^{3}H]narwedine (108) was prepared in tritiated methanol and reduced[19] to give (±)-[^{3}H]galanthamine (109)and (±)-[^{3}H]epigalanthamine (110).





Aqueous solutions of precursors (pH ca. 6) were fed to vigorously growing Chlidanthus fragrans plants in early summer, through wicks of untreated cotton passed through the fleshy leaves near soil level. Two sets of experiments were performed, the first allowing 2 days and the second 7 days for metabolism. Precursor solutions were taken up by the plants in 2 days. Plants were worked up in usual manner. The total bases were separated preparatively on Alumina PF_{254} plates using the solvent system ethyl acetate-methanol (95:5). From each experiment different alkaloid fractions for narwedine, galanthamine, epigalanthamine lycorine, and chlidanthine were isolated. To effect further purification of chlidanthine fraction, it was mixed with inactive precursor and the mixture separated by a second chromatographic run. Final purification was achieved by crystallisation and the radiochemical purity checked using the derived methyl ether methiodide.

In both the experiments, incorporation of galanthamine but not of epigalanthamine into chlidanthine (see Table 2) was observed, supporting the proposed relative stereochemistry for the latter alkaloid. Additionally, narwedine was efficiently

TABLE 2

Incorporations (%) of Precursors into Chlidanthine

Experiment		(±)-Galanthamine	(±)-Epigalanthamine	(±)-Narwedine
1.	2 days	1.08	0.005	-
2.	7 days	0.081	0.009	0.069

converted (7 day experiment) into galanthamine (7.7% incorporation). Thus demonstrating the long suspected[14] precursor-product relationship of these two alkaloids.

No suitable degradative sequence was available for the location of tritium in small quantities of labelled chlidanthine. However, useful evidence that biosynthesis in Chlidanthus fragrans had occurred without "scrambling" of tritium was obtained by examination of radioactive galanthamine obtained from the plants, to which (±)-[^{3}H]narwedine was fed. Oxidation[19] of the galanthamine (relative molar activity 1.00) with chromic acid was carried out. Chromatography of the oxidation product on alumina gave the radioactive keto-aldehyde and inactive (±)-narwedine. After crystallisation from methanol (±)-narwedine showed relative molar activity 0.01. The tritium therefore must have resided at C-2, C-4 or C-3, location at the latter position being however unlikely. The keto-aldehyde, which was isolated as a by-product of this oxidation, was crystallised from methanol. It showed relative molar activity 0.32 after one crystallisation. The partial retention of tritium in this material supports the idea that the keto-aldehyde is formed partly racemic from oxidation of galanthamine (see chapter 2).

CHAPTER 5

EXPERIMENTAL

Melting points were taken on a Kofler hot-stage apparatus. I.r. spectra were run on Perkin Elmer 237 and 257. U.v.spectra were taken on Unicam SP 800 instrument. N.m.r. spectra were run at 60 MHz on Perkin Elmer R10 spectrometer. Optical rotation was measured on Bendix NPL automatic polarimeter. Mass spectra were measured with A.E.I. MS9 and MS 12 spectrometers, with an ionising potential of 70 ev, by direct insertion of samples. Accurate mass measurements were obtained from Physico-Chemical Measurements Unit (Aldermaston). Thin layer chromatography (t.l.c.) was carried out on alumina GF_{254} and silica gel GF_{254}, 0.25 mm. thickness. These were prepared by recommended procedures and dried at 120° for 8 hr. T.l.c. observations were made in common under u.v. lamp. However, for qualitative t.l.c. Dragondroff reagent was used as indicator for detection of alkaloids and iodine for Pummerer's ketone series.

Isolation of (-)-galanthamine from 'King Alfred' daffodils. (General procedure of Fales, Giuffrida and Wildman.[12])

'King Alfred' daffodils (resting bulbs, 400 g.) were macerated and extracted with 1% ethanolic tartaric acid (2x250 ml.). The extract was filtered and the filtrate evaporated to a small bulk (75 ml.), diluted with water (150 ml.) and acidified with 2N-hydrochloric acid (20 ml.). The acid solution was washed with chloroform (4 x 30 ml.), and the washings back extracted with 2N hydrochloric acid (4 x 20 ml.). The combined acid extracts were made alkaline with aqueous sodium hydrogen carbonate and extracted with chloroform (8 x 20 ml.). The chloroform extract was dried over anhydrous sodium sulphate

and the solvent removed under vacuum.

The crude extract (2.058g.) was chromatographed on grade III neutral alumina (80 g.). Elution with ethyl acetate-benzene (1:9) gave fractions rich in galanthamine. However, it was accompanied by another alkaloid galanthine. Repeated chromatography proved unsatisfactory to separate galanthamine from galanthine. The separation was finally achieved by using a gradient elution technique of increasing percentage of ethyl acetate in benzene. The elution of the alkaloids was followed in the u.v. at 280 nm. and on t.l.c. using alumina GF_{254} plates developed in the ethyl acetate-methanol (95:5) solvent system. Galanthamine containing fractions were all combined. Galanthamine (0.095g.) crystallised from ether in needles and gave m.p. 129°-30°. The alkaloids galanthine and haemanthamine were also isolated.

A different variety of daffodils 'Double Narcissi Inglescombe' was also used for isolation of galanthamine using the above procedure. However, in this case galanthamine was accompanied by another alkaloid pluviine. The separation was achieved by preparative t.l.c. on alumina PF_{254}. Final purification of galanthamine was done by crystallisation from ether. From 'Inglescombe' variety of daffodils galanthamine, galanthine and pluviine were obtained, galanthine being the major alkaloid. Haemanthamine was obtained in small amounts.

Oxidation of (-)-galanthamine.

(a) (-)-Galanthamine (50 mg.) in dry ethanol-free chloroform (15 ml.) was shaken with activated manganese dioxide[38] (500 mg.)

for 2 hr. at room temperature. The reaction mixture was chromatographed on grade III neutral alumina (10g.). Elution with ethyl acetate-benzene (1:9) gave the keto-aldehyde (48) (7mg.) while ethyl acetate-benzene (1:4) gave (±)-narwedine (21) (30mg.).

(b) To a solution of (-)-galanthamine (47 mg.) in acetone (15 ml.) containing water (3 ml.) was added 6N-sulphuric acid (0.1 ml.) followed by chromic oxide (12 mg.) in acetone (3 ml.). The mixture was left overnight and next day the solvent evaporated. The residue was dissolved in water, treated with a slight excess of sodium hydrogen carbonate and extracted with chloroform. The extract was evaporated and the residue was chromatographed on grade III neutral alumina (10g.). Elution with ethyl acetate-benzene (1:9) gave the keto-aldehyde (48) (10 mg.) while ethyl acetate-benzene (1:4) gave (±)-narwedine (21) (26 mg.). Chromtaography was followed on t.l.c. (alumina GF_{254}) using solvent system ethyl acetate-methanol (95:5). Typical R_f values for galanthamine, narwedine and keto-aldehyde were 0.46, 0.49 and 0.74 respectively.

The latter method was preferred for large scale oxidations. Chromic acid oxidation of (-)-galanthamine (0.50 g.) gave the keto a-dehyde (48) (65 mg.) and (±)-narwedine (21) (0.30 g.). The keto-aldehyde was crystallised from ether or methanol as needles, m.p. 153-160° which gave specific rotation $[\alpha]_D$-168° (c 0.12 in MeOH). Repeated crystallisation from methanol gave material with m.p. 190-200° with specific rotation $[\alpha]_D$-263° (c 0.31 in MeOH).

Spectral data for the keto-aldehyde:

Mass spectrum: M^+ at m/e 301.129

($C_{17}H_{19}NO_4$ requires m/e 301.131.)

U.v. spectrum: λ_{max}^{MeOH} 238, 279, 322 nm.

(ϵ 11600, 10400, 6500).

I.r. spectrum: $\nu_{max}^{CHCl_3}$ 1720, 1690 $cm.^{-1}$

N.m.r. spectrum: ($CDCl_3$)

τ 0.0	singlet	1H		'CHO'
2.53, 3.07	quartet	2H	8.7 Hz	aryl protons
5.23	distorted triplet	1H	line separations 2.8 and 3.1 Hz	methine proton at position 4a
6.02	singlet	3H		methoxy group
7.75	singlet	3H		N-methyl group

Analysis:

Found C, 67.8; H, 6.35; N, 4.45

$C_{17}N_{19}NO_4$ requires C, 67.7; H, 6.3; N, 4.6%.

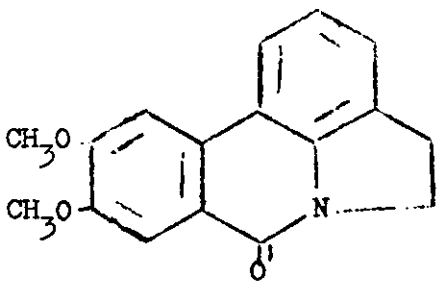
Oxidation of (±)-narwedine.

To a solution of (±)-narwedine (30 mg.) in dry ethanol-free chloroform (15 ml.) was added activated manganese dioxide.[38] The mixture was shaken for 2 hr. at room temperature. After filtration of manganese dioxide, the filtrate was evaporated. The residue showed two spots with R_f 0.74 and 0.49 on t.l.c. on alumina GF_{254} plates developed in ethyl acetate-methanol (95:5) corresponding to the keto-aldehyde and narwedine respectively. It was chromatographed on grade III neutral

alumina (10g.). Elution with ethyl acetate-benzene (1:9) gave the keto-aldehyde (48) (7 mg.). It crystallised from ether in needles, m.p. 153-160°, and had identical u.v. and i.r. spectra to those of the keto-aldehyde obtained in previous experiments. Elution with ethyl acetate-benzene (1:4) gave (±)-narwedine (21) (15 mg.), crystallised from ethanol in needles, m.p. 187-190°.

Oxidation of an impure sample of galanthamine.

Separation of galanthamine from other alkaloids, pluviine and galanthine, was difficult (see above). An attempt was therefore made to oxidise the crude mixture directly to give narwedine which is easier to crystallise than galanthamine. To a solution of the impure (-)-galanthamine (100 mg.) in dry, ethanol-free chloroform (60 ml.) was added activated manganese dioxide[38] (1g.). The reaction mixture was shaken for 2 hr. After working up the reaction mixture as described earlier, the residue (90 mg.) was chromatographed over grade III neutral alumina (10 g.). The chromatography was followed on t.l.c. on alumina GF_{254} plates developed in solvent system ethyl acetate-methanol (95:5), as well as in the u.v. The first few fractions (5 mg.) showed a u.v. spectrum identical to that of the keto-aldehyde. From the remaining fractions, crystallisation of (±)-narwedine from ethanol was attempted, but a compound different from narwedine was obtained in fine needles, m.p. 272-74° (decomp.). The compound was identified as the lactam (A), presumably derived from pluviine, by comparison of u.v. , i.r. spectra and m.p. with that of the authentic sample[12] provided by Dr.H.P.Tiwari.



(A)

Attempted oxidation of Pummerer's ketone (31).

Pummerer's ketone[42,43] was treated with activated manganese dioxide[38] by the method described earlier. The residue after evaporation of the solvent was tested on t.l.c. against Pummerer's ketone, but only one spot, corresponding to the parent material was observed. Oxidation had not taken place and Pummerer's ketone was recovered in quantitative yield. The mixed m.p. of the reaction product with an authentic sample of Pummerer's ketone showed no depression; (mixed m.p. 124° (lit.,[42] m.p. 124°).

Oxidation of (-)-lycoramine.

(-)-Lycoramine (50 mg.) was oxidised with activated manganese dioxide[38] by the method described earlier. The residue after evaporation of the solvent was chromatographed over grade III neutral alumina (10 g.). Elution with ethyl acetate-benzene (4:6) gave an oil (10 mg.), which did not crystallise. The u.v. spectrum showed bands at λ_{max}^{EtOH} 236, 278, 322 nm. I.r. spectrum in chloroform showed bands at ν_{max} 1725, 1670 $cm.^{-1}$

The oil (10 mg.) was dissolved in methanol (1 ml.). To the cooled solution was added sodium borohydride (2 mg.). The mixture was left overnight. Next day the residue obtained

after evaporation of methanol was dissolved in water and extracted with chloroform. The chloroform extract was washed well with water and dried over anhydrous sodium sulphate. Evaporation gave a residue, which showed a u.v. band at λ_{max}^{EtOH} 287 nm. The i.r. spectrum in chloroform showed a band at ν_{max} 1670 $cm.^{-1}$

Hydrogenation[15] of Pummerer's ketone in the presence of sodium ethoxide.

Pummerer's ketone[42,43] (2.1 g., 1 mole) in dry ethanol (75 ml.) containing dissolved sodium (2.3 g., 10 moles) was hydrogenated over 10% palladised charcoal. Two moles of hydrogen were rapidly consumed. There was no further uptake. The catalyst was removed by filtration and the alkaline solution was diluted with 5% aqueous hydrochloric acid (250 ml.). Extraction with ether, removal of ether under vacuum and crystallisation of the residue from aqueous ethanol gave the keto-phenol (49), m.p. 175-76°.

Attempted hydrogenation of keto-aldehyde (48) in the presence of sodium ethoxide.

Keto-aldehyde (30 mg., 1 mole) in dry ethanol (0.75 ml.) containing dissolved sodium (30 mg., 10 moles) was hydrogenated over 10% palladised charcoal (3 mg.). The reaction was followed on alumina GF_{254} plates developed with ethyl acetate-methanol (95:5). After 3 hr. two spots with R_f values 0.6 and 0.4 and one faint spot, R_f 0.74, corresponding to keto-aldehyde were observed. After 4 hr. the reaction appeared to be complete.

The catalyst was filtered off and the solvent evaporated. The residue was dissolved in the minimum quantity of water. The The solution was filtered to get rid of insoluble impurities. To the filtrate was added solid carbon dioxide until pH7 was obtained. The filtrate became turbid and a compound precipitated out. It was extracted with chloroform. The chloroform extract was washed well with water and dried over anhydrous sodium sulphate. Evaporation of solvent gave a residual gum (23 mg.). This was chromatographed on grade III neutral alumina (5 g.). Elution with benzene separated a mixture of three compounds. The major product (8 mg.) showed one spot on t.l.c. The u.v. spectrum of this compound showed bands at λ_{max}^{EtOH} 230, 285 nm. The i.r. spectrum showed bands at $\nu_{max}^{CHCl_3}$ 3560, 1720 cm.$^{-1}$ However, in the n.m.r. spectrum three proton singlet due to aromatic methyl group was absent.

Hydrogenation of veratraldehyde.

Veratraldehyde (30 mg.) in ethanol was hydrogenated over 10% palladised charcoal (3 mg.). The reaction was followed on alumina GF_{254} plates developed with ethyl acetate-methanol (95:5). The reaction was complete in 0.5 hr. The catalyst was filtered off and the filtrate evaporated under vacuum. The oily residue (25 mg.) obtained showed one spot on t.l.c. with R_f 0.79. The u.v. spectrum showed a band at λ_{max}^{EtOH} 285 nm. The i.r. spectrum showed the absence of a band, ν_{max} 1680 cm.$^{-1}$ for an aromatic aldehyde group. The n.m.r. spectrum in deuteriochloroform showed three proton singlet due to methyl group at τ 7.7.

Attempted hydrogenation of keto-aldehyde (48) in the presence of sodium ethoxide under different experimental conditions.

The keto-aldehyde (40 mg., 1 mole) in dry ethanol (1.5 ml.) was hydrogenated over 10% palladised charcoal (4 mg.). The reaction was followed by t.l.c. (as above). When one mole of hydrogen was taken up, the reaction mixture showed one spot on t.l.c. with R_f 0.64, different from the keto-aldehyde (R_f0.74). The u.v. spectrum of the reaction mixture was different from that of the keto-aldehyde. It showed a band at λ_{max}^{EtOH} 285 nm. To the reaction mixture was then added freshly prepared sodium ethoxide [10 moles - prepared by dissolving sodium (30 mg.) in dry ethanol (1.5 ml.)] and a fresh batch of catalyst. Hydrogenation was continued. It was difficult to note the uptake of hydrogen. The reaction mixture was followed by t.l.c. and left overnight under hydrogen. Next day reaction mixture showed one spot with R_f 0.74, the same as for the keto-aldehyde; but the u.v. spectrum of the reaction mixture was different from that of the keto-aldehyde. It showed bands at λ_{max}^{EtOH} 232, 260, 290, 305 nm. The catalyst was filtered off and the solvent evaporated. The residue was dissolved in water (2 ml.) and the solution acidified with dry carbon dioxide until pH7 was reached. The solution became turbid and was extracted with chloroform. The extract was washed well with water and dried over anhydrous sodium sulphate. Evaporation of solvent gave a gum (29 mg.). This was chromatographed on grade III neutral alumina (5g.). Elution with ethyl acetate-benzene (1:9) gave a major fraction (11 mg.) which showed in

i.r. spectrum bands at $\nu_{max}^{CHCl_3}$ 3540, 1720 cm.$^{-1}$ Elution with ethyl acetate-benzene (3:7) gave the fraction (5 mg.). The compounds were not identified on the basis of spectral data.

Isolation of alkaloid chlidanthine from the crude extract of Chlidanthus fragrans.

The total bases (1.5 g.) isolated[18] from resting Chlidanthus fragrans bulbs were treated with a small amount of ethanol. The crystalline mass (0.150g.) which slowly separated was triturated with 2N-sodium hydroxide. Filtration and neutralisation of the filtrate with 2N-hydrochloric acid followed by addition of sodium hydrogen carbonate gave a precipitate of crude chlidanthine. Crystallisation from methanol gave plates (70mg.), m.p. 240-242°. (lit.,[30] m.p. 238-239°). The alkali insoluble material (30 mg.), presumably lycorine, was not investigated further.

Evaporation of the mother liquors from total bases gave a gum (1.7 g.) which was chromatographed on grade III neutral alumina (50g.). The chromatography was followed by t.l.c. on alumina GF_{254} plates developed with ethyl acetate-methanol (95:5). Elution with ethyl acetate-benzene (1:9) gave an oily fraction (0.180g.), which on crystallisation from benzene gave needles, m.p. 210-212°, presumably tazettine, (lit.,[18] m.p. 208-210°). This alkaloid was not investigated further. Later fractions eluted with ethyl acetate-benzene (1:9) gave a negligible amount of galanthamine (5mg.). Elution of the column with chloroform-ethanol (9:1) gave crude chlidanthine (0.10g.). Further purification of crude chlidanthine was achieved by dissolution in and reprecipitation

from alkali. Chlidanthine (20mg.) crystallised from methanol as plates, m.p. 240-242°. Chlidanthine gave specific rotation $[\alpha]_D$-135.5°(c 0.2 in EtOH) (Lit.[30], $[\alpha]_D$-140°).

U.v. spectrum: λ_{max} 287 (ϵ 2960), $\lambda_{inf.}$ 230 nm. (ϵ 9770) in ethanol shifting to $\lambda_{max.}$ 300 (ϵ 3350), $\lambda_{inf.}$ 245 nm. (ϵ 9080) upon addition of sodium hydroxide.

Mass spectrum: M^+ at m/e 287.150 ($C_{17}H_{21}NO_3$ requires m/e 287.152).

Fragment ions at 212.080 ($C_{14}H_{12}O_2$ requires 212.084) and 202.084 ($C_{12}H_{12}NO_2$ requires 202.087).

Acetylation of chlidanthine (55)

To a solution of chlidanthine (0.018 g.) in pyridine (0.01 ml.) was added acetic anhydride (0.1 ml.). The solution was left overnight at room temperature. After evaporation of pyridine and acetic anhydride under vacuum, the residue was treated with water and basified with sodium hydrogen carbonate. The solution was extracted with chloroform. The chloroform extract wash washed well with water and dried over anhydrous sodium sulphate. Evaporation of the solvent gave an oil (0.011 g.), which slowly crystallised in needles, m.p. 98-99°. A suitable solvent for recrystallisation was not found.

Mass spectrum: m/e 329.162 ($C_{19}H_{12}NO_4$ requires m/e 329.163).

I.r. spectrum: ν_{max} 1765 cm.$^{-1}$ in chloroform.

N.m.r. spectrum: see chapter 3, page 29.

Preparation of Pummerer's ketone (59)

a) p-Cresol (14g.) was oxidised with potassium ferricynide

(82 g.) according to the method of Pummerer and his co-workers.[42] The alkali insoluble product (12 g.) was chromatographed on grade III neutral alumina (300 g.). Elution with benzene gave the ketone (2.5 g.) which crystallised from benzene in needles, m.p. 124° (Lit.[42] m.p. 125°).

b) *p*-Cresol (10.8 g., 0.1 mole) was oxidised with sodium persulphate (0.1 mole) according to the method of Bacon, Grime and Munro.[43] An aqueous solution (1 l.) containing *p*-cresol (10.8 g., 0.1 mole), sodium persulphate (0.1 mole) and silver nitrate (0.01 mole) was left for 24 hr. at room temperature. The precipitate was filtered and treated with ether. The ether extract was dried and evaporated, after treatment with alkali. A pale yellow, crystalline compound (2.8 g.) was chromatographed on grade III neutral alumina (100 g.). Elution with benzene gave the ketone (2g.), which crystallised from benzene in needles, m.p. 124° (Lit.[42] m.p. 125°).

Reduction of Pummerer's ketone (59)

Reduction was carried out by the method of Tiwari (H.P.Tiwari, Ph.D. thesis, London University, 1965). Pummerer's ketone (0.500g.) in dry ether (70 ml.) was added to a suspension of lithium aluminium hydride (1.0 g.) in dry ether (30 ml.) at room temperature over a period of one hour with stirring. After another 6 hr. excess lithium aluminium hydride was decomposed with water and ether decanted off. The residual paste was washed with ether (30 ml.). The total ether portion was washed with water and dried over anhydrous sodium sulphate. Evaporation of the solvent gave an oil, which showed two spots (R_f 0.33 and 0.16) on t.l.c. on an alumina GF_{254} plate developed with ethyl acetate-benzene (1:9). It showed

no carbonyl absorption band in i.r.spectrum. The oil was chromatographed on grade III neutral alumina (10 g.). Elution with ethyl acetate-benzene (1:9) separated the two alcohols. The alcohol (60) eluted first from the column as an oil, which slowly crystallised on long standing. Attempts to recrystallise the alcohol (60) failed. The latter fraction gave the alcohol (61) which crystallised from light petroleum (b.p. 60-80°) in needles, m.p. 80-81°. In the i.r. spectrum in carbon tetrachloride the oily alcohol (60) showed a band at ν_{max} 3560 cm.$^{-1}$ and the alcohol (61) showed a band at ν_{max} 3610 cm.$^{-1}$

Hydrogenation of Pummerer's ketone (59) (ref. 15)

Pummerer's ketone (2g.) was hydrogenated in ethanol over 10% palladised charcoal (0.20 g.). One mole of hydrogen was consumed within one hour; there was no further uptake. After removal of catalyst by filtration, the solvent was removed in vacuum and the residue (1.9g.) was crystallised from light petroleum (b.p. 40-60°) in prisms, m.p. 80° (lit.[15] m.p. 80°). Dihydro-Pummerer's ketone (67) showed one spot with R_f 0.70 on t.l.c. on an alumina GF_{254} plate developed with ethyl acetate-benzene (1:9). The n.m.r. spectrum in deuteriochloroform showed that hydrogenation had taken place. Signals due to olefinic protons were absent.

Reduction of dihydro-Pummerer's ketone (67)

Dihydro-Pummerer's ketone (67) (0.50 g.) in dry ether (70 ml.) was added to a suspension of lithium aluminium hydride (1.0 g.) in dry ether (30 ml.) at room temperature over a period of one hour with stirring. After another 6 hr., excess lithium aluminium hydride

was decomposed with water and ether decanted off. The residual paste was washed with ether (30 ml.). The total ether portion was washed with water, dried over anhydrous sodium sulphate and the ether evaporated. The product showed no carbonyl band in the i.r. spectrum. It showed two spots on an alumina GF_{254} plate developed with ethyl acetate-benzene (1:9). The product (0.479 g.) was chromatographed on grade III neutral alumina (30 g.). The chromatography was followed by t.l.c. Elution with ethyl acetate-benzene (1:9) separated the two alcohols. The first fractions gave the alcohol (68) as an oil (0.300g.), which slowly crystallised in needles on long standing, m.p. 59-61°. Attempts to recrystallise the alcohol (68) failed. The latter fractions gave the alcohol (69), which was crystallised from light petroleum (b.p. 60-80°) in needles, m.p. 71° (lit.[16] m.p. 71°). The alcohol (68) showed a band at ν_{max} 3600 cm.$^{-1}$ and the alcohol (69) showed a band at ν_{max} 3650 cm.$^{-1}$ in the i.r. spectra in chloroform.

Analysis of the alcohol (68)

Found: C, 77.00; H, 8.47.

$C_{14}H_{18}O_2$ requires C, 77.06; H, 8.2%.

Attempted methylation of the allylic alcohols (60) and (61) with methyl iodide in the presence of anhydrous potassium carbonate.

To a solution of the oily alcohol (60) in dry acetone (60 ml.) was added anhydrous potassium carbonate (0.60 g.) and methyl iodide (0.6 ml.). The reaction mixture was heated under reflux overnight. Next day it was filtered and the filtrate

evaporated. The residue was extracted with chloroform and the chloroform extract washed well with water and dried over anhydrous sodium sulphate. Evaporation gave an oil. The n.m.r. and i.r. spectra were identical with those of the parent alcohol (60). An attempt to methylate the crystalline alcohol (61) under the condition likewise gave only recovered starting material.

Methylation of the allylic alcohols (60) and (61) with methyl iodide in the presence of freshly prepared silver oxide.

The oily alcohol (60) (50 mg.) was heated overnight under reflux in methyl iodide (15 ml.) in the presence of freshly prepared silver oxide (0.50 g.). The reaction was followed by t.l.c. from time to time (see table 3). Next day the solution was filtered and the filtrate evaporated. The residue, obtained as an oil, showed one spot with R_f 0.70 on an alumina GF_{254} plate developed with ethyl acetate-benzene (1:9). It was chromatographed on grade III neutral alumina (5 g.). Elution with ethyl acetate-benzene (1:9) gave an oil (62) (45 mg.), which slowly crystallised after sublimation (80-120°, 1.5 mm. Hg.), m.p. 41°. The n.m.r. spectrum in deuteriochloroform showed a three-proton singlet at τ 6.65.

Analysis

Found = C, 78.2; H, 7.8.

$C_{15}H_{18}O_2$ requires C, 78.2; H, 7.9%

TABLE 3

RESULTS OF THE THIN LAYER CHROMATOGRAPHY

Plates used for t.l.c. - alumina GF_{254} (heated at 110°)
Plates were developed in 10% ethyl acetate-benzene mixture.
Observations were made under u.v. lamp.

EXPERIMENT	1 HOUR	2 HOURS	3 HOURS	4 HOURS	OVERNIGHT
1.Methylation of the oily alcohol (60)	One faint spot R_f=0.70. Second spot R_f= 0.33 corresponding to the parent compound. Conclusion:- Reaction proceeded 10%.	Spot with R_f=0.70 became more prominent than the spot appeared at the end of 1 hour. Second spot with R_f=0.33 became slightly faint. Conclusion:- Reaction proceeded 20%.	Spot with R_f=0.70 became more prominent than the spot appeared at the end of 2 hrs. Second spot with R_f 0.33 became fainter than the spot appeared at the end of 2 hours. Conclusion:- Reaction proceeded 30%.	Spot with R_f=0.70 became more prominent than the spot appeared at the end of 3 hrs. Second spot with R_f=0.33 became fainter than the spot appeared at the end of 3 hrs. Conclusion:- Reaction proceeded 40%.	One prominent dark spot with R_f=0.70. Spot with R_f=0.33 almost disappeared. Conclusion:- Reaction was complete.
2.Methylation of the crystalline alcohol (61)	One faint spot R_f=0.73 Second spot R_f= 0.16 became slightly faint. Conclusion:- Reaction proceeded 10%.	Spot with R_f=0.73 became more prominent than the spot appeared at the end of 1 hr. Second spot with R_f=0.16 became slightly faint. Conclusion:- Reaction proceeded 20%.	Spot with R_f=0.73 became more prominent than the spot appeared at the end of 2 hrs. Second spot with R_f=0.16 became fainter than the spot appeared at the end of 2 hours. Conclusion:- Reaction proceeded 30%.	Spot with R_f=0.73 became more prominent than the spot appeared at the end of 3 hrs. Second spot with R_f=0.16 became fainter than the spot appeared at the end of 3 hours. Conclusion:- Reaction proceeded 40%.	One prominent dark spot with R_f=0.7. Spot with R_f=0.16 almost disappeared. Conclusion:- Reaction was complete.

Methylation of the crystalline alcohol (61) (50 mg.) under similar conditions gave an oil (63) (40 mg.), which crystallised from light petroleum (b.p. 60-80°) as plates, m.p. 90°. The n.m.r. spectrum in deuteriochloroform showed a three-proton singlet at τ 6.6. (For t.l.c. observation see table 3).

Analysis

Found: C, 78.2; H, 8.0.

$C_{15}H_{18}O_2$ requires C, 78.3; H, 7.9%.

Acetylation of the allylic alcohols (60) and (61)

To the oily alcohol (60) (0.260g.) in pyridine (0.2 ml.) was added acetic anhydride (1 ml.). The reaction was kept overnight at room temperature. Next day the solution was evaporated in vacuum and water was added to the residue. The solution was extracted with ether, the ether extract washed well with water and dried over anhydrous sodium sulphate. Evaporation gave an oil (c.240g.), which showed one spot (R_f 0.66) on t.l.c. (as above). The oil was chromatographed on grade III neutral alumina (10 g.). Elution with ethyl acetate-benzene (1:9) gave an oil (64) (0.180 g.), which slowly crystallised on sublimation in needles, m.p. 67°. The n.m.r. spectrum in deuteriochloroform showed a singlet due to the acetyl group at τ 8.0 . The i.r. spectrum in chloroform showed a band at ν_{max} 1720 cm.$^{-1}$

Analysis

Found: C, 74.1; H, 7.4.

$C_{16}H_{18}O_3$ requires C, 74.4; H, 7.0%.

Similarly acetylation of the crystalline alcohol (61)

(0.200 g.) gave the corresponding acetate (65) (0.180 g.) with R_f 0.70 and m.p. 63°. The n.m.r. spectrum in deuteriochloroform showed a singlet due to the acetyl group at τ 7.92. The i.r. spectrum in chloroform showed a band at ν_{max} 1725 cm.$^{-1}$

Analysis

Found: C, 74.3; H, 6.8.

$C_{16}H_{18}O_3$ requires C,74.4; H,7.0%.

Hydrolysis of both the acetates (64) and (65) gave the alcohols (60) and (61) respectively.

Epimerisation of the allylic alcohol (60)

A solution of the oily alcohol (60) (0.200 g.) in dioxan (10 ml.) and 4% (w/v) hydrochloric acid (10 ml.) was heated under reflux for 3 hr. The colour of the reaction mixture gradually changed to yellow. The reaction was followed by t.l.c. (see table 4). After 3 hr., the cooled reaction mixture was treated with a slight excess of sodium hydrogen carbonate and extracted with ether (4 x 20 ml.). The ether extract was washed well with water and dried over anhydrous sodium sulphate. Evaporation of ether gave an oil (0.15 g.), which showed two spots, R_f 0.58 and 0.16, on an alumina GF_{254} plate developed with ethyl acetate-benzene (1:9). The oil was chromatographed on grade III neutral alumina (15 g.). Elution with ethyl acetate-benzene (1:9) separated two compounds. The first fraction obtained as an oil, was further purified by sublimation. The sublimate slowly crystallised in needles, m.p. 82°. This material was identified

TABLE 4

RESULTS OF THE THIN LAYER CHROMATOGRAPHY

Plates used for t.l.c. - alumina GF_{254} (Heated at 110°)

Plates were developed in 10% ethyl acetate-benzene mixture
Observations were made under u.v.lamp

EXPERIMENT	1 HOUR	2 HOURS	3 HOURS
Epimerisation of the oily alcohol (60)	One faint spot R_f=0.58	Spot with R_f=0.58 became more prominent than the corresponding spot appeared at the end of 1 hour.	Spot with R_f=0.58 became more prominent than the spot appeared at the end of 2 hours.
	Second prominent spot with R_f=0.33 corresponding to the parent compound.	Second spot with R_f=0.33 became fainter than the corresponding spot appeared at the end of 1 hour.	Second spot with R_f=0.33 almost disappeared.
	Third faint spot R_f=0.16 corresponding to the crystalline alcohol (61)	Spot with R_f=0.16 became more prominent than the corresponding spot appeared at the end of 1 hour.	Spot with R_f=0.16 became more prominent than the corresponding spot appeared at the end of 2 hours.
	Conclusion:- Reaction proceeded to 10%.	Conclusion:- Reaction proceeded 50%.	Conclusion:-Reaction was complete.
Epimerisation of the methyl ether (52) of the oily alcohol (60).	One faint spot R_f=0.58. One prominent spot R_f=0.7 corresponding to the parent compound. Third faint spot corresponding to the oily alcohol, R_f=0.33. Fourth faint spot corresponding to the crystalline alcohol (61), R_f=0.16. Conclusion:- Reaction proceeded 10%.	Spot with R_f=0.58 became prominent. Spot with R_f=0.7 became very faint. Spot with R_f=0.33 became prominent. Spot with R_f=0.16 became more prominent than the corresponding spot appeared at the end of 1hr. Conclusion:- Reaction proceeded 50%.	Spot with R_f=0.58 became more prominent than the spot appeared at the end of 2 hours. Spot with R_f=0.7 disappeared. Spot with R_f=0.33 became very faint. Spot with R_f=0.16 became more prominent than the corresponding spot appeared at the end of 2 hours. Conclusion:- Reaction was complete.

as the phenol (66) by n.m.r. examination (see Chapter 3). The second fraction, obtained as an oil (0.070 g.), crystallised from light petroleum (b.p. 60-80°) in needles, m.p. 80°. The i.r. and n.m.r. spectra of this compound were identical with those of the alcohol (61); there was no depression of m.p. of a mixture with authentic (61).

Epimerisation of the methyl ether (62) of the alcohol (60)

A solution of the methyl ether of the alcohol (60) (0.100 g.) in dioxan (5 ml.) and 4% (w/v) hydrochloric acid (5 ml.) was heated under reflux for 3 hr. Work-up, as above, gave the phenol (66) (0.022 g.) and the alcohol (61) (0.032g.).

Rearrangement of the allylic alcohol (60)

A solution of the alcohol (60) (0.250g.) in dioxan (3 ml.) and concentrated hydrochloric acid (3 ml.) was heated under reflux for 3 hr. The cooled solution was treated with a slight excess of sodium hydrogen carbonate and extracted with ether. The ether extract was washed well with water and dried over anhydrous sodium sulphate. Evaporation gave an oil (66) (0.20g.), which was purified by sublimation. The sublimate had m.p. 82°. The compound had identical i.r. and n.m.r. spectra to those of the phenols obtained in previous epimerisation experiments.

Preparation of O-methylcodeine methiodide (73) from codeine[47]

To an ice-cold solution of codeine (1.0 g.) in 1N-sodium hydroxide (5 ml.) was added dimethyl sulphate (0.66 ml.). The solution was shaken vigorously in ice-cold water, until the dimethyl sulphate had disappeared. Then 10N-sodium hydroxide

(0.33 ml.) and dimethyl sulphate (0.33 ml.) were added to the reaction mixture and again it was shaken vigorously. The process was repeated two more times. After 4 hr. of vigorous shaking, to the reaction mixture was added saturated aqueous potassium iodide drop by drop. O-Methylcodeine methiodide separated as an oil, but solidified on cooling. It was crystallised from 80% ethanol in needles (1.36 g.), m.p. 241° (decomp.) (lit.[47] m.p. 257°). However the confirmation of the structure came from examination of the n.m.r. spectrum in hexadeuteriodimethylsulphoxide. The n.m.r. spectrum showed two three-proton singlets at τ 6.6 and 6.23 due to the alkyl O-methyl group and the aryl O-methyl group, and a six-proton singlet due to the $N(Me)_2$ group at τ 6.71.

Attempted methylation of (-)-galanthamine (53)

To an ice-cold solution of galanthamine (50 mg.) in 1N-sodium hydroxide (0.5 ml.) was added dimethyl sulphate (0.1 ml.). The solution was shaken vigorously in ice-cold water, until the dimethyl sulphate had disappeared. Then 10N-sodium hydroxide (0.03 ml.) and dimethyl sulphate (0.03 ml.) were added to the reaction mixture and again it was shaken vigorously. The process was repeated two more times. After 4 hr. of vigorous shaking, to the reaction mixture was added saturated aqueous potassium iodide drop by drop until the precipitation was complete. Galanthamine methiodide (74) (45 mg.) was obtained in crystalline form, which was recrystallised from water in needles, m.p. and mixed m.p. 279°(decomp.).

Preparation of codeine methiodide

To a solution of codeine (0.500g.) in ethanol (5 ml.) was added methyl iodide (0.5 ml.). Codeine methiodide slowly crystallised in needles (0.510 g.). It was recrystallised from methanol in needles, m.p. 270° (decomp.) (lit.m.p. 270°(decomp.).

Attempted methylation of codeine methiodide in dimethylsulphoxide

a) To a stirred suspension of sodium hydride (70 mg., 50% dispersion in oil) in dry dimethylsulphoxide (10 ml.) was added codeine methiodide (0.500 g.) in dry dimethylsulphoxide (5 ml.) under nitrogen at room temperature. The colour of the reaction mixture slowly changed to red. After 10 min. methyl iodide (0.02 ml.) was added. The colour of the reaction mixture slowly changed to pale yellow. After 4 hr., the solvent was evaporated in vacuum and the oily residue washed with light petroleum (b.p. 40-60°). To the residue was added crushed ice and then saturated aqueous potassium iodide. No precipitation of O-methylcodeine methiodide was obtained.

b) To a stirred solution of codeine methiodide (0.240 g.) in dry dimethylsulphoxide (5 ml.) and methyl iodide (0.1 ml.) was added sodium hydride (30 mg., 50% dispersion in oil) under nitrogen at room temperature. The reaction was left overnight at room temperature. Next day the solvent was evaporated and to the residue crushed ice was added. A brown solid separated. The product showed a different R_f value from that of O-methylcodeine methiodide on a silica gel plate developed with n-propanol-water (3:2). The same reaction was also attempted in dry dimethylformamide but without success.

Attempted methylation of codeine (71) with methyl iodide in the presence of silver oxide

To a stirred solution of codeine (0.250 g.) in dry dimethylformamide (5 ml.) was added freshly prepared silver oxide (2.5g.) and methyl iodide (0.2 ml.) under nitrogen at room temperature. The reaction was followed on silica gel plates developed with n-propanol-water (3:2). After 6 hr. the solvent was evaporated and to the residue crushed ice was added. The solution was extracted with dilute ethyl alcohol. Evaporation gave an oil, which appeared to be a mixture of several compounds from t.l.c. observation. They could not be separated preparatively.

Attempted methylation of codeine (71) with methyl iodide and barium oxide

To a cooled solution of codeine (0.500 g.) in dry dimethylformamide (5 ml.) was added barium oxide (2.5 g.) and methyl iodide (1 to 2 ml.). The reaction mixture was stirred at room temperature under nitrogen overnight. The supernatant solution was decanted off and the residue was washed with dimethyl formamide. The combined solutions were evaporated in vacuum and to the oily residue crushed ice was added. A clear solution was obtained. When saturated aqueous potassium iodide was added, no methiodide separated.

Attempted tosylation of the allylic alcohol (61)

To an ice-cold solution of the crystalline alcohol (61) (85 mg.) in dry pyridine (1 ml.) was added toluene-p-sulphonyl chloride (85 mg.). The solution was kept in the refrigerator

for 20 hr. A change in the colour of the reaction mixture from pale yellow to brown and the precipitation of pyridine hydrochloride was observed. The reaction mixture was evaporated in vacuum and to the residue crushed ice was added. The separated pasty residue was extracted with ether. The ether extract was washed well with dilute hydrochloric acid, sodium hydrogen carbonate solution and finally with ice-cold water. Evaporation of ether gave an oil (80 mg.). It showed one spot (R_f 0.8) on an alumina GF_{254} plate developed with ethyl acetate-benzene (1:9). It was purified by chromatography on grade III neutral alumina. The n.m.r. spectrum showed no bands attributable to a tosyl residue.

Reaction of the allylic alcohol (61) with thionyl chloride and then with sodium methoxide

The crystalline alcohol (61) (70 mg.) in benzene (5 ml.) was treated with thionyl chloride (0.5 ml.) at room temperature for 1 hr. with stirring. The reaction was followed on alumina GF_{254} plates developed with ethyl acetate-benzene (1:9). After 1 hr. the solvent was evaporated and traces of thionyl chloride removed by repeated addition and evaporation of benzene. The residue was chromatographed on grade III neutral alumina (10 g.). Elution with benzene gave the allylic chloride (76) (58 mg.) as an oil. It showed one spot (R_f 0.80) on t.l.c. (as above) and on a silical gel plate developed in the same solvent system. The n.m.r. spectrum served to confirm the configuration at C-3. Thirteen of the fourteen C-4 methylene bands were observable (see table 1).

The allylic chloride was heated under reflux in methanol containing an excess of sodium methoxide for 4 hr. (sodium methoxide required for the purpose was prepared by dissolving metallic sodium (0.50 g.) in dry methanol (22 ml.). The solvent was evaporated and the residue was treated with dry ethanol-free chloroform. Insoluble salts were filtered off and evaporation of the filtrate gave an oil. The oil was chromatographed on grade III neutral alumina (5 g.). Elution with ethyl acetate-benzene (1:9) gave the methyl ether (62) (48 mg.). The compound had n.m.r. and i.r. spectra identical with those of the ether prepared directly from the allylic alcohol (60). Examination (n.m.r.) of the total reaction mixture before chromatography showed the presence (ca. 10%)of a second methyl ether, presumably (63). Qualitatively similar results were obtained when the allylic alcohol (61) was treated with thionyl chloride in chloroform in the presence or absence of pyridine.

Reaction of the allylic alcohol (60) with thionyl chloride and then with sodium methoxide

The oily alcohol (60) (148 mg.) in benzene (5 ml.) was treated with thionyl chloride (0.5 ml.) at room temperature for 1 hr. with stirring. The reaction was followed by t.l.c. (as above). After 1 hr. the solvent was evaporated and traces of thionyl chloride removed by repeated addition and evaporation of benzene. The residue, obtained as an oil, was chromatographed on grade III neutral alumina (10 g.). Elution with benzene gave an oil (135 mg.), which appeared to consist of only one component (t.l.c.) But t.l.c. observation on a silica gel plate

developed with benzene indicated that it was actually a mixture of two components with a very little difference in R_f values. Also n.m.r. examination of the compound indicated it to be a mixture of two components with a ratio ca. 3:2. The major component of the mixture was recognised as the allylic chloride (76), but the other component was not positively identified.

The mixture was heated under reflux in methanol containing an excess of sodium methoxide for 4 hr. Work up of the reaction mixture (see above) gave a mixture of the methyl ethers (62) and (63) with a ratio ca. 3:2 (n.m.r. examination of the total mixture). The mixture showed two spots with R_f 0.17 and 0.20 on silica gel plate developed with benzene. The two ethers were separated by preparative t.l.c. on silica gel. The major ether was recognised as (62) and was obtained as an oil (70 mg.). The other ether (45 mg.) was recognised as (63). It was obtained as an oil and crystallised from light petroleum (b.p. 60-80°) as plates, m.p. 90°. Both the others had n.m.r. and i.r. spectra identical with those of the ethers prepared directly from the allylic alcohols (60) and (61). Similar results were obtained when the oily alcohol (60) was treated with thionyl chloride in chloroform in the presence or absence of pyridine.

Epimerisation of (-)-galanthamine[25] (53)

A solution of (-)-galanthamine (250 mg.) in 2% hydrochloric acid (20 ml.-2 ml. of concentrated hydrochloric acid (36%) diluted to 100 ml.) was heated under reflux for 3 hr. The solution was cooled, treated with slight excess of sodium hydrogen carbonate and extracted with chloroform. The

chloroform extract was washed well with water and dried over anhydrous sodium sulphate. Evaporation of chloroform gave a residue, which was chromatographed on grade III neutral alumina (15 g.). Elution with chloroform-ethanol (95:5) gave epigalanthamine (57) (160 mg.) as plates. It recrystallised from benzene in plates, m.p. 188-189°(lit.[25] m.p.188-189°).

Methylation of (-)-epigalanthamine (57)

(-)-Epigalanthamine (50 mg.) in dry ethanol-free chloroform (5ml.) was treated with thionyl chloride (0.1 ml.) with stirring at room temperature for 2 hr. The solvent was evaporated at room temperature and traces of thionyl chloride removed from the crystalline residue by repeated addition and evaporation of chloroform. The residue was heated under reflux for 5 hr. in dry methanol (5 ml.) containing 1$\underline{N}$-methanolic sodium methoxide (0.6 ml.). The methanol was evaporated and the residue extracted with chloroform. Insoluble salts were filtered off and evaporation of the filtrate gave a residue (40 mg.) as an oil. It showed two spots with R_f 0.64 and 0.53 on an alumina GF_{254} plate developed with ethyl acetate-methanol (95:5). The oil (40 mg.) was chromatographed on grade III neutral alumina (8 g.). Elution with benzene, ethyl acetate-benzene (1:9) and ethyl acetate-benzene (2:8) separated the two compounds. The early fractions gave the diene (77) (12 mg.) as an oil which slowly crystallised in needles, m.p. 94-96°.

U.v. spectrum: λ_{max}^{EtOH} 261 nm. (ϵ 4035)

Mass spectrum: M^+ ion at m/e 269.

N.m.r. spectrum: See chapter 3, page 44.

Later fractions gave an oil (10 mg.), which was recognised as galanthamine methyl ether (75). (for the n.m.r. details see chapter 3, page 45).

Preparation of galanthamine methyl ether methiodide (70)

To a solution of galanthamine methyl ether (10 mg.) in dry methanol (0.5 ml.) was added methyl iodide (0.05 ml.) at room temperature. The reaction was left overnight at room temperature. Galanthamine methyl ether methiodide (70) separated as fine needles (12 mg.), m.p. 259°(decomp.). It recrystallised from methanol in needles, m.p. 274° (decomp.).

Specific rotation $[\alpha]_D$-90°(c 0.105 in methanol).

Analysis: Found F, 50.95; H, 6.05; N, 3.15.

$C_{19}H_{26}INO_3$ requires C,51.4; H, 5.9; N, 3.2%.

Methylation of morphine[47] (72)

To an ice-cold solution of morphine (72) (1.0 g.) in 1N-sodium hydroxide (5 ml.) was added dimethyl sulphate (0.66 ml.). The solution was shaken vigorously in ice-cold water, until dimethyl sulphate had disappeared. Then 10N-sodium hydroxide (0.33 ml.) and dimethyl sulphate (0.33 ml.) were added to the reaction mixture and again it was shaken vigorously. The process was repeated two more times. After 4 hr. of vigorous shaking, to the reaction mixture was added saturated aqueous potassium iodide drop by drop. O-Methylcodeine methiodide (73) separated as an oil, but solidified on cooling. It recrystallised from 80% ethanol in needles (1.2 g.), m.p. 241°(decomp.) (lit.[47] m.p. 257°(decomp.)) However the confirmation of the structure came from examination of the n.m.r. spectrum in hexadeuteriodimethyl-

-sulphoxide. Also the mixed m.p. with the sample of methiodide obtained by methylation of codeine, showed no depression. Both methiodides had identical n.m.r. spectra showing two three-proton singlets at τ 6.6 and 6.23 due to the alkyl O-methyl and the aryl O-methyl group and a six-proton singlet due to the NMe_2 group at τ 6.71.

Methylation of Chlidanthine (55)

A solution of chlidanthine (25 mg.) in 1N-sodium hydroxide (0.5 ml.) was shaken vigorously in ice-cold water with dimethyl sulphate (0.1 ml.) until a clear solution was obtained. Methylation was continued by addition at intervals of three further quantities of 10N-sodium hydroxide (0.03 ml.) and dimethyl sulphate (0.03 ml.). After 4 hr. saturated aqueous potassium iodide was added to effect crystallisation of (-)-galanthamine methyl ether methiodide (70) (10 mg.), m.p. 274°(decomp.). The mixed m.p. of this compound with that of the methiodide obtained from galanthamine showed no depression. It gave a specific rotation $[\alpha]_D$-87° (c 0.106 in methanol). The i.r. spectra(KBr disc) of the two methiodides were also identical.

Reaction of the allylic alcohol (60) with trisdimethylamino-phosphine and then with sodium methoxide

The oily alcohol (60) (190 mg.) in dry, ethanol-free chloroform (2 ml.) and dry carbon tetrachloride (2 ml.) was treated[54] with trisdimethylaminophosphine (0.5 ml.) in dry chloroform (3 ml.) with stirring at -35°. The temperature of the reaction mixture was allowed to rise slowly to 20°. After 2 hr. the solvent was evaporated and the residue was chromatographed on grade III neutral alumina (10 g.). Elution with benzene gave the allylic chloride (76)(90 mg.) as an oil. It had identical t.l.c. phenomenon and the n.m.r. spectrum to those of the allylic chloride obtained using the thionyl chloride reagent. Elution with ethyl acetate-benzene (1:9) gave the alcohol (60) (30 mg.).

This allylic chloride (76) (90 mg.) was heated under reflux in methanol containing an excess of sodium methoxide for 4 hr. The work up of the reaction mixture as described earlier gave a mixture of methyl ethers (62) and (63). The two ethers were separated by preparative t.l.c. on silica gel. The major ether obtained as an oil (75 mg.) was recognised as (62). The other ether (5 mg.) was recognised as (63). The two ethers had identical n.m.r. spectra with those of the ethers obtained directly from the allylic alcohols (60) and (61).

Reaction of the allylic alcohol (61) with trisdimethylamino-phosphine and then with sodium methoxide

The crystalline alcohol (61) (118 mg.) in dry, ethanol-free

chloroform (2 ml.) and dry carbon tetrachloride (2 ml.) was treated with trisdimethylaminophosphine (0.3 ml.) in dry chloroform (3 ml.) with stirring at -35°. The temperature of the reaction mixture was allowed to rise slowly to 20°. After 2 hr. the solvent was evaporated and the residue was chromatographed on grade III neutral alumina (8 g.). Elution with benzene gave an oil (90 mg.) which appeared to be a mixture from the n.m.r. examination. One of the components was recognised as allylic chloride (76).

The total mixture was heated under reflux in methanol containing an excess of sodium methoxide for 4 hr. The work up of the reaction mixture as described earlier gave a mixture of methyl ethers (62) and (63). The mixture was separated by preparative t.l.c. on silica gel. The major methyl ether (62) (47 mg.) was obtained as an oil. The other methyl ether (19 mg.) was recognised as (63). Both the ethers had identical n.m.r. spectra with those of the ethers obtained directly by methylation of the allylic alcohols (60) and (61).

Treatment of (-)-galanthamine with the phosphorus reagent

(-)-Galanthamine (65 mg.) in dry ethanol-free chloroform (1 ml.) and dry carbon tetrachloride (0.6 ml.) was treated with tris-dimethylaminophosphine (0.2 ml.) in dry chloroform (1 ml.) with stirring at -35°. The temperature of the reaction mixture was allowed to rise slowly to 20°. After 2 hr. the solvent was evaporated. The residue was heated under reflux for 5 hr. in dry methanol (5 ml.) containing N-methanolic sodium methoxide (0.5 ml.). The solvent was evaporated and the residue extracted

with chloroform. Insoluble salts were filtered off and evaporation of the solvent gave a residue as an oil. It was chromatographed on grade III neutral alumina (10 g.). Elution with benzene gave a fraction as an oil (52 mg.), and elution with ethyl acetate-benzene (1:9) gave a second fraction as an oil (67 mg.). From the n.m.r. spectrum it appeared to be phosphoric trisdimethylamide.

The first fraction (52 mg.) was treated with dilute hydrochloric acid and extracted with ether to get rid of phosphoric trisdimethylamide. The acid solution was basified with sodium hydrogen carbonate and extracted with chloroform. The chloroform extract was washed well with water and dried over anhydrous sodium sulphate. Evaporation gave an oil, which appeared to be a mixture of several compounds from t.l.c. on alumina GF_{254} plate developed with ethyl acetate-methanol (95:5). Attempts to separate this mixture failed.

Biosynthetic experiments

Tritium labelled compounds were counted in a liquid scintillation counter (Beckman CPM 100). The compound was dissolved in a mixture of dry dimethylformamide (0.1 ml.) and liquid scintillator [(5 ml.),made from 2,5-diphenyl-oxazolone (3.8 g.) and dimethyl POPOP scintillator, NE (0.2g.) dissolved in 1 litre of sulphur-free toluene]. The liquid scintillator solution was stored at 0° under nitrogen. The overall efficiency of the counter was determined for each radioactive compound prepared by counting a [1,2-$^{3}H_{2}$]hexadecane

standard in the presence of the inactive compound. Radioactive precursors were counted by dissolving ca. 0.2 - 0.5 mg. in dry dimethylformamide, making up to 5 ml. in a graduated flask and taking 0.1 ml. of this solution for counting. The counter was checked for efficiency-drift by counting the ^{3}H hexadecane standards (sealed under argon) before counting the compound.

Preparation of [^{3}H]methanol

Magnesium methoxide was prepared by dissolving magnesium (0.480 g.) in dry methanol (10 ml.). The excess methanol was distilled off and the magnesium methoxide dried in vacuum at 100°. To the cooled magnesium methoxide (cooled in solid carbon dioxide and acetone) was added tritiated water (0.8 ml., 3.6 mCi/mmole). The mixture was shaken and kept aside for 0.5 hr. Tritiated methanol was distilled over in vacuum.

Preparation of (±)-[^{3}H]narwedine

(±)-Narwedine (60 mg.) and [^{3}H]methanol (0.4 ml.) were heated under reflux in the presence of dry triethylamine (0.01 ml.) for 0.5 hr. (±)-[^{3}H]Narwedine crystallised out in needles after cooling. The solvent was then evaporated off.

Preparation of (±)-[^{3}H]galanthamine and (±)-[^{3}H]epigalanthamine

(±)-[^{3}H]Narwedine (20 mg.) was extracted (soxhlet) into a refluxing suspension of lithium aluminium hydride (40 mg.) in dry ether (20 ml.) during 4 hr. The excess of the reagent was decomposed with ethyl acetate and water. The ether layer was decanted off and the pasty residue extracted with chloroform. Combined ether and chloroform extracts were washed well with water

and dried over anhydrous sodium sulphate. Evaporation of the solvent gave a partially crystalline residue (18 mg.), which showed two spots with R_f 0.46 and 0.33 on an alumina GF_{254} plate developed with ethyl acetate-methanol (95:5) corresponding to galanthamine and epigalanthamine. The residue (18 mg.) was chromatographed on grade III neutral alumina (5 g.). Elution with ethyl acetate-benzene (1:9) gave (±)-[^{3}H]galanthamine (9 mg.), crystallised from ether in needles, m.p. 129-130°. Elution with chloroform-ethanol (95:5) gave (±)-[^{3}H]epigalanthamine (6 mg.) crystallised from benzene in needles, m.p. 188-189°.

Feeding experiments

Known amounts of the precursors, (±)-[^{3}H]narwedine, (±)-[^{3}H]galanthamine and (±)-[^{3}H]epigalanthamine (ca.0.50 mg.) were dissolved in dry dimethylformamide (5 ml.) in three different graduated flasks. 0.1 ml. of these solutions were used for counting.

Activity of the precursors

(±)-[^{3}H]narwedine	a) 1.023 mCi/mmole
	b)4.52 mCi/mmole
(±)-[^{3}H]galanthamine	a) 1.57 mCi/mmole
	b) 3.55 mCi/mmole
(±)-[^{3}H]epigalanthamine	a) 1.57 mCi/mmole
	b) 3.55 mCi/mmole.

Aqueous solutions of precursors (pH ca.6) were fed to Chlidanthus fragrans plants, growing in early summer, through

wicks of untreated cotton passed through the fleshy leaves near soil level. Periods of 2 days (samples "a") and 7 days (samples "b") were allowed for metabolism. The whole plants were taken out and macerated and extracted with 1% ethanolic tartaric acid. The total bases were isolated by the general procedure of Fales and his c-workers.[12] Different solvent systems for the separation of the alkaloids on t.l.c. alumina GF_{254} and silica gel GF_{254} were tried. The ethyl acetate-methanol (95:5) system appeared to be satisfactory in case of alumina GF_{254}. The alkaloids were then separated by preparative chromatography on Merck alumina PF_{254} plates of 0.5 mm. thickness, using the solvent system ethyl acetate-methanol (95:5). Observations were made under u.v.lamp. The typical R_f values were, galanthamine 0.46, epigalanthamine 0.33, lycorine 0.26 and chlidanthine 0.13. Different alumina fractions containing the alkaloids were extracted several times with chloroform-ethanol (95:5) in order to achieve complete elution. Each fraction was dissolved in chloroform (5 ml.) and 0.1 ml. of the solution was pipetted out into a scintillation counting bottle. Evaporation of chloroform in a nitrogen stream gave a residue which was counted in the usual manner using dry dimethylformamide (0.1 ml.) and the liquid scintillator (5.0 ml.). To effect purification chlidanthine fractions were mixed with inactive precursor and the mixture separated by another chromatographic run. Final purification was achieved by crystallisation and the radioactive purity

checked using the derived methyl ether methiodide. The results obtained in the various feeding experiments are tabulated below.

Two day experiment

Precursor	(±)-[^{3}H]Galanthamine
Total ^{3}H activity fed to the plants	0.054 mCi
or	1.118×10^8 disint./min.
Wet weight of the plants	60 g.
Crude alkaloids isolated	97 mg.
Total ^{3}H activity isolated	5.20×10^7 disint./min.
Recovery of ^{3}H activity	$\frac{5.20 \times 10^7 \times 10^2}{1.118 \times 10^8} = 44.07\%$

1st preparative chromatography

Fraction No.	Weight in mg.	Major alkaloid	^{3}H activity (disint./min. x10^7)	% of the total activity fed
1	6	a)fluorescent compound b)galanthamine	0.402	3.41
2	9	galanthamine	5.10	43.22
3	6	lycorine	0.055	0.466
*4	35	a)lycorine b)chlidanthine c)unidentified alkaloid	0.082	0.695
5	5	residue at starting point	0.377	3.2

2nd preparative chromatography of the chlidanthine-rich fraction 4 by diluting with inactive (-)-galanthamine (10 mg.).

Fraction No.	Weight in mg.	Major alkaloid	^3H activity (disint./min. $x10^5$)	% of the activity fed.
1	3	fluorescent compound	0.205	0.0172
2	10	galanthamine	2.5	0.2104
3	1	lycorine	0.45	0.0402
*4	20	a)chlidanthine b)unidentified alkaloid	3.5	0.2945
5	1	residue at starting pt.	0.392	0.0329
6	1	residue at starting pt.	0.162	0.014

Fraction 4 (20 mg.) rich in chlidanthine was diluted with inactive (-)-chlidanthine (15 mg.). Further purification was achieved by crystallisation from methanol. After three successive crystallisations the activity of the alkaloid remained constant.

No.of crystall-isations.	^3H activity of duplicates (disint./min./mmole $x10^7$)	^3H activity of duplicates (disint./min./mg.$x10^4$)
1	a) 1.236 b) 1.240	a) 4.333 b) 4.370
2	a) 1.058 b) 1.096	a) 3.645 b) 3.821
3	a) 1.057 b) 1.058	a) 3.684 b) 3.695

Therefore % incorporation $= \frac{3.69 \times 10^4 \times 35 \times 10^2}{1.118 \times 10^8}$

$= 1.088\%$

Two day experiment

Precursor	(±)-[^{3}H]Epigalanthamine
Total ^{3}H activity fed to the plants	0.043 mCi
or	9.46×10^{7} disint./min.
Wet weight of the plants	50 g.
Crude alkaloids isolated	40 mg.
Total ^{3}H activity isolated	4.52×10^{7} disint./min.
Recovery of ^{3}H activity fed	$\frac{4.52 \times 10^{7} \times 10^{2}}{9.46 \times 10^{7}} = 48.08\%$

1st preparative chromatography

Fraction No.	Weight in mg.	Major alkaloid	^{3}H activity (disint./min.x10^{5})	%of the activity fed
1	2	fluorescent compound	1.78	0.1881
2	1	galanthamine	4.70	0.4968
3	12	epigalanthamine	33.7	3.562
4	6	a)epigalanthamine b)lycorine	282.0	29.81
*5	16	a)lycorine b)chlidanthine c)unidentified alkaloid	18.7	1.97
6	2	residue at starting point	4.55	0.4809
7	1	residue at starting point	4.07	0.4302

Further purification of the chlidanthine-rich fraction 5 (16 mg.) was achieved by chemical separation. The chlidanthine-rich fraction was diluted with inactive (-)-epigalanthamine (10 mg.) and inactive chlidanthine (10 mg.). To get a

homogeneous mixture the alkaloids were dissolved in chloroform. Evaporation of the chloroform gave a gum (36 mg.) which was treated with 2N-sodium hydroxide (0.2 ml.). The insoluble epigalanthamine was filtered off and the filtrate containing chlidanthine was basified with a slight excess of sodium hydrogen carbonate. The aqueous solution was extracted with chloroform several times. Evaporation of the solvent gave crystalline chlidanthine (21 mg.).

a) Counting of alkali insoluble epigalanthamine showed 0.1094% of total ^{3}H activity.

b) Counting of chlidanthine fraction showed 2.07% of total ^{3}H activity.

Chlidanthine (21 mg.) was diluted with inactive chlidanthine (9 mg.) and further purification was achieved by crystallisation from methanol. After even four successive crystallisations the alkaloid showed a drop in the activity.

No.of crystallisations	^{3}H activity of duplicates (disint./min./mmole x 10^{5})	^{3}H activity of duplicates (disint./min./mg.x10^{2})
1	a) 2.821 b) 2.318	a) 9.827 b(8.076
2	a) 1.038 b) 1.119	a) 3.616 b) 3.901
3	a) 0.7302 b) 0.7199	a) 2.544 b) 2.509
4	a) 0.4928 b) 0.4933	a) 1.717 b) 1.719

Therefore % incorporation = $\frac{1.718 \times 10^{4} \times 30 \times 10^{2}}{9.46 \times 10^{7}}$

= 0.0054

Seven day experiment

Precursor (±)-[^{3}H]Galanthamine

Total ^{3}H activity fed to the plants 0.134 mCi

or 2.94×10^{8} disint./min.

Wet weight of the plants 254 g.

Crude alkaloids isolated 0.263 g.

Total ^{3}H activity isolated 1.795×10^{8} disint/min.

Recovery of ^{3}H activity fed = $\frac{1.795 \times 10^{8} \times 10^{2}}{2.94 \times 10^{8}} = 60.89\%$

1st preparative chromatography

Fraction No.	Weight in mg.	Major alkaloid	^{3}H activity (disint./min.x10^{6})	% of the activity fed
1	28	a) fluorescent compound b) galanthamine	14.3	48.64
2	37	a)galanthamine b) lycorine	0.181	0.6180
*3	84	a)lycorine b)chlidanthine c)unidentified alkaloid	1.75	5.95
4	8	residue at starting point	5.45	1.854

2nd preparative chromatography of the chlidanthine-rich fraction 3 (84 mg.).

Fraction No.	Weight in mg.	Major alkaloid	^3H activity (disint./min. $x10^6$)	% of the total activity fed
1	20	a)fluorescent compound b)galanthamine	3.189	1.085
2	3	galanthamine	10.74	3.65
3	11	a)galanthamine b)lycorine	1.709	0.5811
*4	28	a)lycorine b) chlidanthine c)unidentified alkaloid	1.445	0.4916
*5	11	a)chlidanthine b)residue at starting point.	1.329	0.4521

3rd preparative chromatography of the chlidanthine-rich fractions 4 and 5 (39 mg.) by diluting with inactive (-)-galanthamine(10 mg.).

Fraction No.	Weight in mg.	Major alkaloid	^3H activity (disint./min. $x\ 10^5$)	% of the total activity fed
1	12	galanthamine	3.63	0.1235
2	2	lycorine	1.11	0.03981
*3	20	a)lycorine b)chlidanthine c)unidentified alkaloid	7.45	0.2534
*4	6	a)chlidanthine b)unidentified alkaloid	2.96	0.1007
5	3	residue at starting point	7.67	0.2609

Fractions 3 and 4 (26 mg.) rich in chlidanthine were diluted with inactive (-)-chlidanthine (11 mg.). Further purification was achieved by crystallisation from methanol. After three successive crystallisations the activity of the alkaloid remained constant.

No.of crystallisations	^3H activity of duplicates (disint./min./mmole x 10^6)	^3H activity of duplicates (disint./min./mg. x 10^3
1	a) 2.456	a) 8.557
	b) 2.642	b) 9.204
2	a) 1.838	a) 6.595
	b) 1.872	b) 6.583
3	a) 1.816	a) 6.473
	b) 1.761	b) 6.363

Therefore % incorporation = $\frac{6.473 \times 10^3 \times 37 \times 10^2}{2.94 \times 10^8}$

= 0.08147

Seven day experiment

Precursor		$(\pm)$-[^3H]Epigalanthamine
Total ^3H activity fed to the plants		0.112 mCi
	or	2.46×10^8 disint./min.
Wet weight of the plants		60 g.
Crude alkaloids isolated		33 mg.
Total ^3H activity isolated		1.362×10^8 disint./min.
Recovery of ^3H activity fed		$\frac{1.362 \times 10^8 \times 10^2}{2.46 \times 10^8}$ =55.27%

1st preparative chromatography

Fraction No.	Weight in mg.	Major alkaloid	^3H activity (disint./min. x 10^6)	% of the total activity fed
1	2	fluorescent compound	2.67	1.085
2	5	a)galanthamine b)lycorine	58.7	23.86
3	7	a)lycorine b)epigalanthamine	35.2	14.31
*4	8	a)lycorine b)chlidanthine c)unidentified alkaloid	5.15	2.093
*5	11	a)chlidanthine b)unidentified alkaloid	0.73	0.2968
6	4	residue at starting point	3.8	1.545

Further purification of the chlidanthine-rich fractions 4 and 5 (19 mg.) was achieved by chemical separation. They were diluted with inactive (-)-epigalanthamine (10 mg.) and (-)-chlidanthine (8 mg.). To get a homogeneous mixture the alkaloids were dissolved in chloroform. Evaporation of the solvent gave a gum (37 mg.) which was treated with 2N-sodium hydroxide (0.2 ml.). The insoluble epigalanthamine was filtered off and the filtrate containing chlidanthine was basified with a slight excess of sodium hydrogen carbonate. The aqueous solution was extracted with chloroform several times. The chloroform extract was washed well with water and dried over anhydrous sodium sulphate. Evaporation of the chloroform gave crystalline chlidanthine (22mg.).

a) Counting of alkali insoluble epigalanthamine showed 0.647% total activity

b) Counting of the chlidanthine fraction showed 1.85% total activity.

The chlidanthine fraction (22 mg.) was diluted with inactive (-)-chlidanthine (8 mg.). Further purification was achieved by crystallisation from methanol. After even four successive crystallisations the alkaloid activity was still dropping.

No. of crystallisations	^3H activity of duplicates (disint./min./mmole x 10^5)	^3H activity of duplicates (disint./min./mg. x 10^2)
1	a) 12.41	a) 43.22
	b) 12.33	b) 42.95
2	a) 3.102	a) 10.81
	b) 2.952	b) 10.29
3	a) 2.805	a) 9.772
	b) 2.784	b) 9.70
4	a) 2.236	a) 7.787
	b) 2.155	b) 7.509

Therefore % incorporation $= \frac{7.648 \times 30 \times 10^4}{2.46 \times 10^8}$

$= 0.0093$

Seven day experiment

Precursor		(±)-[^{3}H]Narwedine
Total ^{3}H activity fed to the plants		0.173 mCi
	or	3.806 c 10^8 disint./min.
Wet weight of the plants		77 g.
Crude alkaloids isolated		70 mg.
Total ^{3}H activity isolated		1.49×10^8 disint./min.
Recovery of ^{3}H activity fed		$\frac{1.49 \times 10^8 \times 10^2}{3.806 \times 10^8} = 39.13\%$

1st preparative chromatography

Fraction No.	Weight in mg.	Major alkaloid	^{3}H activity (disint./min.x10^6)	% of the total activity fed
1	7	narwedine	1.76	0.4624
2	7	a)narwedine b)galanthamine	69.7	18.31
3	5	galanthamine	49.2	12.93
*4	13	a)lycorine b)chlidanthine	2.55	0.6699
*5	8	a)lycorine b)chlidanthine c)unidentified alkaloid	0.887	0.2330
6	6	residue at starting point	2.41	0.6331

2nd preparative chromatography of the chlidanthine-rich fractions 4 and 5 (21 mg.) by diluting with inactive (±)-narwedine (11 mg.).

Fraction No.	Weight in mg.	Major alkaloid	^3H activity (disint./min. x 10^5)	% of the total activity fed
1	17	a)narwedine b) galanthamine	12.47	0.3275
2	negligible	galanthamine	4.73	0.1243
3	negligible	lycorine	3.54	0.09301
*4	8	chlidanthine	8.92	0.2344
*5	3	a)chlidanthine b)unidentified alkaloid	3.23	0.0848
6	3	residue at starting point	19.65	0.5163

3rd preparative chromatography of the chlidanthine-rich fractions 4 and 5 (11 mg.) by diluting with inactive (-)-galanthamine (10 mg.) and (-)-chlidanthine (9 mg.).

Fraction No.	Weight in mg.	Major alkaloid	^3H activity (disint./min. x 10^4)	% of the total activity fed
1	1	fluorescent compound	1.72	0.0045
2	12	galanthamine	2.7	0.05702
3	1	lycorine	4.97	0.013
*4	16	a)chlidanthine b)unidentified alkaloid	75.7	0.1989
*5	1	a)chlidanthine b)unidentified alkaloid	6.57	0.0172
6	negligible	residue at starting point	5.05	0.0132

Fractions 4 and 5 (17 mg.) rich in chlidanthine were diluted with inactive (-)-chlidanthine (13 mg.). Chlidanthine was further purified by crystallisation from methanol. After three successive crystallisations the activity of the alkaloid remained constant.

No. of crystall-ations	^{3}H activity of duplicates (disint./min./mmole x 10^{6})	^{3}H activity of duplic-ates (disint./min./mg. x 10^{3})
1	a) 2.870	a) 10.03
	b) 3.297	b) 11.49
2	a) 2.379	a) 8.287
	b) 2.292	b) 7.986
3	a) 2.379	a) 8.287
	b) 2.344	b) 8.168

Therefore % incorporation = $\frac{8.287 \times 10^{3} \times 30 \times 10^{2}}{3.806 \times 10^{8}}$

= 0.065

Incorporation of (±)-[^{3}H]narwedine into galanthamine

Fraction 3 (5 mg.) from 1st preparative chromatography containing radioactive galanthamine was diluted with inactive (-)-galanthamine (31 mg.) and (±)-narwedine (34 mg.). The total mixture (70 mg.) was chromatographed on grade III neutral alumina (10 g.). Elution with ethyl acetate-benzene (1:9) and (1:4) separated the two alkaloids.

Fraction No.	Weight in mg.	Major alkaloid	^{3}H activity (disint./min. x 10^{5})	% of the total activity fed
1	30	narwedine	4.037	0.106
*2	11	a)narwedine b)galanthamine	12.99	4.297
3	20	galanthamine	20.81	5.46

2nd column chromatography of the fraction 2 (11 mg.) containing a mixture of (±)-narwedine and (-)-galanthamine in usual manner.

Fraction No.	Weight in mg.	Major alkaloid	^{3}H activity (disint./min.x 10^{6})	% of the total activity fed
1	neglible	narwedine	0.034	0.0088
2	1	a)narwedine b)galanthamine	1.615	0.4242
3	10	galanthamine	9.005	2.365

Thus total galanthamine obtained (30 mg.) was further diluted with inactive (-)-galanthamine (17 mg.) and further purified by crystallisation from ether. After two successive crystallisations the activity of the alkaloid remained constant.

No. of crystallis-ations	^3H activity of duplic-ates (disint./min./ mmole x 10^8)	^3H activity of duplic-ates (disint./min./ x 10^5)
1	a) 1.82	a) 6.34
	b) 1.81	b) 6.32
2	a) 1.784	a) 6.219
	b) 1.781	b) 6.217

Therefore % incorporation into galanthamine

$$= \frac{6.219 \times 10^5 \times 47 \times 10^2}{3.806 \times 10^8}$$

$$= 7.67$$

Oxidation of radioactive galanthamine

Radioactive galanthamine (6 mg.) was diluted with (-)-galanthamine (35 mg.). Oxidation of galanthamine was carried out with chromic oxide in the usual way. After working up the reaction mixture the residue showed two spots with R_f 0.74 and 0.49 corresponding to the keto-aldehyde (48) and narwedine and a very faint spot with R_f 0.46 corresponding to galanthamine on t.l.c. It was chromatographed over grade III neutral alumina (10 g.) Elution with ethyl acetate-benzene (1:9) gave the keto-aldehyde (10 mg.) and elution with ethyl acetate-benzene (1:4) gave narwedine (20 mg.)

a) Counting of the keto-aldehyde (10 mg.) fraction gave 4.191×10^5 disint./min.

b) Counting of the narwedine fraction (20 mg.) gave 3.3×10^4 disint./min.

The keto-aldehyde and narwedine were further purified by crystallisation from methanol. After two successive crystallisations the activity of the alkaloids remained constant.

Compound	No. of crystallisations.	^{3}H activity (disint./min./mg.)
(±)-Narwedine	1	2.2×10^{2}
	2	1.54×10^{2}
ketoaldehyde	1	3.179×10^{4}
	2	2.963×10^{4}
	3	2.769×10^{4}

Thus the oxidation of radioactive galanthamine (relative molar activity 1.00) gave (±)-narwedine (r.m.a. 0.01) and the keto-aldehyde (48) (r.m.a. 0.32). It was therefore possible to get useful evidence that the biogenesis in Chlidanthus fragrans had occurred without scrambling of tritium.

The radioactive purity of the chlidanthine obtained in various feeding experiments described above was checked by preparing the methyl ether methiodide derivative. Radioactive chlidanthine was methylated by dimethyl sulphate in the presence of alkali. After the addition of saturated aqueous potassium iodide the methyl ether methiodide of chlidanthine precipitated out. The derivative was crystallised from methanol as needles. There was considerable difficulty

in counting due to the insolubility of this derivative in the system used [dry dimethylformamide (0.1 ml.) and liquid scintillator (5 ml.)]. The counting was then done in a system, dry dimethylformamide (0.3 ml.) and liquid scintillator (4.8 ml.). There was no significant drop in the activity after two crystallisations from methanol. It was difficult to carry out a 3rd crystallisation due to the small amounts of derivative available. The results are tabulated below. Large variations between duplicates and successive crystallisations were however observed possibly due to precipitation of the sparingly soluble derivative which was obviously not ideal for this purpose.

Chlidanthine derivative from various feedings:	No.of crystallisations:	^3H activity (disint./min./mmole)	^3H activity (disint./min./mg.)	^3H activity of chlidanthine (disint./min./mg.)
1) Two day experiment, precursor - galanthamine	1	2.65×10^4	1.17×10^7	3.68×10^4
	2	1.65×10^4	0.73×10^7	
2) Two day experiment, precursor - epigalanthamine	1	1.26×10^2	5.57×10^4	1.72×10^2
	2	2.14×10^2	9.46×10^4	
3) Seven day experiment, precursor- narwedine	1	4.84×10^3	1.70×10^6	8.28×10^3
	2	3.85×10^3	2.15×10^6	
4) Seven day experiment, precursor - galanthamine	1	4.56×10^3	2.02×10^6	6.47×10^3
	2	5.80×10^3	2.56×10^6	
5) Seven day experiment, precursor- epigalanthamine	1	4.43×10^2	1.96×10^5	7.64×10^2
	2	4.02×10^2	1.78×10^5	

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