# STUDIES IN ALKALOIDS BIOSYNTHESIS OF

a thesis submitted by

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London S.W.7

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## ABSTRACT

The biosynthesis of the Amaryllidaceae alkaloids is reviewed in the light of recent tracer studies. Feeding experiments with $^{14}C$ and $^{3}H$ labelled O-methylnorbelladine have shown this phenol to be a precursor for norpluviine. Various degradations have been worked out to establish the labelling pattern in the alkaloid. On the basis of the results obtained, possible routes for the biosynthesis of norpluviine are discussed. Norpluviine has been shown to be an intermediate in the biosynthesis of galanthine.

The existence of long range proton coupling in narwedine and Pummerer's ketone has been established by n.m.r. studies. The configuration of the allylic alcohols derived from Pummerer's ketone has been determined.

## ACKNOWLEDGEMENTS

The author expresses his deep gratitude to Professor D.H.R. Barton for the privilege of working under his supervision and for the stimulating discussions and help at all stages.

To Dr. G.W. Kirby, the author is indebted for his friendly guidance and unfailing interest throughout the course of this work.

Thanks are also due to Mr. D.R. Aldrich and I. Scobie for invaluable technical assistance and to Mrs. Brown for all her help.

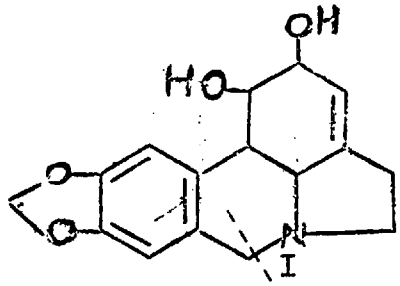
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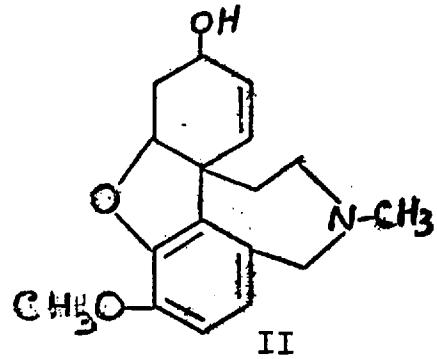
REVIEW OF AMARYLLIDACEAE ALKALOID BIOSYNTHESIS.

Plants of the Amaryllidaceae family produce a large variety of alkaloids of widely diverse functionality and structures. These have been the subject of intensive investigation during the past few years, and an authoritative summary of their chemistry has been published by Wildman.[1]

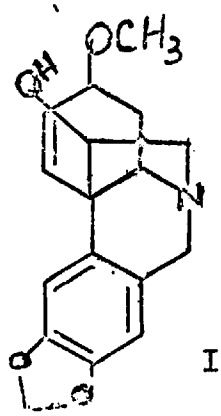
The alkaloids of this family can be broadly divided into three main classes based on their carbon skeleton type. These three main classes are, (a) alkaloids containing a pyrrolophenanthridine skeleton, for example lycorine (I), (b) alkaloids containing a dibenzofuran skeleton, for example, galanthamine (II), and (c) alkaloids containing an ethanophenanthridine ring system, for example, haemanthamine (III).




I

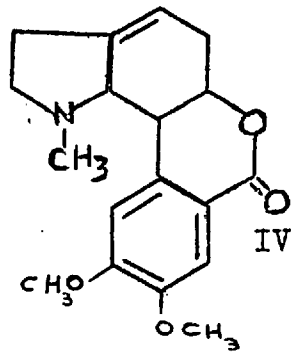



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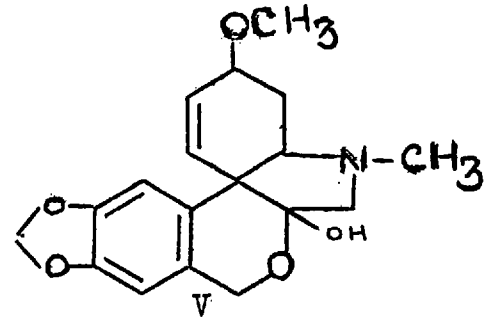



III

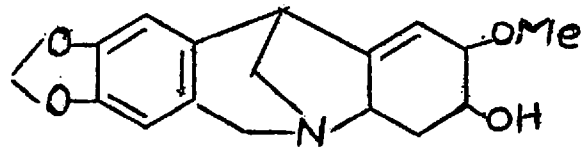
The other classes of alkaloids of this family can, in principle, be derived from these three main classes by process of cleavage, rearrangement, and recyclisation. For example, homolycorine (IV), having a benzpyranoindole skeleton, could be derived by the oxidative cleavage of (I) at the indicated position, and recyclisation. Similarly tazettine (V), one of the most structurally complex Amaryllidaceae alkaloids, could be derived by oxidative cleavage of the haemanthamine type skeleton, followed by an internal oxidation, reduction, and recyclisation. Rearrangements of the carbon skeleton are also possible.[2] The alkaloid montanine (VI) could be derived in this manner.




IV




V



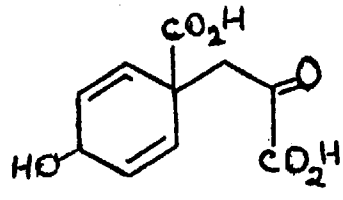

VI

In spite of the apparent diversity in structures of the Amaryllidaceae alkaloids, they are, in fact, very closely related. All these alkaloids contain a common hydroaromatic $C_6$-$C_2$ unit and an aromatic $C_6$-$C_1$ unit. Theories for the biogenesis of these alkaloids have been proposed by Wenkert[3], and by Barton and Cohen[4]. Much work has been done on the biogenesis of these alkaloids and has been discussed in two recent reviews by Battersby[5], and by Barton[6].

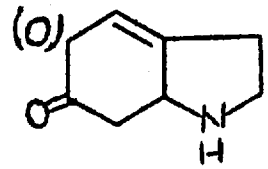
According to Wenkert, these alkaloids are derived from prephenic acid (VII), which by amination and hydration gives rise to pyrroles (VIII) and (IX). Condensation of pyrrole (VIII) with shikimic acid (X) would give the intermediate (XI). A Michael reaction followed by subsequent hydration-dehydration and oxidation-reduction changes could give rise to the lycorine skeleton (XII).

The second pyrrole (IX) by similar condensation with shikimic acid would give the intermediate (XIII), which by similar sequences of reactions could give rise to the haemanthamine skeleton (XIV). Quarternisation of nitrogen, followed by cleavage at bond "a" would lead to the dienone (XV), which by simple changes would give the galanthamine skeleton. Cleavage at bond "b" would lead to the tazettine skeleton.

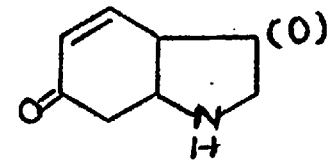
Wenkert's scheme for Biogenesis of Amaryllidaceae Alkaloids.



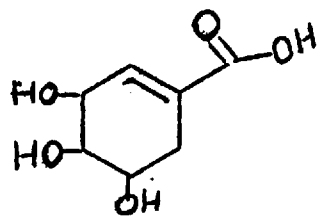
VII



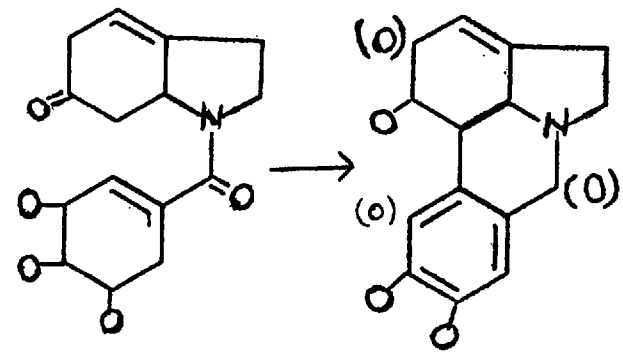
VIII



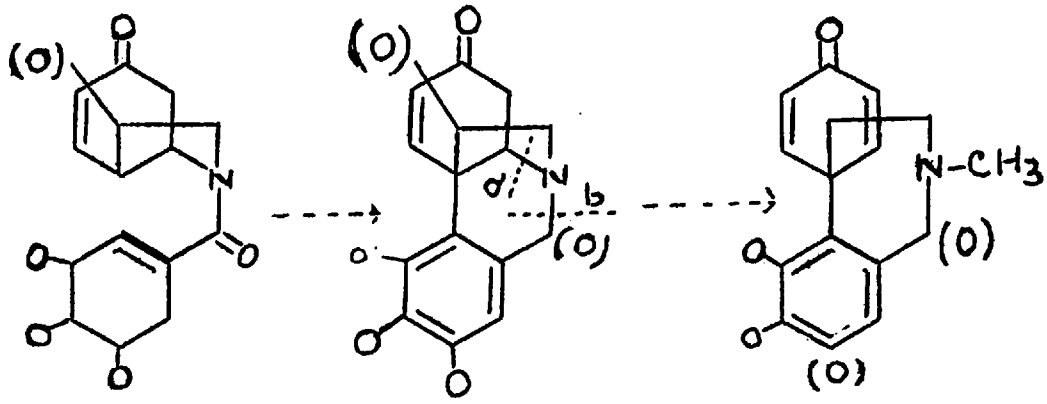
IX



X



XI XII



XIII XIV XV

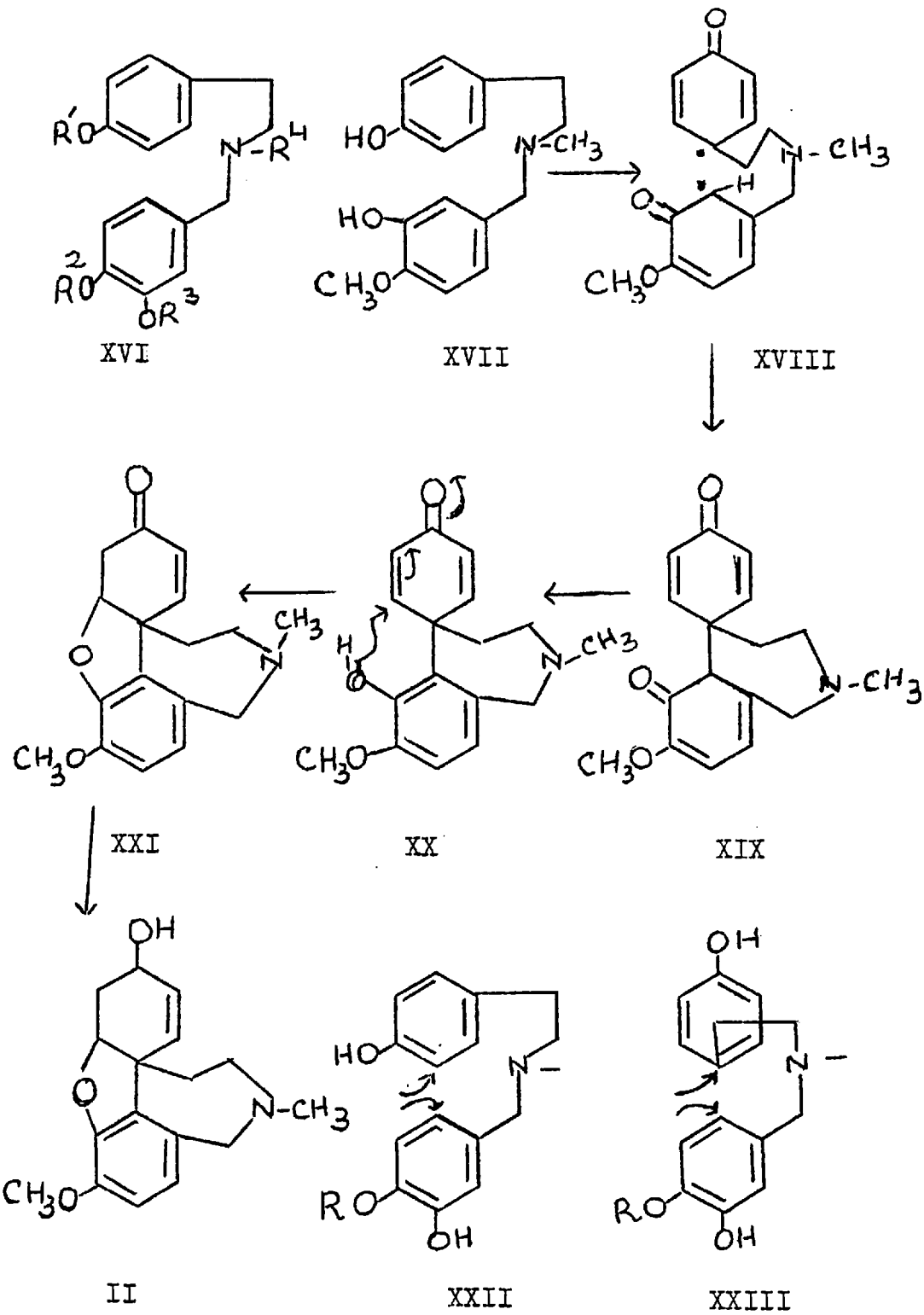
Barton and Cohen, on the other hand, suggested that these alkaloids are formed by the oxidative coupling of the two phenolic rings of a precursor of general type (XVI, where R=H or a suitable blocking group, such as an alkyl group, or part of an enzyme surface). Ortho and para coupling reactions of this precursor can then account for the variety of the structures in the family.

Oxidation of the diphenolic amine (XVII) would give the diradical (XVIII), which on radical pairing would give the dienone (XIX). Aromatisation of the bottom ring, followed by the addition of hydroxyl group across the unsaturated system in (XX) would give the oxide bridge in (XXI). Reduction of the unsaturated ketone (XXI) would then give galanthamine (II).

A similar sequence of reactions involving an ortho-para coupling of the precursor, as indicated in (XXII), would give rise to the lycorine skeleton. The same precursor, written as (XXIII), could give the haemanthamine skeleton by para-para coupling at the indicated positions. The same precursor can, in fact, embrace all the alkaloids in the family.

There is an essential difference between the two biogenetic concepts outlined above. According

Barton and Cohen's scheme for Biogenesis of Amaryllidaceae Alkaloids.

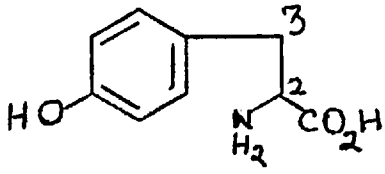


to Wenkert's postulation, the $C_6$-$C_2$ unit of the alkaloids is derived from a hydroaromatic precursor which never becomes aromatic. On the other hand, according to Barton and Cohen, the $C_6$-$C_2$ unit is, in fact, derived from an aromatic precursor.

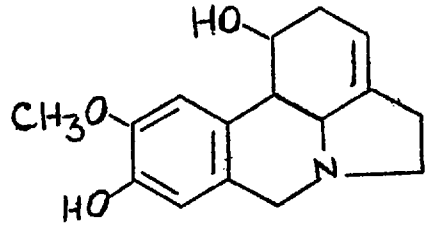
## Origin of the $C_6$-$C_2$ Unit.

The work on the biogenetic origin of the $C_6$-$C_2$ unit of the Amaryllidaceae family started in 1960. In preliminary experiments, Barton and Kirby[7] injected $\left[2\text{-}^{14}C\right]$ tyrosine (XXIV) into 'King Alfred' daffodils. The galanthamine, isolated from these plants, was found to be active. They did not carry out the necessary degradations to establish the labelling pattern in the alkaloid.

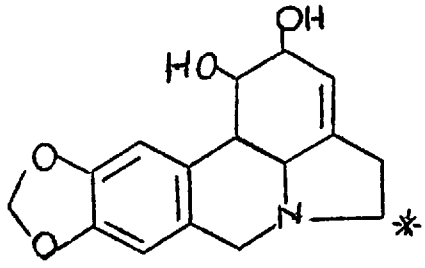
In an independent study, Battersby, Binks, and Wildman[8] isolated radioactive lycorine (XXV, 0.23% incorporation) and norpluviine (XXVI) from 'Twink' daffodils, injected with $\left[2\text{-}^{14}C\right]$ tyrosine. The position of the radioactive label in the lycorine was established as follows.



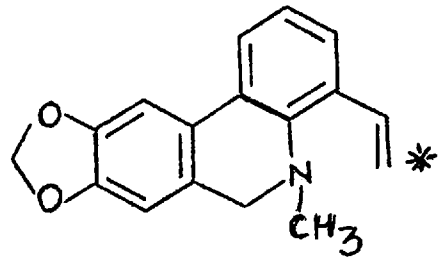
XXIV



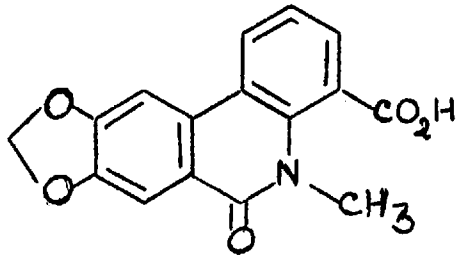
XXVI



XXV



XXVII



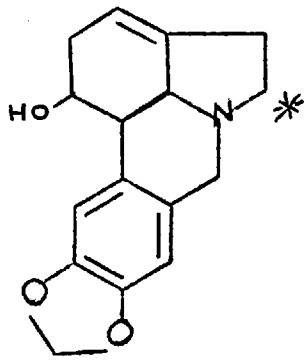
XXVIII

+ $\overset{*}{\text{HCHO}}$

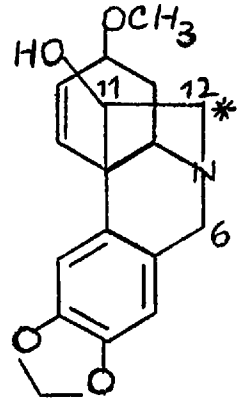
Methylation of the radioactive lycorine gave a mixture of $\alpha$- and $\beta$-lycorine methiodides, which were subjected to Hofmann degradation. The resulting methine (XXVII) was hydroxylated and cleaved to give formaldehyde, collected as its dimedone derivative, containing almost all the activity. The residue, without isolation, was further oxidised to the lactam acid (XXVIII), which, as expected, was virtually inactive. The lack of activity elsewhere, clearly excludes the breakdown of tyrosine, and reincorporation of the fragments.

If the view, that the conversion of prephenic acid into tyrosine in daffodils is irreversible, as seems the case in other systems,[9] is accepted, then the above results establish that tyrosine can provide the $C_6$-$C_2$ unit in accordance with the concept of Barton and Cohen, and contrary to that of Wenkert.

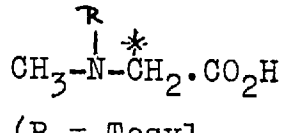
Further evidence for the origin of the $C_6$-$C_2$ unit from an aromatic precursor came from several groups of workers. Battersby, Fales, and Wildman[10] found that [2-$^{14}$C] tyrosine was also incorporated into haemanthamine (XXX, 0.078% incorporation) and caranine (XXIX, 0.79% incorporation). Degradation of radioactive haemanthamine (XXX) established the position of label as indicated.



XXIX

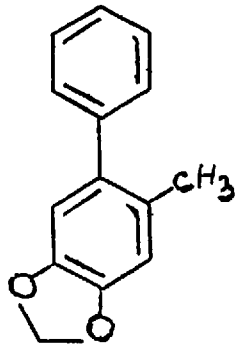


XXX

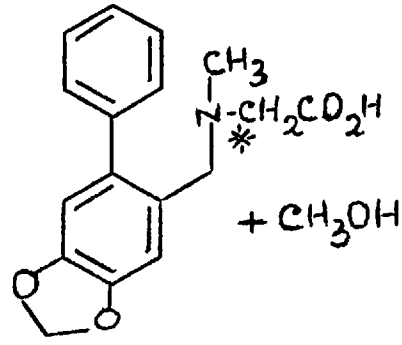


(R = Tosyl

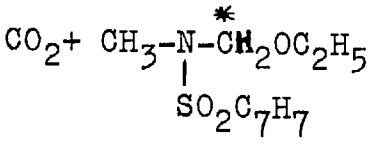
XXXIII



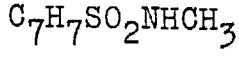
XXXII



XXXI



XXXIV

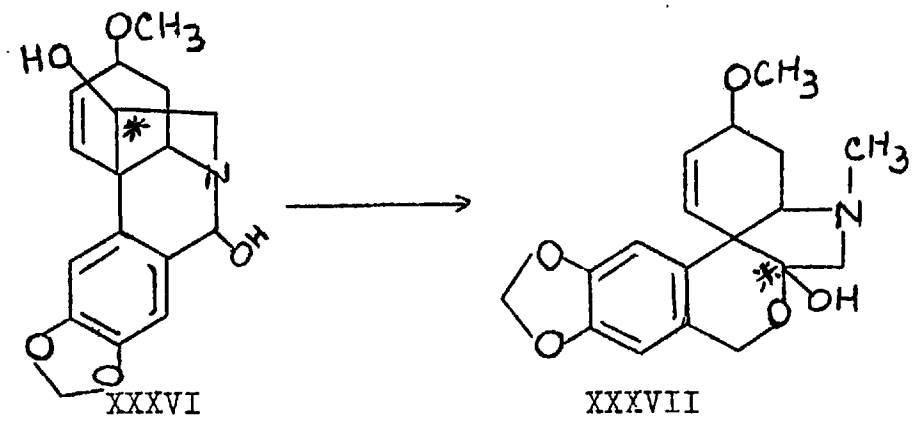


XXXV

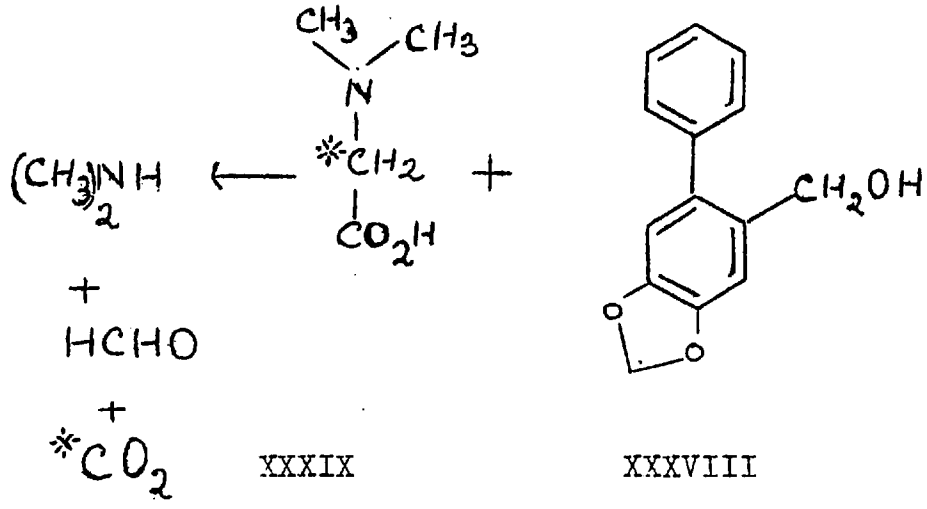


Radioactive haemanthamine was degraded to the acid (XXXI) and methanol by the method of Fales and Wildman.[11] The methanol, counted as its 3,5-dinitrobenzoate, was inactive. Hydrogenolysis of the acid (XXXI) gave the inactive biphenyl (XXXII) and sarcosine, isolated as its tosyl- derivative (XXXIII, R = tosyl), containing almost all the activity. Kolbe electrolysis of (XXXIII) gave carbon dioxide containing no activity, and N-ethoxymethyl-N-methyl-p-toluenesulphonamide (XXXIV), which was hydrolysed to give formaldehyde, containing virtually all the activity, and the inactive N-methyl-p-toluenesulphonamide (XXXV).

In another independent study, Wildman, Fales and Battersby[12] found that [3-$^{14}$C] tyrosine (XXIV), injected into Sprekelia formosissima was incorporated into haemanthamine, haemanthidine (XXXVI, 0.20% incorporation), and tazettine (XXXVII, 0.16% incorporation). Degradation of haemanthamine, as described above, established that all the activity was located at position 11. There was no activity at position 6. The haemanthidine (XXXVI) was converted into tazettine[13], which was degraded as follows.



XXXVI XXXVII



XXXIX XXXVIII

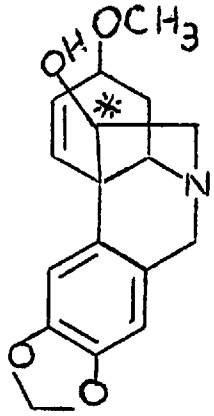
Hofmann degradation of radioactive tazettine gave the inactive 6-phenylpiperonyl alcohol (XXXVIII) and dimethylglycine (XXXIX), containing all the activity. Kolbe electrolysis of (XXXIX) gave radioactive carbon dioxide, containing all the activity and inactive formaldehyde. The same results were obtained from the degradation of biosynthetic tazettine.

Jeffs[15] also observed that [3-$^{14}$C] tyrosine was incorporated into haemanthamine (XL, 0.97% incorporation), haemanthidine (XXXVI, 0.19% incorporation) in Haemanthus natalensis bulbs. Degradation of haemanthamine, as outlined below, established the position of label at the asterisked carbon.

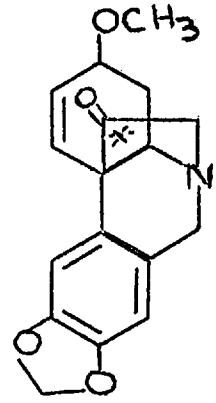
Radioactive haemanthamine (XL) was oxidised to oxohaemanthamine (XLI) and this was cleaved to the glycine derivative (XLII). Decarboxylation of (XLII) with ninhydrin gave carbon dioxide, which contained almost all the activity. The amide (XLIII) was not isolated. The acid (XLII) with N-chlorotoluene-p-sulphonamide at 100° gave carbon dioxide containing half the activity and 6-phenyl-piperonal (XLIV) having no activity.

[3-$^{14}$C] Tyrosine has also been found[16] to be incorporated into lycorine (0.17% incorporation)

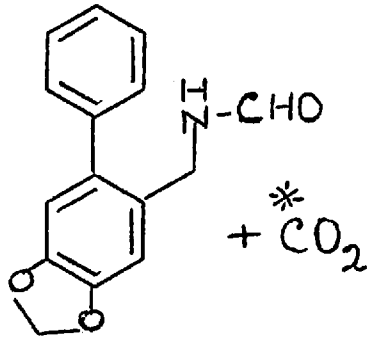
Degradation of Haemanthamine (Jeffs).



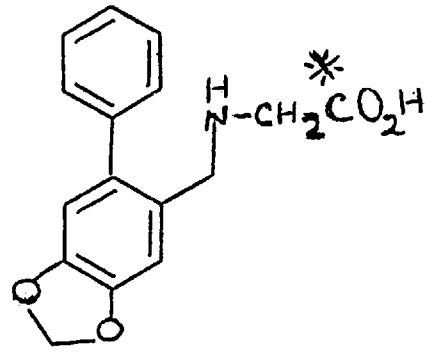
XL.



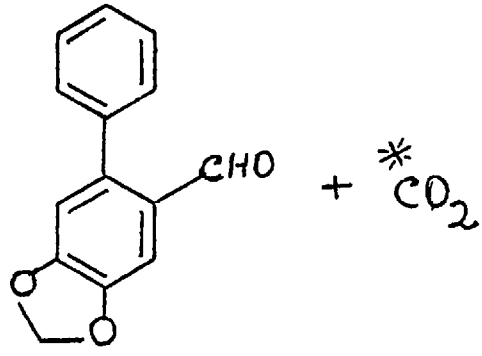
XLI



XLIII

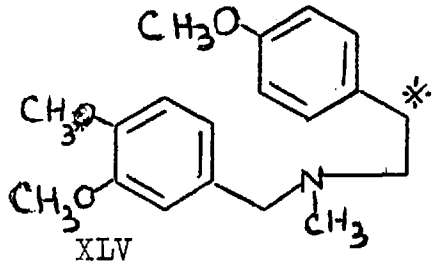


XLII

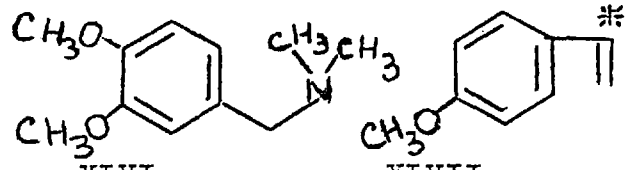


XLIV

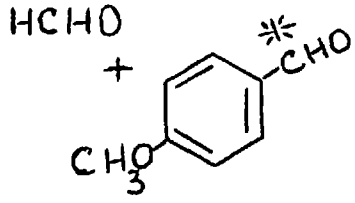
and belladine (XLV, 0.82% incorporation) in Nerine bowdenii. The belladine (XLV) was degraded by the method of Wildman et. al.,[17] as outlined below, to establish the label at the asterisked carbon.



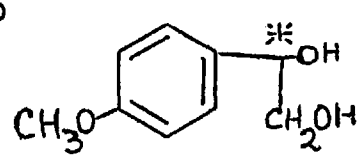
XLV



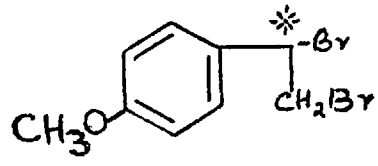
XLVI XLVII



L



XLIX



XLVIII

Belladine (XLV) was subjected to Hofmann degradation to give inactive N,N-dimethylveratryl-amine (XLVI) and p-methoxystyrene (XLVII). p-Methoxy-styrene was converted into the dibromo-derivative (XLVIII), which was then converted into the diol (XLIX). Cleavage of this diol gave anisaldehyde (L), containing all the activity, and formaldehyde having no activity. Radioactive lycorine was degraded as described earlier, and all the activity

was found to be at position 4.

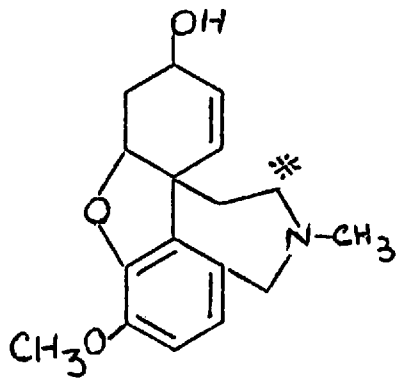
Barton and Coworkers[18] obtained radioactive galanthamine (LI, 0.012% incorporation) and haemanthamine (0.20% incorporation) from 'King Alfred' daffodils injected with [2-$^{14}$C] tyrosine. Radioactive galanthamine (LI) was degraded by the method based on the work of Kobayashi and Uyeo[19], to show the intact incorporation of tyrosine.

Treatment of radioactive galanthamine (LI) with aqueous hydrobromic acid, under reflux, gave apogalanthamine (LII) which was methylated to give the dimethyl ether (LIII). Emde degradation of the corresponding methochloride gave the base (LIV) which was further degraded by Hofmann elimination to give the vinyldiphenyl (LV), containing all the activity of the original alkaloid. Ozonolysis of (LV) gave formaldehyde, isolated as its dimedone derivative, containing all the activity.

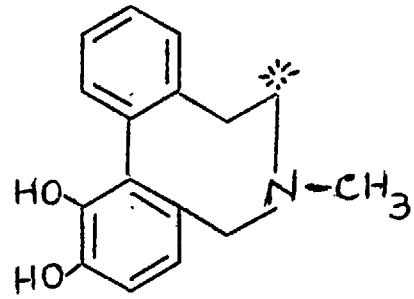
Suhadolnik and Fischer[20] also observed that [2-$^{14}$C] tyrosine was incorporated, intact, into the Amaryllidaceae alkaloids and that it served as a precursor for the $C_6$-$C_2$ unit.

The foregoing results indicate clearly that the hydroaromatic $C_6$-$C_2$ unit in the Amaryllidaceae

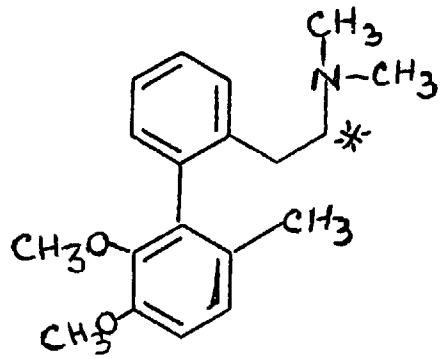
Degradation of Galanthamine (Barton et al.)



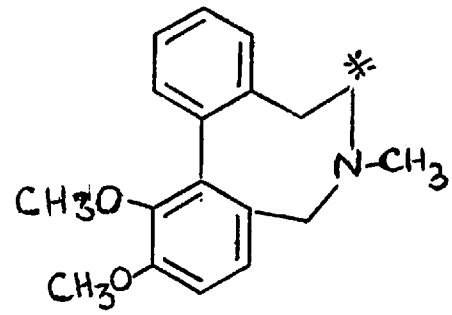
LI



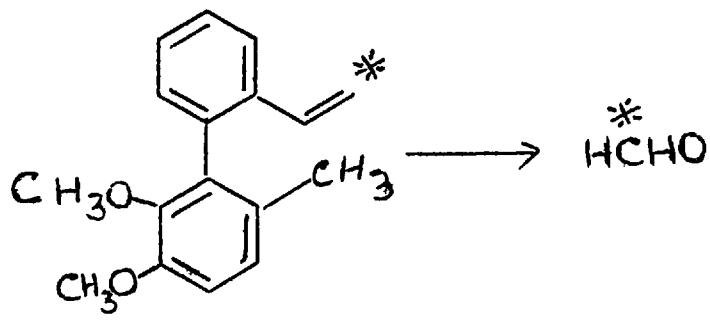
LII



LIV



LIII

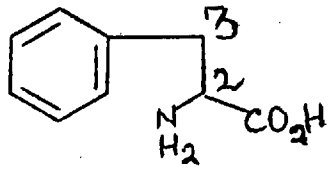


LV

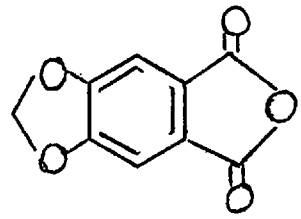
alkaloids originates from tyrosine. They also show that the aromatic $C_6$-$C_1$ unit is not derived from tyrosine.

Origin of the $C_6$-$C_1$ unit.

With the biogenetic origin of $C_6$-$C_2$ unit settled, the attention was diverted towards the $C_6$-$C_1$ unit. Suhadolnik, Fischer, and Zulalian[21] fed $[3\text{-}^{14}C]$ phenylalanine (LVI), $[2\text{-}^{14}C]$ phenylalanine, $[2\text{-}^{14}C]$ tyrosine, and $[3\text{-}^{14}C]$ tyrosine to Narcissus incomperabilis plants. All these compounds, with the exception of $[2\text{-}^{14}C]$-phenylalanine were incorporated into lycorine. The radioactive lycorine obtained from these experiments was degraded to hydrastic anhydride (LVII) according to the method of Warnhoff and Wildman[22].




LVI



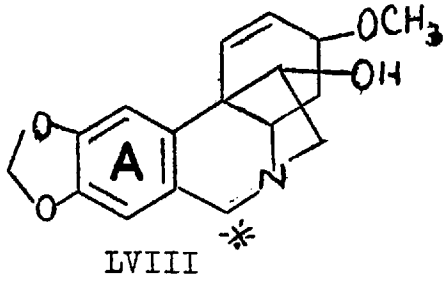
LVII

Hydrastic anhydride obtained by the degradation of lycorine from $[3\text{-}^{14}C]$ phenylalanine feeding alone was found to be active. In other three cases it

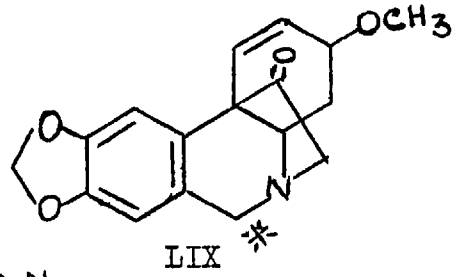
was inactive. These results show that [3-$^{14}$C] phenylalanine can act as precursor for lycorine.

Confirmation for these results came from the work of Wildman, Battersby, and Breuer[16]. They obtained radioactive lycorine (0.095% incorporation) and belladine (0.42% incorporation) from the feeding of [3-$^{14}$C] phenylalanine to Nerine bowdenii. Degradation of these alkaloids, in the manner described earlier, established that phenylalanine can serve as a precursor of ring A and the benzylic carbon atom in both lycorine and belladine, but is unable to provide the $C_6$-$C_2$ unit.

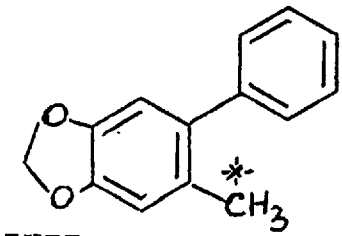
Suhadolnik, Fischer and Zulalian also observed that [3-$^{14}$C] phenylalanine was incorporated into haemanthamine (LVIII). To establish the position of the label at the asterisked carbon, radioactive haemanthamine was degraded as follows



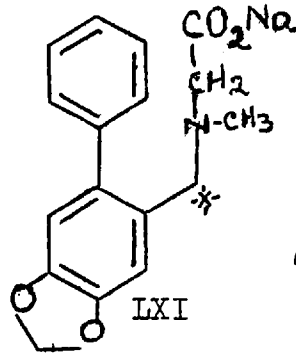

LVIII



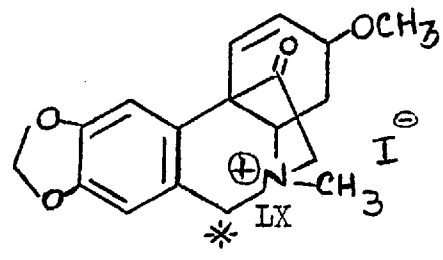

LIX




LXII




LXI




LX

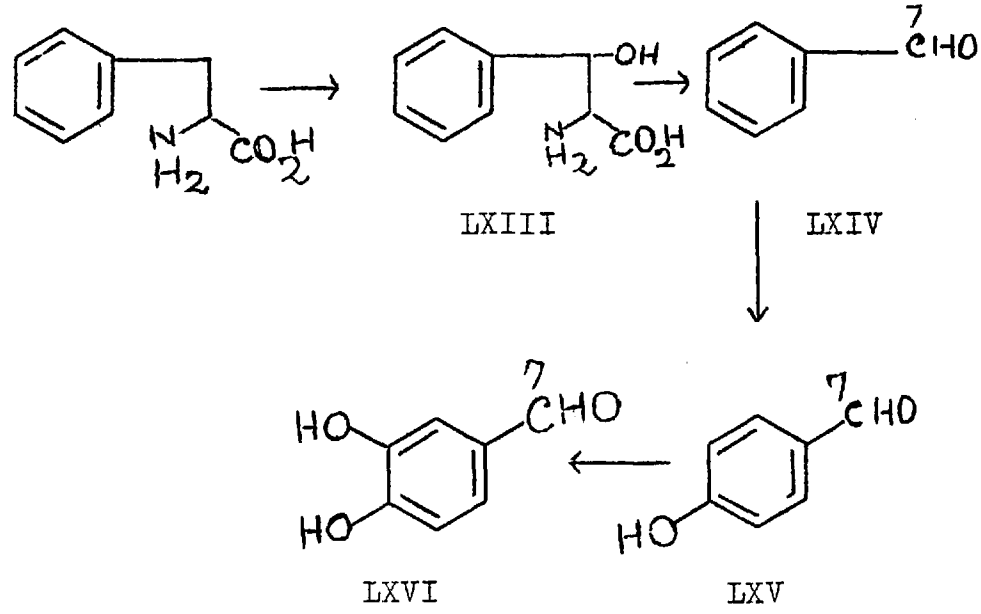
Radioactive haemanthamine (LVIII) was oxidised to oxohaemanthamine (LIX), which was converted into the methiodide (LX), and cleaved to form the glycine salt (LXI). Hydrogenolysis of (LXI) afforded 2-methyl-4,5-methylenedioxybiphenyl (LXII), which contained virtually all the activity. These results also support the origin of ring A and the benzylic carbon atom from phenylalanine.

Two pathways, shown on the page 21, were considered for the biogenetic conversion of phenylalanine into the $C_6$-$C_1$ unit of the Amaryllidaceae alkaloids. According to the first scheme, phenylalanine is converted into protocatechuic aldehyde (LXVI) via phenylserine (LXIII) ⟶ benzaldehyde (LXIV) ⟶ p-hydroxybenzaldehyde (LXV). The second scheme envisages the conversion of phenylalanine into protocatechuic aldehyde through trans- cinnamic acid (LXVII) ⟶ p-coumaric acid (LXVIII) ⟶ caffeic acid (LXIX).

Suhadolnik, Fischer and Zulalian[21] observed that $[^3H]$ protocatechuic aldehyde was incorporated into lycorine. Radioactive lycorine was degraded to hydrastic anhydride, which contained all the activity of the original alkaloid. This proves that phenylalanine is converted to protocatechuic aldehyde before it is incorporated into the alkaloid.

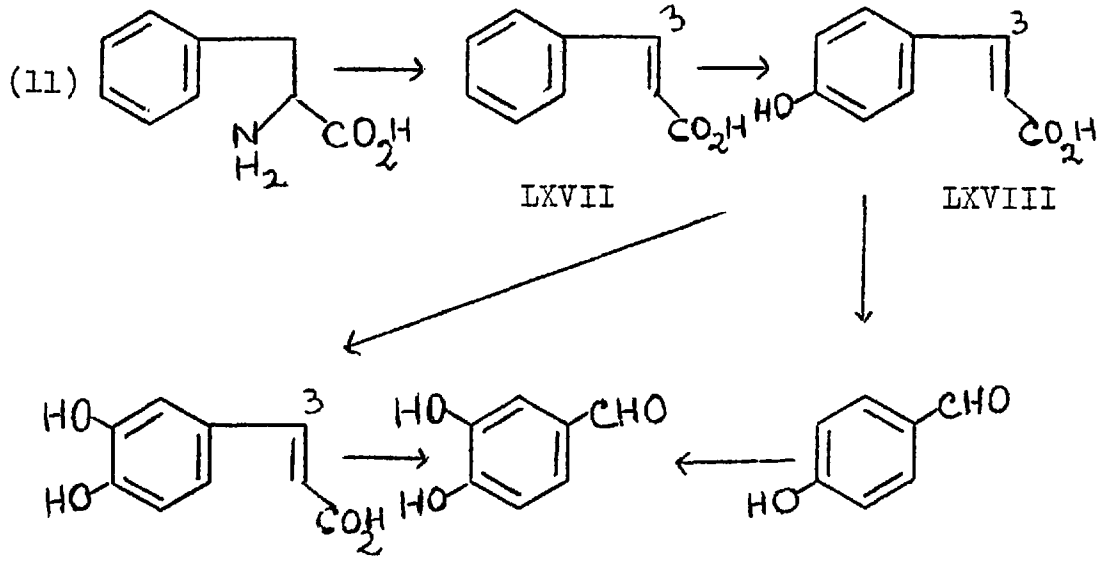
Pathways for the Biogenetic conversion of Phenylalanine into $C_6$-$C_1$ Unit.

(1)




LXIII LXIV LXVI LXV

(11)




LXVII LXVIII LXIX

To decide between the two pathways for the conversion of phenylalanine into protocatechuic aldehyde, these workers[24] fed [3-$^{14}$C] trans-cinnamic acid (LXVII), [3-$^{14}$C] p-coumaric acid (LXVIII), [7-$^{14}$C]-benzaldehyde (LXIV), and [7-$^{14}$C] p-hydroxybenzaldehyde (LXV) to N. pseudonarcissus plants, and also to the floral primordial tissue, from these plants, grown on sterile nutrient agar. The haemanthamine obtained from [3-$^{14}$C] trans-cinnamic acid and [3-$^{14}$C] p-coumaric acid feeding experiments was found to be active. Degradation of the radioactive haemanthamine, as described on page 19, showed that 100% and 86% of the activity from [3-$^{14}$C] cinnamic acid and [3-$^{14}$C] p-coumaric acid respectively resided in 2-methyl-4,5-methylenedioxybiphenyl (LXII). The haemanthamine obtained from the benzaldehydes feeding was inactive. These workers also found that caffeic acid (LXIX), randomly labelled with tritium, was incorporated into lycorine in N. incomparabilis. Threo-DL-phenylserine, on the other hand, was not incorporated.

Suhadolnik, Fischer and Zulalian[24] have also shown that the incorporation of trans-cinnamic acid into haemanthamine does not proceed by amination to form phenylalanine. They hydrolysed the

plant protein from [3-$^{14}$C] trans-cinnamic acid feeding experiment, and isolated the phenylalanine, which was found to be inactive. They also isolated the enzyme, reported[25,26] to be involved in deamination of phenylalanine into cinnamic acid, from the meristematic tissue of N. pseudonarcissus, and found that it deaminated phenylalanine to trans-cinnamic acid.

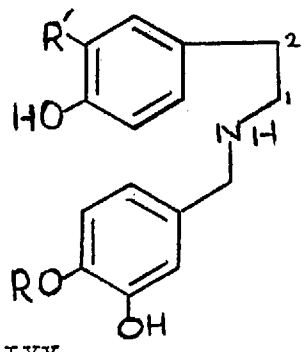
Battersby et al.[27] have also observed the incorporation of sodium trans- [3-$^{14}$C]cinnamate into lycorine in Nerine bowdenii plants. The radioactive lycorine was oxidised to hydrastic acid, which carried all the original activity. They also observed that [carboxyl-$^{14}$C] 3,4-dihydroxybenzoic acid was not significantly incorporated into lycorine, nor was the generally tritium-labelled isovanillin incorporated into lycorine or norpluviine. Barton and coworkers[18] found that 3-hydroxy-4-methoxy-N-methylbenzylamine, labelled at both methyl groups, was incorporated into galanthamine and haemanthamine without randomisation but with loss of the N-methyl group.

These results establish that the $C_6$-$C_1$ unit in the Amaryllidaceae alkaloids is derived from phenylalanine, which is incorporated into the

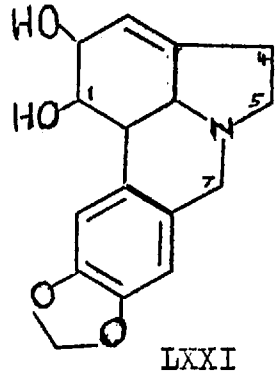
alkaloids via the route, phenylalanine ⟶ trans-cinnamic acid ⟶ p-coumaric acid ⟶ caffeic acid protocatechuic aldehyde.

Aromatic Intermediates.

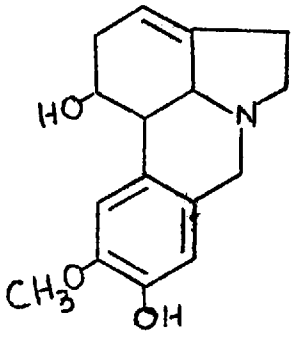
Barton and Cohen[4] proposed that a key step in the biosynthesis of the Amaryllidaceae alkaloids is the oxidative coupling of the aromatic intermediates such as the phenols (LXX, R'=H or OH).




LXX



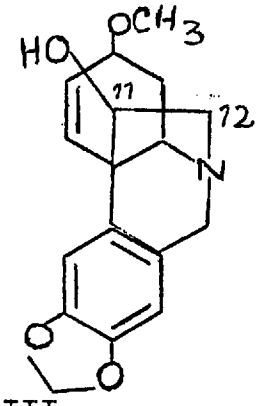

LXXI



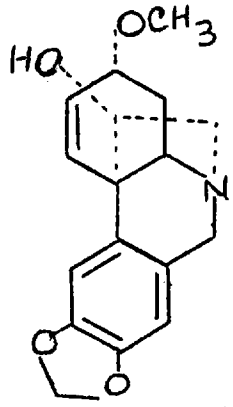

LXXII

In order to test this important proposal Battersby et al.[27,28] synthesised the [1-$^{14}$C]norbelladine (LXX, R=R'=H) and fed it to 'Twink' daffodils. It was incorporated well into lycorine (LXXI, 0.24% incorporation) and into norpluvine (LXXII, 0.74% incorporation). Degradation of the lycorine (page 8 ) gave formaldehyde which contained virtually all the activity. This showed that all the activity in the lycorine (LXXI) was located at the carbon atom 5. A higher incorporation of

norbelladine than that of tyrosine into norpluviine suggests that norbelladine stands closer to norpluviine in the biogenetic pathway. Battersby, Fales and Wildman[10] also observed the incorporation of [1-$^{14}$C] norbelladine into haemanthamine (LXXIII) and crinamine (LXXIV, the epimer of haemanthamine ) in 'Twink' daffodils. Degradation of haemanthamine (page 20 ) established the position of label at the carbon atom 12.

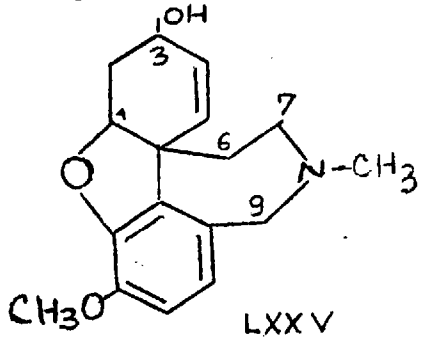



LXXIII

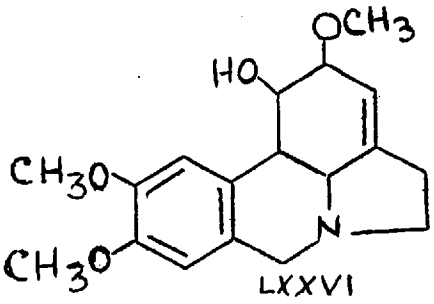



LXXIV

Barton et al.[18,29] also observed the incorporation of [1-$^{14}$C] norbelladine (LXX, R=R'=H) into galanthamine (LXXV, 0.014% incorporation), galanthine (LXXVI, 0.003% incorporation), and haemanthamine (LXXIII, 0.25% incorporation) in 'King Alfred' daffodils.

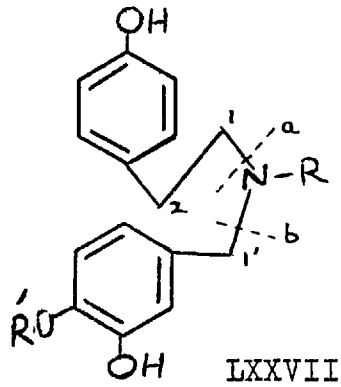



LXXV




LXXVI

Degradation of the radioactive galanthamine (page 17 ) established the position of the radioactivity at the carbon atom 7. These workers also prepared the phenols (LXXVII,a) and (LXXVII, b) labelled with $^{14}C$ in their N-methyl groups.




LXXVII

(a) $R=CH_3$; $R'=H$

(b) $R=R'=CH_3$

(c) $R=H$; $R'=CH_3$

(d) $R=R'=H$

These phenols were incorporated into galanthamine (LXXV) in 'King Alfred' daffodils. In order to prove that no transmethylation had taken place in the plant, the O-methyl and N-methyl groups were cleaved in turn with hydrogen iodide, and their activities determined. In each case, almost all the activity was located in the N-methyl group. It was observed that $\left[1\text{-}^{14}C\right]$ O-methylnorbelladine (LXXVII, C) was incorporated into haemanthamine but not into galanthamine. Thus, of the four biogenetically likely precursor phenols (LXXVII, a-d) only three (LXXVII, a,b and d) were incorporated into galanthamine. This difference in behaviour of closely related phenols of the type (LXXVII) led

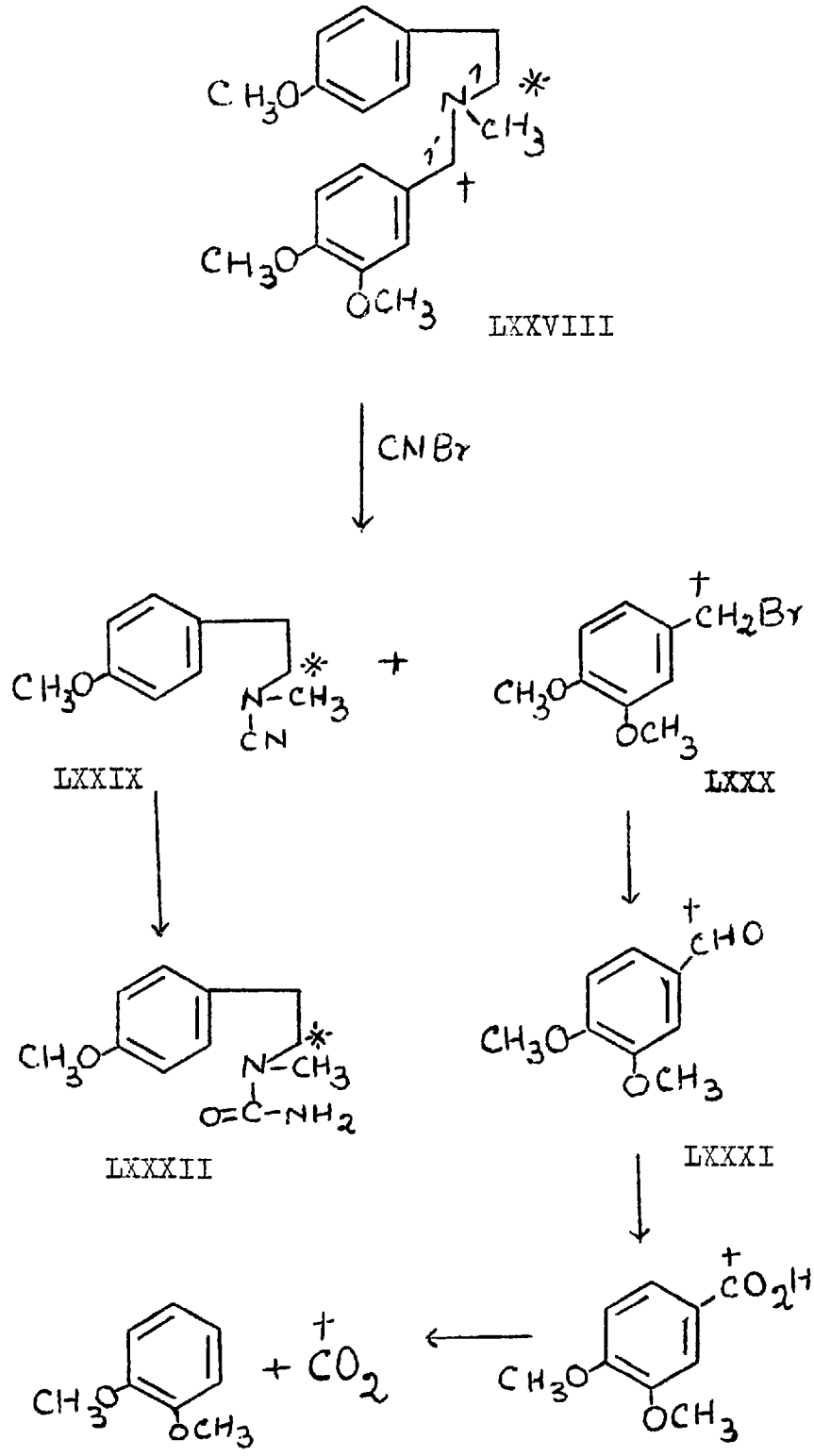
Barton *et al.*[18] to suggest that possibly a definite order of methylation exists in the biosynthesis of galanthamine, thus:

norbelladine ⟶ *N*-methylnorbelladine ⟶ *NO*-dimethyl-norbelladine ⟶ galanthamine.

These results show that norbelladine or its methyl-derivatives can serve as precursors in the biogenesis of the Amaryllidaceae alkaloids. They, however, do not rigorously exclude the possibility of the rupture of the precursors at bonds "a" and/or "b" prior to incorporation.

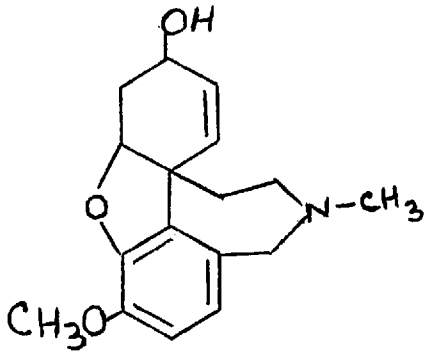
In order to prove that norbelladine is incorporated without any scrambling, Battersby *et al.*, fed the [1,1'-$^{14}$C] norbelladine (LXXVII, d) to *Nerine bowdenii*. It was incorporated into lycorine (LXXI, 0.07% incorporation), crinamine (LXXIV, 0.009% incorporation), and belladine (LXXVIII, 2.64% incorporation). In order to determine the relative amount of activity in the two labelled positions in the precursor, it was methylated to belladine (LXXVIII) which was cleaved by cyanogen bromide to give the bromide (LXXX) and the cyanamide (LXXIX). These were converted respectively into veratraldehyde (LXXXI) and the urea (LXXXII). The activities of these two showed that in [1,1'-$^{14}$C]

Degradation of Belladine (Battersby et al.)

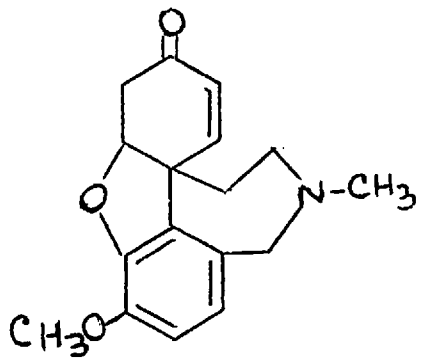


norbelladine, 22% of the activity was at position 1' and 78% at position 1. Each alkaloid was degraded by the standard methods as described earlier. The ratio of activities in the alkaloids was the same as that in the precursor within experimental error.

Further evidence for the intact incorporation of the phenolic precursors of the type (LXXVII) came from the work of Barton et al.[18]. They synthesised the phenol (LXXVII, b) with labels in both O- and N-methyl groups. This was incorporated into galanthamine (LXXXIII). Both the O- and N-methyl groups of the precursor and the alkaloid were cleaved by the modified Zeisel method. The liberated methyl iodide was trapped in triethylamine to give triethylmethylammonium iodide, which was counted. The relative activities of the O-methyl and the N-methyl groups in the precursor, the biosynthetic galanthamine, and the derived ($MnO_2$ oxdn.) narwedine (LXXXIV) were the same. This proved that bond "b"




LXXXIII




LXXXIV

in the precursor was not cleaved in the biosynthesis and, also, that selective demethoxylation does not occur.

To prove that bond "a" in the precursor was not cleaved, the same phenol (LXXVII, b) was prepared with labels in O- and N-methyl groups and at the carbon atom 1. This was also incorporated into galanthamine (LXXXIII). The activities of the O-methyl, N-methyl, and the residue were determined in the precursor, the biosynthetic galanthamine, and the derived narwedine (LXXXIV). The relative activities of the three labelled position in each were identical.

The foregoing results indicate quite clearly that the biogenetically likely precursor phenols of the type (LXXVII) proposed by Barton and Cohen[4] are, in fact, true intermediates in the biogenesis of the Amaryllidaceae alkaloids. These precursors are incorporated intact and without demethoxylation or transmethylation.

That, the phenols of the type (LXXVII) were actually present in the plant, was shown by the intermediate trapping experiments. If the compounds of the type (LXXVII) were the intermediates on the pathway from tyrosine to the alkaloids, they must be present in the plant. If it is so, then the

labelled tyrosine, fed to the plants, will cause their "pool" to become radioactive. A particular substance may then be isolated by using synthetic, non-radioactive material to act as a carrier for the biosynthetic material. Also, if labelled tyrosine was injected into the plant, together with a large amount of an inactive compound X, which is a true biosynthetic intermediate, then two effects should be observed. First, the incorporation of tyrosine should be considerably diminished since the label will become diluted as it passes through the intermediate X. This will hold true providing that the plant cannot convert all of X into alkaloid during the course of the experiment. Second, if X could be reisolated, it should be radioactive.

Battersby *et al.*[27] fed (±)- [2-$^{14}$C] tyrosine to 'Twink' daffodils, and after a period of growth, worked up the plants in the presence of inactive *O*-methylnorbelladine (LXXVII, C) as carrier. Isolation of this base (66% recovery) gave radioactive material which retained its activity after repeated crystallisation as the hydrochloride. This product was methylated with diazomethane to give belladine which was further fractionated by gas-liquid chromatography, and the final product was still active.

Barton et al.[18] fed mixtures of (±)- [2-$^{14}$C]-tyrosine and the inactive phenols (LXXVII, b), (LXXVII, c) and (LXXVII, d) to 'King Alfred' daffodils, and compared the activities of the alkaloids, isolated after one week, with those from plants fed with (±)- [2-$^{14}$C] tyrosine alone. Inhibition of tyrosine incorporation into the alkaloids was observed with all the three phenols. Only the most tractable member (LXXVII, b) was isolated (29% recovery). The phenol was radioactive and retained its activity after dilution with the inactive material and repeated crystallisation. However, the activity of the phenol was very low (0.0016% incorporation based on tyrosine ). Methylation with diazomethane gave belladine which was converted into its methiodide and this was cleaved with sodium amalgam . O-Methylhordenine isolated from the reaction mixture, contained essentially all (99%) of the original activity.

It is of interest to note in this connection that Fales, Mann and Mudd[30] have isolated and purified an enzyme from N. bowdenii bulbs, which catalyses specifically the methylation of norbelladine to yield O-methylnorbelladine.

Origin of Methylenedioxy-groups.

The most interesting result obtained by Barton

*et al.*[18,31] was the incorporation of the [1-$^{14}$C]-*O*-methylnorbelladine (LXXVII, C) into haemanthamine (LXXXVI). This could be explained in one of the two ways. First, the precursor could be demethylated and the methylendioxy-group of haemanthamine formed from a formaldehyde equivalent at a later stage. Second, the methylenedioxy-group could be derived directly by oxidative cyclisation of the methoxyl group. Since demethylation of precursors before alkaloid formation was not observed in the work on galanthamine, they were led to favour the second possibility.

This idea, that the methylenedioxy-group of alkaloids could be derived biogenetically by cyclisation of *O*-methoxyphenols, was first put forward by Sribney and Kirkwood[32]. In the course of their work with protopine, they observed that methionine, but not formate, was a precursor for the methylenedioxy group.

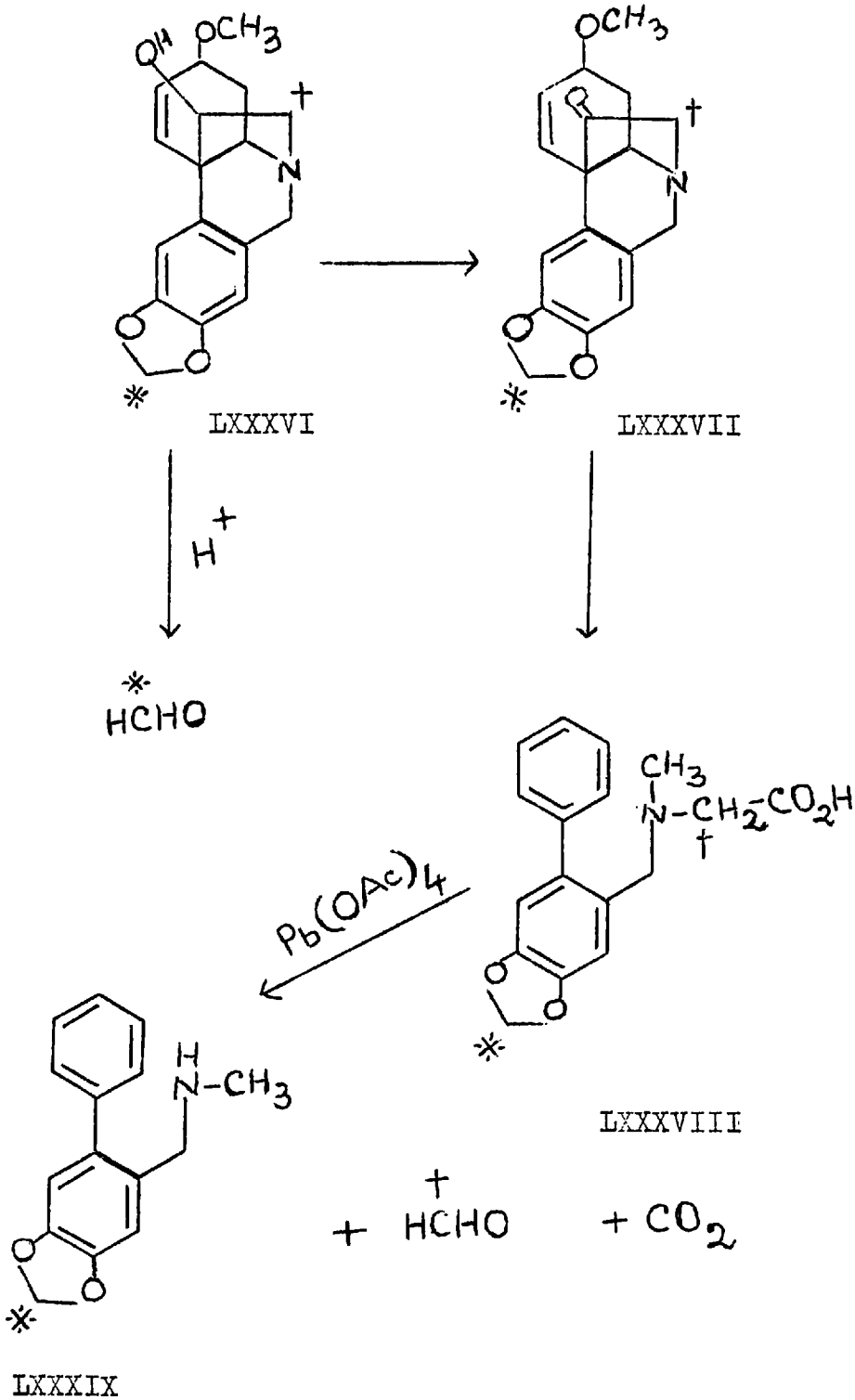
A conclusive proof for this idea came from the work of Barton, Kirby and Taylor[18,31]. They prepared *O*-methylnorbelladine (LXXVII, C) with labels in the *O*-methyl group and at the chain carbon atom 1. The ratio of the labels in the precursor was determined by the cleavage of the *O*-methyl group with hydrogen iodide. The doubly

labelled precursor (4.55% of the total activity in the methoxyl group) was incorporated into haemanthamine (LXXXVI, 1% incorporation) in 'King Alfred' daffodils, but galanthine and galanthamine were both inactive.

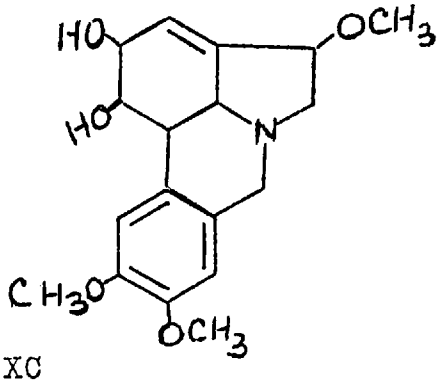
The biosynthetic haemanthamine was degraded as follows. Hydrolysis of the methylenedioxy-group was carried out with 20% sulphuric acid. The formaldehyde, isolated as its dimedone derivative, contained essentially all (4.73%) the activity associated with the methoxyl group of the precursor. To prove that no cleavage had occurred, with resultant scrambling of the labels, the radio-active haemanthamine was converted into the glycine derivative (LXXXVIII) *via* oxohaemanthamine (LXXXVII). The acid (LXXXVIII) was cleaved with lead tetraacetate to give active formaldehyde (94% activity), inactive carbon dioxide and *N*-methyl-6-phenylpiperonylamine (LXXXIX) containing 4.3% of the original activity.

The derivation of methylenedioxy- groups from *O*-methoxyphenols is also supported by the work of Battersby *et al.*[27]. They obtained radio-active lycorine from the plants fed with [methyl-$^{14}$C]-methionine. Hydrolysis of the radio-active lycorine gave formaldehyde containing 95% of the original activity of the lycorine. The same group also

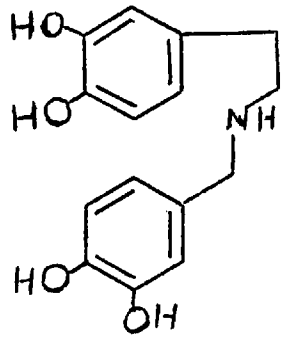
Degradation of Haemanthamine (Barton et al.)



observed that radio-active norpluviine (LXXII) was incorporated into lycorine (LXXI, 10.5% incorporation) as well as into galanthine (LXXVI) and narcissidine (XC). The incorporation of norpluviine




XC

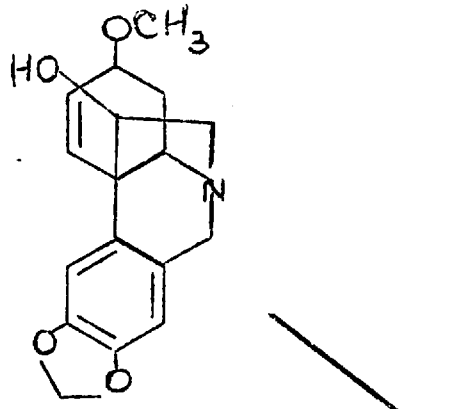



XCI

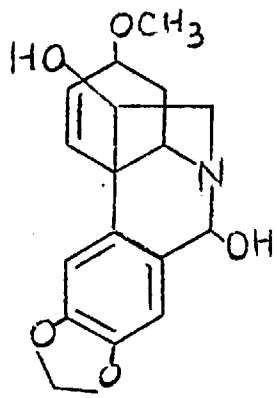
into lycorine, coupled with their observation that the [1-$^{14}$C]hydroxynorbelladine (XCI) was not significantly incorporated into lycorine or norpluviine, led them to postulate that hydroxylation at position 2 occurs at a later stage in the biogenesis of lycorine. That the conversion of norpluviine into lycorine in the plant is irreversible was proved by feeding the radio-active lycorine to the plants. No incorporation into norpluviine was observed. On this basic, these workers proposed that norpluviine is the primary pyrrolophenanthridine alkaloid and that many others are derived from it by oxidative changes.

Experiments on the interconversion of the alkaloids having the ethanophenanthridine skeleton,

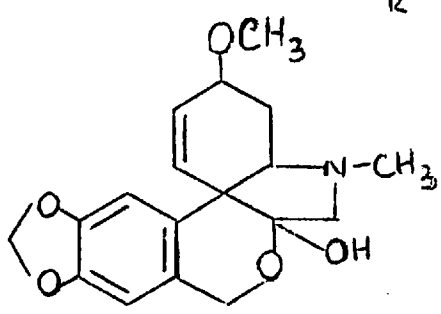
have been carried out by Fales and Wildman.[33] The plants Sprekelia formosissima (L.) produce haemanthamine (XCII), haemanthidine (XCIII), and tazettine (XCIV). The structures of these alkaloids suggest a biological sequence (XCII) ⟶ (XCIII) ⟶ (XCIV). The existence of this sequence in vivo was shown by feeding tritiated haemanthamine to S. formosissima. The specific activities of the biosynthetic haemanthidine and tazettine relative to haemanthamine were measured as a function of time. The rates of incorporation clearly supported the above sequence. Radio-active haemanthidine and tazettine were also fed to the plants. The haemanthidine was incorporated only into the tazettine, whereas the tazettine was not incorporated into the other two alkaloids. This showed that the sequence (XCII) ⟶ (XCIII) ⟶ (XCIV) is essentially an irreversible process. To show that these alkaloids are interconverted without any scrambling of label, each was oxidised to hydrastic acid containing essentially all the activity.



XCII



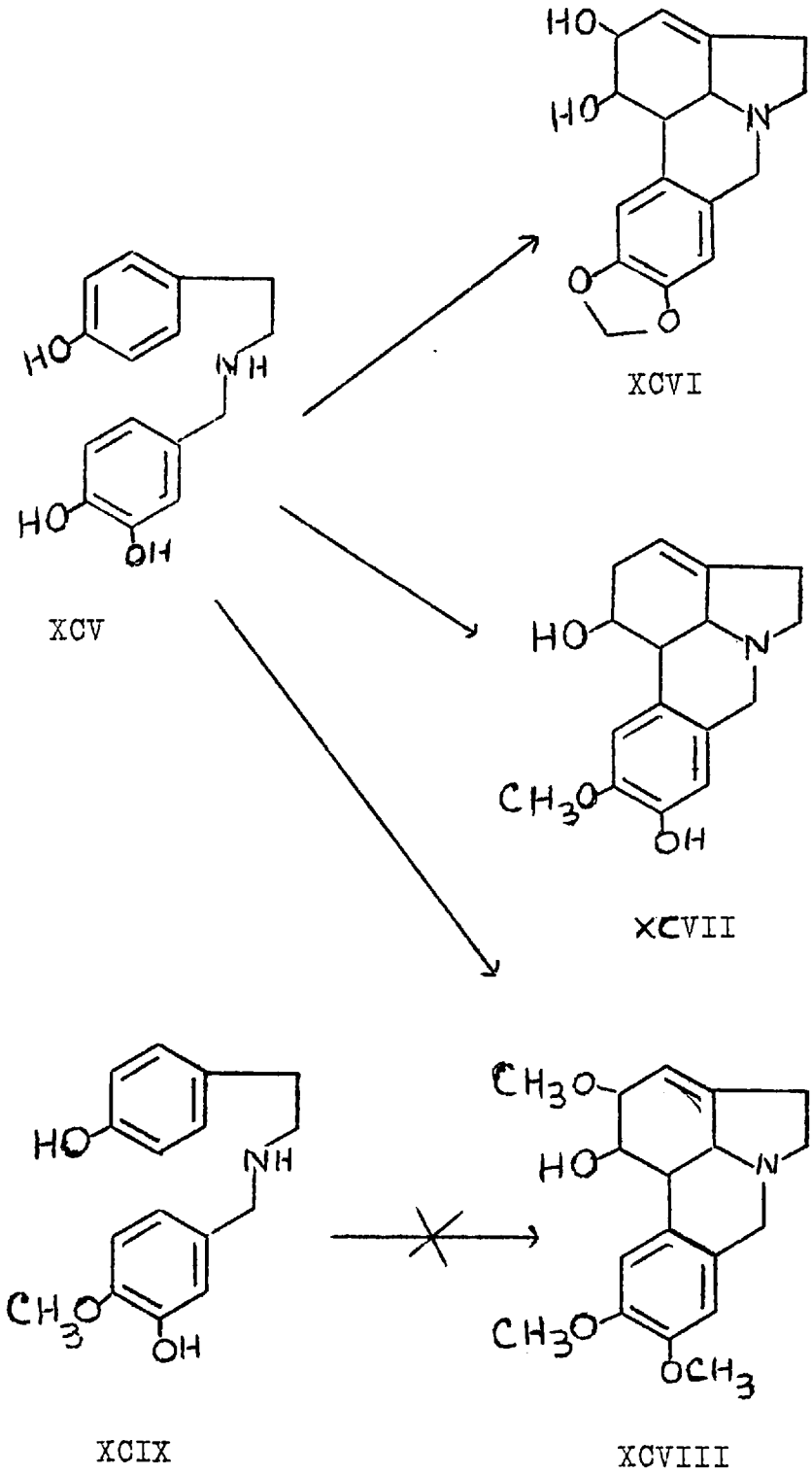
XCIII



XCIV

## BIOSYNTHESIS OF NORPLUVIINE

Norbelladine (XCV)[18,27,28,29] has been shown to be an intermediate in the biosynthesis of lycorine (XCVI), norpluviine (XCVII), and galanthine (XCVIII). O-Methylnorbelladine (XCIX) was not incorporated into galanthine[31] in 'King Alfred' daffodils. It has not been tested as a possible precursor for lycorine and norpluviine. The aim of the present work, described in subsequent pages, was to test if O-methylnorbelladine (XCIX) would be incorporated in lycorine and norpluviine, members of the same structural group as galanthine, and also to study, if possible, the further stages in the biogenetic conversion of this precursor into the alkaloid. For the purpose of feeding experiments 'Texas' daffodils were selected and fed with tritiated O-methylnorbelladine (C) and tritiated [1-$^{14}$C] O-methylnorbelladine (CI).


HO
HO
N
O
O
XCVI
HO
NH
HO
OH
XCV
HO
N
CH3O
OH
XCVII
HO
NH
CH3O
OH
XCIX
CH3O
HO
N
CH3O
OCH3
XCVIII

Synthesis of Precursors.

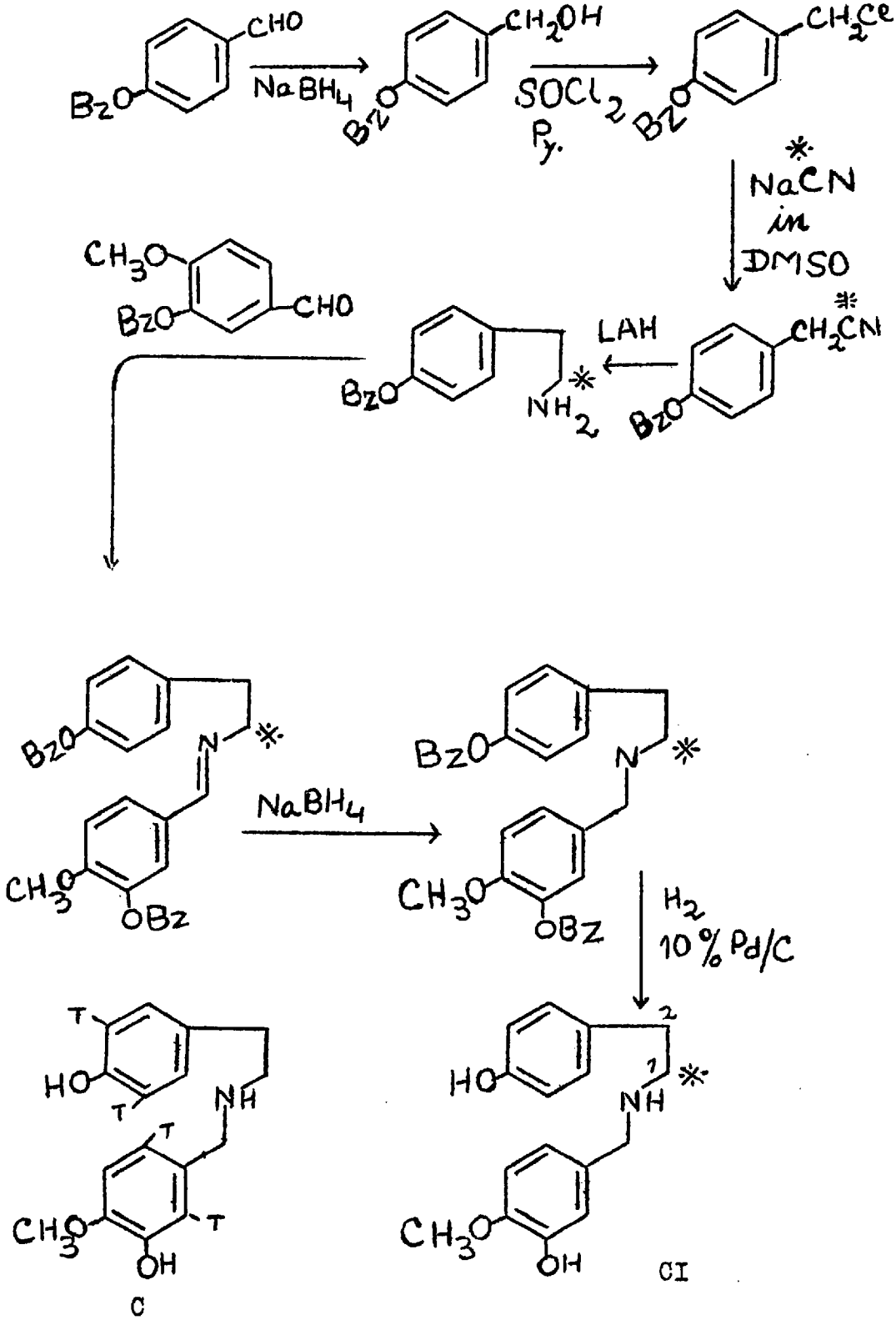
[1-$^{14}$C] O-Methylnorbelladine was synthesised according to the method of Barton et al.[18] as outlined on page 42 .

Tritiated O-methylnorbelladine (C) was prepared by heating the inactive O-methylnorbelladine with potassium tertiarybutoxide and tritiated water at 100° for seven days. The tritium labelled compound was isolated as its crystalline hydrochloride. Introduction of tritium by this method is known to proceed specifically in the positions ortho- and para- to phenolic hydroxyl group.[34]

The precursors were assayed for purity by dilution analysis (see experimental) and also by autoradiography of the circular paper chromatograms. The activity of the tritiated precursor was nearly equal to the calculated value for the exchange of four hydrogen atoms by tritium.

It was necessary to prove that the amount of label in both the aromatic rings of the tritiated precursor was the same. The [$^{3}$H] O-methylnorbelladine (C) was methylated to give belladine methiodide (CII), which was then subjected to the Hofmann degradation. The basic fraction of the

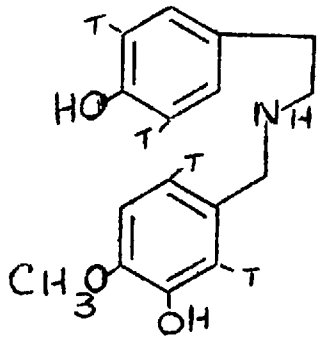
Synthesis of [1-$^{14}$C] O-Methylnorbelladine (Barton et al.[18])



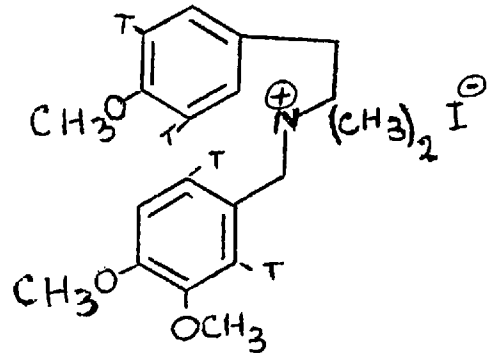
reaction product, N,N-dimethylveratrylamine (CIV) was isolated as its crystalline hydrochloride. The neutral fraction, p-methoxystyrene. (CIII) was ozonolysed and oxidised to give anisic acid (CV). Both these compounds were purified by crystallisation and their activities measured. This degradation was carried out with two samples of [$^3$H] O-methylnorbelladine, synthesised in two different experiments. The counting results, tabulated below, show very clearly that the amount of label in both the aromatic rings was equal.

| Compounds | Activity c.p.m./m.mole | |
|---|---|---|
| | Sample I | Sample II |
| [$^3$H] O-Methylnorbelladine.HCl (diluted) | $1.42 \times 10^6$ | $6.92 \times 10^8$ |
| N,N-Dimethylveratrylamine.HCl | $7.38 \times 10^5$ | $3.46 \times 10^8$ |
| N,N-Dimethylveratrylamine.MeI | $7.56 \times 10^5$ | |
| Anisic acid | $7.74 \times 10^5$ | $3.38 \times 10^8$ |

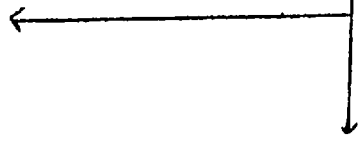
Hofmann degradation of [$^3$H] O-Methylnorbelladine

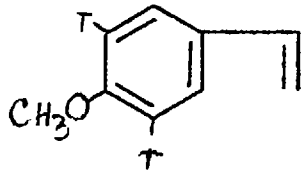


C

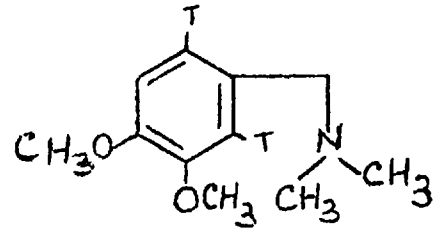


CII

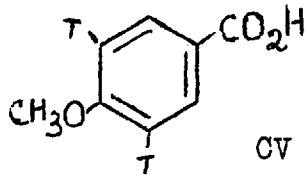


CIII



CIV

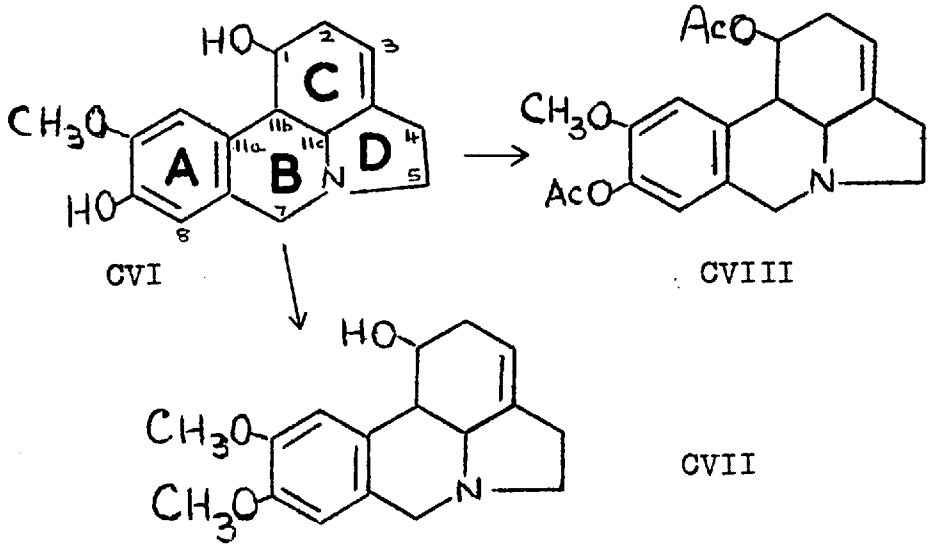


CV

Isolation of Alkaloid.

'Texas' daffodils were worked up according to the general procedure of Fales, Giuffrida and Wildman[35]. The chloroform extract, containing basic fraction, was concentrated to small bulk to give "chloroform insoluble" alkaloids. When this "chloroform insoluble" portion was treated with dilute alkali, it dissolved leaving only a negligible residue. After filtration, the alkaline solution was buffered with sodium bicarbonate and the reprecipitated alkaloid collected. After crystallisation from methanol, the alkaloid (m.p. 239-241°; $[\alpha]_D$-232°) ran as one spot on silica thin-layer plate. Elemental analysis was in accord with the molecular formula $C_{16}H_{19}NO_3$. The acetyl-derivative of the alkaloid showed, in infrared spectrum, the presence of two acetate bands, one phenolic ($\nu_{max.}$ 1760 $cm.^{-1}$) and the other non-phenolic ($\nu_{max.}$ 1723 $cm.^{-1}$). The alkaloid was then methylated with diazomethane to give a dimethyl ether. The physical constants (m.p. 220-222° $[\alpha]_D$-181°) of this dimethyl ether agreed with those of pluviine[36] (CVII) (m.p. 225-227°; $[\alpha]_D$-171°). Although the physical constants of the phenolic alkaloid were in poor agreement with those of

norpluviine[37] (CVI), (m.p. 274-275°(decomp.) $[\alpha]_D$-160°), it was identified as norpluviine by comparison of infrared spectra, and also by taking mixed melting points with a specimen of norpluviine, very kindly provided by Prof. A.R. Battersby. Comparison with the original specimen of Uyeo and Yanihara was not possible. The n.m.r. spectrum of the diacetate of this alkaloid was consistent with the norpluviine diacetate (CVIII).




CVI CVIII CVII

Although Wildman[1] has reported that the major alkaloid from 'Texas' daffodils was lycorine, which is also very sparingly soluble in chloroform, no significant amount of lycorine was obtained from any of our experiments.

Feeding Experiments.

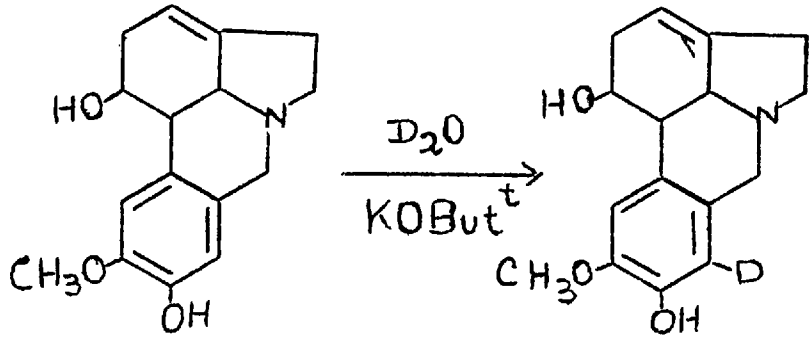
Feeding experiments were carried out with

flowering 'Texas' daffodils. The precursors were dissolved in distilled water and injected directly into the hollow flowering stalks of the plants. The injection of the precursor was followed by the injection of distilled water. The plants were worked up after eight days of the first injection and norpluviine isolated. [$^{3}$H] O-Methylnorbelladine (C) was fed to two different batches of plants in two different seasons. [$^{3}$H, 1-$^{14}$C] O-Methylnorbelladine, made by mixing the tritiated precursor (C) with [1-$^{14}$C] O-methylnorbelladine, was also fed to one batch of plants. In each case, norpluviine was found to be active. The yields and activities of norpluviine, obtained from these experiments, are given in the following table.

| Precursor | Yield of Alkaloid | % Incorporation |
|---|---|---|
| [$^{3}$H] O-Methylnorbelladine HCl. (season 1963) | 0.023% | 0.70 |
| " (season 1964) | 0.18% | 0.73 |
| [$^{3}$H, $^{14}$C] O-Methylnorbelladine HCl. (season 1964) | 0.015% | |

The results obtained from the above experiments prove that O-methylnorbelladine is an intermediate in the biosynthesis of norpluviine.

The position of tritium atoms in biosynthetic norpluviine was determined in the following manner. Inactive norpluviine was acetylated with acetic anhydride and pyridine to give the norpluviine diacetate (CVIII). In the n.m.r. spectrum ($CDCl_3$) of this compound two aromatic protons appeared at 3.1 $\tau$ and 3.2 $\tau$ . This diacetate was heated with deuterium oxide and potassium tertiarybutoxide at 100° for seven days, and the norpluviine obtained was reconverted to the diacetate. In the n.m.r. spectrum of this diacetate, only one aromatic proton (3.1 $\tau$ ) was present. Clearly the other aromatic proton has exchanged with deuterium. This exchangeable proton must be ortho to the phenolic hydroxyl group in norpluviine.




Radio-active norpluviine, obtained from the feeding experiments, was converted to its diacetate,

crystallised and counted. It was then allowed to exchange with water as described earlier. The norpluviine obtained was reconverted to the diacetate, crystallised and counted. There was a drop in the activity. This exchange experiment was repeated till there was no further drop in activity of the compound. The results of this exchange experiment are tabulated below.

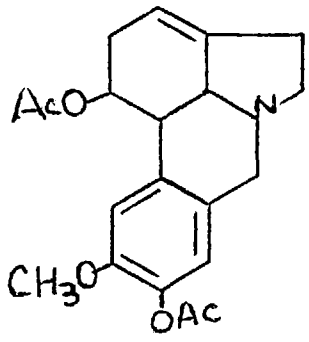
| Compound | Activity(c.p.m./m.Mole) | |
|---|---|---|
| | Season 1963 | Season 1964 |
| Norpluviine diacetate | $4.75X10^5$ | $3.30X10^4$ |
| Norpluviine diacetate after exchange | $1.86X10^5$ | $1.59X10^4$ |
| Norpluviinediacetate after further exchange | $2.36X10^5$ | $1.62X10^4$ |

Half the activity is lost during the exchange experiment. This clearly indicates that the position ortho to the phenolic group contains half of the total activity present in the molecule.

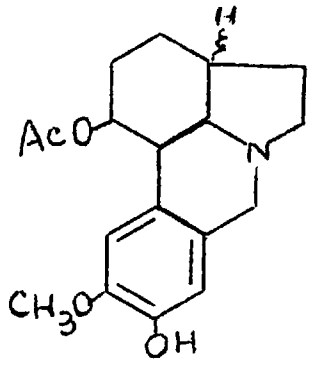
To confirm the position of tritium in the aromatic ring, it was decided to substitute position 8 with bromine. Since norpluviine has a double bond, addition of bromine could also take place. To avoid this, it was decided to reduce the

double bond before attempting the bromination of the aromatic ring. The readily soluble norpluviine diacetate was chosen for this purpose. Catalytic reduction gave two stereoisomeric compounds, which could not be separated by column chromatography. However, the mixture crystallised from benzene as plates showing a wide melting point range. The infrared and n.m.r. spectra of this compound indicated that along with the reduction of the double bond, the phenolic acetyl group was removed to give a mixture of the dihydrophenols (CIX). The n.m.r. spectrum of this compound showed, as expected, two unequal acetate and methoxy bands, suggesting a 60:40 mixture of the two stereoisomers. Bromination of this compound was carried out in acetic acid. The product was purified by column chromatography on alumina, which separated the two epimers, one of which crystallised readily. In the n.m.r. spectrum of this bromo-derivative (CX), only one aromatic proton was present. The substituted proton corresponded to the exchangeable proton of norpluviine.

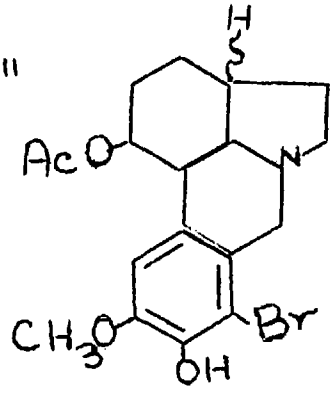
When the reduction was carried out on radioactive norpluviine diacetate, and the resulting dihydromonoacetate (CIX) counted, about half the




CVIII




CIX

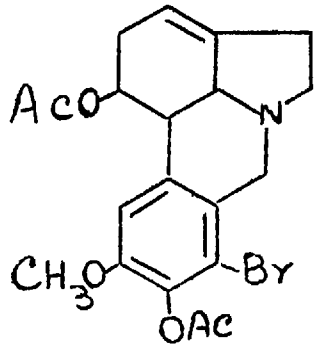



CX

activity was lost, presumably due to the exchange, catalysed by the catalyst. When the dihydromonoacetate (CIX) was brominated there was a further drop in activity. The activity left in the brominated dihydromonoacetate (CX) was much less than half of the activity present in norpluviine diacetate. The results obtained are tabulated below.

| Compounds | Activity (c.p.m./m.mole) |
|---|---|
| Norpluviine diacetate .. | $3.75 \times 10^5$ |
| 1-Acetyl-3,3a-dihydronorpluviine | $1.77 \times 10^5$ |
| 1-Acetyl-8-bromo-3,3a-dihydronorpluviine | $1.08 \times 10^5$ |

Since the results from the above experiments were anamolous, possibly due to the exchange of the allylic proton on the catalyst, selective bromination of norpluviine itself was attempted under mild conditions. One mole equivalent of bromine solution in ethanol was added slowly to a solution of norpluviine hydrochloride in ethanol. The resulting salt was converted to the free base and acetylated to give 8-bromonorpluviine diacetate (CXI). This was purified by chromatography on alumina. In the n.m.r. spectrum of this compound, only one aromatic proton was present. The other aromatic proton had, obviously, been substituted with bromine. This substituted proton corresponded to the exchangeable proton of norpluviine. The olefinic proton was still present, indicating that no addition of bromine to the double bond had taken place.




CXI

Biosynthetic norpluviine diacetate was hydrolysed with 50% potassium hydroxide at room temperature

for an hour to give norpluviine. Bromination of this was carried out as described above, and the product counted. As expected, in agreement with the exchange experiment, half of the total activity in the molecule was lost due to the substitution by bromine. The results of this experiment are tabulated below.

| Compound | Activity (c.p.m./m.mole) |
|---|---|
| Norpluviine diacetate | $4.89 \times 10^4$ |
| 8-Bromonorpluviine diacetate | $2.49 \times 10^4$ |

In order to make sure that during the transformations — norpluviine ⟶ norpluviine diacetate ⟶ norpluviine — no loss in activity took place due to exchange, radioactive norpluviine diacetate was hydrolysed to norpluviine with 50% potassium hydroxide at room temperature for one hour. A portion of this norpluviine was then reacetylated to give the diacetate, which was recrystallised and counted. There was no loss in activity during these operations. Also, in order to show that the conditions used for the isolation of the alkaloid from the plant did not cause any loss of activity, another portion of the norpluviine,

obtained from the hydrolysis of the diacetate, was kept in N-hydrochloric acid overnight at room temperature. It was then acetylated and the diacetate counted. Again, there was no drop in the activity. The results are tabulated below.

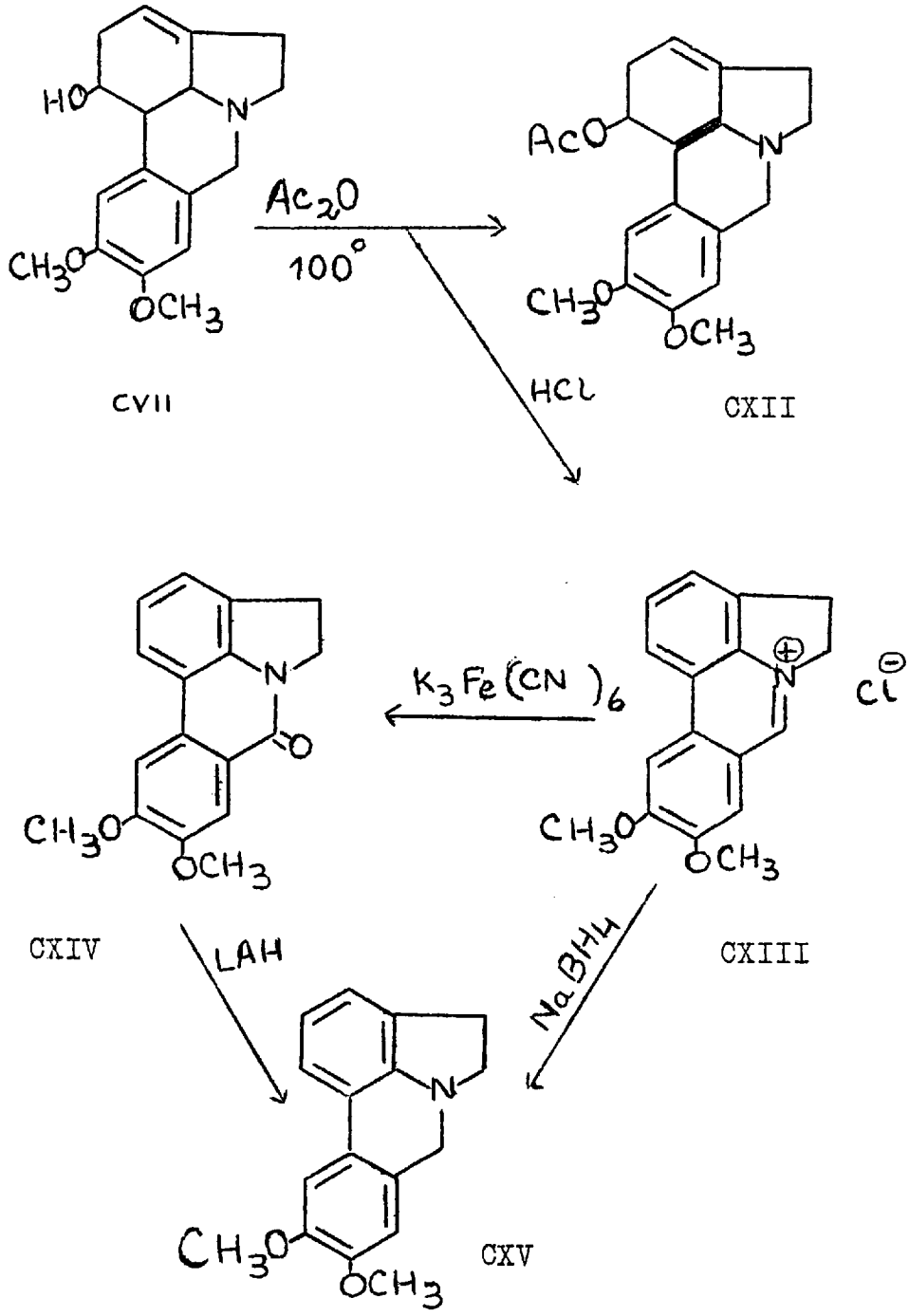
| Compounds | Activity(c.p.m/m.mole) |
|---|---|
| Norpluviine diacetate | $4.89X10^4$ |
| Norpluviine diacetate, after hydrolysis and reacetylation. | $4.79X10^4$ |
| Norpluviine diacetate, after keeping norpluviine in N.HCl and reacetylating. | $4.92X10^4$ |

It remained to locate the position of the remaining activity in the molecule. To find out if position 2 in norpluviine had any tritium, it was decided to introduce a hydroxyl group at that position by oxidation with seleniumdioxide and then to oxidise it to the corresponding ketone. With this in view, norpluviine diacetate was subjected to seleniumdioxide oxidation. The product, however, was a complex mixture which could not be separated.

The problem of finding the position of activity in ring C was finally solved in the following manner. If ring C was aromatised then any

tritium present at benzylic position 11b would be lost completely. Loss may also occur from position 2, depending on the stereochemistry of elimination and the magnitude of the isotope effect. The aromatisation of ring C in norpluviine (CVI) was carried out by methylating it to pluviine (CVII) and then following the procedure of Boit et al.[36]. They observed that the major product obtained on heating pluviine with acetic anhydride was pluviine acetate (CXII). Some anhydromethylpseudolycorinium chloride (CXIII) was also obtained from the reaction mixture in about 25% yield. When oxidised with potassium ferricyanide this compound was converted to 3,4-dihydro,9,10-dimethoxypyrrolo(de)-7-phenanthridone (CXIV). The lactam (CXIV) when reduced with lithium aluminium hydride gave anhydromethylpseudolycorine (CXV). These reactions are outlined on page 56 .

$^{3}H$ Norpluviine was methylated with diazomethane to give $[^{3}H]$-pluviine. This was then heated for 2½ hours with acetic anhydride and worked up according to the method of Boit et al.[36] to give pluviine acetate and anhydromethylpseudolycoriniumchloride. Pluviine acetate obtained was purified by chromatography, crystallised and counted.

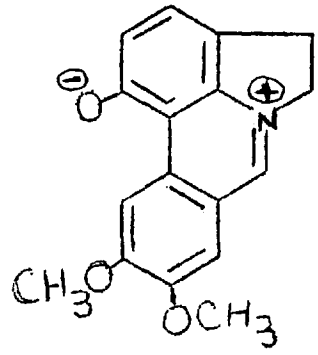

HO
N
CH3O
OCH3
Ac2O
100°
AcO
HCl
CVII
CXII
K3Fe(CN)6
Cl⊖
CXIV
LAH
NaBH4
CXIII
CXV

The anhydromethylpseudolycorinium chloride oxidised with potassium ferricyanide to give the lactam (CXIV) which was purified by chromatography, crystallised and counted. The lactam had the same molar activity as pluviine and pluviine acetate, which proved that there was no tritium at the benzylic position 11b in biosynthesised norpluviine. The counting results are as follows.

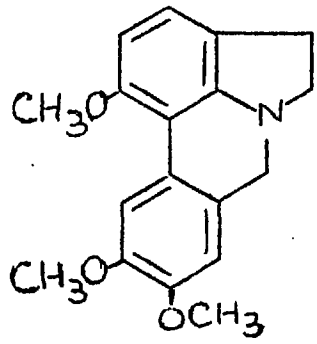
| Compounds | Activity (c.p.m./m.mole) |
|---|---|
| Pluviine | $7.60 \times 10^4$ |
| Pluviine Acetate | $7.51 \times 10^4$ |
| 3,4-Dihydro,9,10-Dimethoxypyrrolo(de)-7-phenanthridone (CXIV) | $7.73 \times 10^4$ |

It was, of course, suspected that the activity in ring C should be located at position 2. To obtain a final proof of this, it was decided to remove the tritium, if any, from that position, either by exchange or by substitution. Boit et al.[36] observed that pluviine, when oxidised with benzophenone in presence of potassium in tertiary butanol gives a red coloured phenolbetaine (CXVI) in low yield. This phenolbetaine could be

methylated and reduced to give 1-methyl anhydro-methylpseudolycorine (CXVII).



CXVI



CXVII

Any tritium present in norpluviine at position 2, would be lost by exchange during the formation of betaine (CXVI). When the oxidation was carried out with inactive pluviine, the yield of betaine was extremely low, and was further reduced on methylation. As the supply of the alkaloid was limited this approach was abandoned.

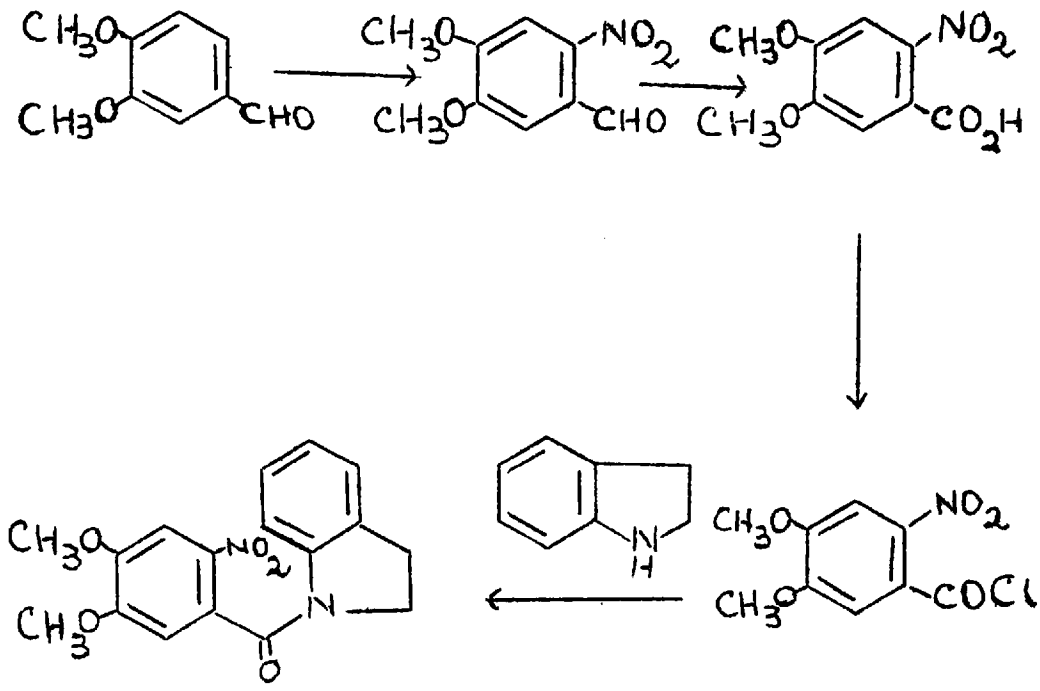
It wasthen sought to substitute position 2 in anhydromethylpseudolycorine (CXV) with bromine. This derivative (CXV) could be obtained by reducing the lactam (CXIV) with lithium aluminium hydride[36]

or by reducing anhydromethylpseudolycorinium chloride (CXIII) with sodium borohydride. Since the supply of the alkaloid was limited and the yield of anhydromethylpseudolycorinium chloride was low, the lactam (CXIV) was synthesised, for further studies, by the method of Fales, Giuffrida and Wildman[35] as outlined on page 60 . All the steps leading to the amine (CXVIII) proceeded in high yields. The final Pschorr cyclisation gave the lactam (CXIV) in only 25% yield.

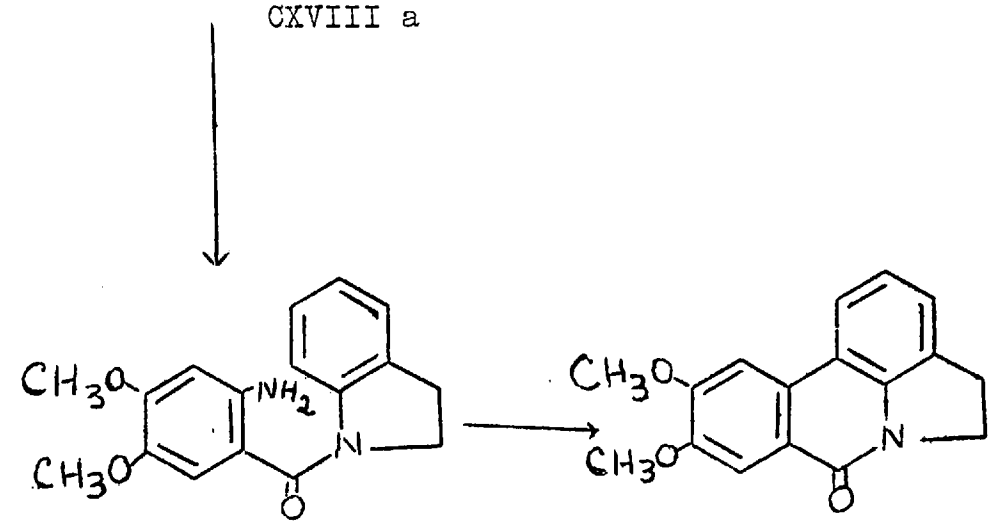
The lactam (CXIV) was reduced with lithium aluminium anhydride to give anhydromethylpseudolycorine (CXV). Attempted bromination of this, under various conditions, always oxidised the benzylic position 7 to give back the lactam (CXIV). No substitution product was obtained.

Bromination of the lactam (CXIV) did not go in chloroform or acetic acid at room temperature. However, when bromination was carried out in acetic acid, containing sodium acetate, at 100°, one mole of bromine was absorbed to give the monobrominated lactam (CXIX) which was purified by chromatography on alumina. In the n.m.r. spectrum of this compound (in $CF_3COOH$) four signals, each corresponding to one proton, were present in the

Synthesis of lactam (CXIV) (Wildman et al.)



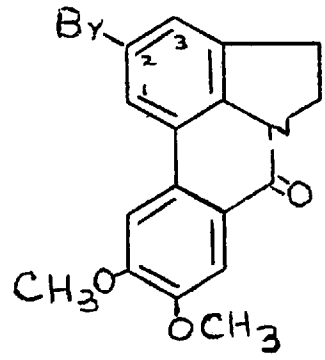
CXVIII a



CXVIII

CXIV

aromatic region. No fine splitting due to meta-coupling between the protons at $C_1$ and $C_3$ was observed but the lines were broad. The n.m.r. spectrum of the lactam (CXIV) also in $CF_3COOH$, showed a broad, partially resolved multiplet with an area corresponding to 5 aromatic protons. Thus the n.m.r. spectrum of the bromo-derivative supports the substitution at position 2.




CXIX

Bromination of the radioactive lactam was carried out as described above in the presence of excess sodium acetate, which would prevent any exchange induced by liberated hydrogen bromide. The 2-bromolactam (CXIX) was purified by chromatography, crystallised and counted. As expected, half of the activity was lost due to the substitution by bromine. The results are tabulated below.

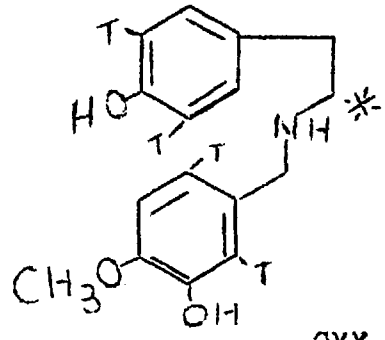
| Compounds | Activity (c.p.m./m.mole) |
|---|---|
| Pluviine | $1.81 \times 10^4$ |
| Pluviine Acetate | $1.77 \times 10^4$ |
| Lactam (CXIV) | $1.90 \times 10^4$ |
| 2-Bromolactam (CXIX) | $9.40 \times 10^3$ |

Confirmation of these results was sought by feeding O-methylnorbelladine labelled with tritium as before and with $^{14}C$ at position 1. In this way it was proposed to prove definitely that half the total tritium in the precursor is lost during its conversion to norpluviine in plants.

Experiments with Pentalabelled Precursor (CXX).

The pentalabelled precursor (CXX) was prepared by mixing solutions of $[^3H]$ O-methylnor-

belladine (C) and [1-$^{14}$C] O-methylnorbelladine (CI) hydrochlorides and allowing the precursor to crystallise out. The ratio $^{3}H/^{14}C$, in the precursor



CXX

was known from the separate activities of each labelled specimen. This ratio was also confirmed by counting the mixed precursor at the maximum efficiency settings for $^{3}H$ and $^{14}C$ in the scintillation counter. The efficiency of the counter at these settings was previously determined by counting [$^{3}H$] - and [$^{14}C$]-hexadecane standards (see experimental) at each of these settings. At maximum efficiency settings for tritium, the efficiency of the counter for $^{3}H$ was 17% and for $^{14}C$ 34%. At maximum efficiency settings for $^{14}C$ the efficiencies were 6.1% and 71% respectively. The total activities due to tritium and $^{14}C$ in any compound could be calculated from the following equations,

$$\frac{X}{100} \times 17 + \frac{Y}{100} \times 34 = \text{Counts at } {}^{3}H \text{ settings}$$

$$\frac{X}{100} \times 6.1 + \frac{Y}{100} \times 71 = \text{Counts at } {}^{14}C \text{ settings}$$

where X and Y represent the activities of tritium and $^{14}C$ respectively.

A known weight of the pentalabelled precursor was injected into the flowering stalks of 'Texas' daffodils. The plants were worked up after eight days in the usual manner and norpluviine isolated. As in earlier experiments, no appreciable amount of lycorine was obtained.

The following sets of experiments were carried out with norpluviine obtained from the above feeding.

(i) Bromination. Radioactive norpluviine was brominated to give 8-bromonorpluviine, which was acetylated and counted for tritium and $^{14}C$ and the ratio $^{3}H/^{14}C$ determined.

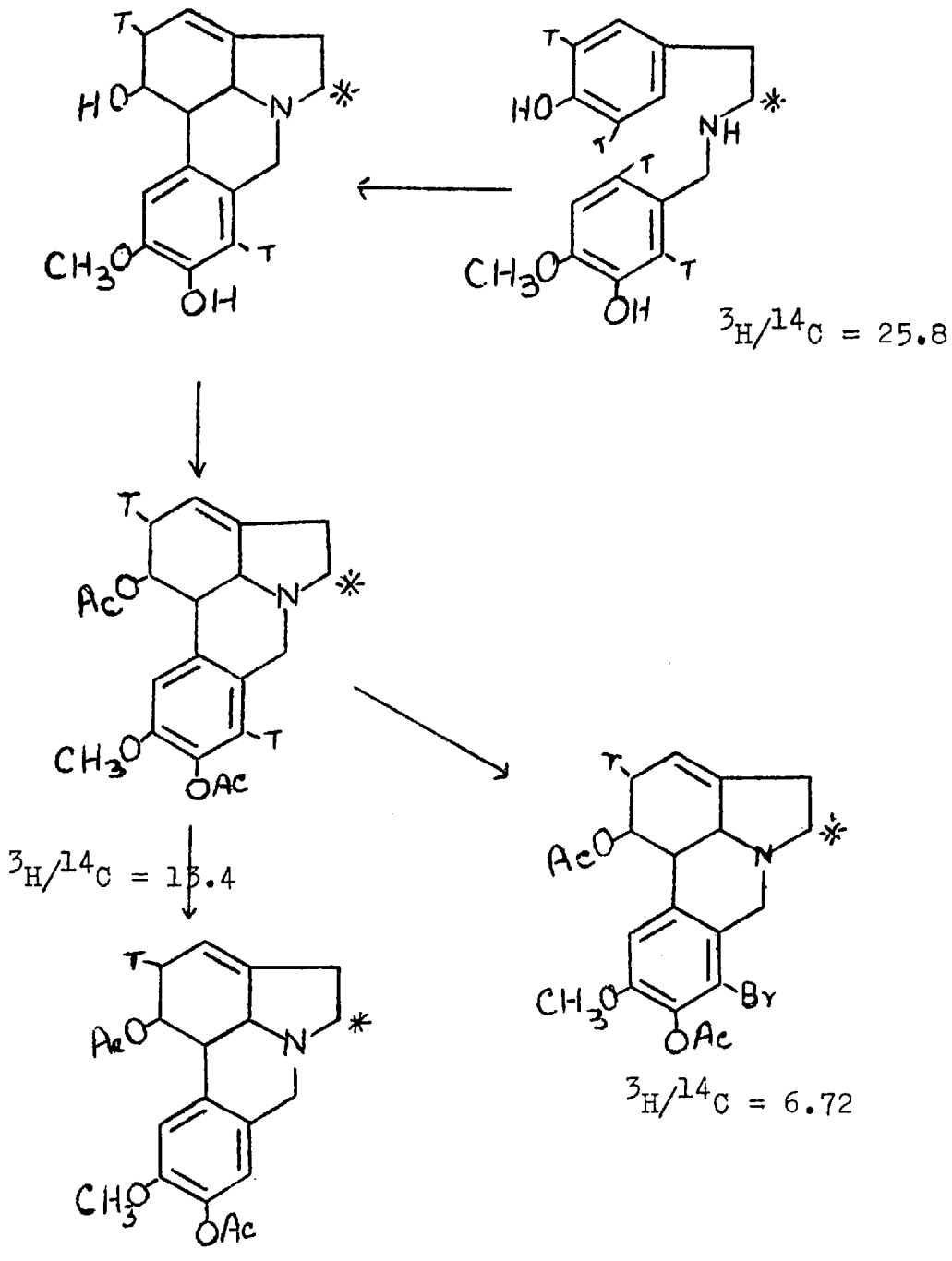
(ii) Aromatisation to Lactam (CXIV). Radioactive norpluviine was methylated to give pluviine, which was heated with acetic anhydride to give pluviine acetate and anhydromethylpseudolycorinium chloride (CXIII). Anhydromethylpseudolycorinium chloride was oxidised with potassium ferricyanide to give the

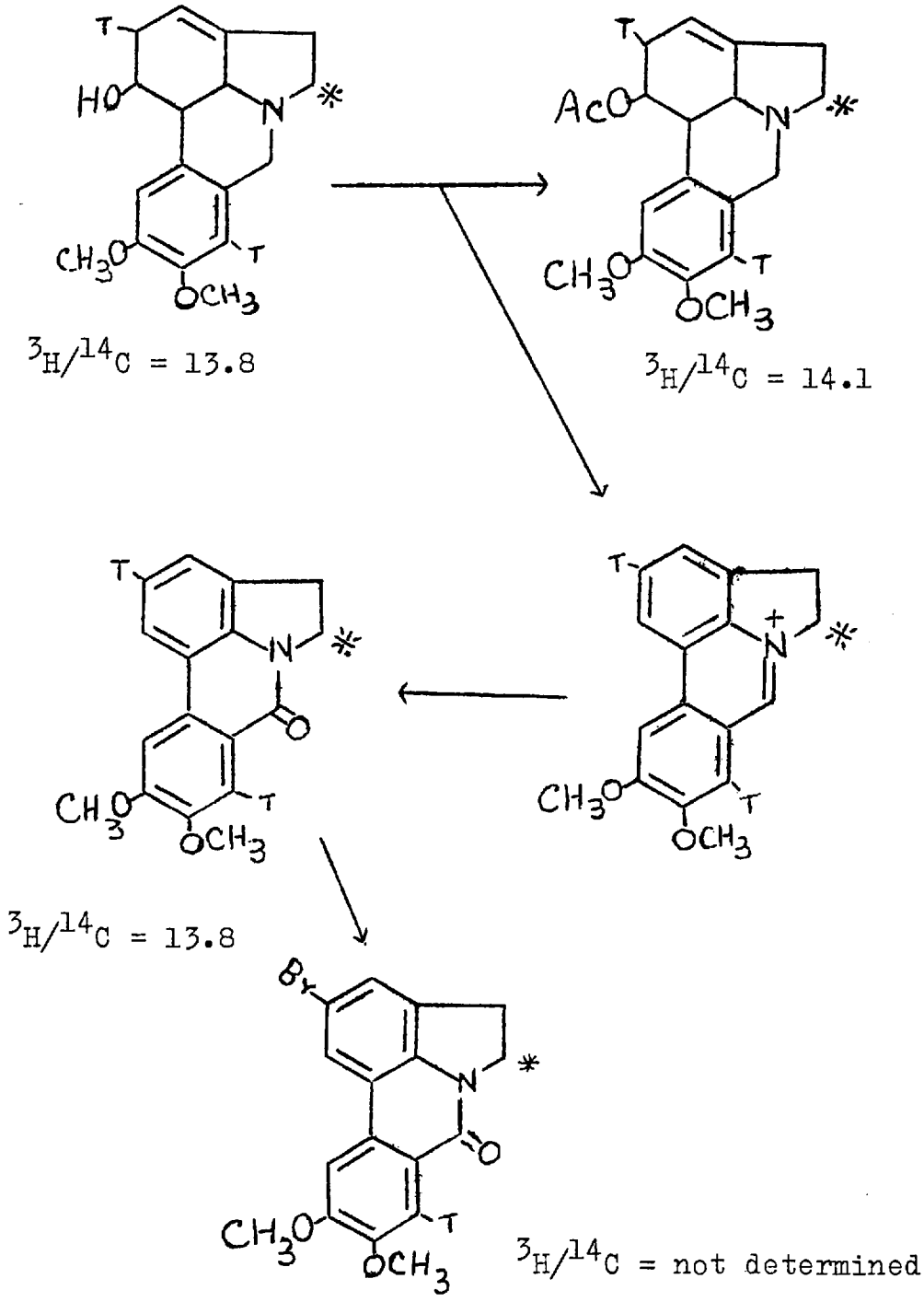
lactam (CXIV). The $^{3}H/^{14}C$ ratio was determined for pluviine, pluviine acetate, and the lactam (CXIV).

The results obtained from the above series of reactions are tabulated below.

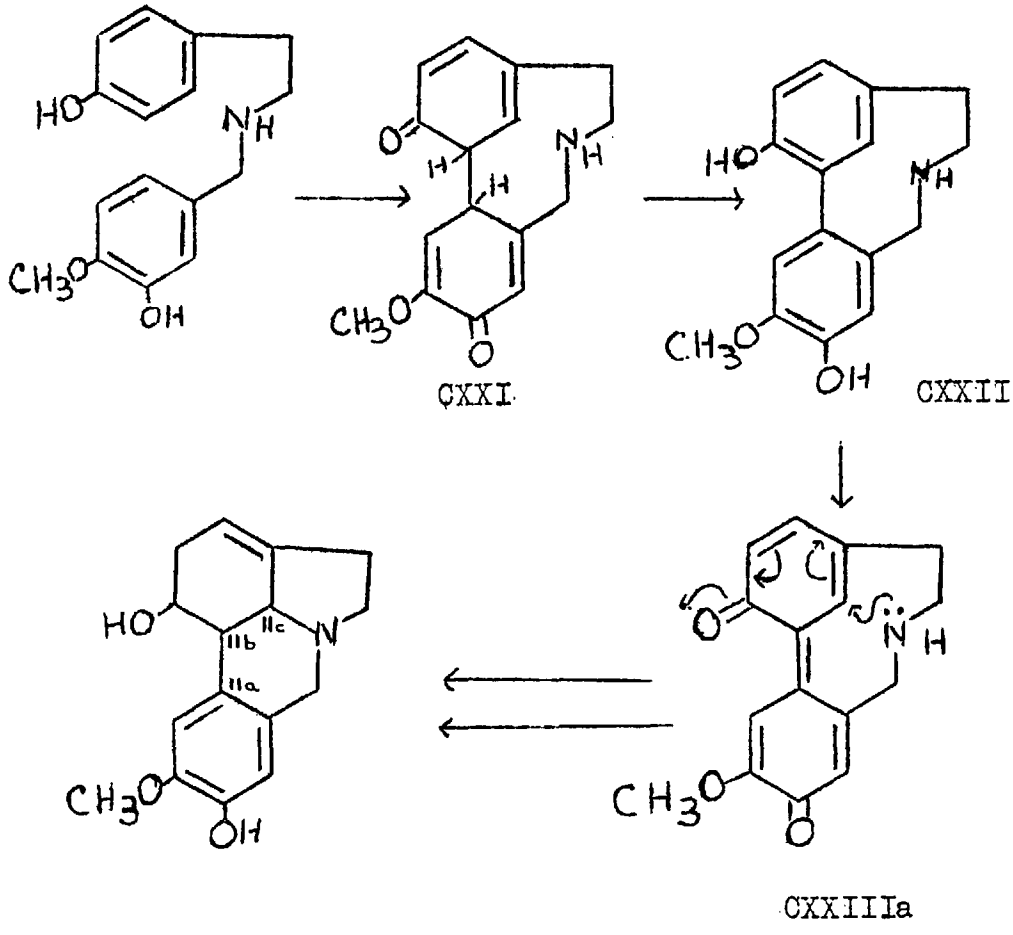
| Compounds | Activity at $^{3}H$ setting (d.p.m./m. mole) | Activity at $^{14}C$ setting (d.p.m./m. mole) | $^{3}H/^{14}C$ |
|---|---|---|---|
| Precursor(diluted specimen) | $2.01X10^{8}$ | $7.80X10^{7}$ | 25.8 |
| Norpluviine diacetate | $2.55X10^{5}$ | $1.89X10^{4}$ | 13.4 |
| 8-Bromonorpluviine diacetate | $1.41X10^{5}$ | $2.12X10^{4}$ | 6.72 |
| Pluviine | $1.82X10^{5}$ | $1.32X10^{4}$ | 13.8 |
| Pluviine Acetate | $1.76X10^{5}$ | $1.25X10^{4}$ | 14.1 |
| Lactam. (CXIV). | $1.68X10^{5}$ | $1.22X10^{4}$ | 13.8 |

The results of all these experiments are represented in the charts on pages 65,66. These results prove conclusively that O-methylnorbelladine is a specific precursor for norpluviine in 'Texas' daffodils. They also throw some light on the further stages in the biogenesis of this alkaloid. Barton and Cohen[4] proposed the following biogenetic scheme for the conversion of the precursor to the alkaloid.

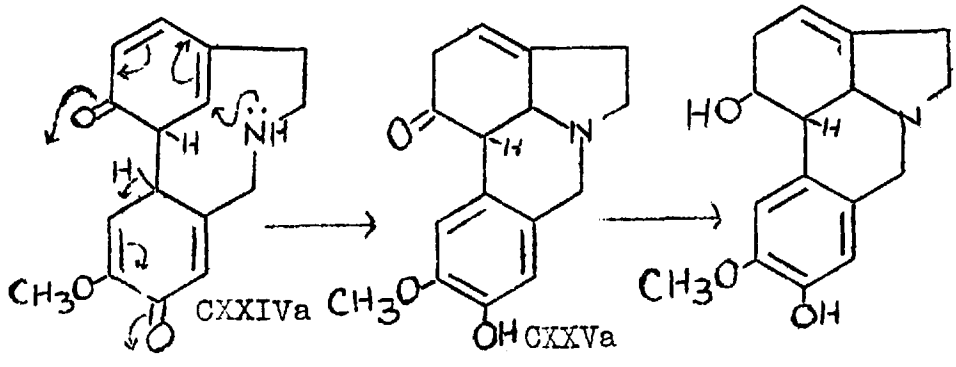

$^3H/^{14}C$ = 25.8
$^3H/^{14}C$ = 13.4
$^3H/^{14}C$ = 6.72


T
HO
N
*
CH3O
T
OCH3
3H/14C = 13.8
T
AcO
N
*
CH3O
T
OCH3
3H/14C = 14.1
T
N
+
*
CH3O
T
OCH3
T
N
*
O
CH3O
T
OCH3
3H/14C = 13.8
Br
N
*
O
CH3O
T
OCH3
3H/14C = not determined

Barton and Cohen's scheme.




This original scheme was modified by Barton[6] as follows,

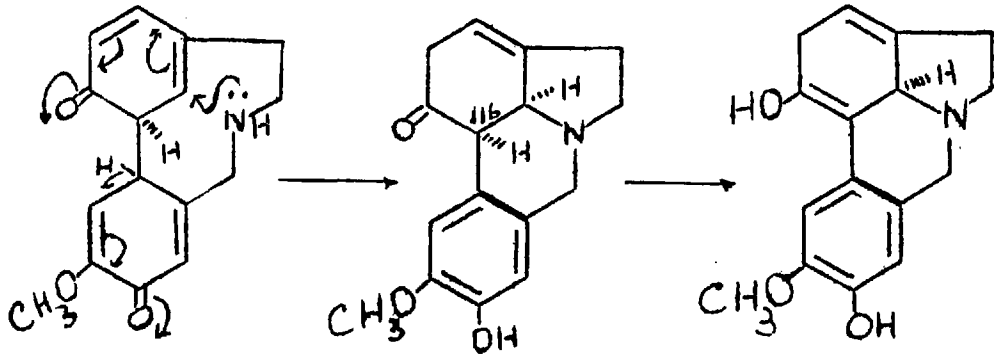


This modification was based on the assumption that in the bis-dienone (CXXIVa), the medium size nitrogenous ring has a special conformational driving force for closure. If it could be accepted that this driving force was enough, the ring closure by vinylogous β-addition would give the enone (CXXVa). The lower dienone ring of (CXXIVa) attains aromaticity in the usual way. The enone (CXXVa) is then reduced to give norpluviine.

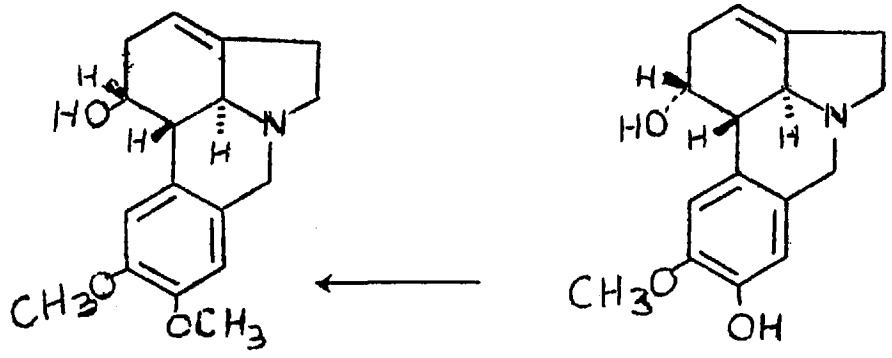
The results of the experiments, described above, rule out the short route proposed by Barton, since it would require retention of tritium at position $11_b$. They are, however, in full accord with the original scheme of Barton and Cohen.

The results, however, do not rule out the possibility of the ring closure by vinylogous β-addition to give enone (CXXVa) as proposed by Barton, provided the tritium atom at position $11_b$ is lost at some later stage. Examination of a Dreiding model of the dienone (CXXIVa) suggests that the vinylogous β-addition of nitrogen to form the five membered ring should be cis with respect to $C_{11b}$-$C_{11c}$ single bond. This cis addition would result in a wrong relative configuration at positions $11_b$ and $11_c$ for norpluviine, which must have

the same relative and absolute configuration as pluviine[38] (CXXVIà). The dienone (CXXIVà) could, however, give rise to the correct relative configuration, if the configuration at position $11_b$ is inverted through enolisation. The scheme is outlined below.



CXXVIIa



CXXVIa

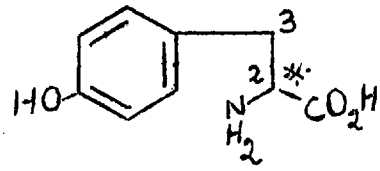
In this scheme, the hydrogen atom at the benzylic position $11_b$ in the enone (CXXVIIà) is lost during inversion of configuration through enolisation. This is in accord with the observations, that in biogenetic conversion of precursor into norpluviine, tritium atom at that position is lost.

It is, however, difficult to choose between the original scheme of Barton and Cohen and the new scheme outlined above. The only conclusive proof can come by synthesising the labelled diphenol (CXXIIà) and feeding it to the plants.

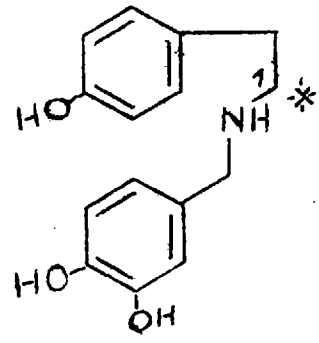
## Biosynthesis of Galanthine

In the course of their biogenetic studies with 'King Alfred' daffodils, Barton and his coworkers[7,18,29] injected [2-$^{14}$C] tyrosine (CXXIII), [1-$^{14}$C] norbelladine (CXXIV) and [1-$^{14}$C] O-methylnorbelladine (CXXV) into the plants. None of these compounds was incorporated into galanthine (CXXVI) in appreciable quantities. The incorporations of these compounds into galanthine, as reported by them, are as follows.

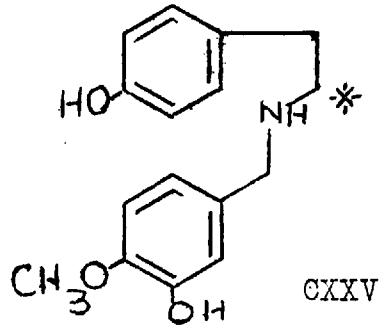
| Precursor | | % Incorporation into galanthine |
|---|---|---|
| [2-$^{14}$C] | Tyrosine | 0.007 |
| [1-$^{14}$C] | Norbelladine | 0.0036 |
| [1-$^{14}$C] | O-Methylnorbelladine | 0.000 |



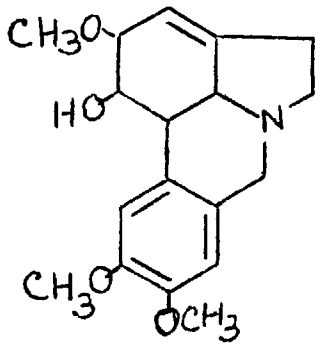
CXXIII



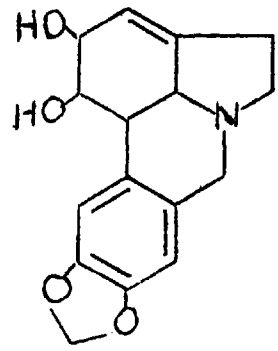
CXXIV



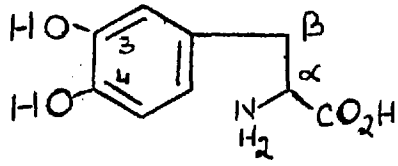
CXXV



CXXVI



CXXVII

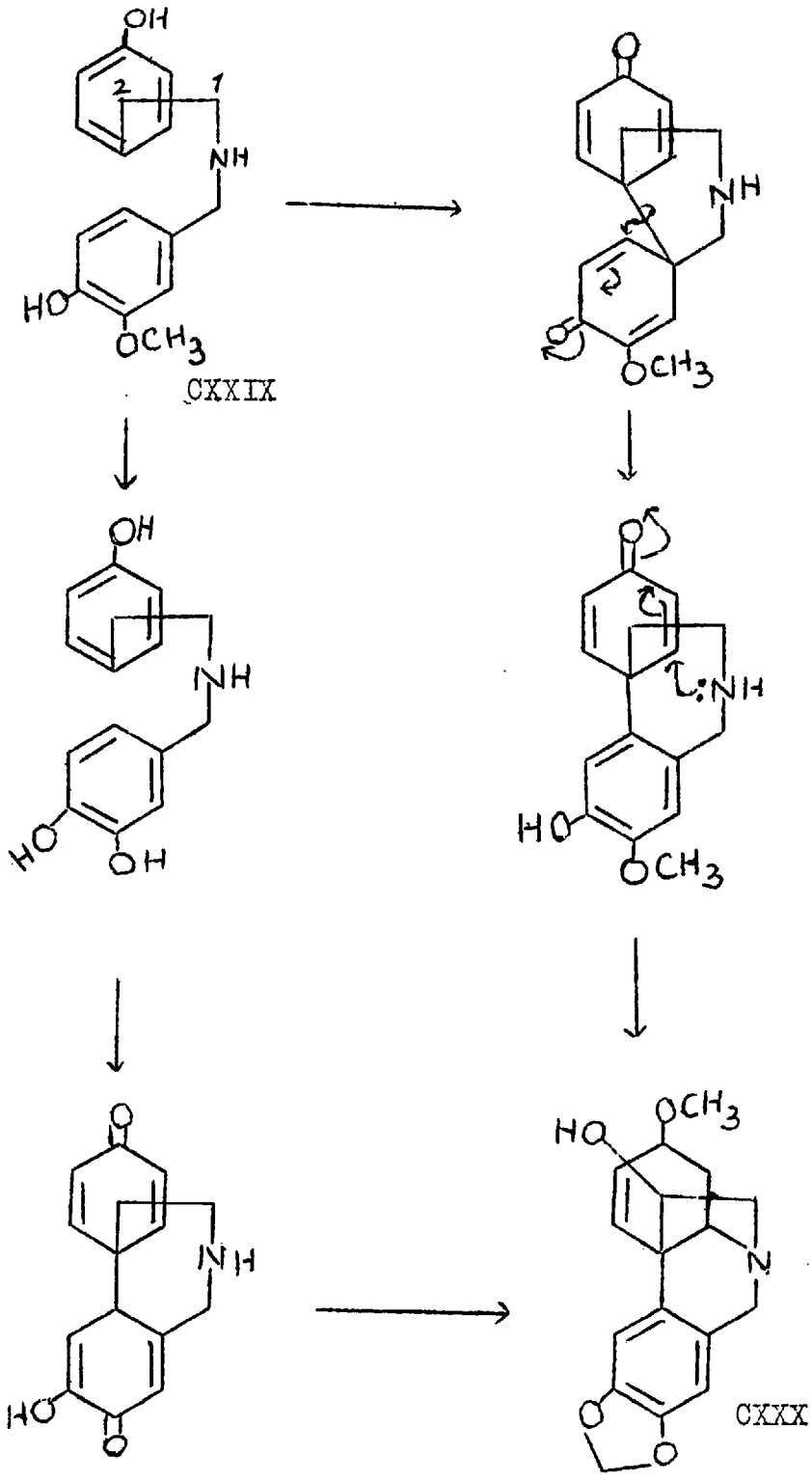


CXXVIII

The purpose of the present experiments was to look for an efficient precursor of galanthine. From several considerations 4-hydroxy-3-methoxy-N-(3-hydroxy-4-methoxybenzyl) phenethylamine (CXXXVIII) appeared to be a good potential precursor of galanthine. In this compound, two phenolic groups, one in each aromatic ring, are suitably situated for the oxidative coupling[4], and the two methoxyl groups are in right positions for galanthine. This compound was synthesised and fed

to the plants. Tyrosine is known to provide the $C_6$-$C_2$ unit of galanthine[39] and lycorine[7,8,21] (CXXVII). 3,4-Dihydroxyphenylalanine (CXXVIII) had not been tested as a possible precursor of the dioxygenated $C_6$-$C_2$ unit in galanthine or lycorine. [$\alpha$-$^{14}$C] 3,4-Dihydroxyphenylalanine was, therefore, fed to the plants.

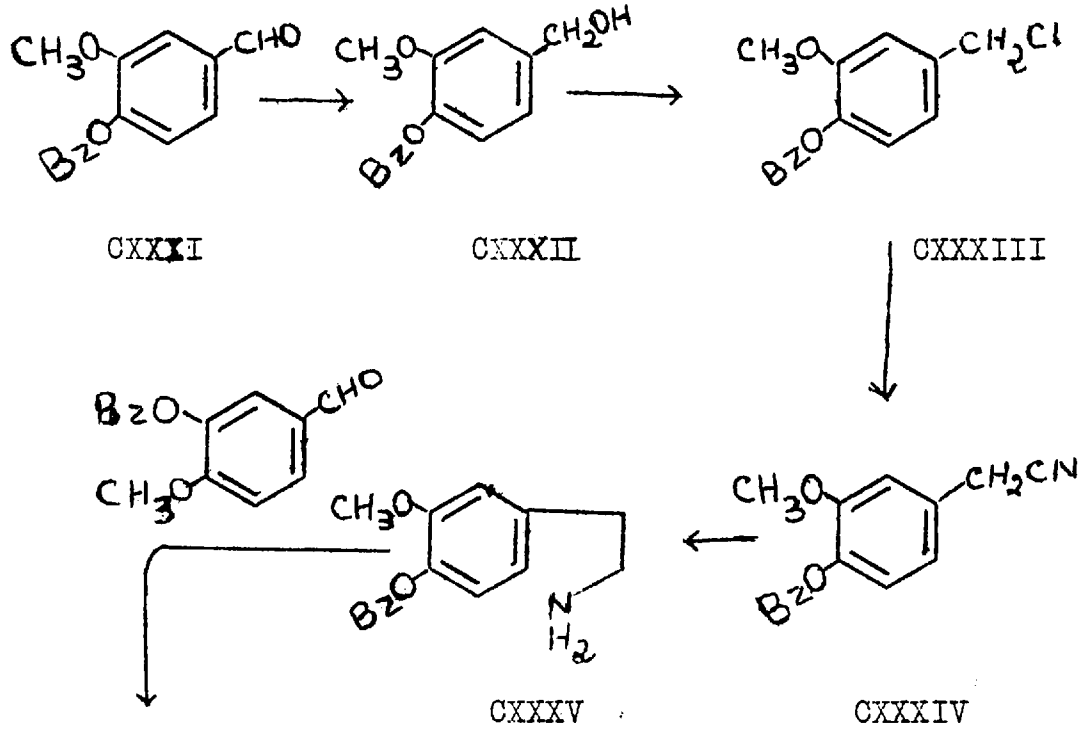
O-Methylnorbelladine has been shown[18,31] to be an efficient precusor of haemanthamine (CXXX). The isomeric O-methyl norbelladine (CXXIX) could also serve as a precursor of haemanthamine and other Amaryllidaceae alkaloids if it is assumed that a dienone-phenol rearrangement, outlined below, takes place in the plant, or if the compound is enzymatically demethylated to give norbelladine which would then be incorporated. To test for these possibilities, [1-$^{14}$C] 4-hydroxy-N-(4-hydroxy-3-methoxybenzyl)phenethylamine (CXXIX) was synthesised and fed to 'King Alfred' daffodils.


OH
2
1
NH
HO
OCH3
CXXIX
O
NH
2
2
OCH3
OH
NH
HO
OH
O
NH
HO
OCH3
O
NH
HO
O
OCH3
HO
N
O
O
CXXX

Synthesis of Precursors.

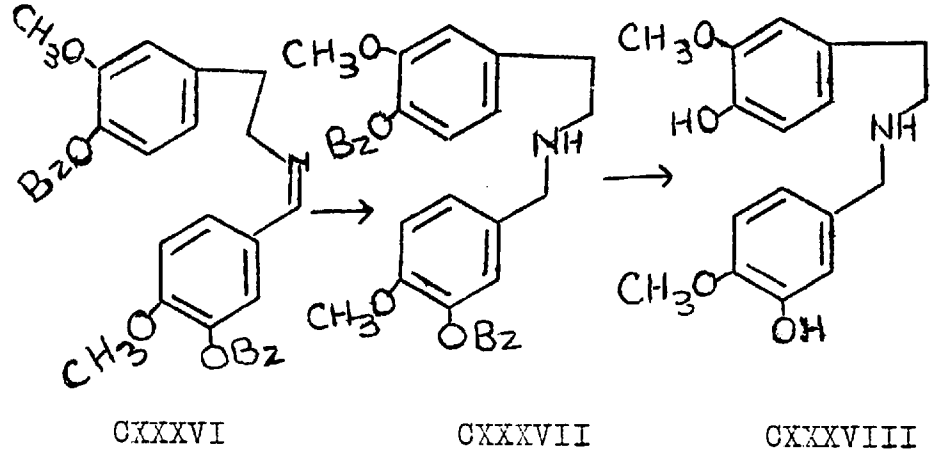
The precursors were synthesised and labelled according to the annexed scheme. The amine (CXXXVIII) was synthesised as follows. O-Benzyl vanillin (CXXXI) was reduced with sodium borohydride to the corresponding alcohol (CXXXII) which was then converted to the corresponding benzyl--chloride (CXXXIII). This chloride with sodium cyanide in dimethyl sulphoxide gave the nitrile (CXXXIV) which was reduced with lithium aluminium hydride to give 4-benzyloxy-3--methoxy-phenethylamine (CXXV). This amine was allowed to condense with O-benzylisovanillin to give imine (CXXXVI), which was reduced with sodium borohydride to give the amine dibenzyl ether (CXXXVII). Hydrogenolysis of this amine gave the amine (CXXXVIII), which was isolated as its crystalline hydrochloride.

This isomeric O-methylnorbelladine (CXXIX) was synthesised as follows. O-Benzyltyramine (CXXXIX), prepared by the method of Barton and his coworkers[18] was allowed to condense with O-benzylvanillin to give the imine (CXL), which was reduced with sodium borohydride to the amine dibenzylether (CXLI).

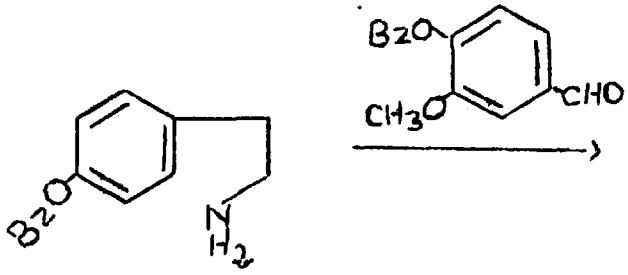


CXXXI CXXXII CXXXIII

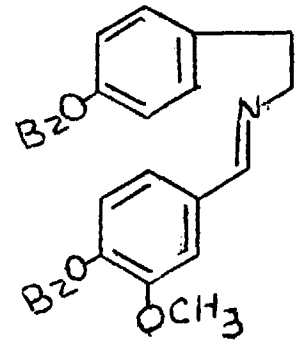
CXXXV CXXXIV



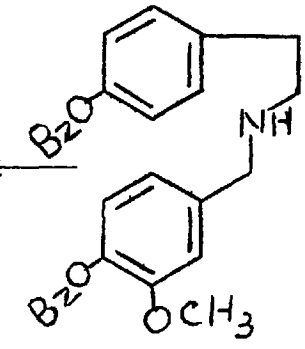
CXXXVI CXXXVII CXXXVIII



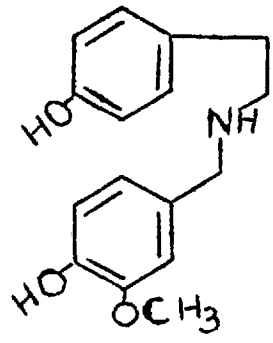
CXXXIX



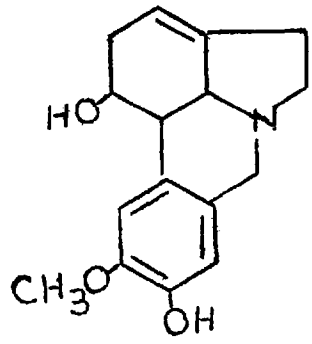
CXL



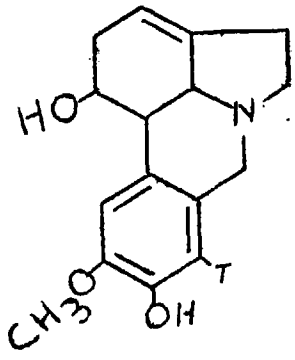
CXLI



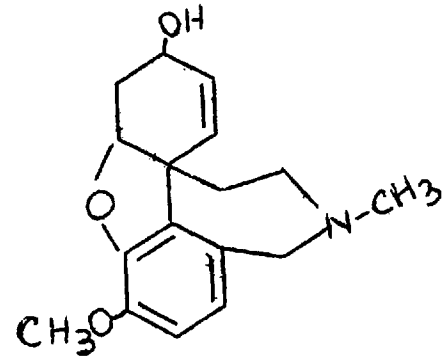
CXXIX



CXLII



CXLIII



CXLIV

Hydrogenolysis of this gave the amine (CXXIX), which was isolated as its crystalline hydrochloride.

Radioactive precursors were prepared by using [$^{14}$C] NaCN and carrying out the syntheses as described above.

Tritium labelled norpluviine (CXLIII) was obtained by heating natural norpluviine (CXLII) with tritiated water and potassium tertiarybutoxide at 100$^{o}$ for seven days. Preliminary experiments were carried out with $D_2O$. Examination of the n.m.r. spectra of the starting material and the product showed that only one of the two aromatic protons exchanged under the reaction conditions. This exchanged proton must be at position ortho to the phenolic hydroxyl group, because under the conditions used, the exchange takes place specifically at ortho- and para- positions to the phenolic hydroxyl group.[34]

The precursors were shown to be pure, both chemically and radiochemically, by dilution analysis, and also by autoradiography of the circular paper chromatograms.

Tracer Studies.

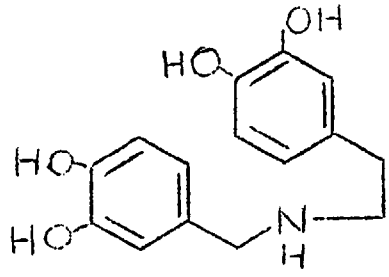
All the biogenetic studies were carried out with 'King Alfred' daffodils. The plants were

worked up, and alkaloids isolated according to the method used by Barton and his coworkers[18]. Nearly equal quantities of galanthamine (CXLIV), galanthine (CXXVI), and haemanthamine (CXXX) were obtained. No significant quantities of other alkaloids present, were obtained from the plant material used.

In the feeding experiments, flowering 'King Alfred' daffodils were injected with [$\alpha$-$^{14}$C] 3,4-dihydroxyphenylalanine, [1-$^{14}$C] 4-hydroxy-3-methoxy-N-(3-hydroxy-4-methoxybenzyl)phenethylamine (CXXXVIII), [1-$^{14}$C] 4-hydroxy-N-(4-hydroxy-3-methoxybenzyl)phenethylamine (CXXIX), and [$^{3}$H] norpluviine (CXLIII). Feedings were accomplished by direct injection of the aqueous solutions of the hydrochlorides of precursors into the hollow flowering stalks of the plants. The injections were made at about pH 6, and were followed by a further injection of distilled water. The plants were worked up after eight days in each case, the alkaloids isolated, and their activity measured. The alkaloids obtained from the feeding experiments of the first three precursors were inactive. In case of [$^{3}$H] norpluviine feeding, only galanthine was found to be active, although the amount of

incorporation (0.0056%) was quite low.

The lack of incorporation of 3,4-dihydroxyphenylalanine into galanthine, galanthamine or haemanthamine indicates that this does not provide the $C_6$-$C_2$ unit of the Amaryllidaceae alkaloids. The lack of incorporation of [1-$^{14}$C] 4-hydroxy-3-methoxy-N-(3-hydroxy-4-methoxybenzyl)phenethylamine (CXXXVIII) and also of 3,4-dihydroxyphenylalanine into galanthine suggests that introduction of the fourth hydroxyl group in this alkaloid occurs at a late stage. This is in agreement with the observation of Battersby et al.[27], that the hydroxynorbelladine (CXLV) is not incorporated into lycorine. In accord with Battersby et al.[27] the



CXLV

biogenetic conversion of norpluviine into galanthine was observed. All these results can best be accommadated by the suggestion[27] that norpluviine is an intermediate in the biosynthesis of lycorine group of alkaloids, and it is hydroxylated to give lycorine and galanthine.

The lack of incorporation of isomeric O-methylnorbelladine (CXXIX) into any of the three alkaloids - galanthine, galanthamine, or haemanthamine - suggests that a dienonephenol rearrangement does not take place in the biosynthesis of these alkaloids. This lack of incorporation also rules out the enzymatic demethylation of the precursor into norbelladine in plants. This proves that O-methylnorbelladine is a specific precursor of haemanthamine. This specificity of the precursor is also proved by the observation of Fales, Mann and Mudd[30] that the enzyme isolated from the flowering bulbs of Nerine bowdenii methylates norbelladine para to the side chain $-CH_2-NH-$ to give O-methylnorbelladine more readily than the meta position to give the isomeric O-methylnorbelladine. The ratio of para to meta methylation by this enzyme was found to be 22:1.

## EXPERIMENTAL

Melting points were taken on the Kofler block. Unless specified to the contrary, the ultraviolet absorption spectra refer to ethanol, infrared absorption spectra to chloroform, and n.m.r. spectra to deuterochloroform ($CDCl_3$) solutions. The n.m.r. spectra were taken on Varian A-60 instrument.

Counting methods.

Compounds labelled with $^{14}C$ were counted as thin films (ca. 0.5 mg. per $cm.^2$) and were not corrected for self-absorption or back-scattering. A gas-flow (methane) proportional counter was used, giving a background of ca. 15 counts per minute. Tritium activities were measured using a Tritium Scintillation Counter (Isotope Developments Ltd., Type 6012 A), samples being dissolved in dimethylformamide (0.2 ml.) and liquid scintillator (Nuclear Enterprises Ltd., Type N.E. 213) (1.2 ml.). The counting procedure was calibrated using standard [1,2-$^3H$] n-hexadecane, efficiencies of ca. 17% being customary. Samples containing both $^3H$ and $^{14}C$ were counted by scintillation method at two different voltage settings, one being optimal for $^3H$ and the other for $^{14}C$. It was possible to

discriminate between the two isotopes in this way and no additional error was introduced as the $^{3}H/^{14}C$ ratio was large (more than 7) in all the compounds.

O-Methylnorbelladine (CI). (Method of Barton[18] et al.)

O-Benzyltyramine (1.25 g.) in methanol (10 ml.) was allowed to condense with O-benzylisovanillin (1.43 g.) in methanol (10 ml.). The intermediate imine in methanol was reduced *in situ* with excess potassium borohydride for 3 hours. The solvent was removed under reduced pressure, residue diluted with water, and the product extracted into ether. The ether extract was washed with water, dried, and the solvent evaporated off. The amine dibenzyl ether was isolated as its hydrochloride (1.64 g.). The dibenzyl ether hydrochloride was hydrogenolysed in ethanol in presence of 10% palladium-carbon catalyst (1 g.) to give O-methylnorbelladine hydrochloride which crystallised from ethanol-ether as plates (0.92 g.), m.p. 205-206° (lit.,[18] m.p. 205-207°).

Belladine Methiodide (CII).

O-Methylnorbelladine hydrochloride (0.2 g.) was treated with excess methyl iodide (5 ml.) and anhydrous potassium carbonate (1 g.) in dry acetone (20 ml.) under reflux for 16 hours. The solution was filtered while hot and the residue washed with hot acetone (4 X 15 ml.). Acetone was evaporated off and the product crystallised from

ethanol to give plates (195 mg.), m.p. 223-224° (Lit.[44] m.p. 224-225.5°).

Hofmann degradation of Belladine Methiodide (CII)

Belladine methiodide (CII, 110 mg.) was heated with 20% aqueous potassium hydroxide solution (10 ml.) at 100° for ten hours. The solution was cooled and extracted with ether (5 X 6 ml.). The ether extract was washed with water, and extracted with 2N-hydrochloric acid (3 X 4 ml.). The ether portion was washed with water (2 X 3 ml.), dried, and ether evaporated. The residue, p-methoxystyrene (18 mg.) was dissolved in ethyl chloride (2 ml.) and ozonolysed at -40° for ½ hour. The ozonide was oxidised with hydrogen peroxide and the resulting anisic acid was crystallised from ethanol into needles (10 mg.), m.p. 183-184° (Lit.,[43] m.p. 184°).

The total acidic portion was basified with sodium carbonate and extracted with chloroform (5 X 4 ml.). The chloroform extract was washed with water (2 X 4 ml.), dried, and the solvent evaporated off. The residue was dissolved in ether (5 ml.) and 6N-hydrochloric (0.2 ml.) added. The precipitated N,N-dimethylveratrylamine (CIV) hydrochloride was collected by filtration and

recrystallised from ethanol-ether as plates (26 mg.), m.p. 204°. (Lit.,[45] m.p. 202-203°). N,N-Dimethylveratrylamine was also isolated as its methiodide, m.p. 179-180° (decomp.). (Lit.,[46] m.p. 179.5-180.5° (decomp.) )

[1-$^{14}$C] O-Methylnorbelladine (CI). (Method of Barton et al.[18])

[$^{14}$C] Sodium cyanide (0.5 mc., 1.5 mg.) was diluted with inactive sodium cyanide (10.2 mg.) dissolved in dimethylsulphoxide (1 ml.) and excess p-benzyloxybenzyl chloride (72.0 mg.) added. The solution was heated at 100° for 3 hours, diluted with water, and extracted with ether, carrier nitrile (50 mg.) being added in the first ether portion. The ether extract was washed with water, dried, and ether evaporated off. The residue was reduced with lithium aluminium hydride, and the [2-$^{14}$C] O-benzyltyramine isolated as its crystalline hydrochloride (74 mg.)

[2-$^{14}$C] O-Benzyltyramine hydrochloride (18 mg.) was diluted with inactive material (34 mg.) and the free base liberated. The base was then allowed to condense with O-benzylisovanillin (70 mg.). The intermediate imine was reduced *in situ* with excess potassium borohydride. The resulting amine

dibenzyl ether was hydrogenolysed as its hydrochloride in presence of 10% palladium-carbon catalyst. The product, O-methylnorbelladine hydrochloride crystallised from ethanol-ether as plates (15 mg.), m.p. and mixed m.p. 204-206°. A portion of the precursor was diluted with a known weight of inactive material, crystallised, and counted.

| Precursor (1.2 mg.) diluted | Activity (c.p.m/m.mole) |
|---|---|
| with inactive material (48.7 mg.) | $1.48 \times 10^4$ |
| (recrystallised) | $1.43 \times 10^4$ |

[$^3$H] O-Methylnorbelladine (C)

O-Methylnorbelladine hydrochloride (50 mg.) potassium tertiarybutoxide (75 mg.), and tritiated water (0.5 ml.) were taken in a Carius tube and nitrogen was bubbled through the solution for 10 minutes. The tube was then sealed and heated at 100° for seven days. The contents of the tube were dissolved in 2N-sodium hydroxide and the solution buffered with sodium bicarbonate. The precipitated amine was centrifuged off and washed with water (6 X 3 ml.). It was then dissolved ir ether and 4N-hydrochloric acid (0.2 ml.) added.

The precipitated hydrochloride was centrifuged off, washed with ether (3 X 4 ml.), and recrystallised from ethanol-ether to give plates (35 mg.), m.p. and mixed m.p. 205-207°. The precursor was diluted with inactive material, recrystallised, and counted.

| Precursor(1.6 mg.), diluted | Activity (c.p.m./mg.) |
|---|---|
| with inactive material (100.1 mg.) | $1.11 \times 10^5$ |
| (recrystallised) | $1.09 \times 10^5$ |

$\frac{[^3H + 1\text{-}^{14}C]\ \underline{O}\text{-Methylnorbelladine}}{[^3H]\ \underline{O}\text{-Methylnorbelladine hydrochloride}}$ ( CXX )

(6.6 mg.) in methanol (1 ml.) was mixed with $[1\text{-}^{14}C]$ O-methylnorbelladine hydrochloride (11.0 mg.) in methanol (1 ml.), and inactive O-methylnorbelladine hydrochloride (14.4 mg.) in methanol (1 ml.). The solution was concentrated, cooled and ether added. The amine hydrochloride crystallised as plates (28 mg.), m.p. and mixed m.p. 205-206°. The precursor was diluted with inactive material, recrystallised and counted only at tritium maximum efficiency settings.

| Precursor (1.1 mg.), diluted with | Activity (c.p.m./mg.) |
|---|---|
| inactive material (48.9 mg.) | $2.90 \times 10^4$ |
| (recrystallised) | $2.84 \times 10^4$ |

Hofmann degradation of [$^3$H] O-methylnorbelladine.

[$^3$H] O-Methylnorbelladine (225 mg.) was methylated to belladine methiodide, which was heated with 20% potassium hydroxide to give p-methoxystyrene (20 mg.) and N.N-dimethylveratrylamine (40 mg.). p-Methoxystyrene was ozonolysed and the ozonide oxidised with hydrogen peroxide to give anisic acid, N.N-dimethylveratrylamine was converted into both, its methiodide and its hydrochloride, which crystallised from ethanol-ether. The activities of all these compounds were measured and were as follows.

| Compounds | Activity (c.p.m./m.mole) | |
|---|---|---|
| | Sample I | Sample II |
| O-Methylnorbelladine hydrochloride | $1.49\times10^6$ | $6.99\times10^8$ |
| (recryst.) | $1.47\times10^6$ | $6.92\times10^8$ |
| Belladine methiodide | $1.42\times10^6$ | $6.78\times10^8$ |
| (recryst.) | $1.43\times10^6$ | $6.79\times10^8$ |
| Anisic acid | $7.80\times10^5$ | $3.41\times10^8$ |
| (recryst.) | $7.74\times10^5$ | $3.38\times10^8$ |
| N,N-Dimethylveratrylamine methiodide | $7.56\times10^5$ | |
| (recryst.) | $7.49\times10^5$ | |
| N,N-Dimethylveratrylamine hydrochloride | $7.38\times10^5$ | $3.50\times10^8$ |
| (recryst.) | $7.34\times10^5$ | $3.46\times10^8$ |

Counting of [$^3$H] Hexadecane Standard with inactive O-methylnorbelladine hydrochloride.

[$^3$H] Hexadecane standard was weighed out in two counting tubes. A known weight of O-methylnorbelladine was added to one of them. Dimethylformamide (0.2 ml.) and the scintillating solution (1.2 ml.) was added to each of the tubes and then their activities were measured. There was no drop in the activity of the standard due to the presence of inactive substance as indicated from the counts below.

| | Specific Activity |
|---|---|
| [$^3$H] Hexadecane Standard | $8.95 \times 10^2$ c.p.m./mg. |
| [$^3$H] Hexadecane Standard containing O-methylnorbelladine hydrochloride (0.75 mg.) | $8.91 \times 10^2$ c.p.m/mg. |

Similar checks were made with other inactive compounds, but none of them was found to quench the activity of the [$^3$H] hexadecane standard.

Isolation and Characterisation of Norpluviine (CVI)

Thegeneral procedure used was that of Fales, Giuffrida, and Wildman[35]. The 'Texas' bulbs (wet weight 500 g.) were macerated and extracted with 1% tartaric acid in ethanol (2 X 200 ml.). The extract

was filtered, evaporated to small bulk (ca. 75 ml.) and diluted with water (150 ml.) and 2N-hydrochloric acid (15 ml.). The acid solution was washed with chloroform (3 X 30 ml.) and the washings back extracted with 2N-hydrochlorid acid (4 X 20 ml.). The combined acid extracts were made alkaline with sodium bicarbonate, and extracted with chloroform (10 X 30 ml.). The chloroform extract was dried, concentrated to a small bulk, and cooled. The 'chloroform insoluble' portion was filtered off and washed with chloroform (3 X 10 ml.). The solid dissolved in N-sodium hydroxide solution leaving only a negligible amount of insoluble material. The solution was filtered, and the filtrate buffered with sodium bicarbonate. The precipitated alkaloid was collected by filtration, washed with water (4 X 15 ml.), and dried. Crystallisation with methanol gave plates (90 mg.), m.p. 239-241°; $[\alpha]_D$ -232° (C, 0.056 in methanol), $\lambda_{max.}$ 284 mμ (log ε 3.77) (Lit.,[37] m.p. 274-275°; $[\alpha]_D$ -160° (C=0.15 in methanol).

The alkaloid had its infrared spectrum (nujol) superimposable on the infrared spectrum (nujol) of a sample of norpluviine from Prof. Battersby. The melting point of the alkaloid did not show a depression when mixed with a sample of norpluviine.

(Found: C, 70.30; H, 7.04; N, 5.25; O, 17.11% calculated for $C_{16}H_{19}O_3N$: C, 70.31; H, 7.01; N, 5.13; O, 17.56%).

Pluviine (CVII)

A suspension of norpluviine (280 mg.) in methanol (25 ml.) was shaken for four hours with ethereal diazomethane (10 ml.). The solvent was removed under reduced pressure, and the residue was dissolved in methanol (15 ml.). A further quantity of diazomethane (10 ml.) was added and the solution kept for 10 hours in dark. The solvent was then evaporated, and the crude product chromatographed over alumina (grade V, 20 g.). Elution with benzene-ethylacetate (4:1) gave the product which crystallised from benzene to give cubes (320 mg.), m.p. 220-222°; $[\alpha]_D$-197° (C. 0.198 in ethanol) and -165° (C. 0.345 in chloroform). $\lambda_{max.}$ 282.5 mμ (log ε 3.60). (Lit.,[36] Constants for pluviine, m.p. 225-227°. $[\alpha]_D$-151° (C. 0.42 in chloroform); -170.5° (C=0.18 in ethanol); -181° (C. 0.3 in ethanol)[37]. The product was acetylated with acetic anhydride and pyridine to give the acetyl derivative, m.p. 182-184°(Lit.,[36] m.p. for pluviine acetate 184°).

Norpluviine Diacetate. (CVIII)

Norpluviine (90 mg.) was shaken with acetic anhydride (2 ml.) and pyridine (0.5 ml.) for four hours. The solution was then poured in ice cold water (150 ml.), made alkaline with sodium bicarbonate, and extracted into ether (3 X 20 ml.) The ether extract was washed with water (3 X 10 ml.), dried, and the ether evaporated off in vacuum. The residue was chromatographed on alumina (grade V, 20 g.) in benzene. Elution with benzene-ethylacetate (9:1) gave the diacetate which crystallised from petroleum ether (60-80°) as cubes (105 mg.), m.p. 150-151°. $\lambda_{max}$ 279.3 mμ (log ε 3.98). $\nu_{max}$ 1723 $cm.^{-1}$ (non-phenolic acetate), 1760 $cm.^{-1}$ (phenolic acetate.). N.M.R. spectrum showed singlets at 3.1 τ and 3.2 τ (one proton each), a broad band at 4.61 τ (one proton), and singlets at 6.21 τ, 7.70 τ and 8.14 τ .(each equal to three protons). (Found: C, 66.86; H, 6.44; N, 4.13; O, 22.27%. $C_{20}H_{23}O_5N$ requires C, 67.21; H, 6.49; N, 3.92; O, 22.38%).

Deuteration of norpluviine diacetate

Norpluviine diacetate (115 mg.) was heated with potassium tertiarybutoxide (1.2 g.) and deuterium oxide (1 ml.) at 100° for seven days. The contents

were then dissolved in N-sodium hydroxide (4 ml.) and then excess sodium bicarbonate was added. The precipitated material was filtered off, washed with water, and dried. It was then acetylated with acetic anhydride and pyridine at room temperature and the product crystallised from petroleum ether (60-80°). N.M.R. spectrum showed a singlet at 3.13 $\tau$ (one proton, aromatic).

1-Acetyldihydronorpluviine (CIX)

Norpluviine diacetate (135 mg.) in ethanol (20 ml.) containing 6N-hydrochloric acid (0.5 ml.) was hydrogenated over 10% palladium-carbon catalyst (40 mg.). Nearly two moles of hydrogen were absorbed and the uptake of hydrogen was complete in ca. three hours. The catalyst was filtered off and the solvent evaporated off. The residue was treated with sodium bicarbonate and extracted with chloroform (3 X 6 ml.). The chloroform extract was dried and the solvent evaporated off. The product showed two very closely running spot on the thin-layer chromatography plate. These two products could not be separated by column chromatography. The mixture crystallised from benzene as shining plates (82 mg.), m.p. 175-180°. $\nu_{max.}$ 1723 $cm.^{-1}$ and 3550 $cm.^{-1}$ ($CHCl_3$). The n.m.r. spectrum showed singlets at

3.32 $\tau$ and 3.40 $\tau$ (one proton each), a broad band at 4.13 $\tau$ (one proton, -O-C-H), a singlet at 6.20 $\tau$ (3 protons, $-O-CH_3$) and two singlets at 8.04 $\tau$ (3 protons) and 8.15 $\tau$ (2 protons). (Found: C, 68.22; H, 7.44; N, 4.54%. $C_{18}H_{23}O_4N$ requires C, 68.12; H, 7.31; N, 4.41%).

The compound seems to be a mixture of the two epimers, present in the ratio of 3:2.

1-Acetyl-8-bromodihydronorpluviine (CX)

A solution of bromine (80 mg., one mole) in acetic acid (3 ml.) was added slowly with stirring to a solution of 1-acetyldihydronorpluviine (88 mg., mixture of the two epimers) in acetic acid (2 ml.). The solution was kept at room temperature for one hour, diluted with water (20 ml.), buffered with sodium bicarbonate, and extracted with chloroform (4 X 8 ml.). The chloroform extract was dried and the solvent evaporated off. The product still showed two close running spots on thin-layer chromatography plate. However, when the crude product was chromatographed on alumina (grade V, 20 g.) and eluted with benzene-ethylacetate (4:1), the two bromo-epimers were separated. The earlier fractions gave a solid, which crystallised from

benzene-petrol into needles (34 mg.), m.p. 189-190°. $\nu_{max.}$ 1723 cm.$^{-1}$ and 3550 cm.$^{-1}$ ($CHCl_3$). The n.m.r. spectrum of the product showed singlet at 3.3 $\tau$ (one proton), a broad band at 4.15 $\tau$ (one proton, -O-C-H), a singlet at 6.25 $\tau$ (3 protons, -O-$CH_3$), and a singlet at 8.14 $\tau$ (3 protons, -CO-$CH_3$).
(Found: C, 54.58; H, 5.92%. $C_{18}H_{22}BrNO_4$ requires C, 54.56; H, 5.56%).

8-Bromonorpluviine Diacetate (CXI)

A standard solution of bromine in ethanol was added slowly with stirring to a solution of norpluviine hydrochloride (100 mg.) in ethanol (10 ml.). Addition of bromine solution was stopped after one mole equivalent of bromine had been added. The solution was kept for a further half hour and then ethanol was evaporated off. The residue was diluted with water, buffered with sodium bicarbonate and extracted with chloroform (6 X 15 ml.) The chloroform extract was dried and the solvent evaporated off. The residue was treated with acetic anhydride (2 ml.) and pyridine (0.5 ml.) at room temperature for three hours. The solution was diluted with cold water (15 ml.), buffered with sodium bicarbonate, and extracted with ether

(3 X 8 ml.). The ether extract was washed with water (4 X 8 ml.), dried, and the ether evaporated off. The residue was chromatographed on alumina (grade V, 20 g.). Elution with benzene-ethylacetate (19:1) gave the bromodiacetate which crystallised from petroleum ether (60-80°) as cubes (90 mg.), m.p. 124-125°. $\nu_{max.}$ 1723 $cm.^{-1}$ and 1760 $cm.^{-1}$ ($CHCl_3$). The n.m.r. spectrum showed a singlet at 3.18 $\tau$ (one proton, aromatic), two broad bands at 4.05 $\tau$ (one proton, -O-C-H) and 4.62 $\tau$ (one proton, -C=C-H), a singlet at 6.25 $\tau$ (3 protons, $-O-CH_3$) and two singlets at 7.66 $\tau$ and 8.08 $\tau$ (each equal to 3 protons, $-CO-CH_3$). (Found: C, 54.63; H, 4.86; N, 3.20%. $C_{20}H_{22}BrNO_5$ requires C, 55.04; H, 5.05; N, 3.21%).

Attempted Hydroxylation of Pluviine Acetate with $SeO_2$

Pluviine acetate (37 mg.) was heated with selenium dioxide (15 mg.) in acetic acid for four hours. The reaction mixture was filtered to remove the liberated selinium, filtrate diluted with water, buffered with sodium bicarbonate, and extracted with chloroform (3 X 5 ml.). The chloroform extract was dried and manganese dioxide

added. After one hour's shaking, manganese dioxide was filtered off, and the solvent removed under vacuum. The product showed at least 6 spots on thin-layer chromatography plate. The infrared spectrum of the product did not show a carbonyl absorption band. The phenolic acetate band was also not present in the infrared spectrum.

Phenolbetaine (CXVI). (Method of Boit et al.[36])

Pluviine (150 mg.) was refluxed with benzophenone (450 mg.), tertiary butanol (6 ml.), and potassium (90 mg.) under nitrogen for six hours. The reaction mixture was then acidified with N-hydrochloric acid and the solvent evaporated off. The residue was dissolved in a little water, made ammoniacal, and filtered. The dark red precipitate was washed with chloroform and the residue recrystallised from ethanol to give the phenolbetaine as dark red needles (12 mg.) which did not melt up to 300°C.

1-Methoxyl-anhydro-methylpseudolycorine. (CXVII)

The phenolbetaine (10 mg.) in dimethylformamide (1 ml.) was methylated with methyl iodide (0.5 ml.). After refluxing for 2 hours the solvent was evaporated off and the residue in methanol was reduced

with sodium borohydride for one hour. The reaction mixture was diluted with water and extracted with chloroform. The chloroform extract was washed with water, dried and the solvent evaporated off. The product was chromatographed on alumina (grade V, 10 g.). Elution with benzene-ethylacetate (9:1) gave 1-methoxyl-anhydro-methylpseudolycorine which crystallised from ethanol as needles (6 mg.), m.p. 216-217° (Found: C, 67.16; H, 6.24%. $C_{18}H_{17}O_4N$ with one mole of $C_2H_5OH$ requires C, 67.21; H, 6.49%).

Pluviine Acetate (CXII) and Anhydro-methylpseudolycorinium chloride. (CXIII). (Method of Boit et al.[36])

Pluviine (130 mg.) and acetic anhydride (4 ml.) were heated at 100° for 3 hours in presence of oxygen. The reaction mixture was cooled, diluted with water, and evaporated to dryness. The residue was dissolved in N-hydrochloric acid (1 ml.), made ammoniacal, and extracted with chloroform (4 X 5 ml.). The chloroform extract was dried and the solvent evaporated off. The residue was chromatographed on alumina (grade V, 15 g.). Elution with benzene-ethylacetate (9:1) gave the pluviine acetate which crystallised from methanol as prisms (70 mg.), m.p. 183-184°. (Lit.,[36] m.p. 184°).

The ammoniacal solution, left after extraction with chloroform, was acidified with N-hydrochloric acid, concentrated, and cooled. Anhydro-methylpseudolycorinium chloride (CXIII) crystallised as needles. These were collected by filtration and recrystallised from ethanol as pale yellow needles (42 mg.), m.p. 225-226° (decomp.). (Lit.,[36] m.p. 225° (decomp.) ).

3,4-Dihydro-9,10-dimethoxypyrrolo(de)-7-phenanthridone (CXIV) ( Method of Boit et al.[36] )

Anhydro-methylpseudolycorinium chloride (20 mg.), potassium ferricyanide (40 mg.), and potassium hydroxide (40 mg.) in water (3 ml.) were heated at 100° for ¾ hour, and the voluminous precipitate extracted with chloroform (4 X 3 ml.). The chloroform extract was washed successively with 5% hydrochloric acid and a 5% solution of sodium carbonate. After drying, the solvent was evaporated off, and the residue chromatographed on alumina (grade V, 15 g.). Elution with benzene-ethylacetate (9:1) gave the lactam (CXIV) which crystallised from ethanol as needles (10 mg.), m.p. 273-274°. (Lit.,[35] m.p. 272-274°). $\nu_{max.}$ 1645 $cm.^{-1}$ The n.m.r. spectrum (in $CF_3COOH$) showed a broad partially resolved band at 2.45 $\tau$ (area equal to 5 protons,

aromatic) and two singlets at 5.88 $\tau$ and 6.04 $\tau$ (each equal to 3 protons, two $-O-CH_3$ groups).

Synthesis of the Lactam (CXIV). (Method of Fales, Giuffrida and Wildman[35])

6-Nitroveratric acid (7 g.) in anhydrous benzene (50 ml.) and thionyl chloride (10 ml.) were refluxed for two hours. Evaporation of the solvent under reduced pressure gave the crystalline acid chloride. This was directly treated with a chilled solution of indolene (5 ml.) in pyridine (8 ml.). After the initial reaction had subsided, the contents were warmed on waterbath for fifteen minutes, cooled, digested with methanol, filtered, and washed with cold methanol. The amide (CXVIII, a) crystallised from ethanol as long needles (6.5 g.), m.p. 182-183° (Lit.,[35] m.p. 180-183°).

A suspension of the amide (CXVIII, a 1.5g.) in ethanol-ethylacetate (1:1, 50 ml.) was hydrogenated using 10% palladium-carbon catalyst (1 g.). The solution, when warmed, absorbed 3 moles of hydrogen and the uptake was complete in ca. three hours. The catalyst was filtered off and the solvent evaporated off. The amine (CXVIII) crystallised from ethanol as needles (1.3 g.), m.p. 157-158°. (Lit.,[35] m.p. 157-158°).

The amine (CXVIII, 1g.) in 0.7N-sulphuric acid (25 ml.) was cooled to 0° and a chilled solution of sodium nitrite (0.4 g.) in water (2 ml.) was added over a period of one hour. The diazotised product was left at room temperature for ½ hour. Copper powder (100 mg.) was added and the contents were heated on steambath till the evolution of nitrogen stopped. The solid from the reaction mixture was extracted into chloroform (5 X 15 ml.), and the chloroform extract was washed successively with sodium carbonate solution and water. After drying, the solvent was evaporated off, the residue triturated with benzene and filtered. The residual solid was chromatographed on alumina (grade V, 50 g.). Elution with benzene-ethylacetate (9:1) gave the desired lactam (CXIV) which crystallised from ethanol-chloroform as needles (230 mg.), m.p. 273-275°.

Anhydro-methylpseudolycorine (CXV).

(i) Method of Boit et al.[36]

The lactam (CXIV, 60 mg.) in tetrahydrofuran (2 ml.) was added to a suspension of lithium aluminium hydride (100 mg.) in refluxing tetrahydrofuran (5 ml.) over a period of ½ hour. After

further one hour, the lithium aluminium hydride was decomposed with water, tetrahydrofuran layer decanted off, and the residue washed with tetrahydrofuran (3 X 4 ml.). The total tetrahydrofuran portion was evaporated to dryness and the residue dissolved in chloroform. The chloroform solution was washed with water, dried, and the solvent evaporated off. The product crystallised from ethanol as needles (45 mg.), m.p. 173-175° and 260-272° (Lit.[36], m.p. 174-177°, also[35] 145° and 260-270°).

(ii) Borohydride reduction of Anhydro-methylpseudolycorinium chloride. (CXIII)

Anhydro-methylpseudolycorinium chloride (CXIII,20mg.) in methanol (2 ml.) was reduced with excess sodium borohydride (20 mg.) at room temperature for one hour. The crystalline product was filtered off, washed with methanol, and recrystallised from ethanol to give needles (9 mg.), m.p. 173-176° 265-270°.

Attempted Bromination of Anhydro-methylpseudolycorine

Bromine (16 mg.) in chloroform (1 ml.) was added to anhydro-methylpseudolycorine (27 mg.) in

Chloroform (2 ml.) over a period of ½ hour. Chloroform was then evaporated off, the residue treated with sodium carbonate solution, and extracted with chloroform. The chloroform extract was washed with water, dried, and the solvent evaporated off. The residue crystallised from ethanol to give needles (20 mg.), m.p. 273-275°. The infrared spectrum of the product in chloroform was identical with that of the lactam (CXIV) in chloroform. Similar results were obtained when the bromination was attempted in acetic acid.

1-Bromo-3,4-dihydro-9,10-dimethoxypyrrolo(de)-7-phenanthridone. (CXIX)

The lactam (CXIV, 70 mg.) and sodium acetate (140 mg.) in acetic acid (4 ml.) were heated on the waterbath in a flask fitted with a reflux condenser. Bromine (40 mg.) in acetic acid (2 ml.) was added over a period of ½ hour. The solution was then cooled and diluted with an excess of cold water. The precipitate was collected by filtration, washed with water, dried, and chromatographed on alumina (grade V, 20 g.). Elution with benzene-ethylacetate (9:1) gave the bromolactam (CXIX) which crystallised from ethanol-chloroform as needles (84 mg.), m.p. 286-287°. $\nu_{max.}$ 1645 cm.$^{-1}$

The n.m.r. spectrum showed 4 singlets at 2.27 $\tau$ 2.43 $\tau$ , 2.65 $\tau$ , and 2.77 $\tau$ (each equal to one proton, the four aromatic protons), and a partially resolved broad band at 5.96 $\tau$ (equal to 6 protons, two $-O-CH_3$ groups).
(Found: C, 56.69; H, 3.92; N, 3.75%. $C_{17}H_{14}BrNO_3$ requires C, 56.44; H, 3.88; N, 3.88%).

TRACER STUDIES

'Texas' daffodils were used for the tracer studies.

(a) [$^3$H] O-Methylnorbelladine.

[$^3$H] O-Methylnorbelladine hydrochloride (19 mg., 0.05 mc.) in water (5 ml.) was injected into the hollow flower stalks of four plants, distilled water being injected after the final injection of the precursor. After eight days the whole plants (wet weight 410 g.) were worked up in the usual way. The chloroform extract on being reduced to a small bulk gave 96 mg. of the insoluble material. This was treated with N-sodium hydroxide solution, filtered, and the filtrate buffered with sodium bicarbonate. Norpluviine (86 mg.) was collected by filtration, washed with water, dried, and crystallised from methanol. The activity of the alkaloid was measured and the

percentage of incorporation calculated.

| Alkaloid | Activity (c.p.m./m.mole) |
|---|---|
| Norpluviine | $3.21 \times 10^6$ |
| (recryst.) | $3.16 \times 10^6$ |

Incorporation of O-methylnorbelladine into norpluviine = 0.70%.

Norpluviine diacetate. (CVIII)

Radioactive norpluviine (70mg.) was acetylated with acetic anhydride and pyridine. The diacetate was purified by chromatography and crystallised from petroleum ether (60-80°) as cubes (65 mg.), m.p. 149-150°.

Exchange reaction of Norpluviine Diacetate

Radioactive norpluviine diacetate (60 mg.) and potassium tertiarybutoxide (57 mg.), in water (5 ml.) were heated at 100° for seven days. The solution was buffered with sodium bicarbonate and the precipitated norpluviine reacetylated. The activity of this compound was measured and the exchange experiment was repeated to ensure the complete exchange at position 2. The counting results were as follows.

| Compounds | Activity (c.p.m./m.mole) | Relative Activity |
|---|---|---|
| Norpluviine diacetate | $4.70 \times 10^5$ | |
| (recryst.) | $4.75 \times 10^5$ | 1.00 |
| (after exchange) | $1.86 \times 10^5$ | |
| (after repeating the exchange experiment) | $2.41 \times 10^5$ | |
| (recryst.) | $2.36 \times 10^5$ | 0.51 |

1-Acetyldihydronorpluviine (CIX)

Radioactive norpluviine diacetate (157 mg.) was hydrogenated as its hydrochloride using 10% palladium-carbon catalyst. The 1-acetyldihydronorpluviine was purified by chromatography and crystallised from benzene as plates (63 mg.), m.p. 176-180°.

1-Acetyl-8-bromodihydronorpluviine.(CX)

1-Acetyldihydronorpluviine (40 mg.) was brominated in acetic acid. The bromo-derivative was purified by chromatography and crystallised from benzene-petrol as needles (18 mg.), m.p. 189-190°.

| Compounds | Activity (c.p.m./m.mole) | Relative Activity |
|---|---|---|
| Norpluviine diacetate | $3.89 \times 10^5$ | 1.00 |
| (recryst.) | $3.75 \times 10^5$ | |
| 1-Acetyldihydronorpluviine | $1.86 \times 10^5$ | |
| (recryst.) | $1.77 \times 10^5$ | 0.48 |
| 1-Acetyl-8-bromodihydronorpluviine | $1.12 \times 10^5$ | |
| (recryst.) | $1.08 \times 10^5$ | 0.30 |

8-Bromonorpluviine diacetate (CXI)

Radioactive norpluviine diacetate (188 mg.) in ethanol (5 ml.) was treated with 50% aqueous potassium hydroxide solution (2 ml.) at room temperature. After one hour, the solution was buffered with sodium bicarbonate and the precipitated norpluviine (120 mg.) was collected by filtration, washed with water, and dried. In order to see if any loss in activity has taken place due to exchange during hydrolysis, the precipitated norpluviine (14 mg.) was reacetylated to the diacetate and its activity measured. Also in order to check if any activity is lost due to exchange during the workup of the plants, norpluviine (15 mg.) was kept in 2N-hydrochloric acid for 24 hours, the free base liberated, and acetylated to give the diacetate, whose activity was also measured.

The bulk of the precipitated norpluviine (80 mg.) was brominated in ethanol. The crude product was acetylated and the 8-bromonorpluviine diacetate purified by chromatography. It crystallised from petroleum-ether (60-80°) as needles (54 mg.), m.p. 123-125°.

| Compounds | Activity (c.p.m./mole) | Relative Activity |
|---|---|---|
| Norpluviine diacetate | $4.94 \times 10^4$ | |
| (recryst.) | $4.89 \times 10^4$ | 1.00 |
| (after hydrolysis and reacetylation) | $4.85 \times 10^4$ | |
| (recryst.) | $4.79 \times 10^4$ | 0.97 |
| (after keeping the norpluviine in acid and reacetylation) | $4.86 \times 10^4$ | |
| (recryst.) | $4.92 \times 10^4$ | 1.01 |
| 8-Bromonorpluviinediacetate | $2.36 \times 10^4$ | |
| (recryst.) | $2.49 \times 10^4$ | 0.51 |

Pluviine (CVII)

Radioactive norpluviine (24 mg.) diluted with inactive material (82 mg.) was methylated with ethereal diazomethane. The crude product was chromatographed to give pluviine which crystallised from methanol as plates (85 mg.), m.p. 222-224°.

Pluviine Acetate (CXII) and Anhydro-methylpseudolycorinium chloride (CXIII).

Pluviine (80 mg.) was acetylated by heating with acetic anhydride according to the procedure of Boit et al.[36] Pluviine acetate was purified by chromatography and crystallised from methanol as prisms (45 mg.), m.p. 184°.

The other product of the reaction, the anhydro-methylpseudolycorinium chloride was crystallised from ethanol as pale yellow needles (12 mg.), m.p. 223-225°

(decomp.).

3,4-Dihydro,9,10-dimethoxypyrrolo(de)-7-phenanthridone. (CXIV).

Anhydromethylpseudolycorinium chloride (12 mg.) in water (2 ml.) was heated with potassium ferricyanide (35 mg.) and potassium hydroxide (35 mg.) for ½ hour on the waterbath. The oxidation product was extracted in chloroform. The crude product obtained was chromatographed on alumina. The lactam (CXIV) crystallised from ethanol as needles (7 mg.), m.p. 272-274°.

| Compounds | Activity (c.p.m/m.mole) | Relative Activity |
|---|---|---|
| Pluviine | $7.74 \times 10^4$ | |
| (recryst.) | $7.60 \times 10^4$ | 1.00 |
| Pluviine Acetate | $7.62 \times 10^4$ | |
| (recryst.) | $7.51 \times 10^4$ | 0.98 |
| The lactam (CXIV) | $7.64 \times 10^4$ | |
| (recryst.) | $7.73 \times 10^4$ | 1.01 |

The feeding experiment with [3]-O-methylnorbelladine was repeated in the following flowering season. O-methylnorbelladine hydrochloride ( 5.4 mg., 0.09 mc.) in water (5 ml.) was injected into the hollow flower stalks of four plants. The plants (wet weight 380 g.) were worked up after eight days in the usual way and gave radioactive norpluviine (68 mg., $1.35 \times 10^7$ c.p.m./m.mole, 0.73% incorporation).

The two sets of experiments, carried out with the radioactive norpluviine diacetate and the radioactive pluviine, from the previous feeding experiment, were repeated. Also the lactam (CXIV) was converted to 2-bromo-derivative (CXIX) as follows and its activity measured.

2-Bromo-3,4-dihydro-9,10-dimethoxypyrrolo(de)-7-phenanthridone (CXIX).

The radioactive lactam (CXIV, 10 mg.) in acetic acid (1 ml.) was brominated in presence of excess sodium acetate (50 mg.) at steam-bath. The product was purified by chromatography on alumina and crystallised from ethanol-chloroform as needles (7 mg.), m.p. 284-285°.

| Compounds | Activity (c.p.m./m.mole) | Relative Activity |
|---|---|---|
| SET I | | |
| Norpluviine diacetate | $3.34 \times 10^4$ | |
| (recryst.) | $3.30 \times 10^4$ | 1.00 |
| (after exchange) | $1.59 \times 10^4$ | |
| (after further exchange) | $1.58 \times 10^4$ | |
| (recryst.) | $1.62 \times 10^4$ | 0.49 |
| 8-Bromonorpluviine diacetate | $1.59 \times 10^4$ | |
| (recryst.) | $1.68 \times 10^4$ | 0.51 |

| Compounds | Activity (c.p.m./m. mole) | Relative Activity |
|---|---|---|
| SET II | | |
| Pluviine | $1.88 \times 10^4$ | |
| (recryst.) | $1.81 \times 10^4$ | 1.00 |
| Pluviine Acetate | $1.84 \times 10^4$ | |
| (recryst.) | $1.77 \times 10^4$ | 0.98 |
| The lactam (CXIV) | $1.82 \times 10^4$ | |
| (recryst.) | $1.90 \times 10^4$ | |
| The 2-bromolactam (CXIX). | $9.51 \times 10^3$ | |
| (recryst.) | $9.40 \times 10^3$ | 0.53 |

Counting of $[^3H]$ - and $[^{14}C]$ Standard.

Both the $[^3H]$ - and the $[^{14}C]$ standards were counted at the voltage settings for the maximum efficiency of tritium (settings I, 1560 V/1700 V) and that of $^{14}C$ (settings II, 1300 V/1400 V). The specific activities of these standards at these settings were as follows.

$[^3H]$ Standard (Specific activity $4.00 \times 10^3$ d.p.m./mg.)

Specific activity at Settings I = $6.82 \times 10^2$ c.p.m./mg.

Efficiency of the counter = 17%

Specific activity at Settings II = $2.46 \times 10^2$ c.p.m./mg.

Efficiency of the counter = 6.1%

[$^{14}$C] Standard. (Specific activity 2.15X10$^3$ d.p.m./mg.)

Specific activity at Settings I = 7.32 X 10$^2$ c.p.m./mg.

Efficiency of the counter = 34%

Specific activity at Settings II = 1.54 X 10$^3$ c.p.m./mg.

Efficiency of the counter = 71%

(b) [$^3$H + 1 -$^{14}$C] O-Methylnorbelladine (CXX)

[$^3$H] O-Methylnorbelladine hydrochloride (6.6 mg., sp. activity 4.35 X 10$^7$ d.p.m./mg.) in ethanol (0.5 ml.) and [1-$^{14}$C] O-methylnorbelladine hydrochloride (11.0 mg., sp. activity 1.04 X 10$^6$ d.p.m./mg.) in ethanol (1.0 ml.) were mixed and the precursor was crystallised by adding ether to give [$^3$H+1-$^{14}$C] O-methylnorbelladine hydrochloride (15 mg., $^3$H/$^{14}$C ratio 25.4). The precursor (CXX) was diluted with inactive material and the activity of the diluted precursor was measured at settings I and II. From these values the ratio of $^3$H/$^{14}$C in the precursor was calculated from the following simultaneous equations.

$$\frac{17x}{100} + \frac{34y}{100} = \text{Counts at settings I,}$$

$$\frac{6.1x}{100} + \frac{71y}{100} = \text{Counts at settings II}$$

Where x and y are activities due to tritium and $^{14}$C respectively.

The [$^{3}$H+1-$^{14}$C] O-methylnorbelladine hydrochloride (14 mg.) in water (5 ml.) was injected into the hollow flower stalks of three plants. Distilled water was injected after the final injection of the precursor. After eight days, the plants (wet weight 350 g.) were worked up in the usual way and the radioactive norpluviine (35 mg.) isolated. The following two sets of experiments were carried out with the radioactive norpluviine and the activities of the products measured. The ratio $^{3}H/^{14}C$ was determined in each case.

Set I

Norpluviine Diacetate. (CVIII)

Radioactive norpluviine (12.0 mg.) was diluted with the inactive material (95.0 mg.) and acetylated in the usual way to give the radioactive norpluviine diacetate (120 mg.). It was crystallised from petroleum ether (60-80°) and its activity measured at the two settings.

8-Bromonorpluviine Diacetate (CXI)

The radioactive norpluviine diacetate was hydrolysed and the norpluviine obtained was brominated in ethanol. The product was acetylated and the 8-bromonorpluviine diacetate was purified by chromatography. It was crystallised from benzene-petrol and its activity measured at the two settings.

Set II.

Pluviine. (CVII)

The radioactive norpluviine (15 mg.) diluted with inactive material (90 mg.) was methylated with diazomethane. The product was purified by chromatography and the pluviine crystallised from methanol as plates (100 mg.). The activity of the compound was measured at both the settings.

Pluviine Acetate (CXII)

Pluviine (90 mg.) was heated with acetic anhydride for 3 hours to give pluviine acetate (35 mg.) and anhydro-methylpseudolycorinium chloride (20 mg.). The pluviine acetate was purified by chromatography, crystallised, and its activity measured at both the settings.

3,4-Dihydro-9,10-dimethoxypyrrolo(de)-7-phenanthridone (CIV)

The anhydro-methylpseudolycorinium chloride (20 mg.) obtained from the above experiment, was oxidised with potassium ferricyanide in presence of alkali. The lactam (CXIV) was purified by chromatography, crystallised, and its activity measured at the two settings.

The results from the two sets of experiments are tabulated on p. 116 .

| Compounds | Activity (d.p.m./m.mole) | | $^{3}H/^{14}C$ |
|---|---|---|---|
| | Settings I | Settings II | |
| Precursor (diluted) | | | |
| (recryst.) | $2.01 \times 10^{8}$ | $7.80 \times 10^{7}$ | 25.8 |
| Norpluviine diacetate | $2.65 \times 10^{5}$ | $1.79 \times 10^{4}$ | 14.5 |
| (recryst.) | $2.55 \times 10^{5}$ | $1.89 \times 10^{4}$ | 13.4 |
| 8-Bromonorpluviine diacetate | $1.46 \times 10^{5}$ | $2.31 \times 10^{4}$ | 6.35 |
| (recryst.) | $1.41 \times 10^{5}$ | $2.12 \times 10^{4}$ | 6.72 |
| Pluviine | $1.76 \times 10^{5}$ | $1.38 \times 10^{4}$ | 12.8 |
| (recryst.) | $1.82 \times 10^{5}$ | $1.32 \times 10^{4}$ | 13.8 |
| Pluviine Acetate | $1.94 \times 10^{5}$ | $1.42 \times 10^{4}$ | 13.7 |
| (recryst.) | $1.76 \times 10^{5}$ | $1.25 \times 10^{4}$ | 14.1 |
| The lactam (CXIV) | $1.78 \times 10^{5}$ | $1.38 \times 10^{4}$ | 12.8 |
| (recryst.) | $1.68 \times 10^{5}$ | $1.22 \times 10^{4}$ | 13.8 |

4-Benzyloxy-3-methoxybenzyl Alcohol (CXXXII)

O-Benzylvanillin (2 g.) in methanol (10 ml.) was shaken with potassium borohydride (0.5 g.) at room temperature for one hour. The solvent was removed under reduced pressure. Excess borohydride was decomposed by addition of water and the product extracted into ether (5 X 6 ml.). The ether extract was washed with water (2 X 5 ml.), dried ($Na_2SO_4$), and the solvent evaporated. The alcohol crystallised from ether-light petroleum as needles (1.6 g.), m.p. 70-71°.
(Found: C, 73.91; H, 6.53%. $C_{15}H_{16}O_3$ requires C, 73.77; H, 6.56%).

4-Benzyloxy-3-methoxybenzyl Chloride. (CXXXIII)

The corresponding alcohol (1.0 g.) in benzene (10 ml.) was added slowly to a solution of thionyl chloride ( 5 ml.) in boiling benzene (10 ml.) containing pyridine (0.1 ml.). The solution was heated under reflux for one hour, cooled, and treated with ice-water. The benzene layer was washed successively with N-sodium hydrogen carbonate and water, dried, and the solvent removed under vacuum. The chloride crystallised from ether-light petroleum as needles (0.75 g.), m.p. 73-74°.
(Found: C, 68.41; H, 5.89%. $C_{15}H_{15}ClO_2$ requires

C, 68.58; H, 5.71%).

4-Benzyloxy-3-methoxybenzyl Cyanide.(CXXXIV)

The corresponding chloride (0.5 g.) in freshly distilled dimethylsulphoxide (2 ml.) was added to excess sodium cyanide (0.15 g.) in the same solvent (5 ml.). The solution was heated at 100° for three hours with stirring. The reaction mixture was diluted with water, and the product extracted into ether (4 X 4 ml.). The ether extract was washed with water (4 X 3 ml.), dried, and the solvent removed under vacuum. The nitrile crystallised from petroleum (60-80°) as plates (0.3 g.), m.p. 67-68°.

(Found: C, 76.05; H, 5.92; N, 5.03%. $C_{16}H_{15}NO_2$ requires C, 75.90; H, 5.93; N, 5.53%).

4-Benzyloxy-3-methoxyphenethylamine (CXXXV).

4-Benzyloxy-3-methoxybenzyl cyanide (0.54 g.) in anhydrous ether (20 ml.) was added slowly over a period of one hour to a suspension of lithium aluminium hydride (2 g.) in ether (20 ml.) under reflux. After a further one hour, the excess lithium aluminium hydride was decomposed with water, the ether layer decanted off, and the aqueous layer washed with further quantities of

ether (2 X 10 ml.). The total ethereal extract was washed with water, dried, and the solvent removed under vacuum. The amine was converted into its hydrochloride (0.28 g.) using ethanolic hydrogen chloride. The amine hydrochloride crystallised from ethanol-ether as plates, m.p. 173-174°. (lit.[40] 173-175°).

4-Benzyloxy-3-methoxy-N-(3-benzyloxy-4-methoxybenzyl) phenethylamine. (CXXXVII)

The amine (CXXXV, 2.84 g.) in methanol (5 ml.) was added to O-benzylisovanillin (2.56 g.) in methanol (5 ml.) and the solution allowed to stand at room temperature for one hour. The crystalline intermediate imine (CXXXVI) was filtered off, and recrystallised from benzene to give needles, m.p. 101-103°.

(Found: C, 77.69; H, 6.59; N, 2.86%. $C_{33}H_{31}NO_4$ requires C, 77.34; H, 6.46; N, 2.91%).

In general, the condensation product in methanol was directly reduced with excess potassium borohydride for two hours at room temperature. Excess solvent was removed under vacuum, water added, and the product extracted into ether. Concentrated hydrochloric acid (1 ml.) was added, the crystalline hydrochloride (3.7 g.) filtered off,

and recrystallised from ethanol-ether as plates, m.p. 140°.
(Found: C, 71.61; H, 6.68; N, 2.78%. $C_{31}H_{33}O_4N$. HCl requires C, 71.60; H, 6.54; N, 2.70%).

4-Hydroxy-3-methoxy-N-(3-hydroxy-4-methoxybenzyl) phenethylamine (CXXXVIII)

The dibenzyl ether (CXXXVII, 0.28 g.) in ethanol (15 ml.) containing concentrated hydrochloric acid (0.1 ml.) and 10% palladium/carbon catalyst (60 mg.) was hydrogenolysed. The uptake of hydrogen was complete after Ca. forty minutes. The catalyst was filtered off, and the solvent removed under vacuum. The amine hydrochloride (0.12 g.) was crystallised from ethanol-ether as plates, m.p. 195°.
(Found: C, 60.10; H, 6.48; N, 3.91%. $C_{17}H_{21}NO_4$. HCl requires C, 60.09; H, 6.48; N, 4.12%).

4-Hydroxy-N-(4-hydroxy-3-methoxybenzyl) phenethylamine (CXXIX). (Method of Dr. M.N. Afzal)[41]

O-Benzyltyramine (CXXXIX, 0.23 g.) in methanol (5 ml.) was added to O-benzylvanillin (0.22 g.) in methanol (5 ml.), and the solution was allowed to stand for 2 hours. The intermediate imine (CXL) in methanol was directly reduced with excess potassium borohydride (500 mg.) for 4 hours at room temperature. The excess methanol was evaporated under reduced

pressure, water added, and the product extracted into ether. Evaporation of ether gave the dibenzyl ether of the amine (CXLI), which was converted to its crystalline hydrochloride and then hydrogenolysed in ethanol (10 ml.) over 10% palladium-carbon catalyst (50 mg.). The catalyst was filtered off and the solvent evaporated. The amine (CXXIX) hydrochloride crystallised from ethanol-ether as plates, m.p. 170° (lit.[41] 170-171°).

Radiochemical Synthesis of Precursors.

[1-$^{14}$C] 4-Hydroxy-3-methoxy-N-(3-hydroxy-4-methoxybenzyl) phenethylamine.

[$^{14}$C] Sodium cyanide (1.5 mg; 0.5 ml.) was diluted with sodium cyanide (8 mg.), dissolved in freshly distilled dimethylsulphoxide (1.0 ml.), and excess 4-benzyloxy-3-methoxybenzyl chloride (70 mg.) in dimethylsulphoxide (1 ml.) added. The solution was heated at 100° for 3 hours, cooled and diluted with water (10 ml.). The product was extracted into ether (4 X 3 ml.), carrier nitrile (40 mg.) being added in the first ether portion. The ether extract was washed with water (3 X 2 ml.) and dried.

After evaporation of ether in vacuum, the residue was dissolved in anhydrous ether (5 ml.),

and added slowly to a suspension of lithium aluminium hydride (400 mg.) in ether (5 ml.) under reflux during one hour. After a further hour, excess lithium aluminium hydride was decomposed with water, ether layer decanted off, and the residue washed with further quantities of ether (4 X 2 ml.), carrier amine (32 mg.) being added in the first ether portion. The total ether extract was washed with water (3 X 3 ml.), and concentrated hydrochloric acid (0.1 ml.) added. The precipitated amine hydrochloride (50 mg.) was washed thoroughly with ether (4 X 3 ml.) and dried. The free base was obtained by adding 4N sodium hydroxide (1 ml.) and extracting the base into ether. The ether extract was washed with water, dried, and ether evaporated to give the amine.

To the amine (22 mg.) in methanol (1 ml.) was added excess O-benzylisovanillin (40 mg.) in methanol (2 ml.), and the solution allowed to stand for two hours. Excess sodium borohydride (100 mg.) was then added, and the solution left for a further two hours at room temperature. The solvent was then evaporated under reduced pressure, water (4 ml.) added, and the product extracted into ether (3 X 3 ml.). The ether extract was washed with water (2 X 2 ml.),

dried, and 4N hydrochloric acid (0.1 ml.) added. The precipitated hydrochloride was centrifuged off, washed with ether (3 X 2 ml.), and recrystallised from ethanol-ether. Hydrogenolysis was carried out in ethanol (5 ml.) using 10% palladium-carbon catalyst (20 mg.). The catalyst was filtered off, washed with ethanol (2 X 2 ml.), and the total filtrate evaporated. The product recrystallised from ethanol-ether as plates (20 mg., 0.036 ml.), m.p. and mixed m.p. 193-195°. The precursor was diluted with a known amount of inactive material, recrystallised, and counted.

Precursor (1.2 mg.), diluted

with inactive material (99.8 mg.) - $1.26 \times 10^7$ c.p.m/m. mole

recrystallised - $1.28 \times 10^7$ c.p.m/m. mole

[1-$^{14}$C] 4-Hydroxy-N-(4-hydroxy-3-methoxybenzyl) phenethylamine.

[1-$^{14}$C] O-Benzyltyramine (18 mg.), prepared by the method of Barton and coworkers,[18] was allowed to stand for two hours at room temperature. Excess sodium borohydride (70 mg.) was added, and the solution allowed to stand for further four hours. Excess methanol was evaporated under reduced pressure, water (4 ml.) added, and the product

extracted into ether (4 X 2 ml.). The ether extract was washed with water (2 X 2 ml.), dried, and 4N hydrochloric acid (0.1 ml.) added. The precipitated amine hydrochloride was centrifuged off, washed with ether (3 X 2 ml.), and recrystallised from ethanol-ether. Hydrogenolysis was carried out in ethanol (5 ml.) using 10% palladium-carbon catalyst (20 mg.). The catalyst was removed by filtration and washed with ethanol (3 X 2 ml.). The total ethanol filtrate was evaporated, and the product crystallised from ethanol-ether as plates (18 mg; 0.044 ml.) m.p. and mixed m.p. 170-172°. The precursor was diluted with a known amount of the inactive material, recrystallised, and counted.

Precursor (1.4 mg.), diluted with
inactive material (46.6 mg.) - $7.86 \times 10^4$ c.p.m./m.mole
recrystallised - $7.80 \times 10^4$ c.p.m./m.mole

[$^3$H] Norpluviine (CXLIII)

Tritiated water (0.5 ml.) was added to norpluviine (CXLII, 100 mg.) and potassium tertiary butoxide (85 mg.) in a Carius tube, and nitrogen was bubbled through the contents for 10 minutes. The tube was carefully sealed, and heated at 100° for seven days. Excess sodium bicarbonate was added, and the precipi-

tated norpluviine centrifuged off. It was washed thoroughly with water (3 X 3 ml.), dissolved in N-hydrochloric acid (4 ml.), and reprecipitated with sodium bicarbonate. The precipitate was washed again with water (4.X 3 ml.), and dried to give tritiated norpluviine (66 mg; sp. activity $8.74 \times 10^6$ c.p.m./mg.). This was diluted with a known quantity of inactive norpluviine, recrystallised and counted.

| | |
|---|---|
| [$^3$H] Norpluviine (1 mg.), diluted with inactive norpluviine (99 mg.) | $2.40 \times 10^7$ c.p.m./m.mole |
| recrystallised | $2.36 \times 10^7$ c.p.m./m.mole |

Autoradiography of Precursors.

The precursors were run on circular paper chromatograms in n-butanol:acetic acid:water system (4:1:5). The dried paper was exposed for one day to X-ray film (Kodirex). Development of the film showed that the bands were coincident with those obtained by spraying the paper with Pauli[42] reagent. No radioactive impurities were shown to be present.

Isolation of Alkaloids. (General procedure of Fales Giuffrida and Wildman[35]).

'King Alfred' daffodils (whole plants; wet weight 400 g.) were macerated and extracted with

1% ethanolic tartaric acid (2 X 250 ml.). The extract was filtered, the filtrate evaporated to a small bulk (ca. 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 2 N-hydrochloric acid (4 X 20 ml.). The combined acid extracts were made alkaline with aqueous sodium carbonate and extracted with chloroform (8 X 20 ml.). The chloroform extract was dried, and the solvent removed under vacuum. The crude basic fraction (640 mg.) was chromatographed on alumina (grade III; 50 g.) using a gradient elution technique of increasing percentage of ethylacetate in benzene. The elution of the alkaloid was followed in U.V. at 280 mμ . The following fractions were obtained from the column.

Fractions.

1) Fluorescent Artefact.

2) Gum (15 mg.), which did not crystallise.

3) Gum (120 mg.), which crystallised with ether to give galanthamine (100 mg., 0.25%), m.p. 128-130°.

4) Gum (130 mg.), which crystallised with methanol to give galanthine (90 mg., 0.22%), double m.p.

75-90°, 164-165°.

5) Gum (180 mg.), which crystallised with ethylacetate to give haemanthamine (140 mg., 0.035%), m.p. 199-201°.

The alkaloid yields are based on the wet plant weight.

Feeding Experiments.

Flowering 'King Alfred' daffodils were injected with [$\alpha$-$^{14}$C] 3,4-dihydroxyphenylalanine (CXXVIII), [1-$^{14}$C] 4-hydroxy-3-methoxy-N-(3-hydroxy-4-methoxybenzyl)phenethylamine (CXXXVIII), [1-$^{14}$C]-4-hydroxy-N-(4-hydroxy-3-methoxybenzyl)phenethylamine (CXXIX), and [$^{3}$H] norpluviine (CXLIII) as their hydrochlorides, dissolved in water (10 ml.) at about pH 6. Each compound was injected into the hollow flowering stalk of 4 plants. One mililitre of the solution was injected each time and after the final injection, distilled water was injected. The plants were worked up after eight days of the first injection, and the alkaloids separated. The results from the feeding experiments were as follows.

(a) [$\alpha$-$^{14}$C] 3-4-Dihydroxyphenylalanine (4 mg., 0.02 m.c.)

Plants wet weight - - - 430 g.

Crude basic fraction - - - 0.632 g.

| Alkaloids | Activity |
|---|---|
| Galanthamine (104 mg.) | Inactive |
| Galanthine (85 mg.) | Inactive |
| Haemanthamine (112 mg.) | Inactive |

(b) [1-$^{14}$C] 4-Hydroxy-3-methoxy-N-(3-hydroxy-4-methoxybenzylphenethylamine. (16.5 mg., 0.03 m.c.)

Plants wet weight 625 g.

Crude basic fraction 0.960 g.

| Alkaloids | Activity |
|---|---|
| Galanthamine (127 mg.) | Inactive |
| Galanthine (115 mg.) | Inactive |
| Haemanthamine (154 mg.) | Inactive. |

(c) 1-$^{14}$C 4-Hydroxy-N-(4-hydroxy-3-methoxybenzyl)phenethylamine (14 mg., 0.034 m.c.)

Plants wet weight 532 g.

Crude basic fraction 0.827 g.

| Alkaloids | Activity |
|---|---|
| Galanthamine (122 mg.) | Inactive |
| Galanthine (103 mg.) | Inactive |
| Haemanthamine (150 mg.) | Inactive |

(d) $^{3}$H Norpluviine. (22.4 mg., 0.09 m.c.)

Plants wet weight 684 g.

Crude basic fraction 1.098 g.

| Alkaloids | Activity (c.p.m./m.mole) |
|---|---|
| Galanthamine (170 mg.) | Inactive |
| Haemanthamine (220 mg.) | Inactive |
| Galanthine (120 mg.) | $2.74 \times 10^4$ |
| recrystallised | $2.82 \times 10^4$ |
| Galanthine hydrochloride | $2.87 \times 10^4$ |
| recrystallised | $2.90 \times 10^4$ |

Incorporation in Galanthine 0.006%

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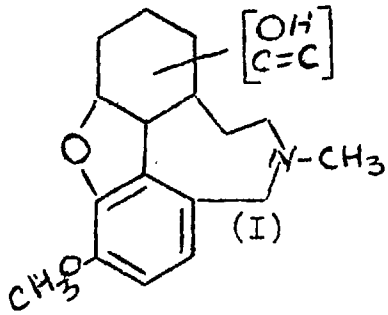
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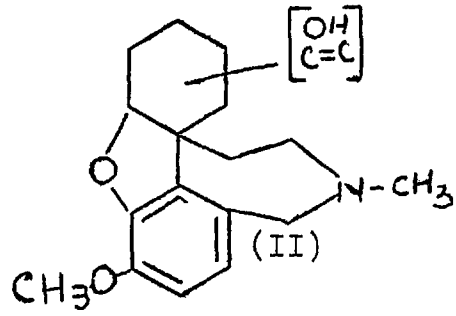
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## CHEMISTRY OF GALANTHAMINE AND PUMMERER'S KETONE

The alkaloid galanthamine $C_{17}H_{21}NO_3$, which has the dibenzofuran ring system, has been investigated by a large group of workers.[1] By 1956, galanthamine could be represented by partial structures (I) and (II).

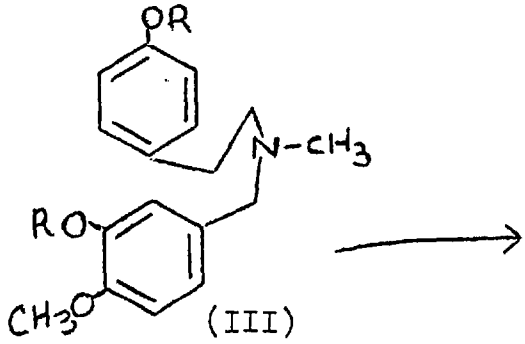



(I)

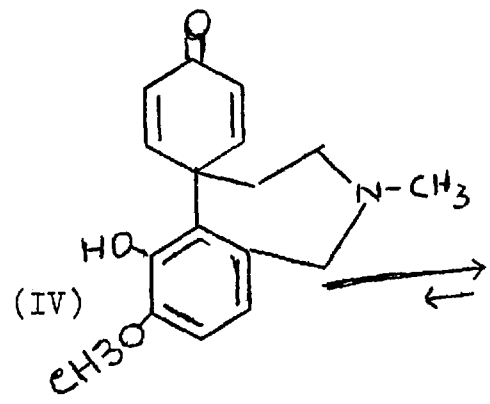



(II)

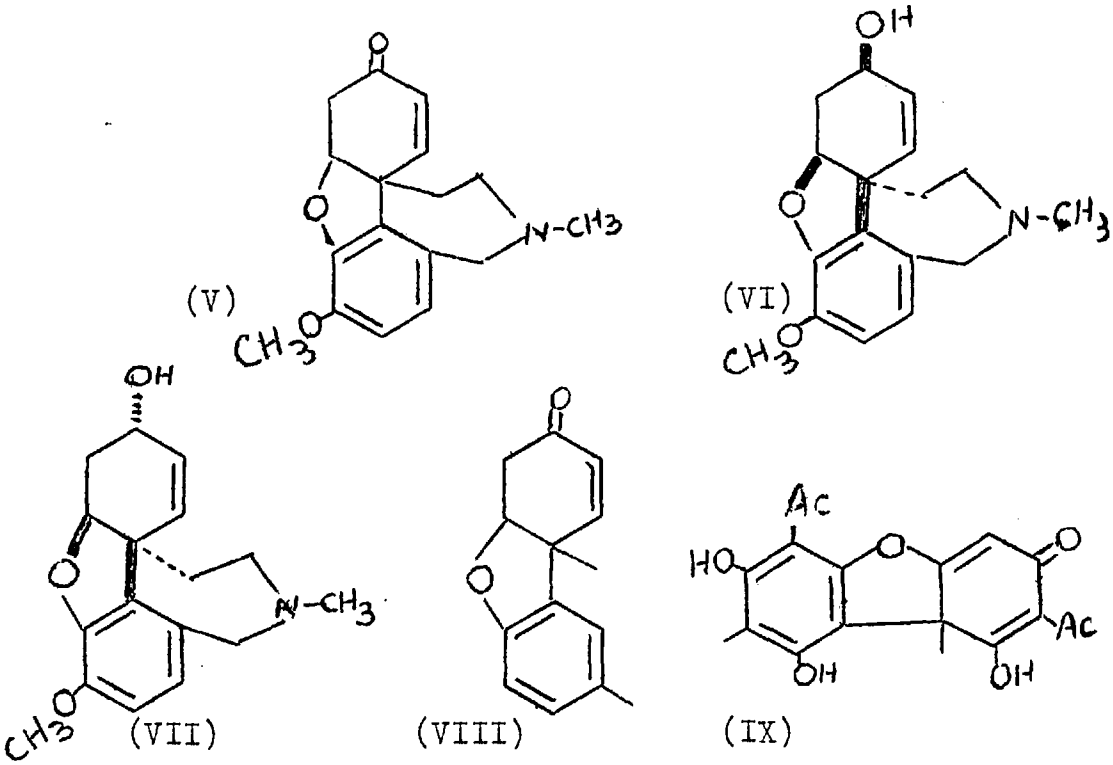
In 1957, Barton and Cohen[2] proposed the correct structure for galanthamine (VI). This was based on the concept of biogenesis, indicated by the formulae (III) - (VI), reactions which have analogy in the synthesis of Pummerer's Ketone[3] (VIII) and biosynthesis of Usnic acid[4] (IX).




(III)




(IV)



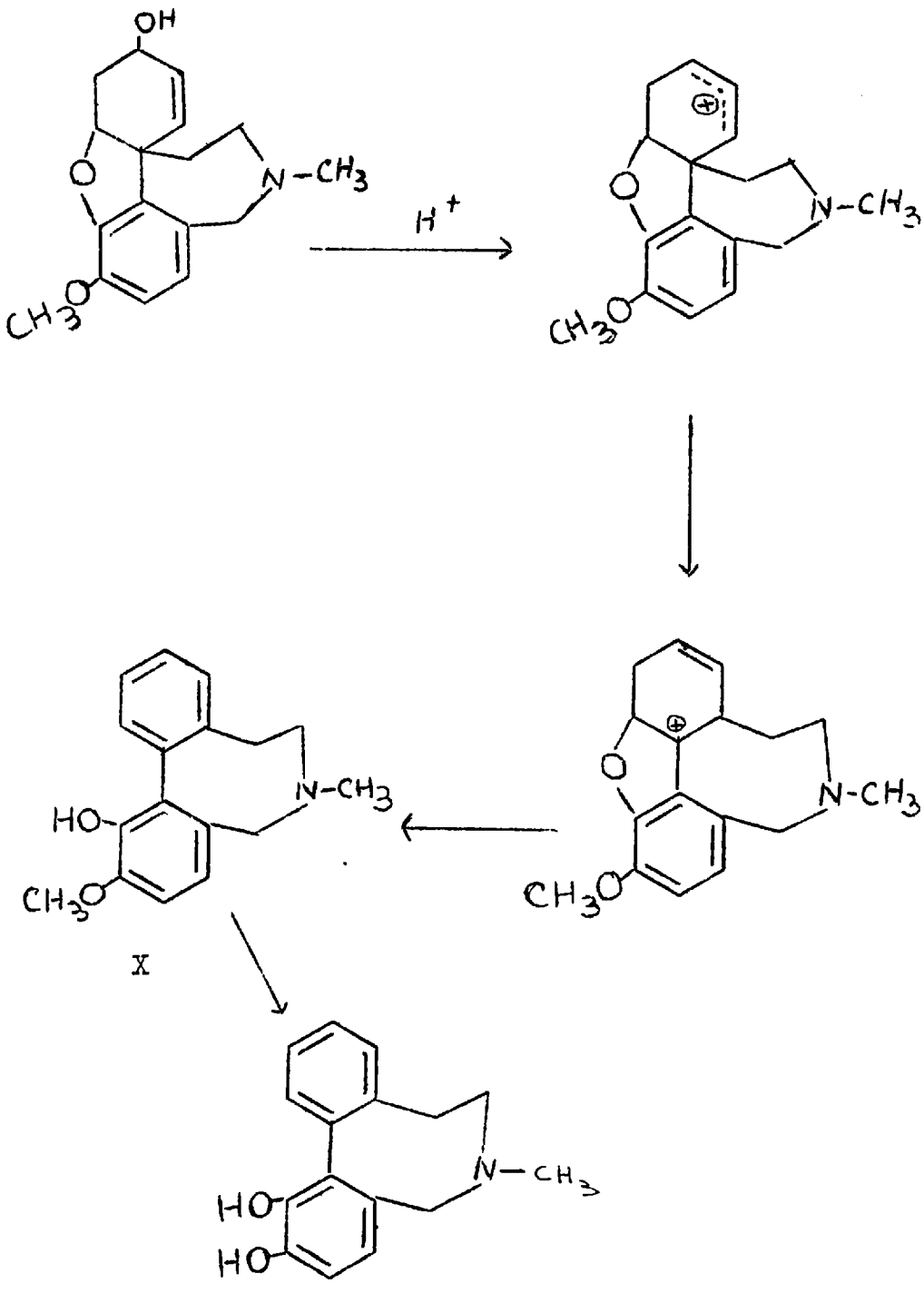

These proposals were strengthened by the subsequent isolation of the alkaloids belladine [5]. (III, R=Me) and narwedine [6]. (V). The proposed structure for galanthamine was confirmed by Uyeo [7]. The final proof for the structure (VI) came from the total synthesis of (-)-galanthamine by Barton and Kirby [8]; following the proposed [2] biogenetic route, using the radio-active diphenol (III, R=H) as the precursor.

In the course of their studies, Barton and Kirby [8] reduced (±)-narwedine with lithium aluminium hydride and obtained two stereoisomeric alcohols, m.p. 121-123°, and m.p. 190°, which were readily

separated by chromatography. Both these alcohols reconverted into narwedine by oxidation with manganese dioxide. The lower melting alcohol was identified as (±)-galanthamine by comparing its infrared spectrum with that of (-)-galanthamine. The other isomer must therefore be (±)-epigalanthamine (VII). There was a marked difference in the physical properties of the two stereoisomers. Galanthamine was much more soluble in non-polar solvents and was much more easily eluted from alumina. (±)-Galanthamine showed a hydroxy frequency band at 3575 $cm.^{-1}$ in infrared in dilute chloroform solution. This was about 55 $cm.^{-1}$ lower than the main hydroxyl absorption band of (±)-epi-galanthamine (3630 $cm.^{-1}$). These factors led them to believe that galanthamine had an intramolecular hydrogen bonding. These factors, along with the observation that (-)-narwedine racemises but does not epimerise led them to the conclusion that in narwedine (and hence in galanthamine and epigalanthamine) the oxide ring has the more stable cis-fused configuration. The hydrogen bonding shown by (±)-galanthamine was between the hydroxyl group and the oxygen of the oxide ring, which requires that the two groupings must lie cis to each other. Also, by interpreting the difference in rotations of

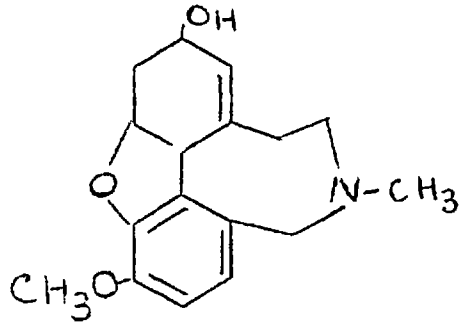
(-)-galanthamine [$(\alpha)_D$-120°] and (-)-epigalanthamine [$(\alpha)_D$-243°] according to Mills's[9] generalisation, they gave the absolute configuration of galanthamine, represented by (VI). Epigalanthamine must have the absolute configuration shown in (VII). The absolute configuration (VI) for galanthamine has recently been confirmed by the X-ray crystallographic studies of Rogers and Williams.[10]

Galanthamine has been shown to undergo rearrangements on treatment with mineral acids. Proskurnina and Yakovleva[1,a] observed that hydrobromic acid cleaved the ether linkage in galanthamine to give a diphenol apogalanthamine (XI). Uyeo and coworkers[1,d] observed that concentrated hydrochloric acid converted galanthamine to O-methyl apogalanthamine (X). These reactions could be explained as follows.


OH
O
N-CH3
CH3O
H+
O
N-CH3
CH3O
O
N-CH3
CH3O
HO
N-CH3
CH3O
X
N-CH3
HO
HO
XI

Bubewa-Iwanowa [11] has studied the action of dilute mineral acids on galanthamine. On refluxing galanthamine with dilute mineral acids, she obtained an alcohol, m.p. 182-184° to which she assigned the structure (XII). This alcohol, when further refluxed

(XII)



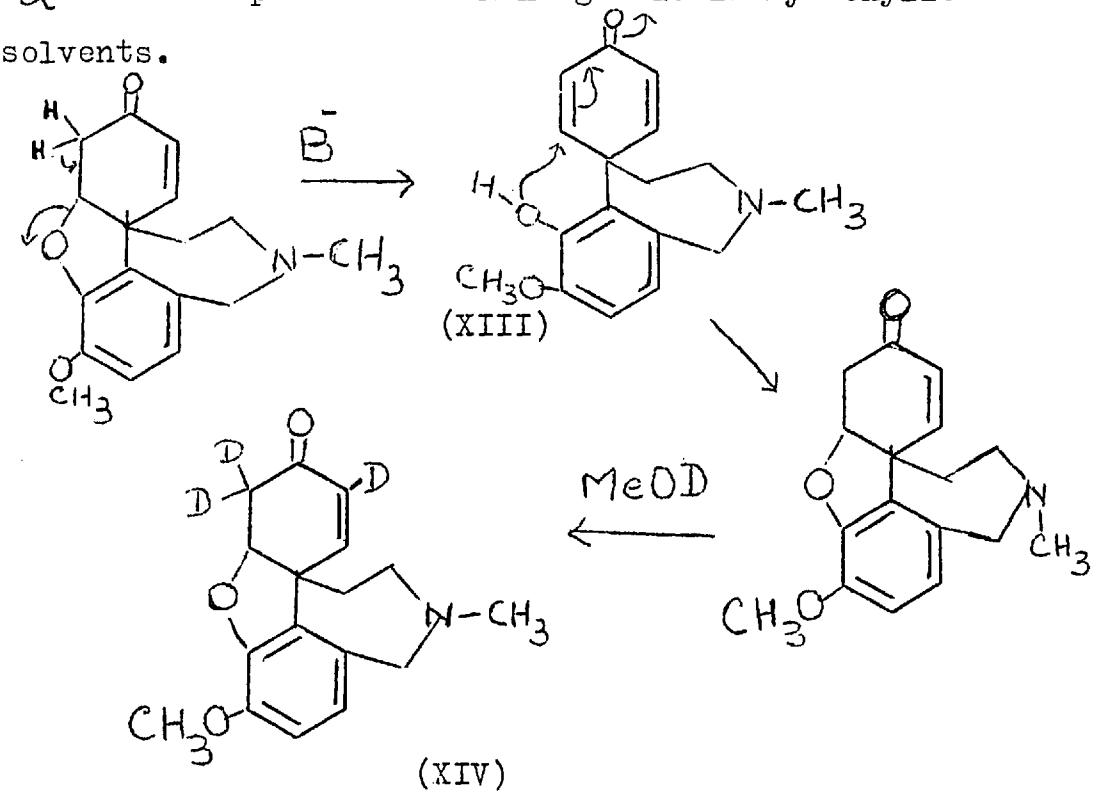

with concentrated hydrochloric acid gave O-methylapogalanthamine (X) and with concentrated hydrobromic acid gave apogalanthamine (XI). Since the proposed structure (XII) for the alcohol was not compatible with mechanistic considerations, the work was repeated according to the method of Bubewa-Iwanowa. The alcohol obtained was purified by chromatography and had the m.p. 188-189°, $[\alpha]_D$ -243° ($CHCl_3$). The infrared spectrum of this alcohol in chloroform was identical to that of (±)-epigalanthamine in chloroform. In the Nuclear Magnetic Resonance (n.m.r.) spectrum, the two vinyl protons (H-C=C-H) appeared as doublets (3.96 $\tau$ and 4.20 $\tau$ J= 10 c.p.s.). The rest of the spectrum was consistent with the structure of epigalanthamine. Oxidation of this alcohol with manganese dioxide in chloroform gave an $\alpha$-$\beta$ -unsaturated

ketone, whose infrared spectrum in nujol was identical to that of (±)-narwedine. Dr. Bubewa-Iwanowa very kindly compared the samples of alcohol and ketone with her compounds and found them identical. She also informed that, in independent studies,[12] she rejected the structure (XII), proposed earlier, and concluded, in agreement with our results, that the isomerisation product of (-)-galanthamine is, in fact, (-)-epigalanthamine (VII).

In the course of this work the n.m.r. spectrum of (±)-narwedine (V) was studied in detail. The $\alpha$-(4.00$\tau$) and $\beta$-olefinic protons (3.05$\tau$) gave the expected AB-quartet ($J_{\alpha\beta}$ = 10.5 c.p.s.). However, each of the $\beta$-components was split further into a doublet (J = 2 c.p.s.). The origin of this splitting was kindly investigated by Dr. D.W. Turner by spin-spin decoupling[13]. The sample was irradiated in the region of the band corresponding to the methine proton (-O-C-H) which appeared as a multiplet at 5.28 $\tau$. This irradiation caused the $\beta$-olefinic proton doublet to collapse to a singlet. The existence of this long-range proton coupling was confirmed in the following way.

Narwedine is known to racemise in hydroxylic solvents[8]. In basic solvents, the benzofuran ring

is opened with the loss of a methylene proton. The alkaloid itself is sufficiently basic to achieve this ring opening. The resulting dienone (XIII) can then reclose, to regenerate (±)-narwedine, the phenolic hydroxyl group adding to either double bond of the dienone group. As a result of this, the methylene protons $\alpha$- to carbonyl group and the $\alpha$-olefinic proton are exchangeable in hydroxylic solvents.



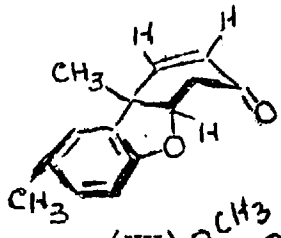

In accordance with this, narwedine was recrystallised from deuteromethanol (MeOD). This gave the trideutero-derivative (XIV) lacking in n.m.r. spectrum the $\alpha$-proton band at 4.00 $\tau$ and

the doublets at 6.96 $\tau$ and 7.16 $\tau$ , ascribable to the methylene group. The $\beta$-(3.08 $\tau$) and the methine proton (5.31 $\tau$) bands now appeared as simple doublets (J = 2 c.p.s.).

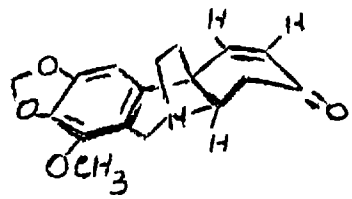
It was decided to examine the n.m.r. spectra of other compounds analogous to narwedine, for this long range proton coupling. The most readily accessible compound, which has a similar structure as narwedine is Pummerer's Ketone [3,a] (VIII). In the n.m.r. spectrum of this compound, as expected, the olefinic protons gave an AB quartet [$\alpha$ , 4.12 $\tau$ ; $\beta$ , 3.58 $\tau$ ; $J_{\alpha\beta}$ = 10.0 c.p.s.] with further splitting (J = 2 c.p.s.) of the $\beta$ -proton bands. As in narwedine the $\alpha$ -components showed no detectable fine splitting. The methine proton [-O-C-H] appeared as a closely spaced sextet (5.35 $\tau$) which could arise from splitting (J $\sim$ 3 c.p.s.) by the methylene group to give a triplet or overlapping double doublet, which was split further (J $\sim$ 2 c.p.s.) by the $\beta$ -olefinic proton. The methylene group gave a partially resolved multiplet (7.12 $\tau$ ), whose general appearance was consistent with the coupling of the two non-equivalent protons between themselves (J = 17.5 c.p.s.) and with the neighbouring methine proton (J $\sim$ 3 or 4 c.p.s.). Pummerer's Ketone was equilibrated with deuteromethanol containing a

trace of alkoxide to give the corresponding trideuteroketone. In the n.m.r. spectrum of this trideuteroketone, the methine (5.38 $\tau$) and $\beta$-olefinic (3.60 $\tau$) proton bands were, as expected, doublets (J = 2.0 c.p.s.). Some broadening of the $\beta$-olefinic bands was observed, presumably caused by coupling with the $\alpha$-deuterium.

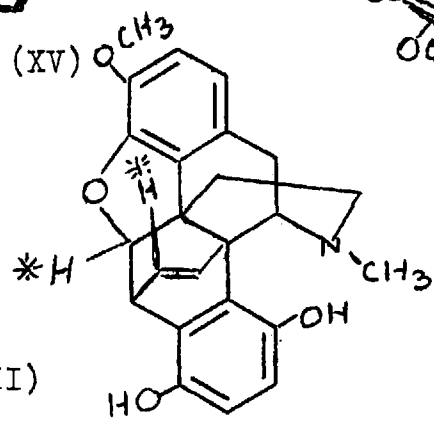
The long range proton coupling discussed here resembles in magnitude that observed between the C-2 and C-6 and the C-3 and C-5 protons of cyclohexa-2,5- dienones [14]. It is likely, therefore, that spin-coupling in these compounds is large when the relevant C-H bonds lie in, or nearly in, the plane containing the intervening carbon atoms. This is possible for narwedine and Pummerer's Ketone if the aryl group is placed in a quasi-equitorial position as shown in (XV) for Pummerer's Ketone.



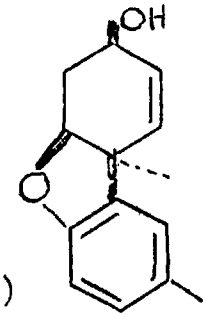
(XV)



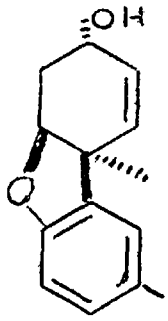
(XVI)



(XVII)




(XVIII)




(XIX)

In contrast the n.m.r. spectrum of Powellinone (XVI), described by Lloyd et.al., [15] shows a simple AB-quartet for the olefinic protons. In agreement with these workers, no significant fine splitting of the olefinic signals was observed. Lack of coupling is consistent with the conformation (XVI) in which the methine proton (-N-C-H) lies outside the median plane of the ring. Rapoport and Sheldrick [16] have observed a long range proton coupling (J$\sim$ 2 c.p.s.) in "thebaine-hydroquinone" (XVII) between the asterisked protons, which appears similar in type to the examples described here.

In order to look for the long range proton coupling in the corresponding alcohol, Pummerer's Ketone was reduced with lithium aluminium hydride. The reduction gave two stereoisomeric alcohols, which were separated by chromatography on alumina. The alcohol (XVIII), eluting first from the column was an oil and showed the hydroxyl absorption band in infrared at 3560 cm.$^{-1}$ in chloroform solution.

The alcohol (XIX), eluting later, was crystalline and had the hydroxyl absorption band in infrared at 3610 $cm.^{-1}$ in chloroform solution.

The examination of the n.m.r. spectra of these alcohols did not show the existence of the long range proton coupling seen in Pummerer's Ketone. The $\beta$ -olefinic proton in each case appeared as simple doublet. In the case of alcohol (XVIII), the $\alpha$-olefinic proton bands were further split by the neighbouring methine -O-C-H proton into a quartet (J = 4 c.p.s.), indicating that the hydroxyl group was quasi-axial.[17] This, along with the facts, that this alcohol is easily eluted off alumina and has the hydroxyl absorption band about 50 $cm.^{-1}$ lower than the other isomer, shows that an intramolecular hydrogen bonding exists in this alcohol (Cf. galanthamine). Clearly, this alcohol has a configuration analogous to that of galanthamine.

In case of crystalline alcohol (XIX), the $\alpha$-olefinic proton appeared as doublet (4.35 $\tau$; J = 10 c.p.s.). The lines of the doublet were broadened but not split by coupling with the neighbouring methine proton, indicating that the hydroxyl group was quasi-equatorial and the dihedral angle was nearer to $90^{\circ}$ (Cf. epigalanthamine)[12]. The doublet due to $\beta$ -olefinic proton

(4.62 $\tau$; J = 10 c.p.s.) was also broad, presumably owing to allylic coupling with the methine proton next to $\alpha$-olefinic proton.

## Experimental

### Epimerisation of Galanthamine [11]. (VI)

A solution of (-)-galanthamine (250 mg.) in 2% hydrochloric acid (20 ml.) was refluxed for three hours, cooled, treated with excess sodium bicarbonate, and extracted into ether (4 X 10 ml.). The ether extract was washed with water (2 X 10 ml.), dried, and the ether evaporated. The residue was chromatographed in benzene on alumina (grade V, 20 g.). Elution of the column with benzene-ethylacetate (3:1) gave (-)-epigalanthamine (160 mg.) which crystallised from benzene as plates, m.p. 188-189°, $[\alpha]_D$-243° (C 1.02 in $CHCl_3$). (lit.[1],[e] 199°, $[\alpha]_D$-222°.). (Found: C, 71.11; H, 7.31; N, 4.77%. Calculated for $C_{17}H_{21}NO_3$ C, 71.05; H, 7.37; N, 4.87%).

The product has its infrared spectrum in chloroform identical with that of (-)-epigalanthamine.

### Oxidation of (-)-epigalanthamine. [11] (VII)

(-)Epigalanthamine (90 mg.) in chloroform (20 ml.) was shaken for two hours with manganese dioxide (900 mg.) at room temperature. Manganese dioxide was filtered off, and the chloroform evaporated under vacuum. The crude product was chromatographed on alumina (grade V, 10g.). Elution with benzene-

ethylacetate (9:1) gave (±)-narwedine (60 mg.), which crystallised from ethanol as needles, m.p. and mixed m.p. 189-190°. The infrared red spectrum (nujol mull) of this compound was identical with that of a specimen of (±)-narwedine obtained by the oxidation of (-)-galanthamine in the same conditions.

Deuteromethanol $^{18}$ (MeOD)

Magnesium was dissolved in carefully dried absolute methanol. Excess methanol was distilled off and the magnesium methoxide produced was dried in vacuum at 100°. Less than one equivalent of deuterium oxide (99.95% deuterium) was then added, and after shaking the mixture for some time, the deuteromethanol was distilled over at 60° in vacuum. N.M.R. spectrum of the alcohol showed that it contained more than 99% of deuteromethanol.

(±)-Trideuteronarwedine (XIV)

(±)-Narwedine (50 mg.) in deuteromethanol (2 ml.) was refluxed for 5 minutes, concentrated, and cooled The crystalline trideutero-derivative was collected by filtration and dried at 50° in vacuo.

Pummerer's Ketone[3,3a] (VIII)

p-Cresol (14 g.) was oxidised with potassium ferricyanide (82 g.) according to the method of Pummerer, Melamed, and Puttfarcken.[3a] The alkali

insoluble product (12 g.) was chromatographed on alumina (grade III) in benzene. Elution of the column with benzene gave the ketone (2.5 g.), which crystallised from ethanol as needles, m.p. 124° (Lit.[3a] 124°).

Deuteration of Pummerer's Ketone

Pummerer's Ketone (200 mg.) in deuteromethanol (2 ml.) and potassium tertiarybutoxide (5 mg.) was refluxed for five minutes and cooled. The trideutero-derivative, which crystallised from the cooled solution, was collected by filtration and dried in vacuo at room temperature.

Reduction of Pummerer's Ketone

Pummerer's Ketone (500 mg.) in anhydrous ether (70 ml.) was added to a suspension of lithium aluminium hydride (1.0 g.) in anhydrous ether (30 ml.) at room temperature over a period of one hour with stirring. After another six hours, excess lithium aluminium hydride was decomposed with water and the ether decanted off, and the residual paste was washed with ether (3 X 10 ml.). The total ether portion was washed with water, dried, and the ether evaporated. The product showed no carbonyl band absorption in infrared spectrum, but showed two spots on thin layer chromatography plate. The crude product was chroma-

graphed on alumina (grade III, 10 g.) in benzene. Elution with benzene-ethylacetate (9:1) separated the two alcohols, elution being followed in U.V. at 280 mμ. The first fraction yielded the alcohol (XVIII), which was an oil ($n_D^{25}$ 1.559) and failed to crystallise. $\nu_{max.}$ 3560 $cm.^{-1}$
(Found: C, 77.41; H, 7.35%. $C_{14}H_{16}O_2$ requires C, 77.75; H, 7.46%).

The later eluting fraction gave alcohol (XIX), which crystallised from petroleum ether (60-80°) into needles, m.p. 80-81°, $\nu_{max.}$ 3610 $cm.^{-1}$
(Found: C, 77.50; H, 7.36%. $C_{14}H_{16}O_2$ requires C, 77.75; H, 7.46%).

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