STUDIES IN ALKALOID BIOSYNTHESIS

a Thesis submitted by

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ABSTRACT

The biosynthesis of alkaloids derived from the 1-benzylisoquinoline skeleton is reviewed in the light of recent tracer studies. The chemistry of the proaporphine alkaloids is briefly described and evidence for the positions of the functional groups in crotonosine is presented.

Hypothetical phenolic precursors for crotonosine have been synthesised containing carbon-14 and tritium. The distribution of tritium in coclaurine has been determined. The resolution of coclaurine is described and the absolute configuration of the enantiomers has been determined. Degradation of the biologically derived crotonosine has been carried out to locate the labelled atoms. The biosynthesis of crotonosine and roemerine is discussed in the light of results from tracer experiments with coclaurine derivatives.

The oxidative coupling step in the synthesis of thebaine from reticuline has been investigated. A direct correlation between the configurations of the benzylisoquinoline and morphine alkaloids has been accomplished.

ACKNOWLEDGEMENTS

I would like to express my deep gratitude to Professor D.H.R. Barton for his stimulating interest and guidance.

I wish to thank Dr. G. W. Kirby for his constant encouragement, stimulating discussions and valuable help.

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CONTENTS

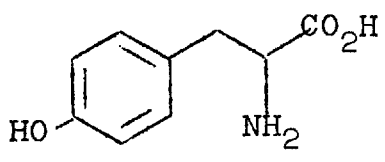
INTRODUCTION

Ever since the structures of alkaloids were elucidated, organic chemists have speculated about the way plants build up these molecules. In the last decade interest in the problem has increased markedly. The number of reviews[1-5] which have appeared in recent years indicates the extent of present interest in the problem. Important ideas of alkaloid biosynthesis have been put forward by Robinson,[1] Schöpf,[9] Barton and Cohen,[8] Woodward,[2,10] Wenkert,[5,11] and Thomas.[12]

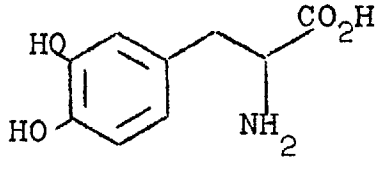
Indeed, real progress in alkaloid biosynthesis began when tracer techniques became available. Currently, with the widespread use of tracers, the field of alkaloid biosynthesis is in a state of rapid growth. Recently, excellent general comprehensive reviews[13-17] describing the tracer experiments, especially covering the earlier stages of biosynthesis have also appeared. Since our interest has been confined to the later stages of biosynthesis, particularly those of the benzylisoquinoline series and since much interesting information has accumulated in this field, an attempt will be made to review the biosynthesis of alkaloids derived from the benzylisoquinoline skeleton.

The Benzylisoquinoline Alkaloids.

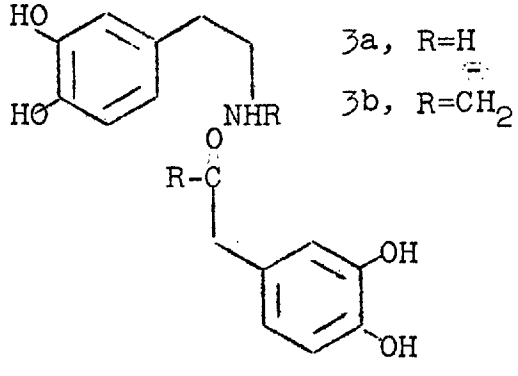
That the 1-benzylisoquinoline skeleton, which should strictly be called 1-benzyl-1,2,3,4-tetrahydroisoquinoline, is derived from two molecules of 3,4-dihydroxyphenylalanine (DOPA) (2) or tyrosine (1) by a scheme such as shown below was first suggested by Winterstein and Trier[18] in 1910. This hypothesis was subsequently elaborated,



(1)

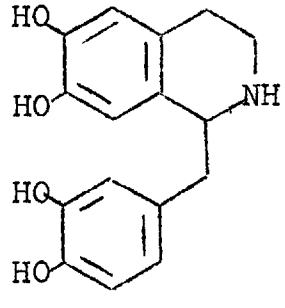


(2)



3a, R=H

3b, R=CH_2



(5)

4a, R=H

4b, R=CO_2H

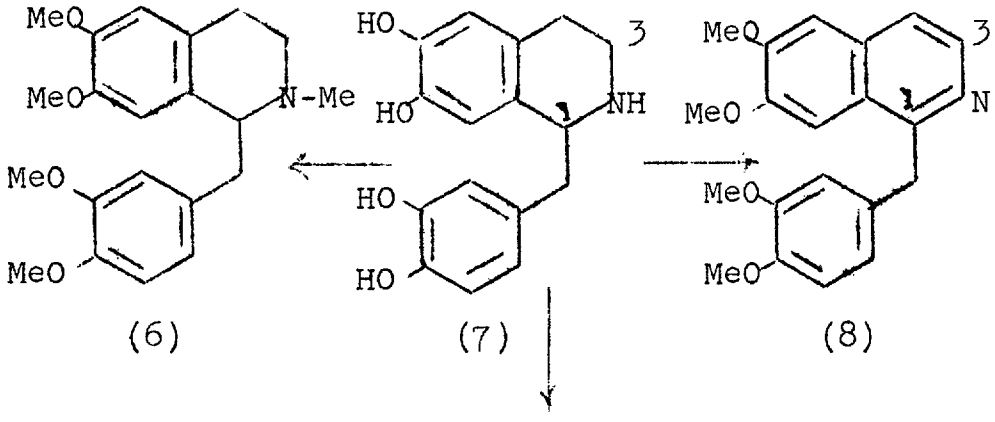
extended and refined mainly by Robinson.[1,6] Decarboxylation of DOPA (2) was thought to give rise to dopamine (3a) while oxidative deamination of a second molecule of the

amino acid could give rise to arylpyruvate (4b) or aryl-acetaldehyde (4a). Condensation of the amine (3a) and the carbonyl compound (4a) by a Mannich reaction could then form norlaudanosoline (5). This classical hypothesis of the isoquinoline skeleton has been amply supported by circumstantial evidence from model syntheses in the laboratory.[19-23]

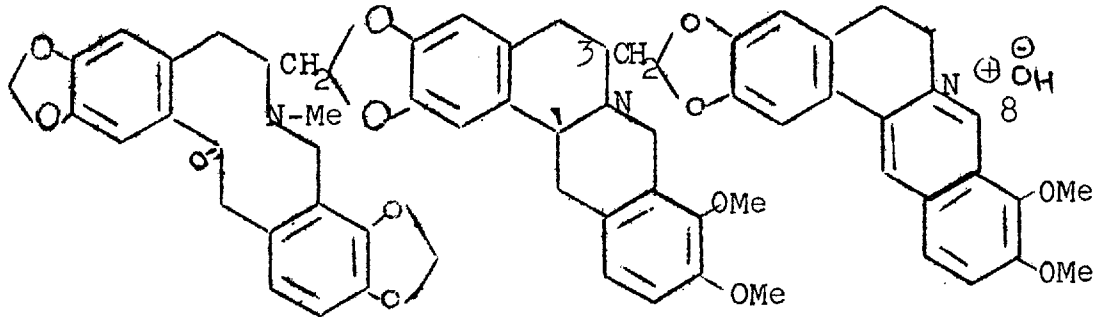
Norlaudanosoline (5) is the key intermediate, which in principle could give rise to a large number of isoquinoline alkaloids. Laudanosine (6) could be formed by simple methylation. Dehydrogenation of the heterocyclic system which might occur by way of an N-oxide[11] could give rise to papaverine (8).

Berberine and related alkaloids were also considered by Robinson[1] to be derived from norlaudanosoline skeleton by condensation with one carbon unit. However, recently a more attractive proposal has been put forward for the biosynthesis of these alkaloids (see later). According to classical theory,[1] condensation with formaldehyde or its biological equivalent could give the canadine skeleton (10). Dehydrogenation of canadine could then afford berberine (11) whereas oxidative modification of the

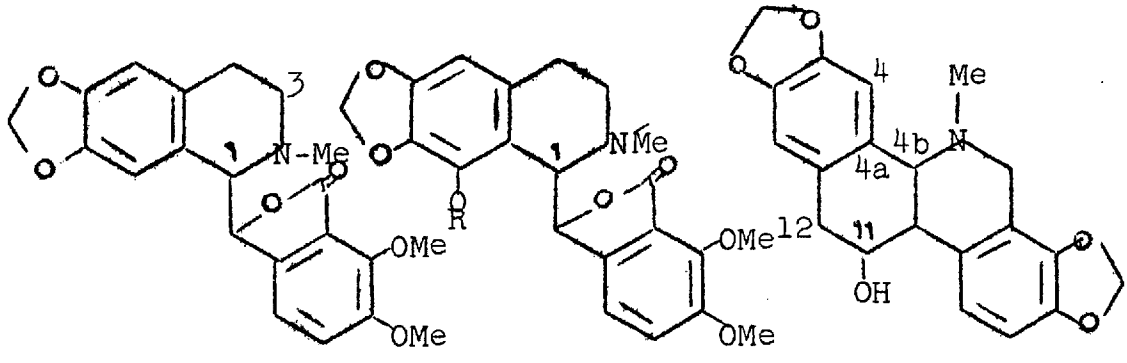
Alkaloids derived from Norlaudanosine.



(6) (7) (8)



(9) (10) (11)



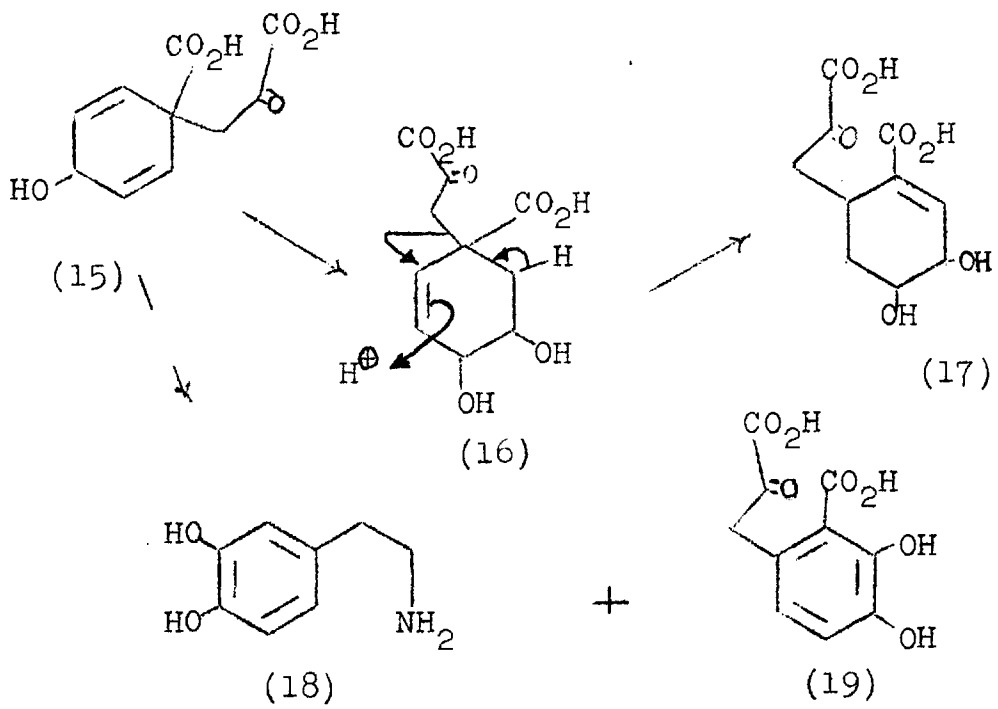
(12) 13a, R=Me (14)

13b, R=H

canadine skeleton could give one possible route to the phthalide isoquinoline alkaloids such as hydrastine (12). Narcotine (13a) and narcotoline (13b) could be formed from an oxygenated canadine skeleton. Oxidative cleavage of a C-N bond in a protoberberine could afford protopine (9), whereas benzophenanthridine alkaloid such as chelidonine (14)[24] could be formed by an oxidative modification of the berberine skeleton (11). In fact, as mentioned by Battersby,[13] it is possible to draw a scheme as illustrated above, for a large number of isoquinoline alkaloids.

Norlaudanosoline (7) in theory could also give rise to cryptowoline, morphine, aporphine, and bisbenzylisoquinoline alkaloids. This aspect of biosynthesis has been discussed in the later part of this review.

Recently an alternative hypothesis for the biosynthesis of the benzylisoquinoline skeleton has been advanced by Wenkert.[5,11] According to this proposal it is not tyrosine but its biochemical precursor, prephenic acid (15)[25,26] which serves directly as the progenitor of the arylethylamine (18) and the arylpyruvate (4b). Condensation of these two units then yields norlaudanosoline.



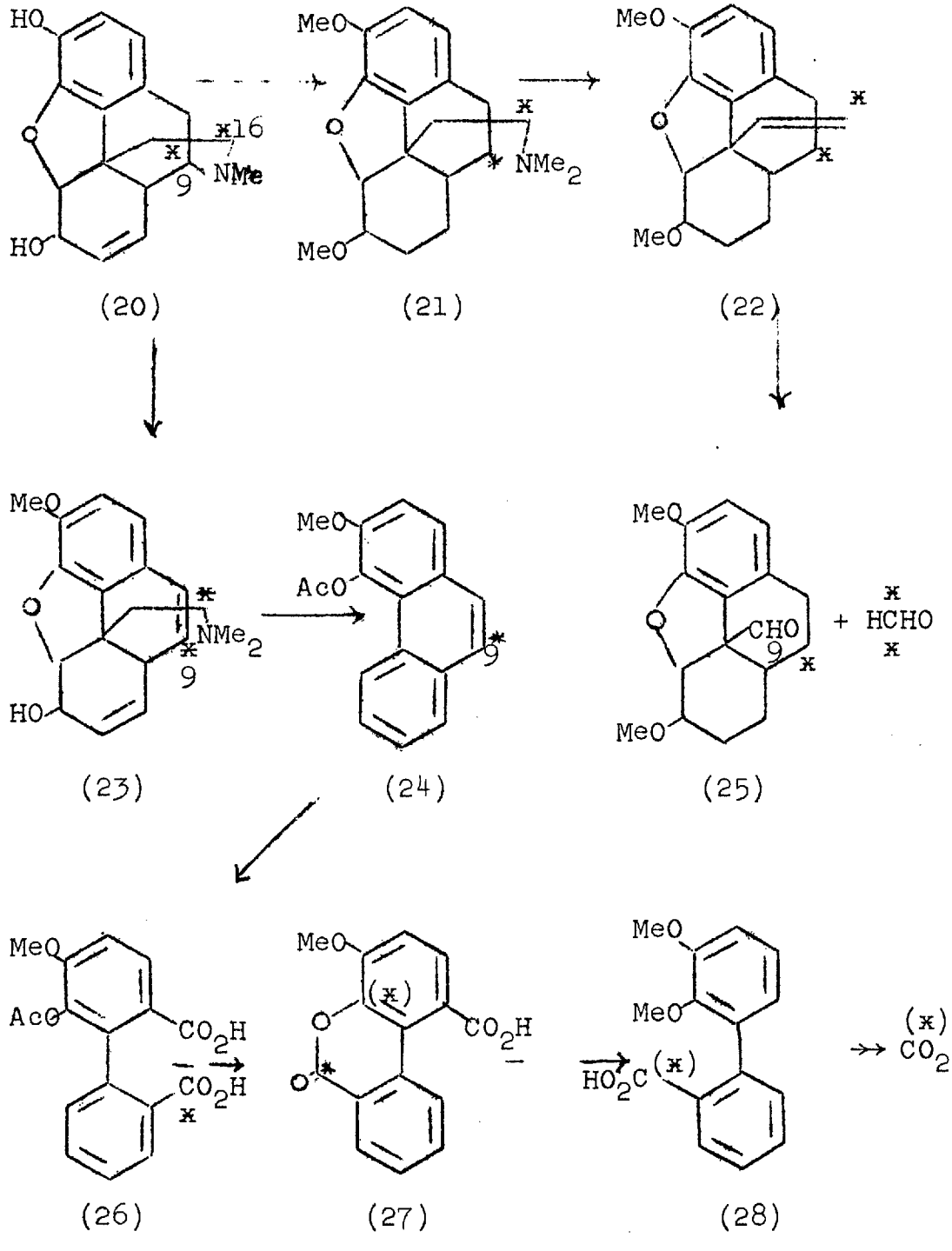
'Berberine bridge' structures were thought to arise not by introduction of a one carbon unit into norlaudanosoline but by interaction of two prephenate derived precursors (18) and (19), one of which contained the one carbon unit prior to condensation.

The first evidence in support of the classical hypothesis was presented by Battersby and Harper.[27] Since tyrosine[28] was known to be converted by the plants into 3,4-dihydroxyphenylalanine, DL- 2-[^{14}C] tyrosine was fed to Papaver somniferum and radioactive papaverine (8)[27] was isolated. Systematic degradation of radioactive papaverine established the labels at positions 1 and 3 in the alkaloid in accord with the theory. In subsequent

experiments[29], 2-[^{14}C] tyrosine was administered to poppies and the more complex benzylisoquinolines such as thebaine (48), codeine (51) and morphine (52) were found radioactive. The biosynthetic morphine[29] was degraded as follows. Methylation, quaternisation, Hofmann degradation and reduction gave the tetrahydrocodeimethine (21). Further Hofmann degradation gave the morphenol derivative (22). Oxidative cleavage of the terminal methylene group then afforded formaldehyde and the aldehyde (25) each containing half the activity of the original morphine. Obviously, the one half of the activity of the radioactive morphine was located at position 16.

The position of the second carbon atom was established as follows. Methylation of the biosynthetic morphine (20)[29] yielded codeine methiodide which was subjected to a Hofmann degradation to give the methine (23). This was treated with sodium ethoxide to give methylmorphol. Oxidation of acetylmethylmorphol (24) by chromium trioxide gave a quinone which was cleaved by peracetic acid to the diphenic acid (26). Hydrolysis of the acetyl group gave the coumarin (27) which was decarboxylated with strong acid. Alkaline hydrolysis in the presence of

Degradation of Morphine (Battersby et al.)



dimethyl sulphate gave the dimethoxy acid (28), which was decarboxylated to give radioactive carbon dioxide containing half the activity of the original alkaloid. Thus the position of the second label was established at position 9 in morphine. Independent work of Leete[30] on morphine confirmed these results.

The above results establish that morphine is biosynthesised from two molecules of tyrosine. Since two molecules of tyrosine would furnish a norlaudanosoline skeleton (7) labelled at positions 1 and 3, the labelling pattern of morphine is in agreement with the scheme.

The first evidence in favour of the classical hypothesis for the phthalideisoquinoline alkaloids such as hydrastine (12) and also for berberine (11) and canadine (10) was presented by Spenser and Gear.[31] DL- 2-[^{14}C]-Tyrosine was administered to Hydrastis canadensis and radioactive hydrastine (12), canadine (10) and berberine (11) were isolated. Degradation of the biosynthetic hydrastine (12) located the activity exclusively at carbon atoms 1 and 3. Since Wenkert's hypothesis[5,11] predicts the activity only at position 3, these results thus disprove this hypothesis and support the classical theory.

Degradation of berberine[31] derived from 2-[^{14}C]tyrosine demonstrated that two tyrosine units were specifically incorporated into the alkaloid. However, in this case, the specific incorporation of radiotyrosine is not of diagnostic value in differentiating the two hypotheses, since both of the prephenate derived precursors (3b and 4b) could also arise by feasible metabolic routes from tyrosine.[32]

Narcotine (13a)[33] and the benzophenanthridine alkaloid chelidonine (14)[34] have also been shown to be specifically derived from two units of tyrosine in accord with the theory.

The foregoing results establish that the 1-benzylisoquinoline skeleton is derived from two molecules of tyrosine or a close biological equivalent and demonstrate indirectly that it is this skeleton which is transformed by the plants into more complex benzylisoquinoline skeletons.

Phenylalanine has been reported to be a much less efficient precursor than tyrosine for berberine,[31] hydrastine[31] and morphine.[29,30] This suggests that in these cases tyrosine is derived directly from prephenic acid, although it is known that in mammalian systems

tyrosine[35] is formed by hydroxylation of phenylalanine. However, it is reported that in certain higher plants[36,37] and Escherechia coli[38] this reaction is not important. In these cases tyrosine[39] is formed directly from prephenic acid.

It is interesting that 2-[^{14}C] tyrosine was incorporated unequally into two "halves" of hydrastine.[31] This unequal utilisation of tyrosine into two "halves" of the benzylisoquinoline skeleton has also been observed in other cases,[34,40] although in some cases[29,30] this inequality is negligible. Recent investigations have shown that in the biosynthesis of morphine,[41,42] berberine,[31] hydrastine[31] and chelidonine[34] only one unit of 3,4-dihydroxyphenethylamine is incorporated. In each case the labelling pattern is consistent and shows that the dopamine contributes to the formation of only the phenethylamine portion of the molecules. On the basis of these results it has been suggested[29,31] that the basic benzylisoquinoline system could be formed by the condensation of a molecule of dopamine with phenylacetaldehyde or phenylpyruvic acid. However, other plausible schemes can also be drawn for the biosynthesis of this skeleton. At this stage it can be said that the early stages of biosynthesis

of the basic benzylisoquinoline are not yet clear. Further investigations in this field could give interesting results.

The first direct evidence that the plants use a 1-benzylisoquinoline as an intermediate on the way to phthalideisoquinoline came from Battersby and McCaldin.[33] DL-[1-^{14}C] Norlaudanosoline (7) was administered to Papaver somniferum and the isolated narcotine (13a) was radioactive. Degradation of the biosynthetic alkaloid showed the label exclusively in the position 1 in narcotine (13a) in accord with the theory. In a subsequent experiment, by using sodium [^{14}C] formate it was demonstrated that the lactone-carbonyl of the alkaloid is derived from the pool of C_1-units in the plant.

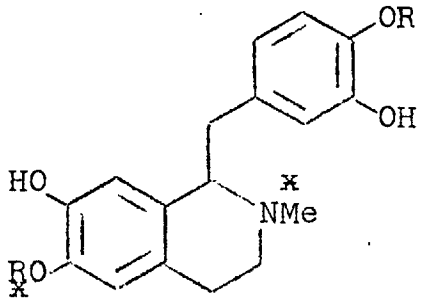
Recently it has been reported that papaverine[43] in Papaver somniferum is derived from [1-^{14}C] norlaudanosoline (7). However, the degradation of the biosynthetic papaverine (8) has not yet been reported.

It has been suggested[16,17,44] that carbon atom 8 of berberine and its relatives known as the 'berberine bridge' could be formed in Nature by oxidative modification of an N-methyl group. Indeed, this was the case. Barton, Hesse and Kirby[45] fed (±)-reticuline (29a) labelled

with ^{14}C in its N-methyl to Hydrastis canadensis. The derived berberine (11) was degraded and all the activity was found at position 8 of the alkaloid. In a separate experiment, when (±)-reticuline labelled both in an O-methyl group (as shown in 29a) and in its N-methyl group was used, the berberine was found to be labelled again at position 8. Further, the activity of the O-methyl group of the precursor was found in the methylenedioxy group of berberine. This experiment confirms the previous one and also provides a second example of the oxidative cyclisation of an O-methoxyphenol to a methylenedioxy benzene.[47]

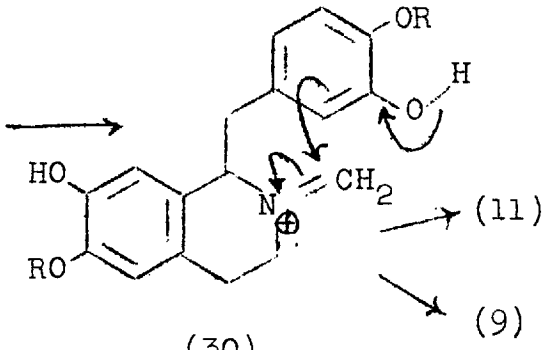
Independently, Battersby's group[46] by using (±)-laudanosoline (29b) labelled with ^{14}C in the N-methyl group, has confirmed the above results. Also indirect evidence for the origin of carbon atom 8 in berberine has been provided by Gupta and Spenser[48] from experiments with [^{14}C]methionine.

(±)-[N-methyl-^{14}C]Reticuline has also been shown to be a precursor of protopine (9)[45] in Dicentra spectabilis. The so-called 'berberine bridge' in this case is also derived from an N-methyl group.



29a, R=Me

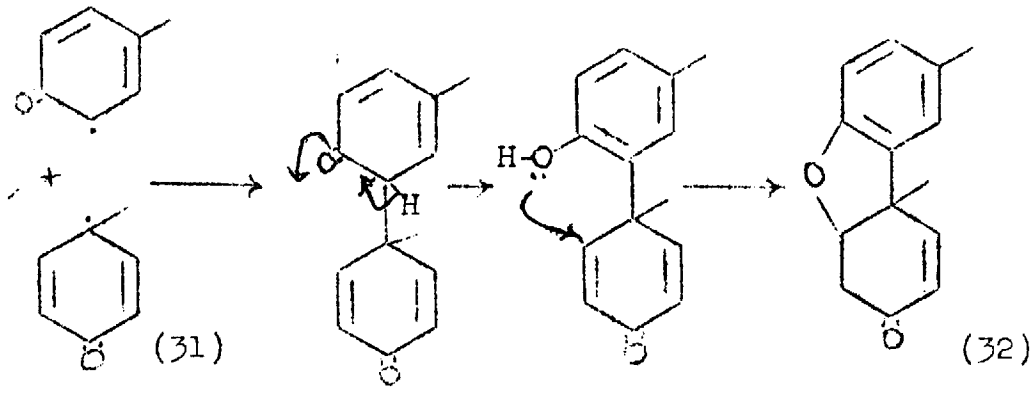
29b, R=H



(30)

The second aspect of the biosynthesis of isoquinoline alkaloids which will now be discussed is the formation of the morphine, aporphine and bisbenzylisoquinoline alkaloids. Many speculations have been advanced to explain the biosynthesis of these alkaloids. The phenol oxidation hypothesis of Barton and Cohen[8] has proved most fruitful.

Barton and his coworkers[50] employing a radical mechanism clarified the structure of a dimer of p-cresol obtained earlier by Pummerer[81] and showed the correct structure of the dimer to be (32).



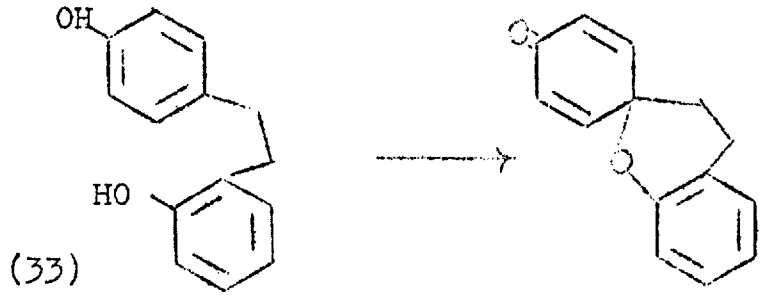
(31) (32)

Dimerisation occurs by recombination of two p-cresolate radicals (31) and the ketone (32) is then formed by intramolecular tautomerisation (β-addition of phenolate anion). Although the possibility that this reaction proceeds via a radical attack on a phenolate anion has not been excluded, it has been shown[51] that the substituted products are not formed by the attack of a phenolate radical on a phenol ether.

The radical coupling mechanism imposes a restriction on the mode of coupling. In principle, by this process, only para and ortho substituted products should result.

Phenolate radicals once generated from the corresponding phenols by some one-electron transfer process can, in principle, couple to give new O-O, O-C or C-C bonds. O-O coupling is thermodynamically unfavourable and no stable diaryl peroxides are at present known.[52] On the other hand, O-C coupling occurs readily, for example, the

diphenol (33), when treated with active manganese dioxide, gives the 6-membered ring ether (34).



(33) (34)

Intramolecular O-C coupling to give a 5-membered ring is also well known.

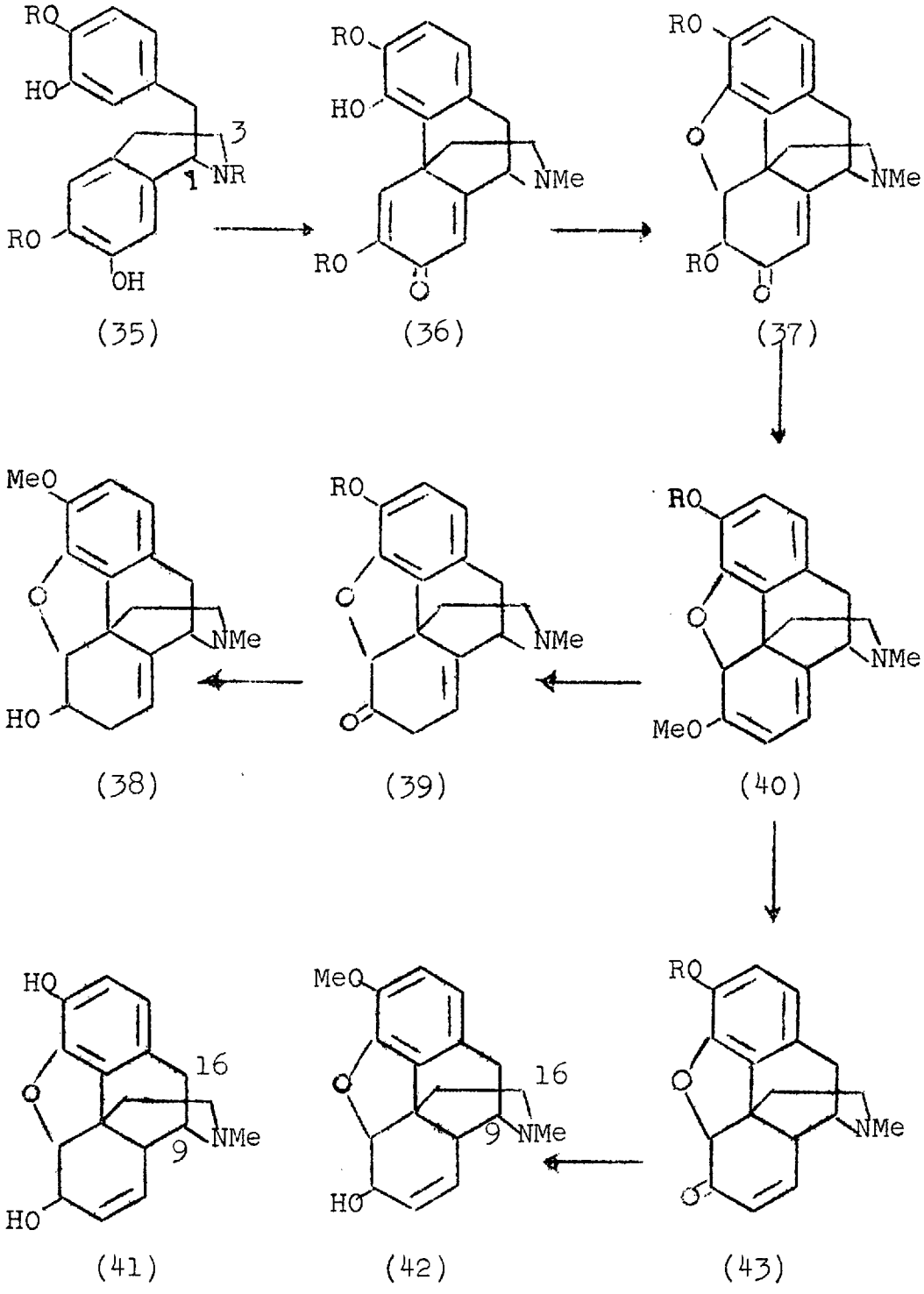
The biogenetic implication of the radical coupling mechanism was recognised by Barton,[8] and by Erdtman.[53] We will now apply these ideas to the remaining group of benzylisoquinoline derived alkaloids.

Morphine Alkaloids.

Gulland and Robinson[54] in 1925, first recognised that if norlaudanosoline (35, R=H) undergoes oxidative condensation, the skeleton of morphine (41) could in principle be obtained. The insight of Robinson led to the correct formulation (41) for this important alkaloid.

Following Barton and Cohen's proposal, oxidation of reticuline (35, R=Me) could give the dienone (36). The oxide bridge could then be formed by addition to the enone

Biogenesis of Morphine Alkaloids (Barton and Cohen).



system to give the compound (37) which by obvious changes could afford thebaine (40, R=Me), oripavine (40, R=H), neopine (38), codeine (42) and morphine (41).

Tracer experiments to verify the biogenetic speculations in the field of morphine alkaloids have been extensive. Early work, as discussed earlier, has provided indirect evidence that a norlaudanosoline skeleton (44) is transformed by poppies into thebaine, codeine and morphine. Direct evidence for this transformation was first presented by Battersby and his coworkers.[29,43] [1-^{14}C] Norlaudanosoline (44) was fed to Papaver somniferum and radioactive morphine, codeine, thebaine, and papaverine were isolated. Degradation of the biosynthetic morphine (52) showed that all the radioactivity was at position 9. In another experiment [3-^{14}C] norlaudanosoline (44) was converted in poppies into morphine (52) and codeine (51) labelled exclusively at position 16. Thus, the incorporation of norlaudanosoline without randomisation of the label was demonstrated. Moreover, the higher incorporation of norlaudanosoline than tyrosine into these alkaloids showed that the benzylisoquinoline is on the direct biosynthetic path.

Tracer studies by both Battersby, Barton and their colleagues has shown that reticuline (45, R=Me) is in fact the precursor which undergoes oxidative cyclisation.

Battersby's group[43] fed [3-^{14}C] norlaudanosoline (44), [3-^{14}C] N-norreticuline (45, R=H) and [3-^{14}C] reticuline (45, R=Me) separately to poppies and isolated specifically labelled thebaine, codeine and morphine. Furthermore, N-norreticuline was incorporated less efficiently than reticuline but more efficiently than norlaudanosoline. However, when [3-^{14}C] N-norlaudanosine was fed to the plants there was virtually no incorporation into morphine. This was not a surprise; obviously, the biosynthesis was blocked by methylation of both phenolic groups. The above results indicate a methylation pattern thus: norlaudanosoline (44) → N-norreticuline (45, R=H) → reticuline (45, R=Me).

The fact that reticuline is a true precursor of thebaine has been firmly proved by Barton's group.[56] In early experiments the doubly labelled reticuline (45, R=Me) (^{14}C in the N-methyl and tritium at position 1) was shown to be specifically incorporated into morphine.[55] The ^{3}H / ^{14}C in the derived morphine was 83% of that in the precursor. Subsequently this was confirmed by the following elegant experiment. Multi-labelled reticuline

(labelled with ^{14}C in both the methoxy groups and the N-methyl group, at C_3, and with ^{3}H at C_1) was administered to Papaver somniferum and multi-labelled thebaine[56] was isolated. The labels were found in the expected positions and their ratio was consistent with intact incorporation of reticuline. These results prove that demethylation of reticuline does not precede its further transformation in the plant into the morphine skeleton.

It is interesting to note that recently reticuline (45, R=Me) isolated earlier from Anona reticulata[57] has also been found to occur in Papaver somniferum.[58] Rapoport and his coworkers[59] by exposing P.somniferum seedlings to $[^{14}C]$ carbon dioxide have also confirmed that reticuline is the direct precursor of thebaine.

That thebaine is the precursor of codeine and morphine has been shown independently by Battersby and Harper,[60] and by Rapoport and his coworkers[61] by using $[2\text{-}^{14}C]$ tyrosine and $^{14}CO_2$ respectively. In each case, there was rapid uptake of activity into thebaine, followed by a rise in codeine then a steady fall in both relative to morphine. Moreover, this was neatly confirmed[61] by using generally ^{14}C labelled alkaloids. When labelled thebaine was fed to poppies radioactive codeine and morphine were isolated.

Feeding of labelled codeine gave radioactive morphine and the isolated thebaine was essentially inactive. Whilst on administration of labelled morphine both thebaine and codeine isolated were inactive. These results not only establish the pathway from thebaine → codeine → morphine but also showed the importance of O-demethylation in the final stages of the biogenetic process. This is contrary to the earlier theory of methylation as the terminal step used by the plants as a deactivating process.[62]

So far, attention has not been drawn to the formation of the oxygen bridge in the morphine alkaloids. Barton and Cohen[8] suggested that the dienone (36) could cyclise to the enone (37) by analogy with the properties of Pummerer's ketone (32).[50] The enone (37) after reduction and dehydration would give thebaine (40, R=Me). An important variant of this scheme was proposed by Battersby[16] and by Ginsburg.[63] It was suggested that the dienone (46) could be reduced to the dienol (49) and the oxide bridge could then be formed by an allylic elimination mechanism[64] (as shown in 49). To test these ideas, it was essential to prepare the important dienone (46) which was unknown at the time. Barton and his coworkers[55] achieved its synthesis from thebaine. It is gratifying

to record that after this synthesis an alkaloid named salutaridine, identical with the synthetic dienone (46), was isolated from a Brazilian source.[65] The dienone was found exclusively in the open form (46). Ring closure to give the enone (37) did not take place in either acidic or basic solutions. Obviously the open form was thermodynamically more stable.

The presence of salutaridine (46) in the opium poppy has also been shown recently by radio-dilution experiments.[56] Since salutaridine exists in the open form and the allylic elimination reaction is known[66,67] to occur in living systems, the biological conversion of salutaridine into thebaine by this process was obviously more attractive. Chemical support[55] for this came when reduction of salutaridine with borohydride afforded two epimeric alcohols (49). Both the alcohols gave thebaine in fair yield in weakly acidic solution (pH 3-4) at room temperature.

Critical tracer experiments with living plants to confirm the scheme have been carried out independently by Barton's and by Battersby's groups.

Barton and his coworkers[56] fed salutaridine (46) labelled with tritium <u>para</u> to the phenolic hydroxyl group, and the two dienols (49) labelled in the same position

and also at the secondary allylic position, to poppies. Both the dienone and dienol-I were incorporated into thebaine in high yield (ca. 7%). The position and the ratio of the labels was determined in the following way. Reconversion of the thebaine derived from the dienol (49) into salutaridine (46) gave the amount of tritium at position 7. Bromination of salutaridine then gave inactive 1-bromosalutaridine. Thus confirming the position and amount of the other tritium label. The results showed that the dienol-I is incorporated intact into thebaine without preliminary oxidation to salutaridine.

Dienol-II was also incorporated into thebaine. However, the efficiency of incorporation for dienol-II was low compared with that of dienol-I. The marked difference in the incorporation of these alcohols strongly suggested that the cyclisation is enzymatically controlled possibly through a phosphorylated intermediate and that dienol-I is the biological precursor of thebaine.

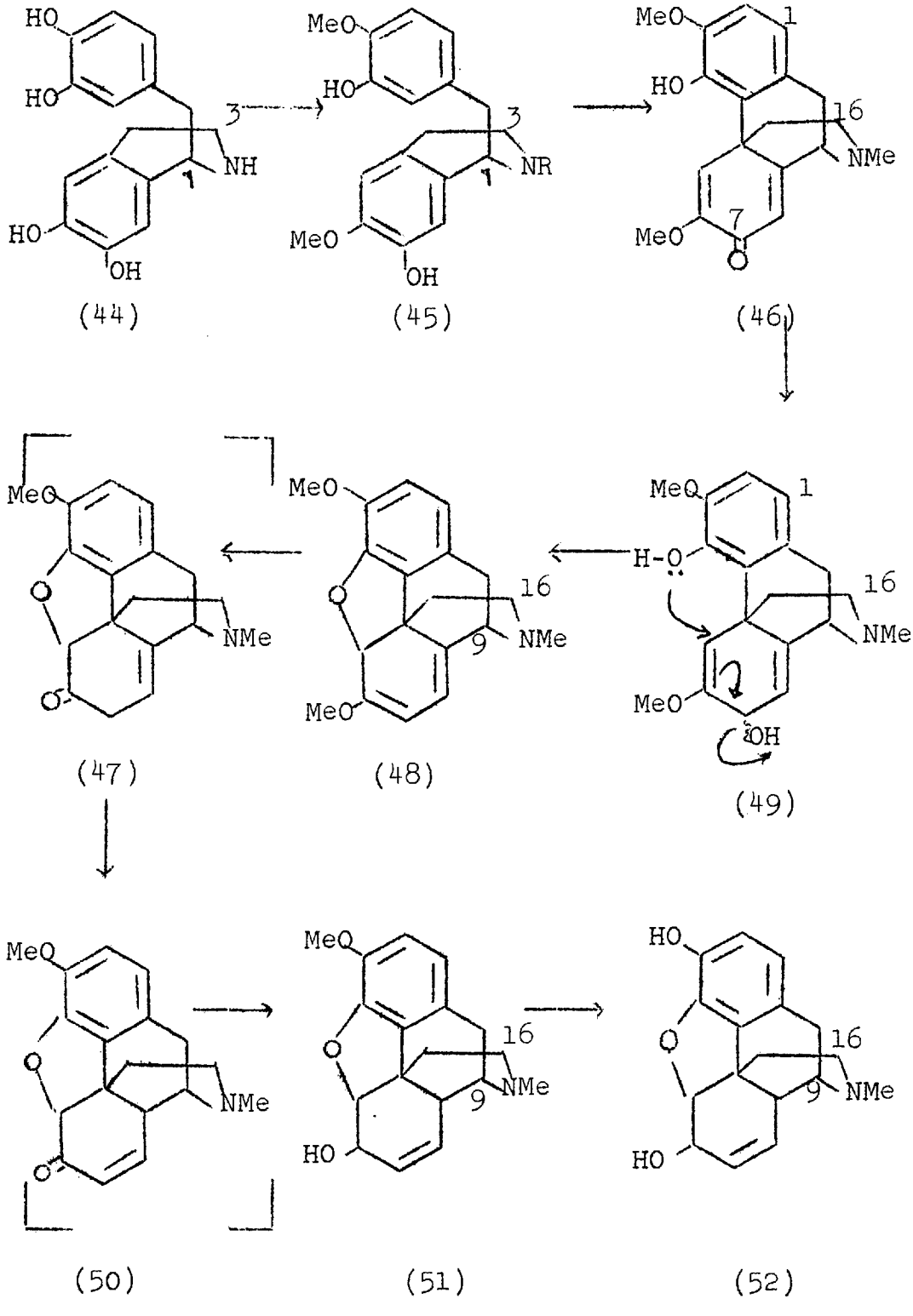
Battersby's group[56] independently confirmed these results. [16-^{14}C] Salutaridine (46), prepared from biosynthetic [16-^{14}C] thebaine (48) and dienol-I (49) labelled with ^{14}C in the same position and with tritium at position 7, were very efficiently incorporated into the morphine

alkaloids and again the dienol-I proved a much better precursor for thebaine than did its epimer. Careful measurements of the $^{3}H / ^{14}C$ ratios in the alkaloids derived from the dienols revealed an interesting effect. The ratio in thebaine was the same within experimental error, as that in the precursor. However, significant loss of tritium occurred during the conversion of thebaine into codeine. This throws some light on the possibilities of intermediates in the transformation and neopinone (47) and codeinone (50) have been suggested[56] for this role.

The important proposals which have stood the test of experiments can now be summarised. Morphine, codeine and thebaine are derived from a suitable benzylisoquinoline. The carbon-carbon bond joining positions 12 and 13 in these alkaloids is formed as a result of phenol oxidation and the oxide bridge is formed by an allylic elimination mechanism. The biosynthetic pathway for the morphine alkaloids is summarised (p.25). The intermediates in the biogenetic pathway which still have not been identified are kept within brackets.

Sinomenine (55)[69] is yet another interesting alkaloid related to the morphine group. Robinson and Sugasawa[68] have considered 'protosinomenine' (53) as a precursor of

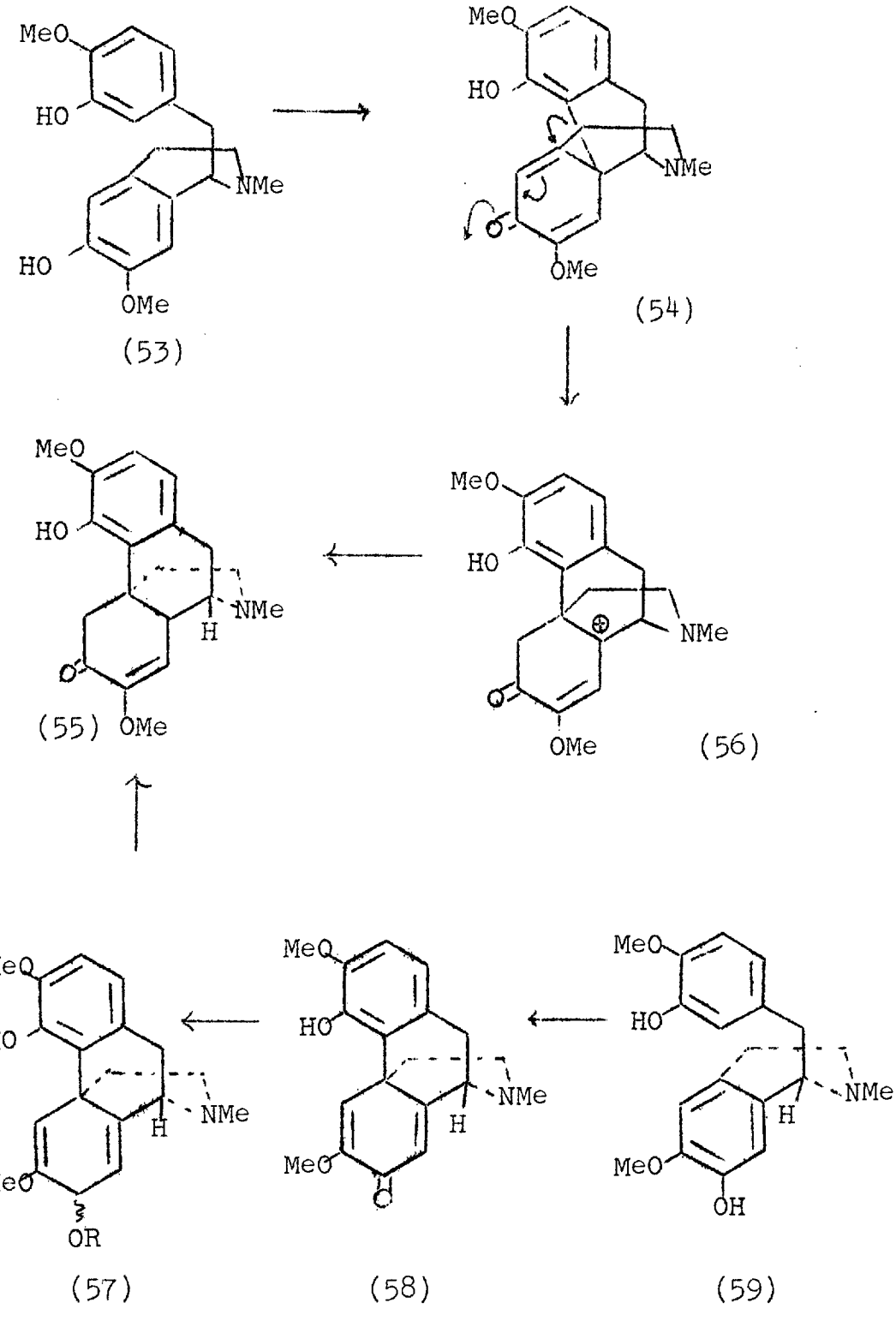
Biosynthesis of Morphine Alkaloids.



this base. A plausible mechanism for the conversion of this precursor into sinomenine has recently been suggested.[70] Thus, oxidative coupling of 'protosinomenine' (53) could give the dienone (54) which could rearrange into the carbonium ion intermediate (56). Direct or indirect reduction of this intermediate would then afford sinomenine (55).

Barton[70] has considered (+)-reticuline (59) as the precursor of sinomenine. It was suggested that oxidative coupling of (+)-reticuline (59) could furnish the dienone (58). Reduction to the corresponding dienol (57), methylation to the methyl ether (57, R=Me), hydrolysis of the vinylic methyl ether and the conjugation of the ethylenic linkage would then furnish sinomenine (55). Indeed, recent tracer experiments have shown[71] that reticuline is incorporated into sinomenine. It is gratifying to note that, after this proposal, an alkaloid sinoacutine[72] identical with the hypothetical intermediate (58) has been found in Sinomenium acutum which produces sinomenine. Tracer experiments have shown[71] that sinoacutine is incorporated into sinomenine in high yield. The correctness of the biogenetic proposal has thus been confirmed.

Biogenesis of Sinomenine (Barton)

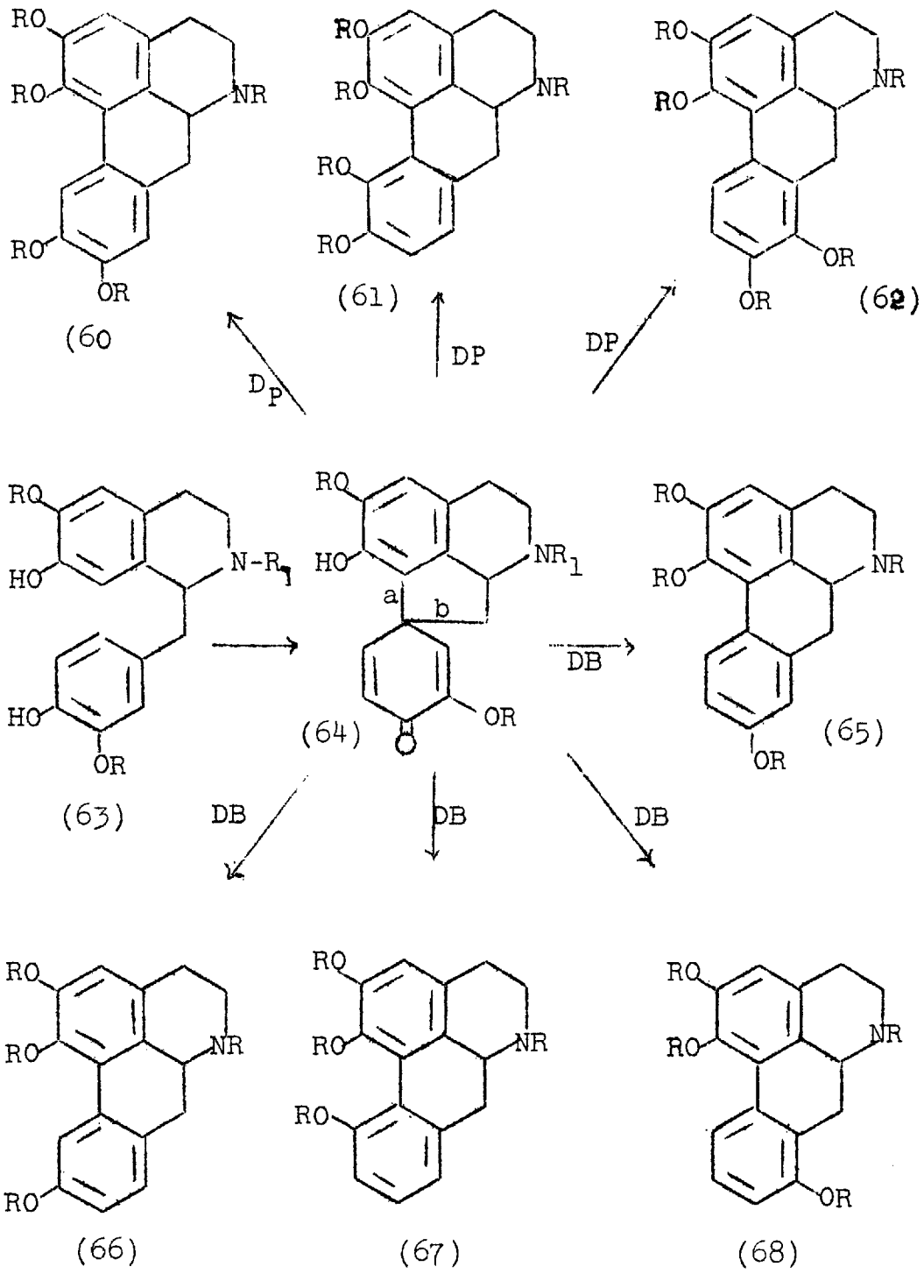


Aporphine Alkaloids.

Gadamer[73] as early as in 1911 suggested that dehydrogenation of laudanosoline (63, R=H, R_1=Me) could give the glaucine skeleton (60). According to the Barton and Cohen's proposal[8] the same benzylisoquinoline (63) by ortho-ortho coupling could give an alkaloid of the corytuberine type (61) whereas ortho-para coupling could account for the glaucine type of base (60). Indeed, recently the latter type of transformation has been accomplished in the laboratory by Franck and Schlingloff.[74] Oxidation of laudanosoline (63, R=H, R_1=Me) methiodide with ferric chloride at room temperature affords the glaucine skeleton (60) in 60% yield.

Many aporphines such as crebanine (62), stephanine (68), isothebaine (67) and laureline (66) types show an unusual oxygenation pattern. It seems that the biosynthesis of these alkaloids would involve rather unlikely precursors. However, Barton and Cohen[8] have accommodated these apparent exceptions under the unifying mechanism of phenol oxidation by assuming a dienone-phenol (DP) rearrangement in the transformation. Boit[75] has more recently elaborated on this idea and has used dienone-phenol (DP) and dienol-benzene (DB) rearrangements to explain the path in a large

Biogenesis of Aporphine Alkaloids.



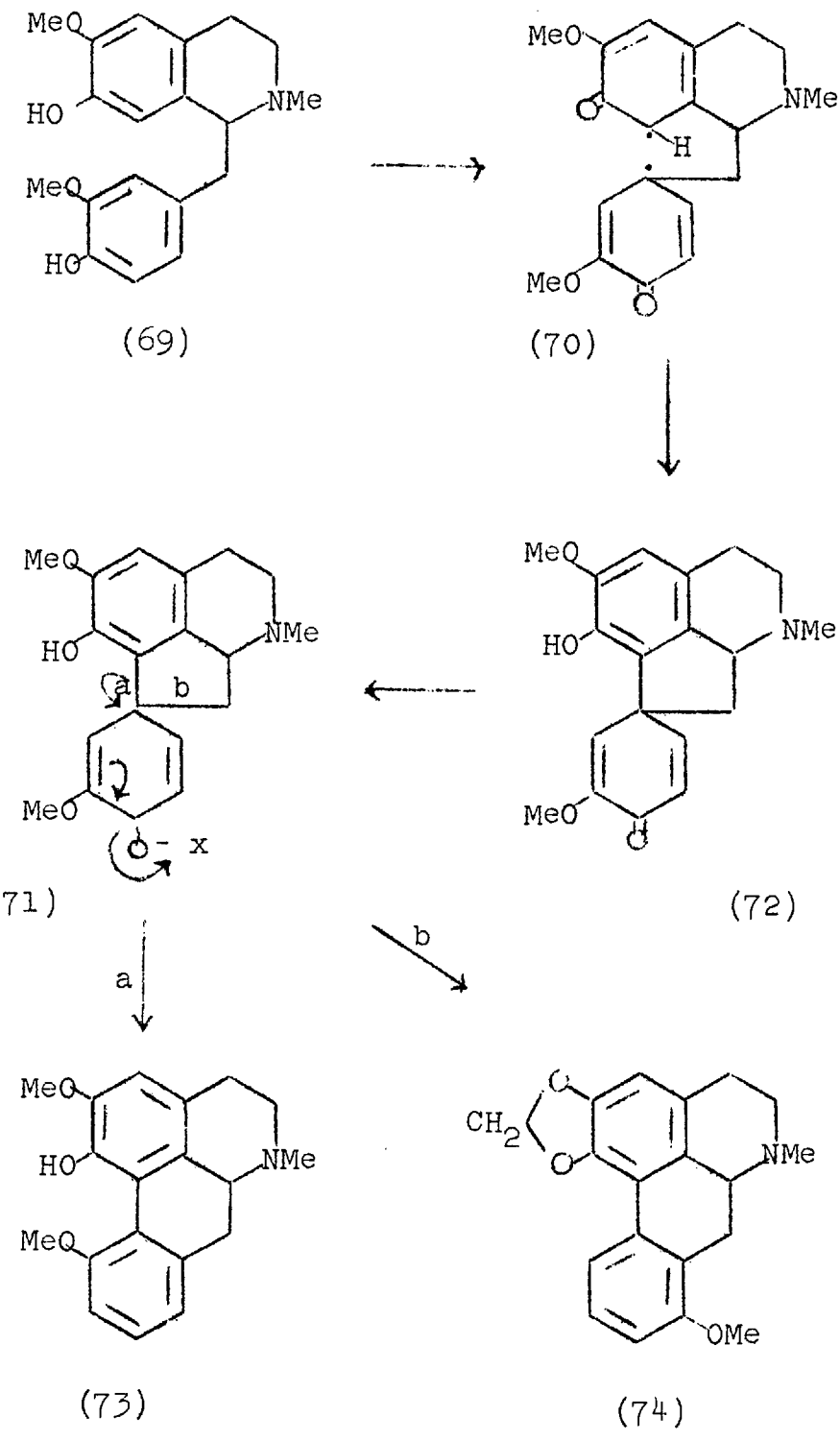
number of aporphines. The general scheme is given on page 29.

Battersby[16] has advanced a biogenetic scheme for the 'abnormal aporphines' isothebaine (73) and stephanine (74). It was suggested that oxidative coupling of the benzylisoquinoline (69) could give the dienone (72). This could be reduced to the dienol (71). The alcohol could then furnish the desired bases by an allylic elimination mechanism. Migration of bond 'a' as shown in (71) would afford isothebaine (73) whereas migration of bond 'b' would give rise to a product close to stephanine (74).

Indeed, it is gratifying to mention that the biosynthetic scheme for isothebaine has recently been confirmed in the laboratory by Battersby and Brown[76] by an elegant synthesis of the alkaloid. The phenol (69) was oxidised with alkaline potassium ferricyanide to give a mixture of dienones. From which two were isolated in crystalline form. Reduction of the dienone (72) with borohydride afforded the isomeric alcohol (71). Dienol-benzene rearrangement[82] finally yielded (±)-isothebaine (73) identical (apart from optical activity) with the natural alkaloid.

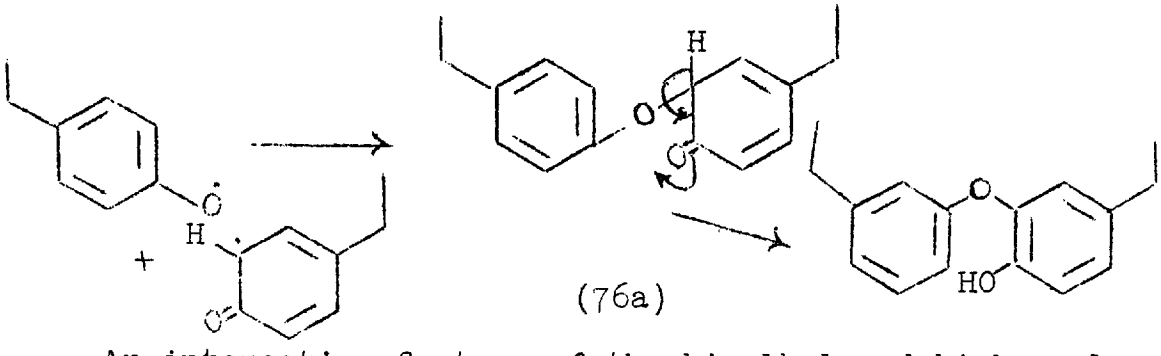
The biosynthesis of aporphine alkaloids such as roemerine and anonaine which lack oxygen function in one of the rings will be discussed in the next chapter.

Biogenesis of Isothebaine (Battersby).



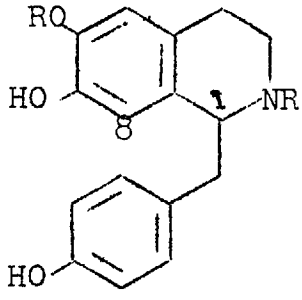
Bisbenzylisoquinoline Alkaloids.

In 1930, Faltis[77] suggested that bisbenzylisoquinoline alkaloids could be formed in Nature from two molecules of the benzylisoquinoline (75) or one of its methylated derivatives. Barton and Cohen[8] have discussed the mechanism by which plants could make these structures. It is thought that diaryl ether links could be formed by carbon-oxygen coupling of resonance stabilised phenolate radicals. For example, the benzyl residue (76a) of a benzylisoquinoline of the oxyacanthine type (76) could be formed as shown below.

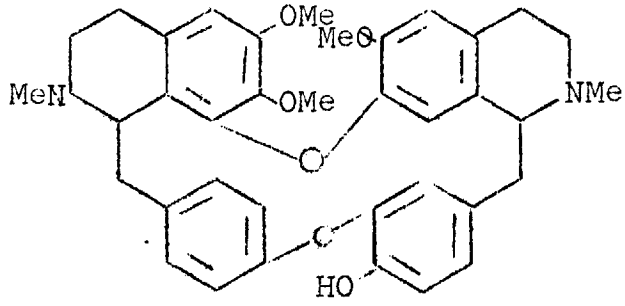


(76a)

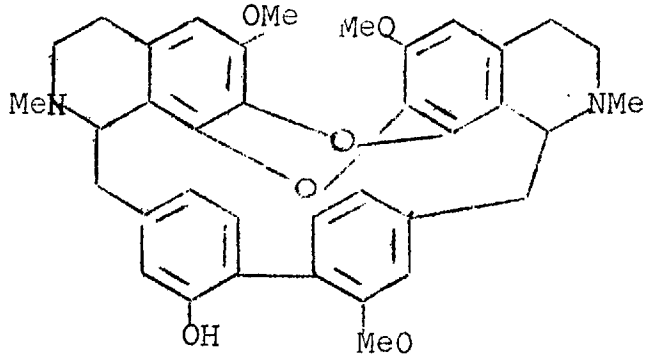
An interesting feature of the bisalkyl and bisbenzylisoquinoline alkaloids is that the two moieties are linked *via* ether bridges. A simple explanation is that the carbon radical at position 8 of the isoquinoline residue which is produced in the oxidation is sterically hindered by substituents at C-1 and C-7. It can apparently condense with a second isoquinoline moiety only if the latter


RO
HO
NR
HO

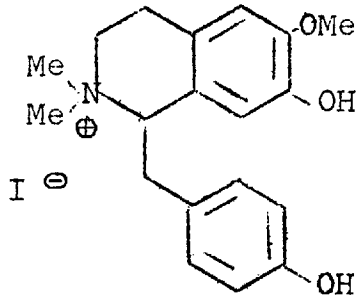
(75)


OMe
MeO
MeN
OMe
NMe
O
HO

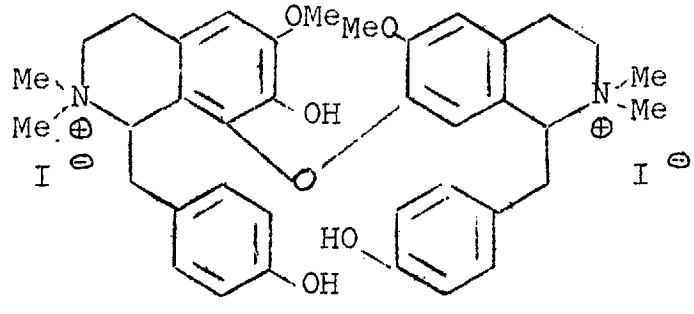
(76)


OMe
MeO
MeN
NMe
O
O
OH
MeO

(77)


OMe
Me
N
Me
OH
I
OH

(78)


OMe
MeO
Me
N
Me
OH
N
Me
Me
I
I
HO
OH

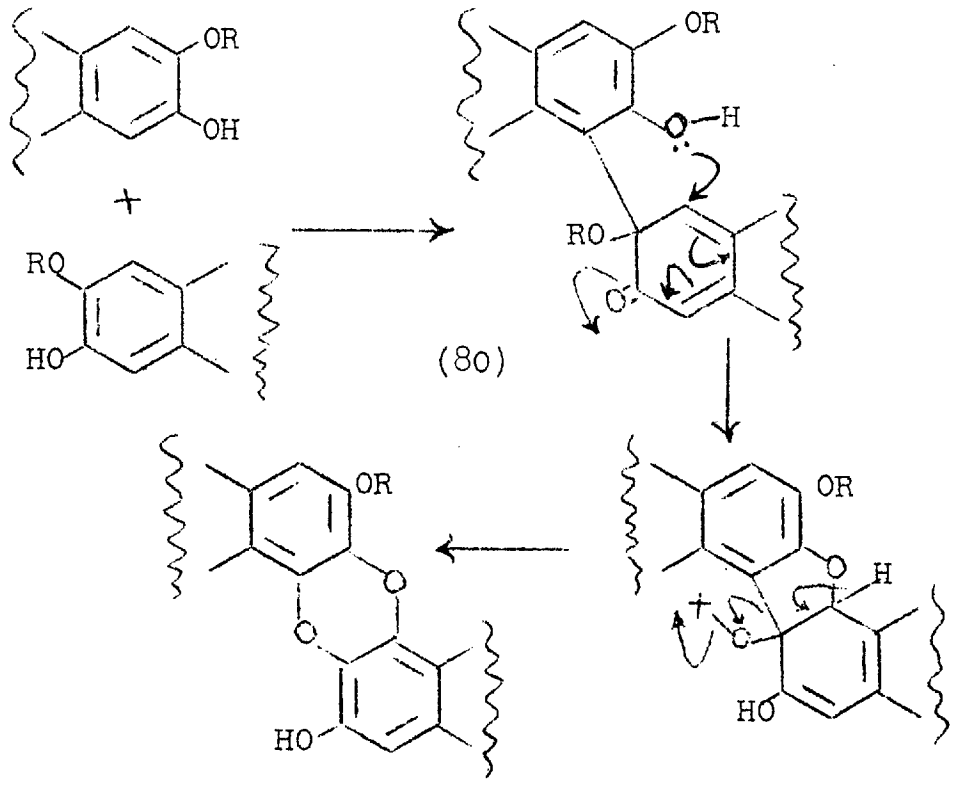
(79)

is held at a distance by an interposed oxygen atom. It is perhaps owing to this reason that carbon-oxygen coupling is preferred rather than carbon-carbon coupling.

Although the theory allows para or ortho coupling to a phenolic group, it is ortho coupling which predominates in the bisbenzylisoquinolines derived from coclaurine, because the para coupling would lead to a dienone intermediate which could not aromatise without rearrangement.

The bases such as tiliacorine (77) which are diphenyl derivatives are obviously formed by carbon-carbon coupling of the corresponding phenolic precursors.

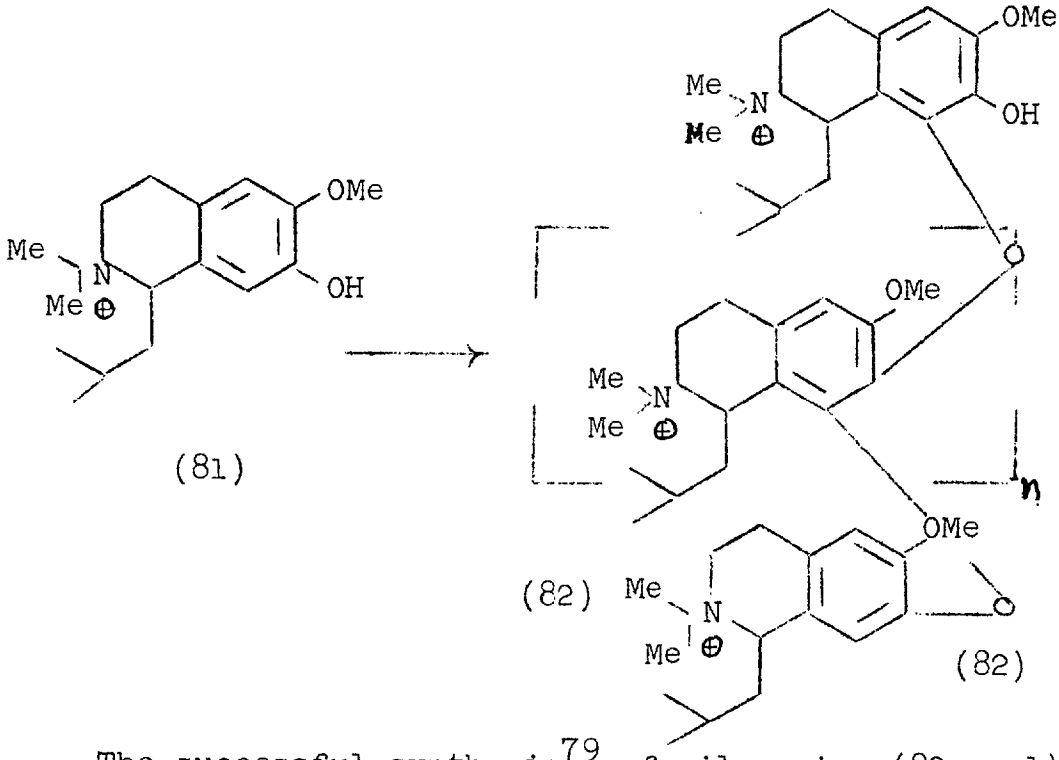
The biosynthesis of dibenzo-1,4-dioxin alkaloids such as micranthine and also tiliacorine (77) apparently appears difficult to explain by C-O coupling. Barton and Cohen[8] have explained the formation of these types of bases by phenol oxidation and an example of such a process is shown below.



The first stage involves carbon-carbon coupling and it is followed by formation of a diaryl ether group by intramolecular reaction. The subsequent migration as shown in (80) is induced by a strong electron attracting residue 'X'.

Recently some very interesting experiments designed to simulate the biosynthesis of bisbenzylisoquinolines have been reported.[78] In one experiment when the phenol (78) was treated with potassium ferricyanide at pH 10, a crystalline product (79) was obtained in 18% yield. The experiment clearly supports the hypothesis that the

biosynthesis of bisbenzylisoquinolines proceeds by intermolecular radical coupling of the benzylisoquinoline units. The structural type represented by the synthetic compound (79) has not yet been found naturally but it should be discovered eventually.



The successful synthesis[79] of pilocerine (82, n=1)[80] an alkaloid from Lophocerus species and of isopilocerine (82, n=0) a degradation product of the same, from lophocerine methiodide (81) by oxidation with potassium ferricyanide presents strong circumstantial evidence for the correctness of the biogenetic theory; direct evidence

by tracer experiments has not been reported but is awaited with great interest.

It can now be summarised that 1-benzylisoquinolines are the precursors of a large number of alkaloids. It is by phenol oxidation that this basic skeleton is transformed by the plants into more complex isoquinoline systems. At least for the generation of the morphine alkaloids, the plants apparently use O-methyl protecting groups to direct coupling in the correct sense. Of course, experiments *in vivo* and *in vitro* have shown that the biosynthesis of alkaloids involves phenol oxidation. The proof that the coupling of phenolate radicals is actually involved is difficult to present. Phenolate radicals certainly exist[83] in Nature and dimerize. However, the basic correctness of the biogenetic theory has been proven. More detailed information of biogenetic processes may come in future from experiments with isolated enzyme systems.

During the last few years a new group of alkaloids has been isolated from natural sources. All the members of this group possess a typical cyclohexadienone system. They rearrange readily to aporphine bases on treatment with mineral acid. Cava and his coworkers[84] have proposed the name proaporphines for this group of alkaloids. Barton and Cohen[8] in 1956 postulated a cyclohexadienone intermediate while explaining the biosynthesis of a number of aporphine alkaloids which display the so-called abnormal hydroxylation pattern. Thus the occurrence in Nature of this group of bases was, in fact, predicted nearly 6 years ago. Since our special interest has been with crotonosine, a typical member of this group, a brief review of the chemistry of this new group of alkaloids is presented here.

Crotonosine and Related Bases.

Crotonosine, the major alkaloid of Croton linearis Jacq., was isolated by Haynes and Stuart[85] in 1963 together with the minor bases linearisine, homolinearisine, and base A. Subsequent work has shown the identity of base A with pronuciferine (15) from the Asiatic lotus, Nelumbo nucifera[86] and with N-methylstepharine from Stephania glabra.[84] Crotonosine[85] contained one methoxyl group and N- and C-methyl groups were absent. The phloroglucinol-

concentrated sulphuric acid test for a methylenedioxy group was negative. This was also true for homolinearisine, linearisine, and base A. Acetylation of crotonosine with acetic anhydride-pyridine gave NO-diacetylcrotonosine.

The infrared spectrum of crotonosine showed absorption at 3220 (>NH), 2600 (bonded OH), 1664 (conjugated carbonyl) and 1622 (-C=C) cm^{-1}. Ultraviolet absorption at 235 mμ in conjunction with a strong absorption band at 1664 cm^{-1} in the infrared region suggested a cross conjugated dienone. Hydrogenation of crotonosine with Adam's catalyst gave a tetrahydro compound having a strong carbonyl band at 1706 cm^{-1}.

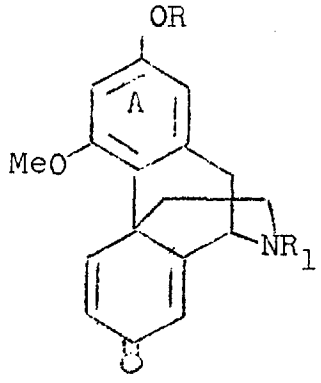
Crotonosine on heating in methanol with 3N-hydrochloric acid yielded an amorphous rearrangement product, O-methylapocrotonosine. Methylation of the product with diazomethane followed by acetylation with acetic anhydride-pyridine gave N-acetyl-OO-dimethylapocrotonosine, while treatment with an excess of methyl iodide in the presence of potassium carbonate afforded NOO-trimethylapocrotonosine methiodide. The rearrangement product, when treated with acetic anhydride-pyridine, gave NO-diacetyl-O-methyl-apocrotonosine. Crotonosine was refluxed with 3N-hydrochloric acid and treatment of the product with an

excess of methyl iodide in the presence of potassium carbonate afforded NO-dimethylapocrotonosine methiodide. The ultraviolet spectra of these crystalline derivatives were characteristic of aporphines.

The rearrangement of crotonosine into apocrotonosine on treatment with acid suggested to Haynes and Stuart[85] that the dienone base had a morphine type of structure. Supporting evidence for this was put forward by acetolysis of crotonosine. This degradation of crotonosine was thought to be analogous to that of the morphine-sinomenine group. Thus on the basis of foregoing evidence a morphine type of structure (1a) was proposed for crotonosine. Evidence for the location of the phenolic hydroxyl and methoxyl groups was obtained from the nuclear magnetic resonance data of N-acetyl-OO-dimethylapocrotonosine. Evidence for the meta orientation of oxygen functions in ring A of crotonosine (1a) came from colour tests with demethylated apocrotonosine.

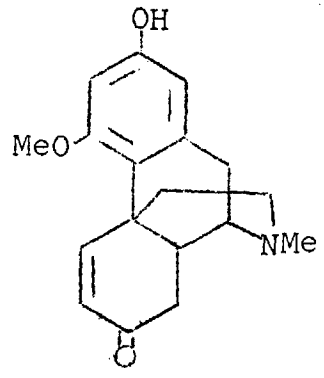
Base A which also had a cyclohexadienone system, was converted by acid treatment and methylation into NOO-trimethylapocrotonosine methiodide. Base A was, therefore, NO-dimethylcrotonosine and formula (1b) was given to it.

Linearisine absorbed one mole of hydrogen in methanol

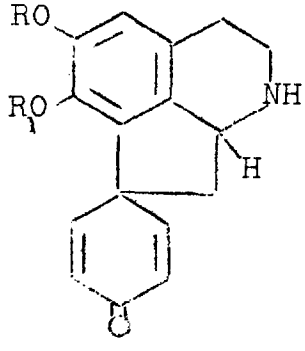


1a, $R=R_1=H$

1b, $R=R_1=Me$

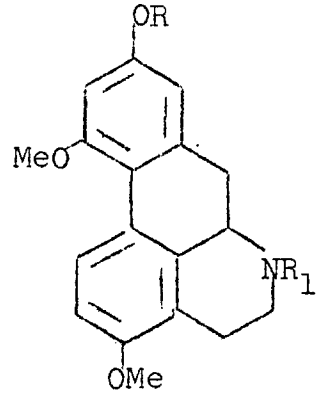


(2)

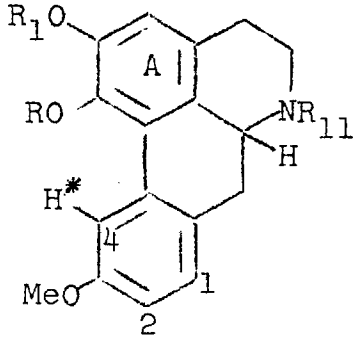


3a, R=Me; R_1=H

3b, R=H; R_1=Me

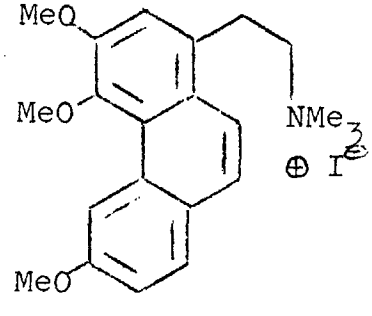


4a, R_1=R=Ac



5a, R or $R_1=R_{11}$=Ac; R_1 or R = Me.

5b, R_1=R=Me, R_{11}=Ac.



(6)

in the presence of 10% palladium on charcoal. With methyl iodide in the presence of potassium carbonate the product gave tetrahydro-NO-dimethylcrotonosine methiodide. The double bond, as shown in the proposed formula (2) for linearisine, was located by means of the u.v. data of the methiodide of the first stage Hofmann degradation product of linearisine. The u.v. absorption of the linearisine methine derivative was similar to that of α-codeimethine and isoeugenol, but different from that of β-codeimethine and thebainone-β-methine.[87]

Recently a more thorough examination of nuclear magnetic resonance data coupled with earlier chemical evidence, showed[88] that crotonosine had, in fact, the formula (3a) or (3b). The former was preferred on biogenetic grounds.

The integrated n.m.r. spectrum of crotonosine revealed the presence of one aromatic hydrogen at 3.42 τ and four olefinic protons. In agreement with this, diacetyltetrahydrocrotonosine showed only one aromatic proton as a singlet at 3.20 τ. The olefinic protons of crotonosine gave multiplets centred at τ values of ca. 2.9 and ca. 3.8 arising from the two overlapping AB quartets from the β and α protons of an unsymmetrical 4,4-disubstituted

cyclohexa 2,5-dienone. Transannular coupling ($J_{\alpha\alpha'}$, 1.5 c.p.s.; $J_{\beta\beta'}$, 2.5 c.p.s.) of the α and β protons was also present. The spectrum of diacetylcrotonosine confirmed these assignments.

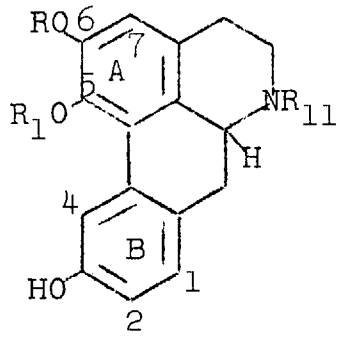
Further inspection of the n.m.r. spectrum of NO-diacetyl-O-methylapocrotonosine formerly regarded as (4a), demonstrated that the deshielded[89] aromatic proton asterisk in (5a), appearing at τ 1.95 showed meta coupling (J, 2.5 c.p.s.) with the upfield components of the AB quartet produced by the remaining ortho protons in the same ring. The spectra of N-acetyl-OO-dimethyl-apocrotonosine (5b) and the phenanthrene (6) were entirely in accord with this interpretation. The former compound showed good agreement in physical constants with N-acetyl-O-methyltuduranine.[90]

Two alternative formulae (3a) or (3b) were possible for crotonosine from the studies reported so far. The former was preferred on biogenetic grounds. However, we investigated this problem and have provided strong evidence in favour of the formula (3b) for crotonosine. Diacetyl-tetrahydrocrotonosine had a lone aromatic proton which appeared as a singlet at τ 3.20. When the compound was heated at 100° for 2 to 3 hours in deuterium oxide in the

presence of potassium tert.-butoxide, deuterated N-acetyl-tetrahydrocrotonosine was obtained. The n.m.r. spectrum of the deuterated compound gave no signal for the lone aromatic proton. The exchange of the aromatic hydrogen with deuterium under these conditions indicated that the exchangeable aromatic hydrogen was next to a hydroxyl function. Furthermore, the n.m.r. spectrum of the deuterotetrahydrocrotonosine, as is expected, did not give any signals for protons α to the carbonyl group.

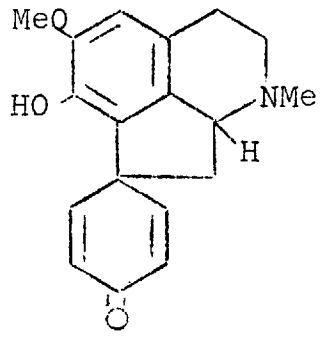
It is interesting to note in these exchange experiments that when the diacetyltetrahydro base was heated in the deuterium oxide in the presence of potassium tert.-butoxide for a longer period (10 to 12 hours) the resulting deuterated compound gave no signal for the N-acetyl group. The i.r. spectrum of the compound, however, confirmed the presence of an amide function (1640 cm^{-1}) in the molecule. Presumably the protons from the acyl group were being exchanged with deuterium under these conditions.

Crotonosine on treatment with 6N-hydrochloric acid afforded apocrotonosine (7a). The n.m.r. spectrum of this compound gave a singlet at τ 3.52 for the aromatic proton at position 7. The protons at C_1, C_2 and C_4 appeared at τ 2.98 (doublet), 3.42 (quartet), and 2.36 (doublet) respectively.

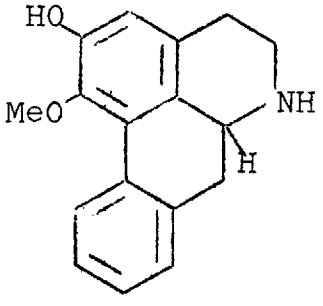


7a, $R=R_{11}=H$; $R_1=Me$

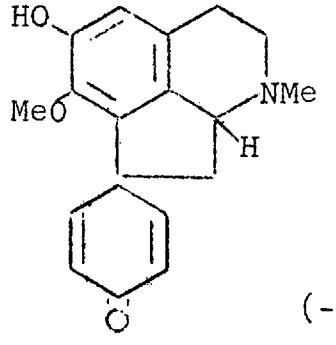
7b, $R=R_{11}=Me$; $R_1=H$



(8)

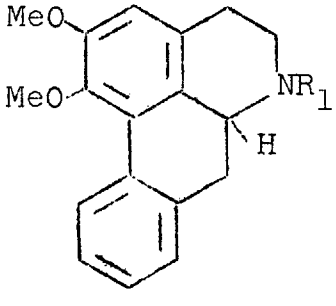


(9)

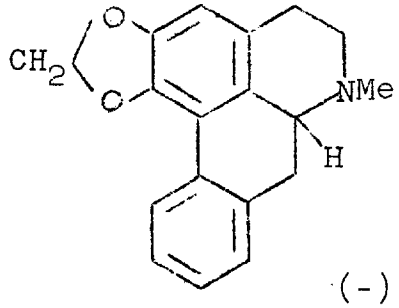


(-)

(10)



(11)



(-)

(12) roemerine

Apocrotonosine, when heated at 100° in deuterium oxide in the presence of potassium tert.-butoxide, for 3 hours gave deuteroapocrotonosine. The n.m.r. spectrum of the deuterated compound again gave no signal for the lone aromatic proton in ring A. Clearly, the aromatic proton at C_7 in apocrotonosine (7a) was next to the hydroxyl function. Of course, under these conditions, the hydrogens in ring B next to the phenolic hydroxyl group were also susceptible to exchange but this took place more slowly. The doublet at τ 2.98 had collapsed into a singlet and the doublet at τ 2.36 and the quartet at τ 3.42 were diminished in intensity. Thus structures (3b) and (7a) were established for crotonosine and apocrotonosine respectively.

Confirmatory evidence for the orientation of the methoxyl and hydroxyl groups in crotonosine (3b) was obtained by repeating the exchange experiments with glaziovine (8)[91] an isomer of N-methylcrotonosine. (A sample of glaziovine was kindly sent to us by Dr. Gilbert for this purpose.)

Glaziovine, like crotonosine, had a cyclohexadienone system. Hydrogenation of the base afforded tetrahydroglaziovine.[91] The lone aromatic hydrogen in the n.m.r.

spectrum of the tetrahydro compound appeared as a singlet at τ 3.67. The deuterium exchange experiments were carried out under the same conditions that were used for tetrahydrocrotonosine. The n.m.r. spectrum of the deuterated compound gave no signals for the protons α to the carbonyl group, as was expected. The lone aromatic proton still gave a singlet at τ 3.67 equivalent to one proton.

Glaziovine (8) was further converted into the apoglaziovine (7b).[91] The aromatic hydrogen at C_7 in apoglaziovine (7b) gave a singlet at τ 3.22. The protons at C_1, C_2, and C_4 appeared at τ 2.79 (doublet), 3.38 (quartet), and 1.92 (doublet) respectively. Deuteration of apoglaziovine (as above) did not affect the intensity of the aromatic singlet at 3.24 τ. Prolonged heating caused the doublet at 2.79 τ to collapse to a singlet. Further, the doublet at 1.92 τ and the quartet at 3.38 τ were considerably diminished in intensity. The experiment showed clearly that the aromatic protons at C_1 and C_4 were being exchanged with deuterium while no such exchange was taking place at C_7. From this it followed that there is no hydroxyl group ortho to the lone aromatic proton in glaziovine.

The deuterium exchange experiments with tetrahydroglaziovine and apoglaziovine were in agreement and provide strong support for the formula (8) for glaziovine. This confirmed the earlier assignment by Gilbert and his coworkers.[91]

The proposed structure (3b) for crotonosine has now been confirmed chemically.[92] N-Methylation of the base with formic acid-formaldehyde afforded (+)-N-methylcrotonosine. Reduction with sodium borohydride and dehydration with acid gave an aporphine (9).

Linearisine and homolinearisine, the minor alkaloids of Croton linearis, have been shown[92] to have the formulae (dihydro-10) and (10) respectively. Linearisine on hydrogenation gave L(-)-dihydrolinearisine $[\alpha]_D$ - 61°, enantiomeric with D(+)-N-methyltetrahydrocrotonosine $[\alpha]_D$ + 59°.

Further examination of homolinearisine has shown that it is in fact L(-)-N-methylcrotonosine (10).

Pronuciferine.

From the Asiatic lotus, Nelumbo nucifera[86] the aporphine bases (-)-nuciferine (11, R_1=Me),[93] (-)-nornuciferine (11, R_1=H),[98] (-)-roemerine (12), and the

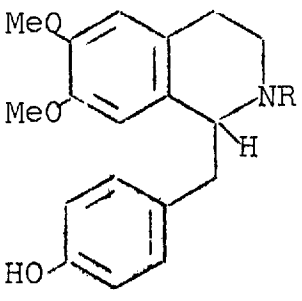
benzylisoquinoline (-)-armepavine (13, R=Me)[99] had been isolated earlier. Recently a careful chromatographic separation of the non-phenolic bases of N.nucifera permitted the isolation of a new base named pronuciferine.[86]

Pronuciferine showed no absorption for NH or OH groups in the infrared spectrum. The bands at 1656, 1634, and 1613 cm^{-1} (KBr) or 1656 and 1618 cm^{-1} ($CHCl_3$) suggested the presence of a cyclohexadienone grouping in the molecule. Confirmation was obtained from the u.v. absorption: λ_{max} 230 and 283 mμ (log. e 4.46 and 3.46).

Reduction of pronuciferine with sodium borohydride afforded an alcohol (14, R=Me), presumably a mixture of epimers which could not be crystallised. However, treatment of the reduction product with dilute mineral acid readily gave a compound identical with natural (-)-nuciferine (11, R_1=Me). The structure (21) was proposed for pronuciferine, and confirmatory evidence was presented by n.m.r. measurements on the base. Pronuciferine is, therefore, identical with the croton Base A and a direct comparison of samples has confirmed this.

DL-Pronuciferine (21)[100] has been synthesised recently by classical routes. The tetrahydro-1-isoquinoline-acetic acid ethyl ester (16) was methylated

(-) Armepavine



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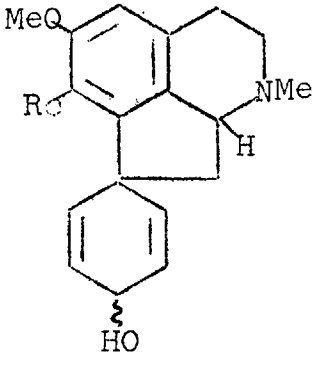

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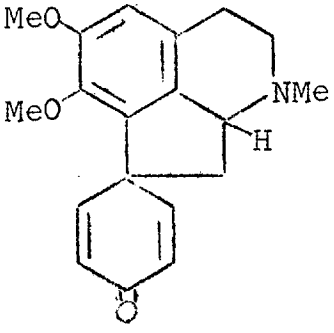

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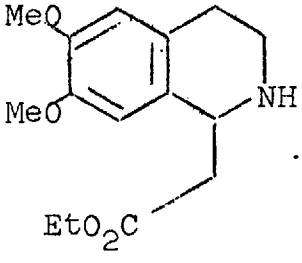

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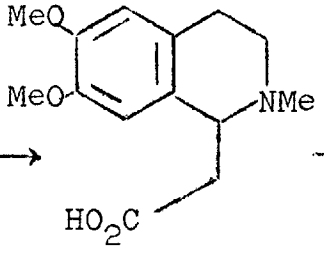

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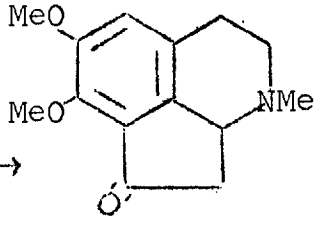

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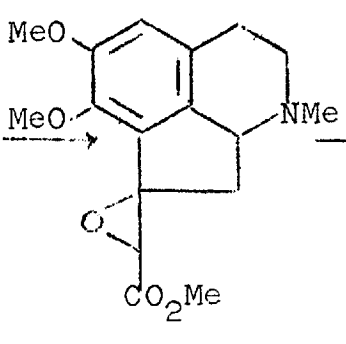

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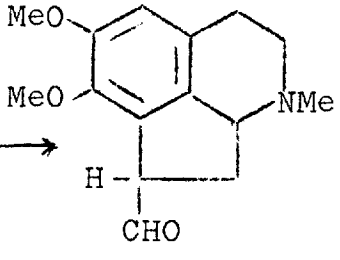

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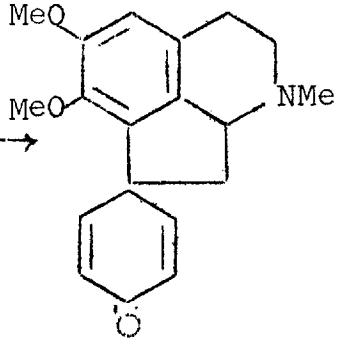


(21)

with formaldehyde-formic acid and saponified to give the N-methyl derivative (17). Cyclisation of (17) with phosphoric acid afforded (18). Treatment with monochloroacetic acid methyl ester in the presence of sodamide in ether gave (19). Hydrolysis by boiling aqueous alcoholic potassium hydroxide afforded the aldehyde (20). The aldehyde was treated with methylethinylketone in the presence of sodium hydride or potassium tert.-butoxide to give DL-pronuciferine (21).

DL-Pronuciferine was reduced with lithium aluminium hydride. The reduction product was treated with dilute mineral acid to give DL-nuciferine which was also prepared by an independent route. The formula (21) for pronuciferine was thus firmly proved.

The co-occurrence of (-)-armepavine (13, R=Me), (+)-pronuciferine (15), and (-)-nuciferine (11, R=Me) in the Asiatic lotus, Nelumbo nucifera, presents circumstantial evidence for the correctness of the biogenetic speculations of Barton and Cohen.

Stepharine and N-Methylstepharine.

In the course of reinvestigating the alkaloids of Stephania glabra Cava and his coworkers[84] in 1964 isolated

two previously[101] unreported bases, base-I and base-II. Base-I was named Stepharine (3, R_1=R=Me). Acetylation of the base with acetic anhydride-pyridine afforded N-acetylstepharine. A formaldehyde-formic acid mixture converted stepharine into base-II. The base-II was, therefore, N-methylstepharine. Direct comparison of the samples establishes the identity of base-II with pronuciferine (21).

Cava and his coworkers[84] have determined the absolute configuration of the proaporphines by an elegant method. For example, reductive cleavage of N-acetylstepharine afforded a product in quantitative yield which was identified with N-acetylnorarmepavine (13, R=Ac). Similar cleavage of pronuciferine (21) with sodium and liquid ammonia containing toluene afforded D-(-)-armepavine (13,R=Me). Since (-)-armepavine is known[102] to have the D (or R) configuration at C_1 then pronuciferine must have the D (or R) configuration at the corresponding asymmetric carbon atom. Furthermore, since pronuciferine had been converted by reduction and acid rearrangement into (-)-nuciferine (11, R=Me), the latter base must belong stereochemically to the D (or R) series. This procedure thus provides a new method for determining the absolute configuration of an aporphine alkaloid which lacks oxygen containing functional

groups on the benzyl derived aromatic ring. Since a large number of aporphine alkaloids may arise in Nature from dienone precursors, it is probable that additional members of the proaporphines will be isolated from plant sources in the near future. The reductive cleavage reaction of Cava and his coworkers exemplified above will thus simplify the determination of both structure and stereochemistry within this group.

Glaziovine.

Gilbert and his coworkers[91] in 1964 isolated two isomeric bases glaziovine (8) and the aporphine (7b) from Ocotea glaziovii. Glaziovine showed infrared absorption characteristic of an α,β-unsaturated ketone and formed an oxime. Catalytic hydrogenation gave a tetrahydro derivative showing carbonyl absorption at 1698 cm^{-1}. Glaziovine, when treated with cold hydrochloric acid, underwent a dienone-phenol rearrangement to give apoglaziovine (7b). This phenol was identical with the aporphine (7b) isolated from the same plant. Apoglaziovine showed ultraviolet absorption typical of an aporphine (7b) oxygenated at positions 3, 5, and 6 and was identified as (-)-3,5-dihydroxy-6-methoxyaporphine.[103] Confirmatory evidence was obtained by n.m.r. data and colour reactions.

Methylation of apoglaziovine with dimethyl sulphate gave 3,5,6-trimethoxyapoglaziovine methosulphate identical with NO-dimethyltuduranine methyl sulphate. The formula (8) was, therefore, proposed for the alkaloid and was proved to be in full accord with the n.m.r. data.

Sodium borohydride reduction of glaziovine (8) gave an alcohol (14, R=H) which was readily dehydrated to 5-hydroxy-6-methoxyaporphine (22, R=H). Methylation of this phenolic base with diazomethane afforded 5,6-dimethoxyaporphine (22, R=Me) identical with (-)-nuciferine (11, R_1=Me).

Mecambrine and Fugapavine.

Slavik[105] and Slavikova[104] in 1960 isolated a number of bases from Meconopsis cambrica and named three of the crystalline bases mecambrine, mecambroline, and mecambridine.

Mecambrine showed ultraviolet absorption at λ_{max} 231 mμ (log. e 4.5), λ_{max} 294 mμ (log. e 3.7) and λ_{min} 270 mμ (log. e 3.4). On heating with dilute mineral acid the base rearranged to mecambroline. Methylation with diazomethane afforded O-methylmecambroline.

Yunusov and his coworkers[106] at about the same time isolated an alkaloid fugapavine from Papaver fugax. Fugapavine possessed a tertiary N-methyl group and a

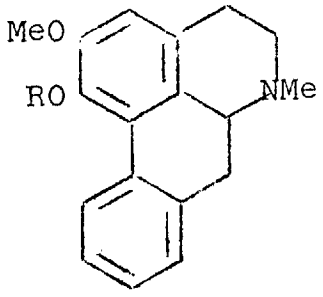
methylenedioxy group. The infrared and ultraviolet spectra of the base were indicative of a conjugated carbonyl and an isoquinoline nucleus. Hydrogenation in the presence of platinum black gave a tetrahydro and a hexahydro compound. The latter had alcoholic properties.

Fugapavine on treatment with mineral acid was transformed into the isomeric phenolic compound, isofugapavine. The rearrangement product when treated with diazomethane afforded O-methylisofugapavine apparently identical with (+)-laureline (27, R=Me). Fugapavine on reduction with lithium aluminium hydride gave an alcohol (presumably this had the formula (25)) which on treatment with acid was dehydrated to give a compound enantiomeric with (-)-roemerine (26).[107] On the basis of the above results the Russian authors[106] have proposed a highly unlikely structure (23) for fugapavine. Isofugapavine is (27, R=H). The knowledge gained from the studies of the proaporphine bases suggests that fugapavine should have the alternative formula (24). All the reported data of the base are consistent with this alternative proposal. Recently, some other workers[91, 108] have shown their dissatisfaction with the Russian proposals for the base.

It is interesting to note that the reported properties of fugapavine from Papaver fugax[106] are in close agreement

with those of mecambrine from Meconopsis cambrica.[104] The latter base has also been isolated recently from Papaver dubium.[109] The acid rearrangement products, isofugapavine and mecambroline, appear to be the same. The O-methyl derivatives of these products show very close similarity and are apparently identical with (+)-laureline (27, R=Me). Thus it is most probable that mecambrine and fugapavine are identical. It would be gratifying to establish their identity by direct comparison and finally to confirm the proposed structure (24) for the bases.

The properties of the proaporphines are summarised on page 59. A very interesting correlation appears between the optical rotation of the so far isolated dienone bases and the rotation of the corresponding acid isomerised aporphines. If the parent dienone has a positive rotation in chloroform or in alcohol, the isomerised aporphine invariably has a negative rotation. Since all (-)-aporphines are known[110,111] to have the D (or R) configuration at position C_1 the proaporphines with positive rotation should also have the D configuration at the corresponding position. Mecambrine (fugapavine) has a negative rotation while the corresponding aporphine shows a positive rotation. If the proposed structure for the



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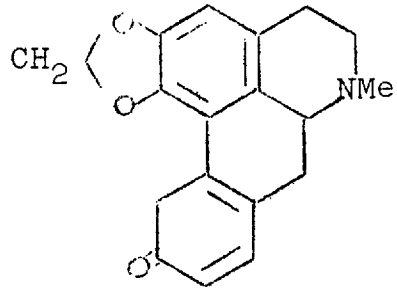

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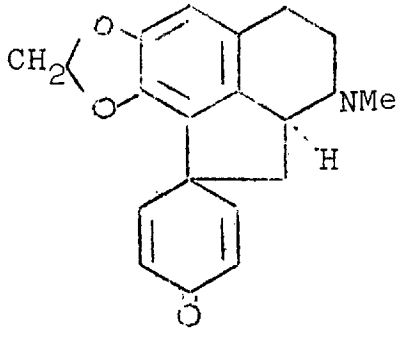

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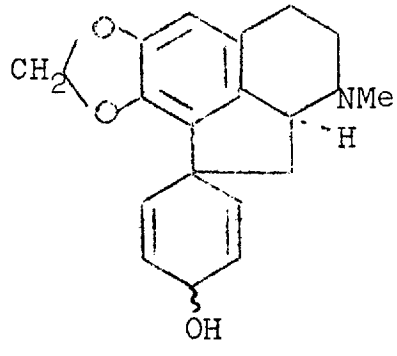

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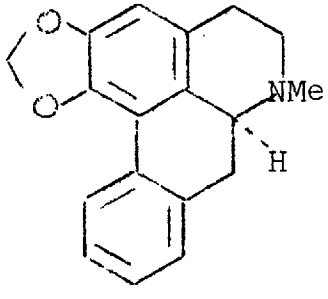

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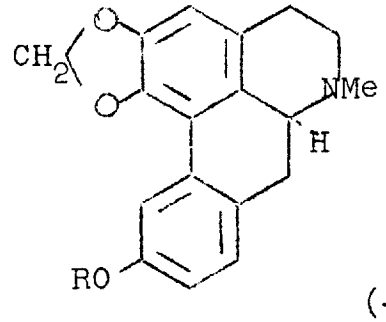
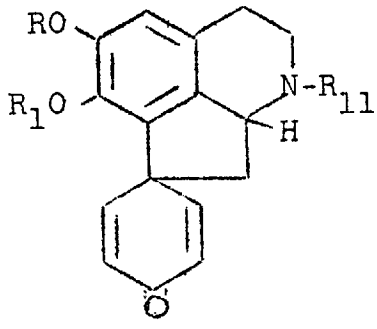


(+)

(27)

dienone base is confirmed, the absolute configuration should be L (or S) as is shown in the formula (24).

It is interesting to note that croton linearis contains the dienone bases of opposite absolute configuration.[92] The occurence of the dienone bases in Nature is an excellent indication of the essential correctness of Barton and Cohen's biosynthetic proposals.



28a, $R=R_{11}=H$; $R_1=Me$

28b, $R=R_1=R_{11}=Me$

28c, $R=R_{11}=Me$; $R_1=H$

28d, $R=R_1=Me$; $R_{11}=H$

Proaporphine Alkaloids.

No.	Name	Source	Structure	m.p.	$[\alpha]_D$	Ref.
1	Crotonosine	Croton linearis	28a	>300°	+180° (MeOH)	85, 88
2	Pronuciferine (Base A; N-methyl-stepharine	Nelumbo nucifera	28b	127-128°	+111.2° (EtOH)	86
		Croton linearis		127-129°	+105.8° ($CHCl_3$)	85
		Stephania glabra			+ 99° ($CHCl_3$)	84
3	Glaziovine	Ocotea glaziovii	28c	235-237°	+70° ($CHCl_3$)	91
4	Stepharine	Stephania glabra	28d	179-181°	+143° ($CHCl_3$)	84

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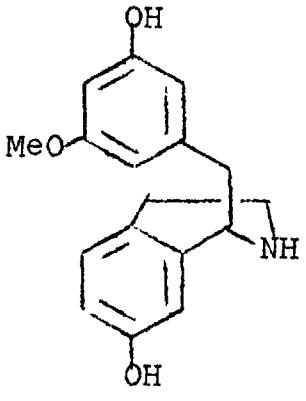
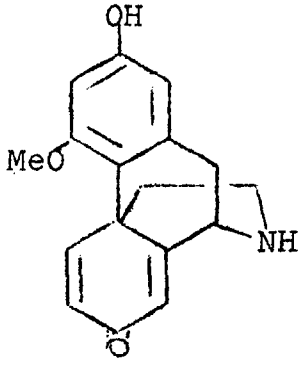
BIOSYNTHESIS OF CROTONOSINE

Introduction.

The purpose of the present investigation was to study the biosynthesis of crotonosine. At the time the work was started the structure (2)[1] was assigned to crotonosine. Obviously the likely precursor for the alkaloid was the benzylisoquinoline (1). An approach to the synthesis of this precursor is given in the experimental section.



(1)



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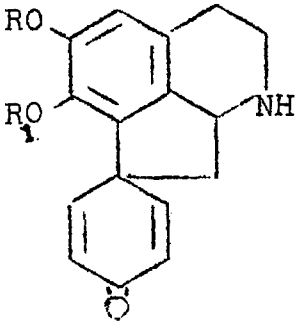


(3)

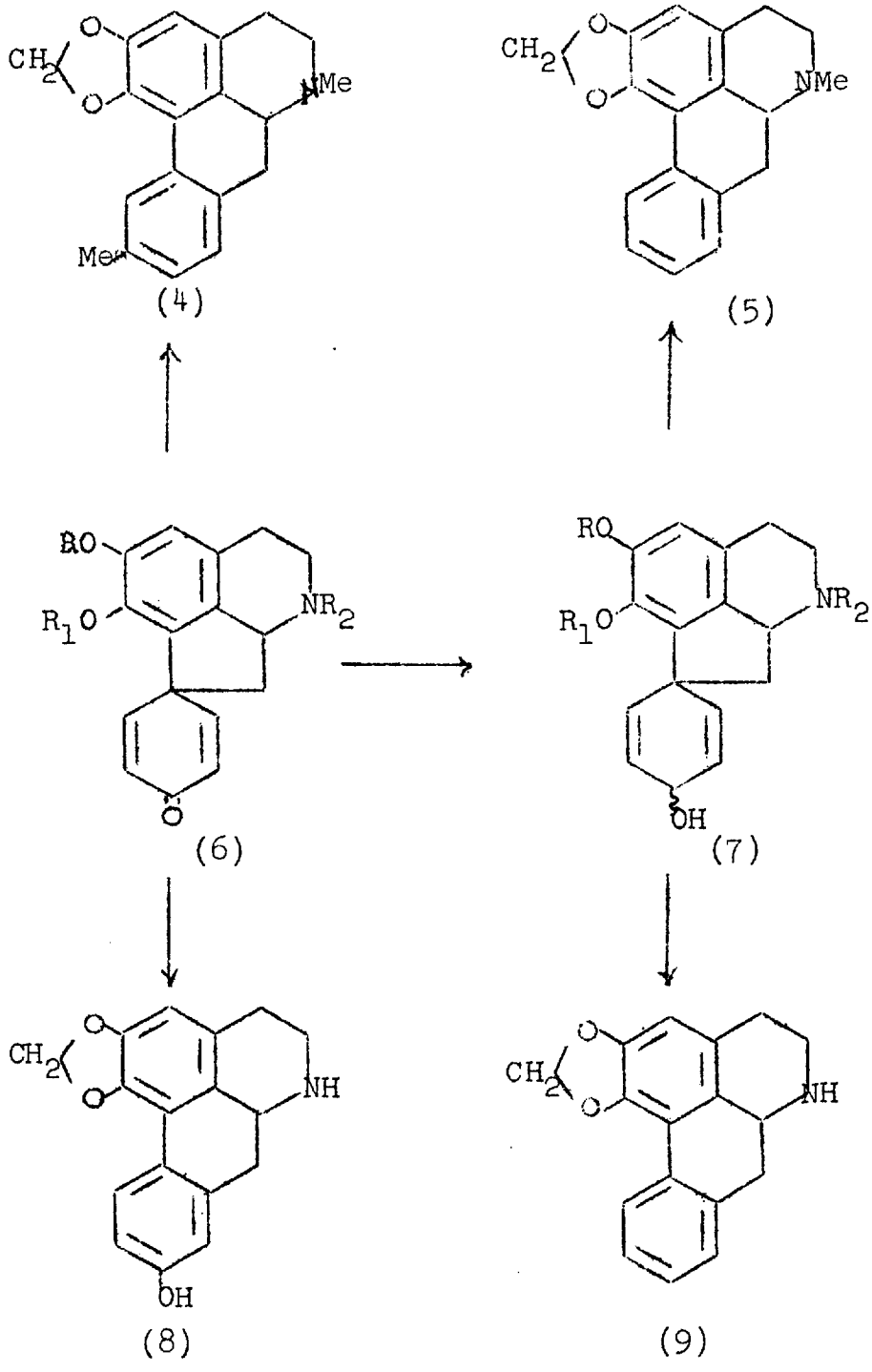
Eventually a more thorough examination of the n.m.r. measurements coupled with the earlier chemical data showed that, in fact, crotonosine[2] had the formula (3a, R=Me, R_1=H or 3b, R_1=Me, R=H). Subsequently by deuterium exchange experiments (see Chapter I) we established formula (3b) for crotonosine.

Biosynthesis

From the biogenetic point of view crotonosine is a very interesting compound. In fact, it is a hypothetical intermediate in the biogenetic scheme of Barton and Cohen,[3] as shown on page 72, for an apparently abnormal group of aporphine alkaloids.[4] The dienol-benzene rearrangement of the alcohol (7) derived from the intermediate (6) could give aporphine bases such as anonaine (9) and roemerine (5) whereas a dienone-phenol rearrangement would give rise to either laureline (4) or anolobine (8). These rearrangements are now well appreciated.[5,6,7] Thus the plausible precursors of crotonosine could also be used in the biosynthetic studies of these alkaloids.

The biosynthesis of crotonosine itself has some interesting features. The positions of the methoxyl and phenolic hydroxyl groups present an interesting problem of interpretation. Oxidative coupling of isococlaurine (10) could give the intermediate bis-dienone (11). A dienone-phenol rearrangement would then give rise to crotonosine (12). The base could also be formed *via* the intermediate (14) from the oxidation of norcoclaurine (15, R=H) by using removable protecting groups for specific coupling. Methylation of the dienone (14) or equivalent intermediate would then provide the *O*-methyl group. It has been argued[6]

Aporphines via Dienone Intermediate.

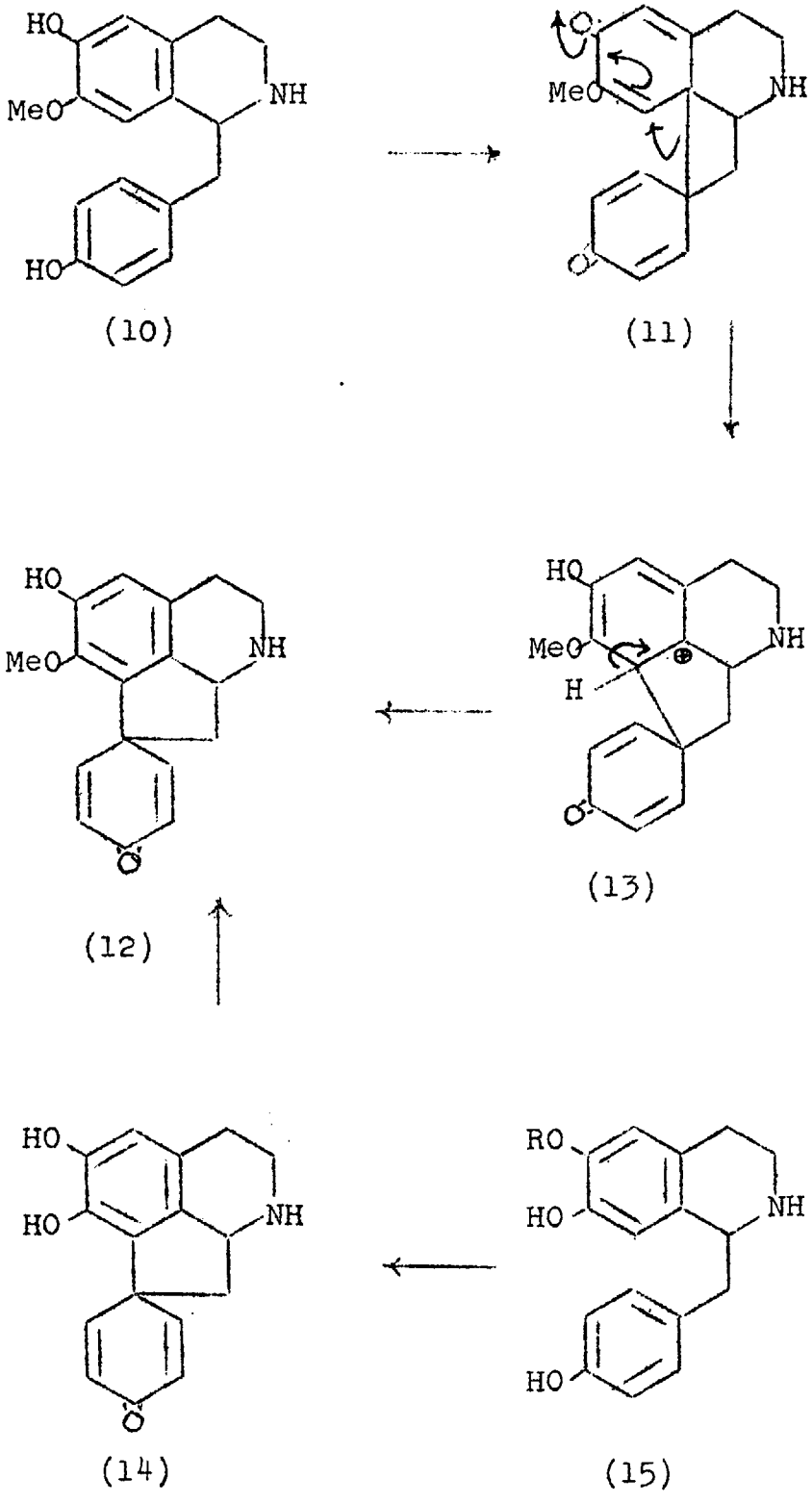


that the existence of the intermediate (11) seems improbable, especially as such a compound should rearrange rapidly as shown by the arrows in (21). Furthermore, four membered rings are not, so far, known to be formed in phenol oxidations. The second explanation, however, is quite possible.

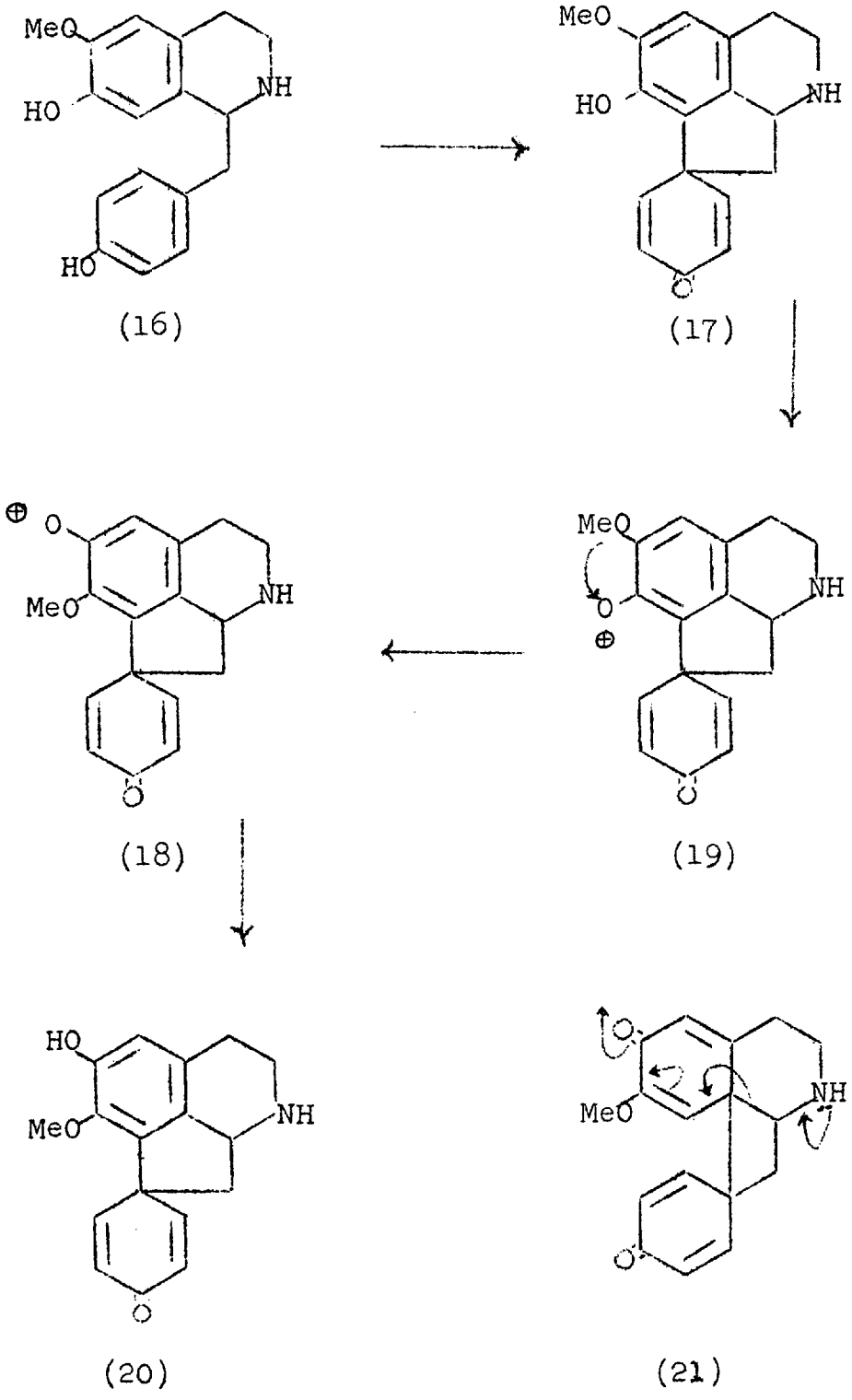
Biosynthetic studies, particularly with the morphine alkaloids[10,11] (see Chapter I) and Amaryllidaceae alkaloids,[8,9] have shown that plants appear to use O-methyl groups for directing the coupling process. If it is true in the case of crotonosine, coclaurine (16) could be the precursor of the base. The involvement of coclaurine could then introduce a novel possibility for the biosynthesis of crotonosine. The first product of coupling of coclaurine (16), that is "isocrotonosine" (17), could "rearrange" to crotonosine (20). A plausible mechanism for such a rearrangement is as follows. Oxidation of the dienone (17) to the corresponding phenoxonium ion (19) could induce methyl migration to (18) which by reduction would give crotonosine (20).

The first task to test these speculations was the preparation of labelled precursors and this was then undertaken.

Biogenesis of Crotonosine.



Biogenesis of Crotonosine (continued).



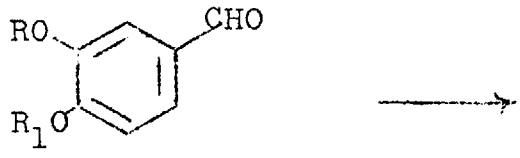
Synthesis of Precursors.

Since the syntheses of some of the compounds were known, the modified procedure in these cases and the syntheses of new compounds have been described only briefly in the sequel.

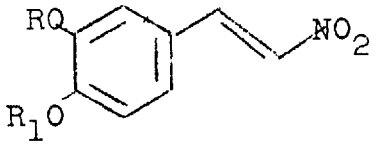
The suitable derivatives (22) of protocatechuic aldehyde have been prepared by standard procedures. The O-methyl label was introduced at the earliest stage of the synthesis by methylation of a suitable monobenzyl-protocatechuic aldehyde with $[^{14}C]$methyl iodide and $[^{3}H]$methyl iodide by a modification of the method of Barton and his coworkers.[12]

The phenethylamines (25) have been prepared via ω-nitrostyrenes (23) from suitable derivatives of protocatechuic aldehyde. Condensation of the aldehydes (22b) and (22c) with nitromethane in the presence of sodium ethoxide afforded the corresponding ω-nitrostyrenes. Difficulties were encountered in the preparation of the ω-nitrostyrene (23a) from OO-dibenzylprotocatechuic aldehyde. The aldehyde in methanol or ethanol, when treated with nitromethane in the presence of sodium or potassium hydroxide in the usual fashion, gave the corresponding saturated nitro-alcohol or a polymerised product. A number of other bases or a mixture of bases (see

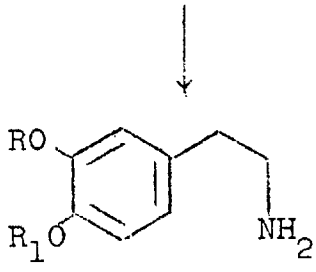
Synthesis of Precursors.



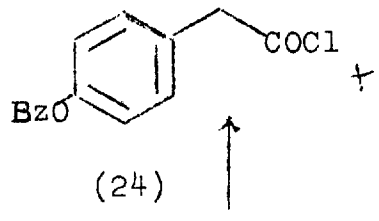
22a, R=R_1=Bz
22b, R=Me; R_1=Bz
22c, R_1=Me; R=Bz



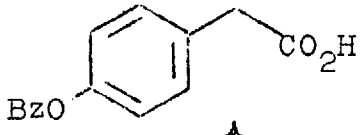
23a, R=R_1=Bz
23b, R=Me; R_1=Bz
23c, R_1=Me; R=Bz



25a, R=R_1=Bz
25b, R=Me; R_1=Bz
25c, R_1=Me; R=Bz



(24)



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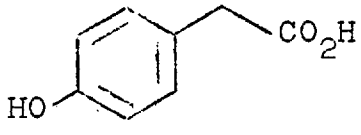
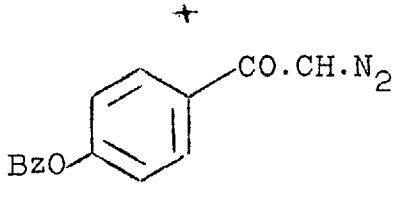
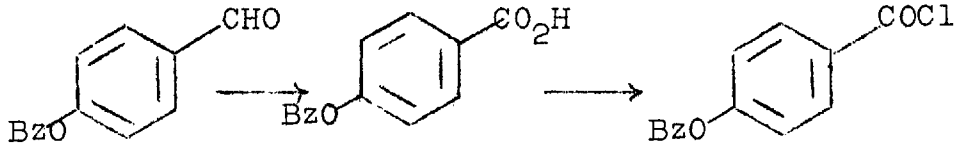


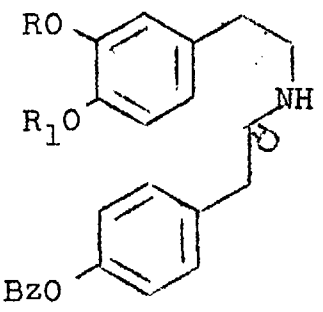


(30)

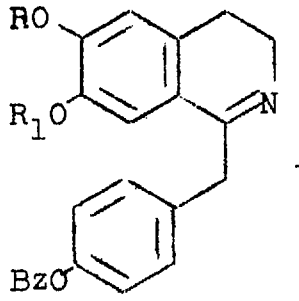


(27) (28) (29)

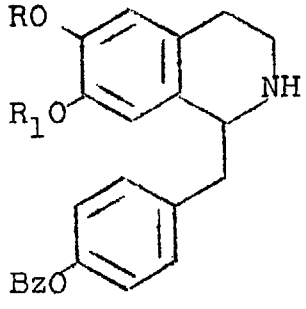
Synthesis of Precursors (continued)



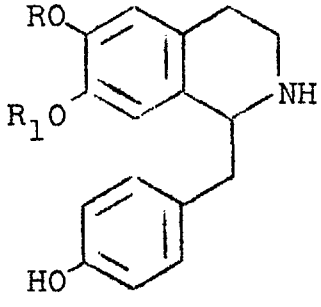
31a, $R=R_1=Bz$
31b, R=Me, $R_1=Bz$
31c, R=Me, R=Bz



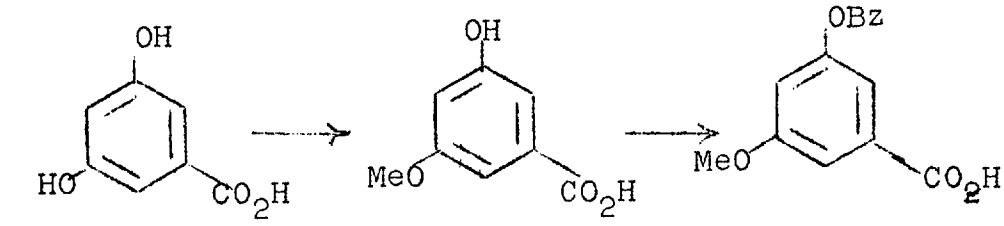
32a, $R=R_1=Bz$
32b, R=Me, $R_1=Bz$
32c, $R_1=Me$, R=Bz



33a, $R=R_1=Bz$
33b, R=Me, $R_1=Bz$
33c, $R_1=Me$, R=Bz



34a, $R=R_1=H$
34b, R=Me, $R_1=H$
34c, $R_1=Me$, R=H



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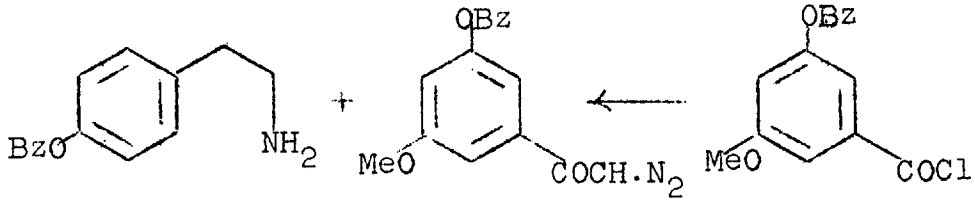
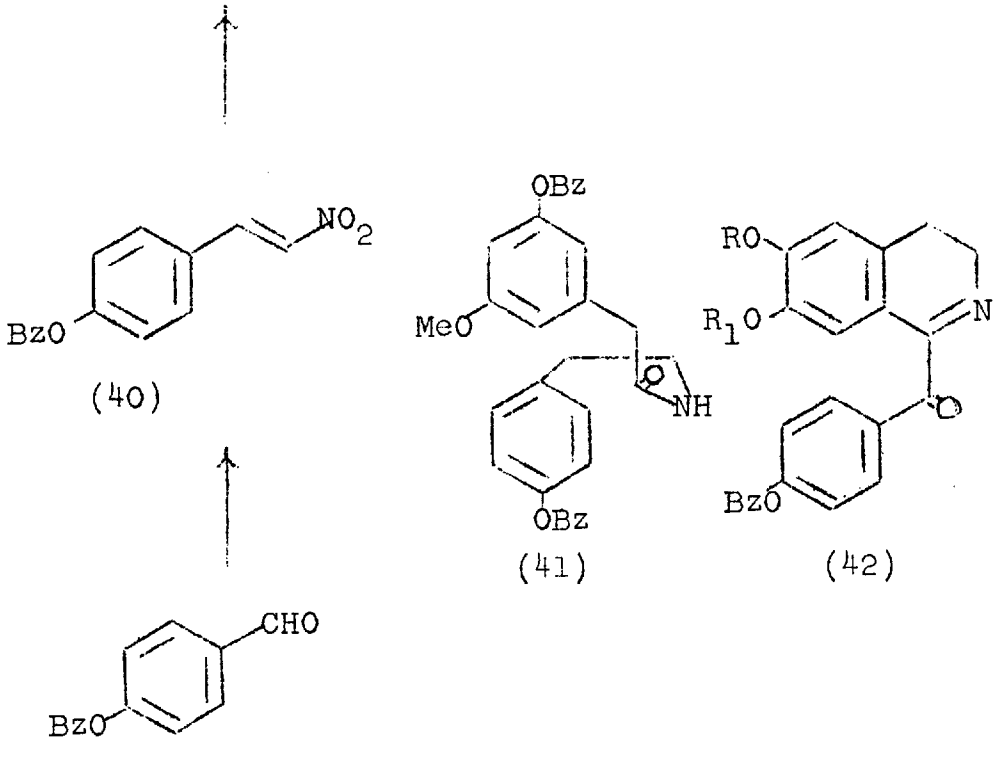


(37) (38) (39)



(40) (41) (42)

Experimental) were also tried but with no success. Temperature variation also did not prove effective. However, when the aldehyde (22a) was treated with purified nitromethane in the presence of methylamine hydrochloride and anhydrous sodium acetate the desired ω-nitrostyrene (23a) was obtained in 90 to 93% yield. Surprisingly, when a mixture of methylamine hydrochloride and ammonium acetate was used the yield of the ω-nitrostyrene was only 25 to 30%. The former method was tried in the preparation of other nitrostyrenes (23) and in almost all the cases the yield of the desired products were much better than by other known procedures.

The ω-nitrostyrenes (23) have been reduced by lithium aluminium hydride to the corresponding phenethylamines (25). In the reduction, variable yields of the amines have been found. However, quite satisfactory yields of the desired products have been obtained by adding slowly a solution of the pure nitrostyrenes in dry tetrahydrofuran to a large excess of lithium aluminium hydride in the same solvent.

The amides (31) have been prepared either by treating p-benzyloxyphenylacetyl chloride with the phenethylamines (25) in the usual way or by a variant of the Wolff

rearrangement[15] (p.123). The diazoketone (30) for this purpose has been prepared from p-benzyloxybenzaldehyde (27) by way of the acid (28) and the acid chloride (29).

The Bischler-Napieralski reaction[13] has been used in the preparation of the dihydroisoquinolines (32) from the amides (31). This reaction proceeded satisfactorily in all the cases except one. In this case (see Experimental) all attempts to prepare the dihydroisoquinoline from the amide (41) did not succeed.

The free bases (32) corresponding to the dihydroisoquinoline hydrochlorides are susceptible to aerial oxidation.[10,14] In some cases oxidation at the benzylic position as shown in (42) has been observed. However, the free bases, liberated from their hydrochlorides with sodium hydrogen carbonate under a nitrogen atmosphere, were safely converted into the corresponding tetrahydroisoquinolines (33) by reduction with sodium borohydride in the usual way. Better yields of the tetrahydro compounds were obtained by carrying out the reaction in ice cold methanol.

The syntheses of the precursors were completed by removal of the protecting groups. Acid catalysed debenzylation is reported[16] to give the phenolic bases sometimes in poor yield. Obviously hydrogenolysis was the method

of choice. Hydrogenolysis of the benzyl ethers (33) in the presence of hydrochloric acid with a reasonable excess of palladium on charcoal in all the cases furnished the desired phenolic precursors (34) in very good yield. Trouble sometimes arose when the benzyl ethers were not sufficiently pure. In that case removal of the catalyst and addition of fresh catalyst afforded the desired product.

Utilising the reactions outlined above, (±)-[O-methyl-^{14}C] coclaurine, (±)-[O-methyl-^{3}H] coclaurine, (±)-coclaurine (34b), (±)-isococlaurine (34c), and (±)-norcoclaurine (34a) hydrochlorides have been prepared.

Exchange experiments.

The next task was to introduce tritium into the nucleus of the inactive precursors. Reactions were known[17] by which the isotopes of hydrogen could be introduced into the aromatic ring. Specific labelling of some simple phenols by exchange reactions were also reported.[18,19] In most of the cases drastic conditions have been used, under which the phenolic bases needed for biosynthetic studies would be destroyed. It was, therefore, important to investigate this reaction.

Inactive (±)-coclaurine, (±)-isococlaurine, and (±)-norcoclaurine were now available. Exploratory exchange experiments with these bases were carried out in deuterium oxide and the reaction was followed by nuclear magnetic resonance measurements. The conditions used were those found valuable in another connection by Mr. L. Ogunkoya.[20] An example of the procedure followed is given below. The n.m.r. spectrum of (±)-coclaurine (43) showed an AB quartet at 3.44 τ and 2.97 τ (J = 8 c/sec.) for the protons ortho and meta to the hydroxyl group in ring B. The ortho and meta hydrogens in ring A appeared at 3.40 τ and 3.42 τ respectively. (±)-Coclaurine hydrochloride in deuterium oxide was heated in the presence of potassium tert.-butoxide (3 mols.) in a sealed tube under nitrogen at 100°. Every 8 hours the n.m.r. spectrum of the resulting product was taken. It was observed that as the time of heating was increased, the doublet at 3.44 τ and the singlet at 3.40 τ gradually disappeared. The complete disappearance of these signals took about 80 hours. The deuterated compound was then isolated and identified as coclaurine hydrochloride. The n.m.r. spectrum of this deuterated (±)-coclaurine (43) gave a singlet at 2.99 τ, equivalent to two hydrogens,

for two meta protons in ring B and a singlet at 3.42 τ, equivalent to one hydrogen, for a meta proton in ring A. The results showed specific labelling at positions ortho to hydroxyl groups.

Surprisingly, in one of the exchange experiments when an old sample of postassium tert.-butoxide (which was later on found to contain potassium carbonate) was used, the deuterium exchange took place almost exclusively at the ortho position in ring A in coclaurine (43). There was practically no exchange of the ortho hydrogens in ring B.

The acid catalysed deuterium exchange reaction was also attempted (see Experimental) with (±)-coclaurine hydrochloride. However, the low solubility of the hydrochloride in hydrochloric acid discouraged further attempts.

The base catalysed exchange reaction, exemplified above, was then attempted with (±)-isococlaurine (34c) and (±)-norcoclaurine (34a). After completion of the reaction the deuterated compounds were isolated and identified. Again the n.m.r. measurements showed specific labelling at the positions ortho to the hydroxyl groups. The standardised procedures for introducing isotopes of hydrogen at specific positions were now available. By

this method, specifically tritium labelled (±)-coclaurine, (±)-isococlaurine, and (±)-norcoclaurine were prepared. The labelled precursors were rigorously purified and the chemical and radiochemical purities were determined by dilution methods. The radio activity was assayed. The results are tabulated below.

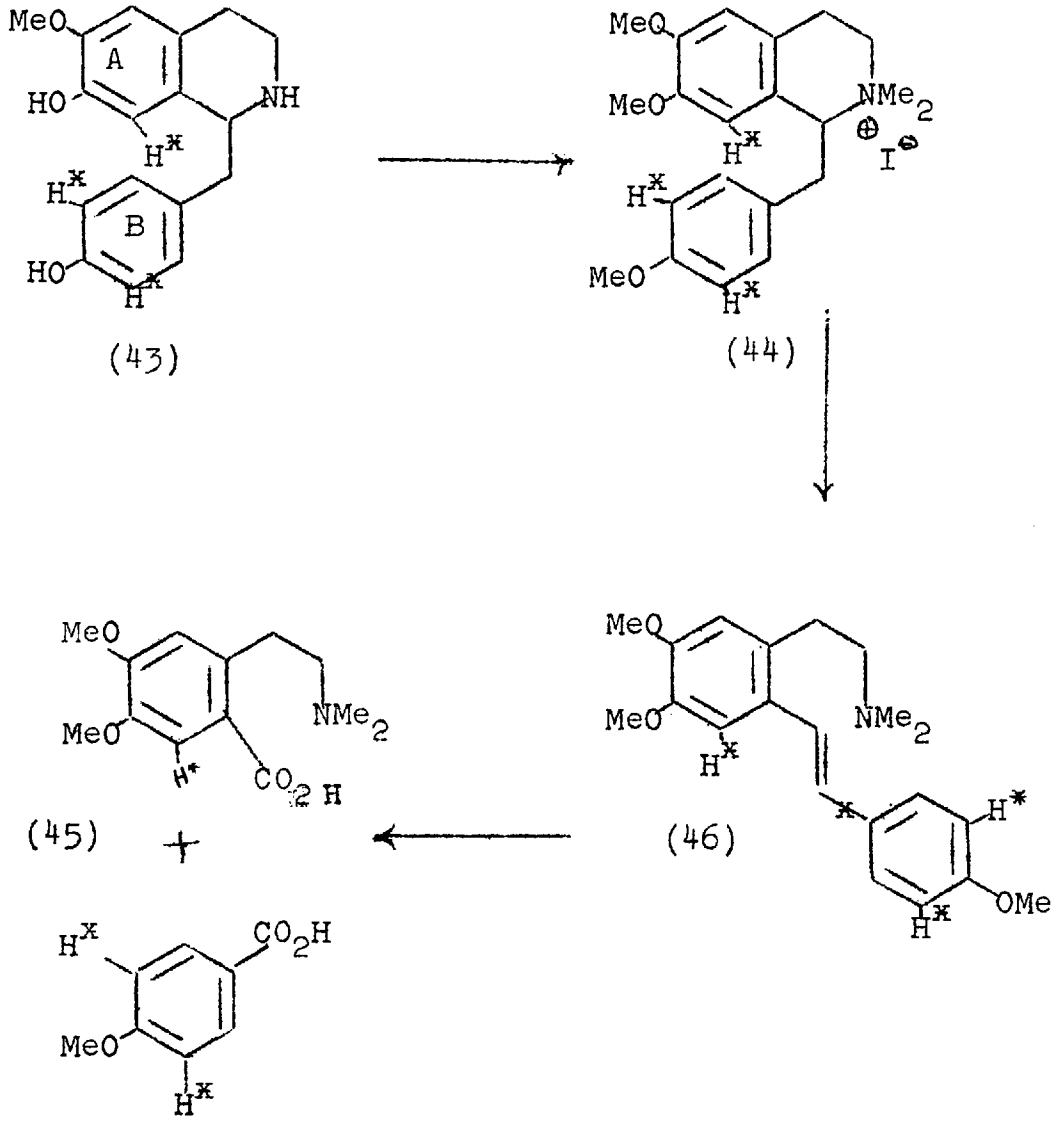
Compound.	Activity. d.p.s./mMole
(±)-Coclaurine Hydrochloride	1.39×10^8
(±)-Isococlaurine Hydrochloride	3.50×10^8
(±)-Norcoclaurine Hydrochloride	3.60×10^8

If one examines the biosynthesis of crotonosine (p. 74) by using these labelled phenolic bases, in each case one expects to lose one tritium at the oxidative coupling step. It was, therefore, decided to determine the distribution of tritium at different positions. This information was obtained by the following degradation of tritium labelled (±)-coclaurine hydrochloride.

Degradation of (±)-Coclaurine.

Methylation of coclaurine (43) with methyl iodide in the presence of potassium hydroxide afforded the quaternary salt (44). Since there was danger of losing

Degradation of (±)-Coclaurine.



tritium under these conditions, an alternative procedure was explored. It was found that treatment of the phenolic base (43), in dry dimethylformamide, with methyl iodide in the presence of sodium hydride at room temperature gave the desired quaternary salt (44) in excellent yield. By this method the radioactive (±)-coclaurine hydrochloride was converted into the corresponding methiodide. Hofmann degradation in the usual way of the active salt (44) gave the radioactive methine (46). The specific molar activity of the active methine was the same as that of coclaurine. Oxidation of the methine (46) with potassium permanganate gave anisic acid in very poor yield. We required an efficient cleavage of the double bond in the radioactive methine so this step was carefully investigated. The trial experiments were carried out with styrene and stilbene. Ozonolysis of styrene and oxidation of the ozonide with alkaline hydrogen peroxide afforded benzoic acid in about 25% yield. Stilbene in methylene chloride at 0° was ozonolysed. Oxidation of the ozonide with alkaline hydrogen peroxide gave benzoic acid (30%). However, oxidation with alkaline silver oxide gave benzoic acid in about 75% yield. This method was then followed for the cleavage of the double bond in the active methine.

Ozonolysis of the methine (46) in methylene chloride at 0° gave the ozonide. Oxidation with alkaline silver oxide afforded radioactive anisic acid (65%). The acid was rigorously purified, the radiochemical purity was established by the usual dilution method, and the specimen was assayed for its radioactivity. The anisic acid contained 64% of the activity of the original coclaurine thus confirming uniformity of labelling in the positions ortho to phenolic hydroxyl groups.

Tracer Experiments.

Croton linearis Jacq, which synthesises crotonosine, grows in the West Indies and was not available for feeding experiments. Professor L. J. Haynes and Dr. K. L. Stuart of the University of the West Indies kindly offered to collaborate with us on this investigation. All the feeding experiments and some of the degradation work was, therefore, carried out by Dr. K. L. Stuart in Jamaica.

In preliminary experiments (±)-[2-^{14}C] phenylalanine was incorporated (0.04%) into crotonosine. In subsequent experiments (±)-coclaurine (34b), (±)-norcoclaurine (34a), and (±)-isococlaurine (34c) hydrochlorides, specifically labelled with tritium in positions ortho to the phenolic hydroxyl groups were separately administered to the plants.

The results of the feeding experiments are tabulated below.

Precursor	Incorporation (%)
(±)-Coclaurine	0.19*, 0.11, 0.20
(±)-Norcoclaurine	0.11, 0.07, .08
(±)-Isococlaurine	0.00*

The asterisk indicates feeding experiments performed in parallel. The incorporations are calculated allowing for the inevitable loss (one third for coclaurine and one quarter for norcoclaurine) of tritium which occurs during cyclisation.

The lack of incorporation of isococlaurine (60) when fed in parallel with coclaurine excludes it as a precursor. Two possibilities remain. Either coclaurine is demethylated to norcoclaurine before incorporation or norcoclaurine is methylated to coclaurine which is then incorporated. In parallel experiments (see Table) (±)-coclaurine was incorporated with a rather higher efficiency than (±)-norcoclaurine. Although these results are not conclusive they suggest that demethylation of coclaurine does not precede incorporation into crotonosine. The lack of incorporation of isococlaurine confirms the phenol coupling idea and further indicates the lack of a demethylation process in the plant.

The utilisation of (±)-coclaurine by the plants in the biosynthesis of crotonosine is obviously the most interesting result. The position of the tritium in the biosynthetic crotonosine was established by Dr. K. L. Stuart in the following way. Reduction of the radioactive alkaloid in the presence of palladium on charcoal gave tetrahydrocrotonosine. Acetylation with acetic anhydride-pyridine afforded NO-diacetyltetrahydrocrotonosine having the same molar activity as the original crotonosine. Base catalysed exchange of this compound in methanol gave essentially inactive tetrahydrocrotonosine, counted as its diacetyl derivative. This, therefore, establishes the tritium atoms exclusively at the expected positions α to the carbonyl group.

It remained to investigate the fate of the methyl group of coclaurine in the biological transformation. For this purpose (±)-coclaurine hydrochloride labelled with ^{14}C in the methoxyl group was mixed with (±)-coclaurine hydrochloride labelled with tritium in the ortho positions. The multilabelled precursor was then fed to the plants. The derived crotonosine (0.98% incorporation) was counted for ^{14}C and ^{3}H by Dr. Stuart. The $^{3}H/^{14}C$ ratio in the precursor and alkaloid is given below.

Compound	Activity (d.p.m./mMole)	
	^{3}H	^{14}C
Crotonosine	2.25×10^{4}	5.4×10^{3}
Diacetylcrotonosine	2.15×10^{4}	5.53×10^{3}

Compound	Ratio ($^{3}H/^{14}C$)
(±)-Coclaurine	1.8:1
Crotonosine	4:1

Clearly the crotonosine still contained ^{14}C but less than expected for simple methyl migration. We attempted to check these values in the following way. The efficiency of the scintillation counter at the maximum efficiency settings for ^{3}H and ^{14}C was determined by using $[^{3}H]$- and $[^{14}C]$-hexadecane standards (see Experimental). At maximum efficiency settings for tritium, the efficiency for the counter for ^{3}H was 13% and for ^{14}C 43%. At maximum efficiency settings for ^{14}C the efficiencies were 5.9% and 70% respectively. Samples containing both ^{3}H and ^{14}C were counted at two different voltage settings, one being optimal for ^{3}H and the other for ^{14}C. However, the $^{3}H/^{14}C$ ratio in the biosynthetic crotonosine was not

sufficiently large for accurate measurements of ^{14}C and ^{3}H activities with our counting equipment. Nevertheless, the crotonosine and diacetylcrotonosine were counted at the optimum setting for ^{14}C. Also a sample of crotonosine was converted into apocrotonosine to check the radiochemical purity. The results of counting are given below.

Compound	Activity (^{14}C) (d.p.m./mMole)
Crotonosine	4.61×10^{3}
Diacetylcrotonosine	4.68×10^{3}
Apocrotonosine Hydrochloride	4.51×10^{3}

The difference in absolute values of these activities from those recorded by Dr. Stuart probably arose from different calibration methods.

It was still important to locate the ^{14}C label in the derived crotonosine. This was done in the following manner. The methoxyl group in the biosynthetic base was cleaved by the Ziesel method. Attempts were made to trap the evolved methyl iodide by methylation of some phenolic compounds in order to get a suitably soluble derivative for counting. However, the method did not prove effective. The active methyl iodide was, therefore, trapped by triethylamine in ethanol in the usual manner. The isolated triethylmethylammonium iodide was counted

in dilute solution and found to carry ^{14}C activity. This was confirmed by the cleavage of the methoxyl group of the radioactive apocrotonosine by the method as above. The results are given below.

Compound	Activity (^{14}C)
$(EtN)_3\overset{+}{N}H_4\bar{I}$ (Crotonosine)	4.11×10^3 d.p.m./mMole
$(EtN)_3\overset{+}{N}H_4\bar{I}$ (Apocrotonosine)	4.13×10^3 " "

The above degradation established ^{14}C activity in the methoxyl group of the derived crotonsine. Since the ratio of the labelled isotopes was anomalous the experiment was repeated. (±)-Coclaurine hydrochloride labelled with tritium in the methoxyl group was mixed with (±)-coclaurine hydrochloride labelled with tritium in the ortho positions and fed to the plants. At this time a very poor incorporation (0.0032%) of the precursor into crotonosine was found and it was not possible to carry out the degradation and obtain meaningful ratios. However, in the coming season the experiment should be repeated.

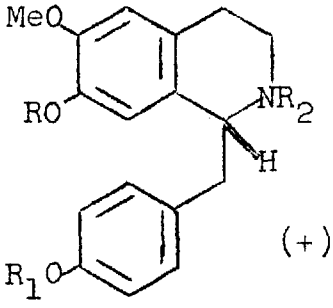
Configuration of Coclaurine.

Since enzymatic reactions are generally stereospecific, the use of labelled optically active precursors of known

configuration in the biosynthetic studies was very attractive.

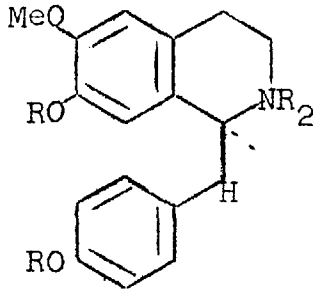
Coclaurine[57] has been isolated from natural sources but the absolute configuration of the base had not been determined. The first task, therefore, was to determine the configuration of the enantiomers. (±)-OO-Dibenzylcoclaurine required for this purpose was synthesised by the route described earlier. Repeated attempts to get a crystalline salt with α-bromocamphor π-sulphonic acid did not succeed. The (±)-dibenzyl ether, when treated with D-camphor-10-sulphonic acid, however, afforded a crystalline salt. Fractional crystallisation of the salt from a variety of solvents resulted in only partial resolution of the racemate. Attempts were then made to resolve the racemate with dibenzoyltartaric acid. (±)-OO-Dibenzylcoclaurine was treated with (+)-dibenzoyltartaric acid. The resulting salt, when subjected to fractional crystallisation gave a crystalline (+)-salt in polymorphic forms with a constant rotation. Decomposition of the salt with 4N-sodium hydroxide afforded (-)-OO-dibenzylcoclaurine. (+)-OO-Dibenzylcoclaurine was obtained by treating the partially resolved dibenzylcoclaurine with (-)-dibenzoyltartaric acid. Hydrogenolysis of the enantiomeric dibenzyl ether with palladium on charcoal in the usual way gave

Configuration Coclaurine.



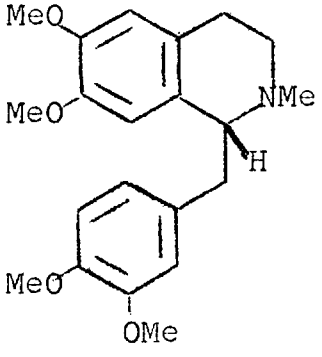
(+)

47a, $R=R_1=Bz$, $R_2=H$
47b, $R=R_1=R_2=H$
47c, $R=R_1=H$, $R_2=Me$
47d, $R=R_1=R_2=Me$

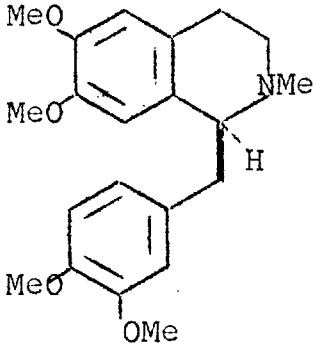


(-)

48a, $R=R_1=Bz$, $R_2=H$
48b, $R=R_1=R_2=H$
48c, $R=R_1=R$, $R_2=Me$
48d, $R=R=R_1=Me$



(49)



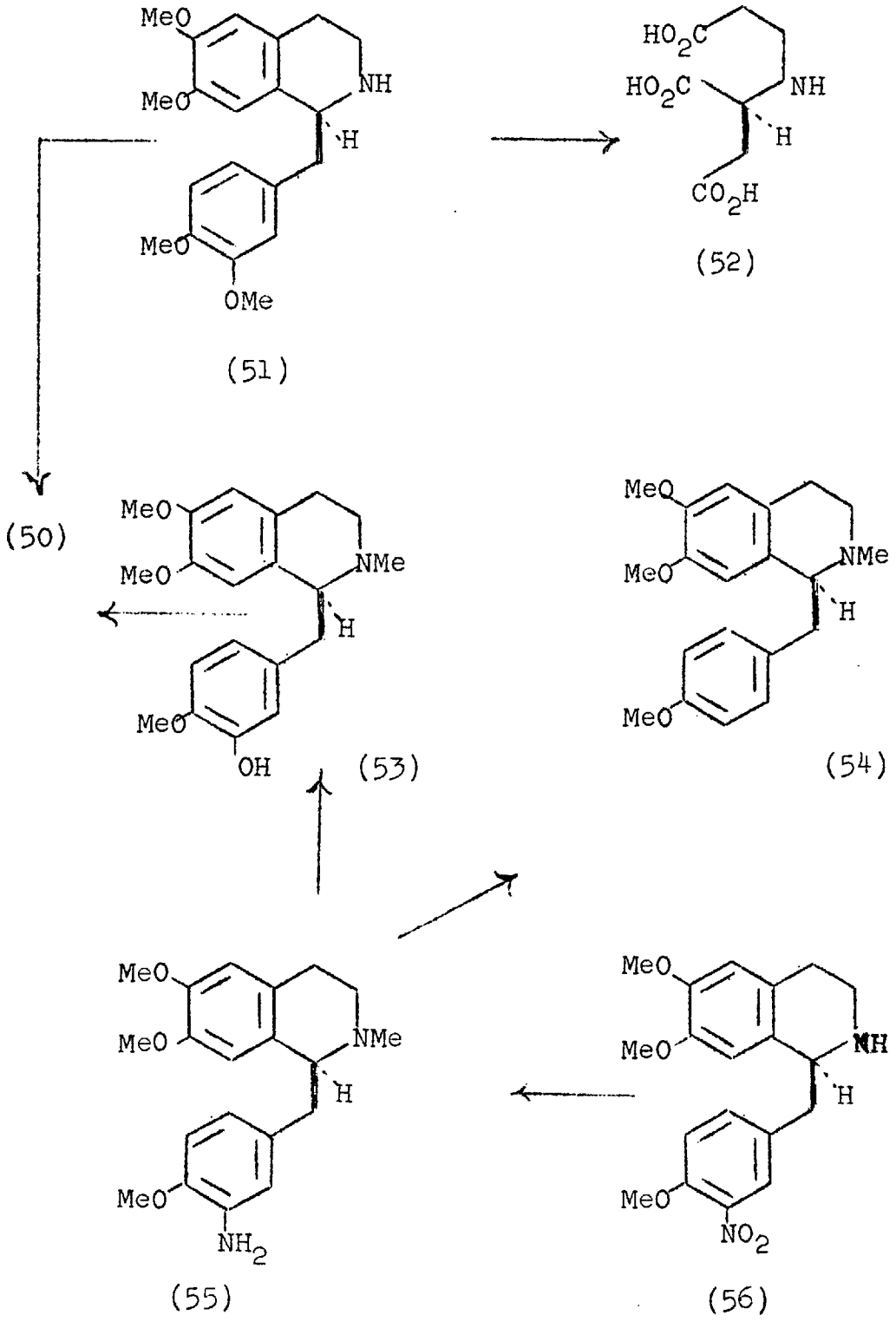
(50)

the corresponding forms of the coclaurine hydrochlorides, isolated in their hydrated forms. Drying over phosphorus pentoxide *in vacuo* afforded the anhydrous salts.

Tomita and Kunitomo[40] in 1962 had correlated the configuration of (+)-*NOO*-trimethylcoclaurine (54) with (+)-laudanosine (50). Ferrari and Delefeu[58] had also reported the same correlation by a different route. (+)-Laudanosine (50) and (+)-*NOO*-trimethylcoclaurine (54) were each obtained from the intermediate aminoisoquinoline (55). The absolute configuration of (+)-laudanosine has been established by Corrodi and Hardegger[59] by chemical correlation of (+)-laudanosine with the amino acid (52) of known absolute configuration.

Our problem was therefore to convert the enantiomers of coclaurine into *NOO*-trimethylcoclaurine. N-Methylation of (+)-coclaurine with formaldehyde-formic acid afforded (-)-*N*-methylcoclaurine. The (+)-enantiomer was likewise obtained from (-)-coclaurine. A similar change in the sign of rotation on *N*-methylation has also been observed by Corrodi and Hardegger[59] when (-)-*N*-norlaudanosine (51) was converted into (+)-laudanosine methiodide and also noted by Ferrari and Delefeu[58] in the N-methylation of the nitroisoquinoline (56).

Correlation of configuration.



The final step was the O-methylation. Treatment of (+)- and (-)-N-methylcoclaurine with diazomethane gave (+)- and (-)-NOO-trimethylcoclaurine respectively. Since (+)-NOO-trimethylcoclaurine has the same absolute configuration as (+)-laudanosine and since a change in the sign of rotation takes place during N-methylation of coclaurine, the configuration of (-)- and (+)-coclaurine must be the same as that of (+)- and (-)-laudanosine respectively. (+)-Coclaurine is therefore formulated as (58). The rotation of coclaurine derivatives are given below.

$[\alpha]_D$ Values of Coclaurine Derivatives

	Compounds	$[\alpha]_D$ in MeOH	
		(+)-Coclaurine	(-)-Coclaurine
1	OO-Dibenzyl-coclaurine-(+)-dibenzoyltartrate	-	+ 72°
2	OO-Dibenzyl-coclaurine-(-)-dibenzoyltartrate	- 72°	
3	OO-Dibenzyl-coclaurine	- 16° + 24.5*	+ 15° - 26.5*
4	OO-Dibenzyl-coclaurine hydrochloride	- 48°	+ 47°
5	Coclaurine hydrochloride	+ 13°	- 14°
6	N-Methylcoclaurine	- 120° (lit.[38] -122°)	+ 123° (lit.[38] +124°)
7	NOO-Trimethyl-coclaurine	- 83° (lit.[40] -85.7°)	+ 83° (lit.[40] +86°)

* In chloroform

Configuration of Crotonosine.

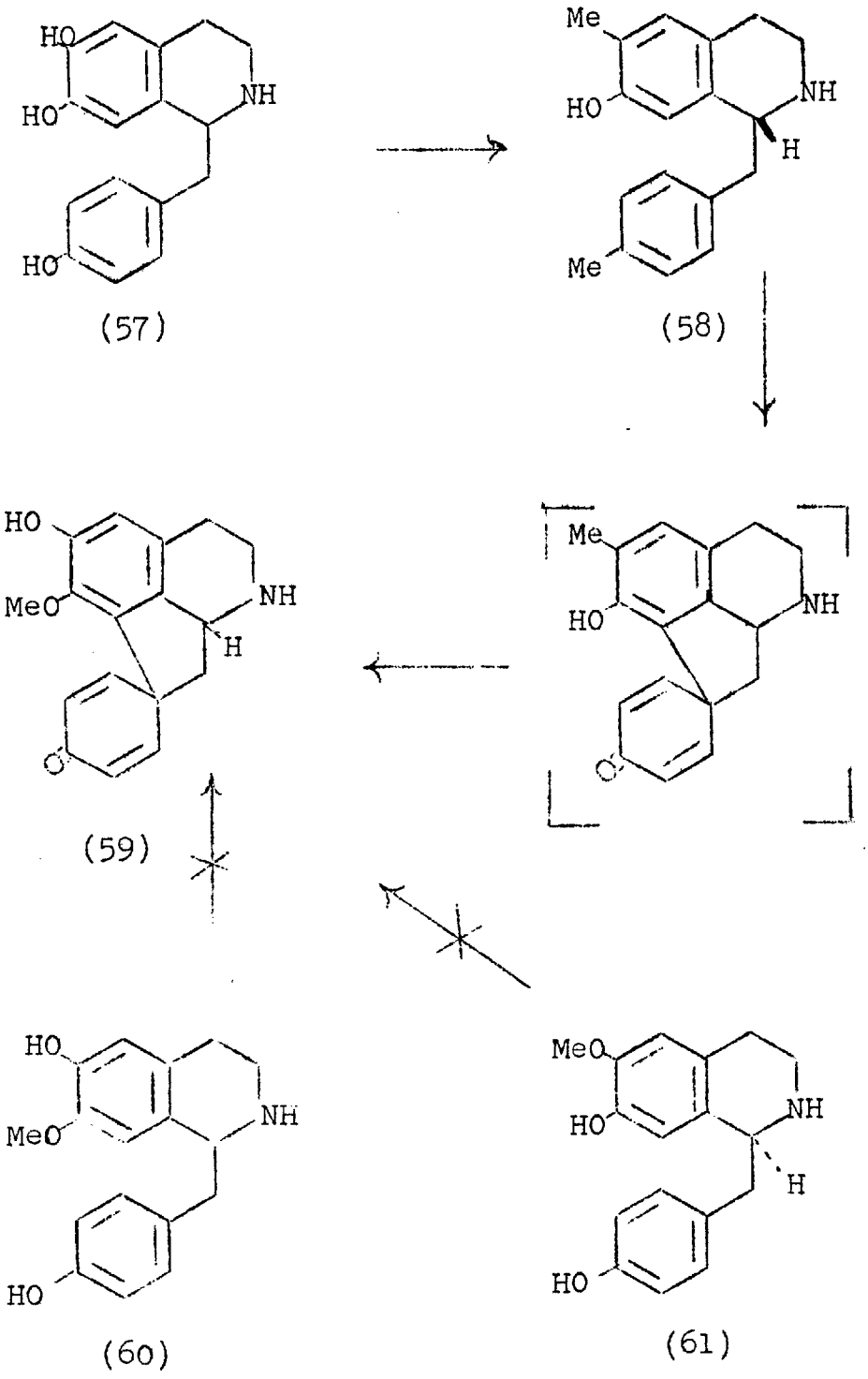
(+)- And (-)-coclaurine were now available. Tritiation in the usual way gave the specifically tritium labelled enantiomers. These were then fed to croton linearis. The results of feeding experiments are given below.

Precursor	Incorporation (%)
(+)-Coclaurine	0.17*
(-)-Coclaurine	0.00*, 0.00*

The asterisk indicates feeding experiments performed in parallel. The incorporations are corrected for loss of one tritium. The above results clearly show that only (+)-coclaurine is an efficient precursor of crotonosine. This confirms the absolute configuration of crotonosine and shows that the biological conversion is stereospecific.

The most probable biosynthetic route to crotonosine (p.101) is summarised below. Norcoclaurine (57) is converted in the plant into (+)-coclaurine (58) which is then incorporated into crotonosine (59). The fate of the methoxyl group of coclaurine in the biological transformation is yet undecided.

Biosynthesis of crotonosine.



Introduction.

(-)-Roemerine was first isolated by Orekhov and his collaborators[61] in 1939 from Roemeria refracta DC. The correct formula (66, R=Me) for the alkaloid was established by Barger and Weitnauer.[62] In subsequent years the base has also been isolated from Cryptocarya angulata,[63] Neolistea sericea,[64] and Nelumbo nucifera.[65,66]

Recently Slavik has isolated (+)-roemerine from Papaver dubium.[67] The isolation of the base provides the first authenticated case of an aporphine alkaloid existing in antipodal forms in Nature.[67] A compound which could be (+)-roemerine has recently been isolated from Papaver fugax.[68]

Biosynthesis.

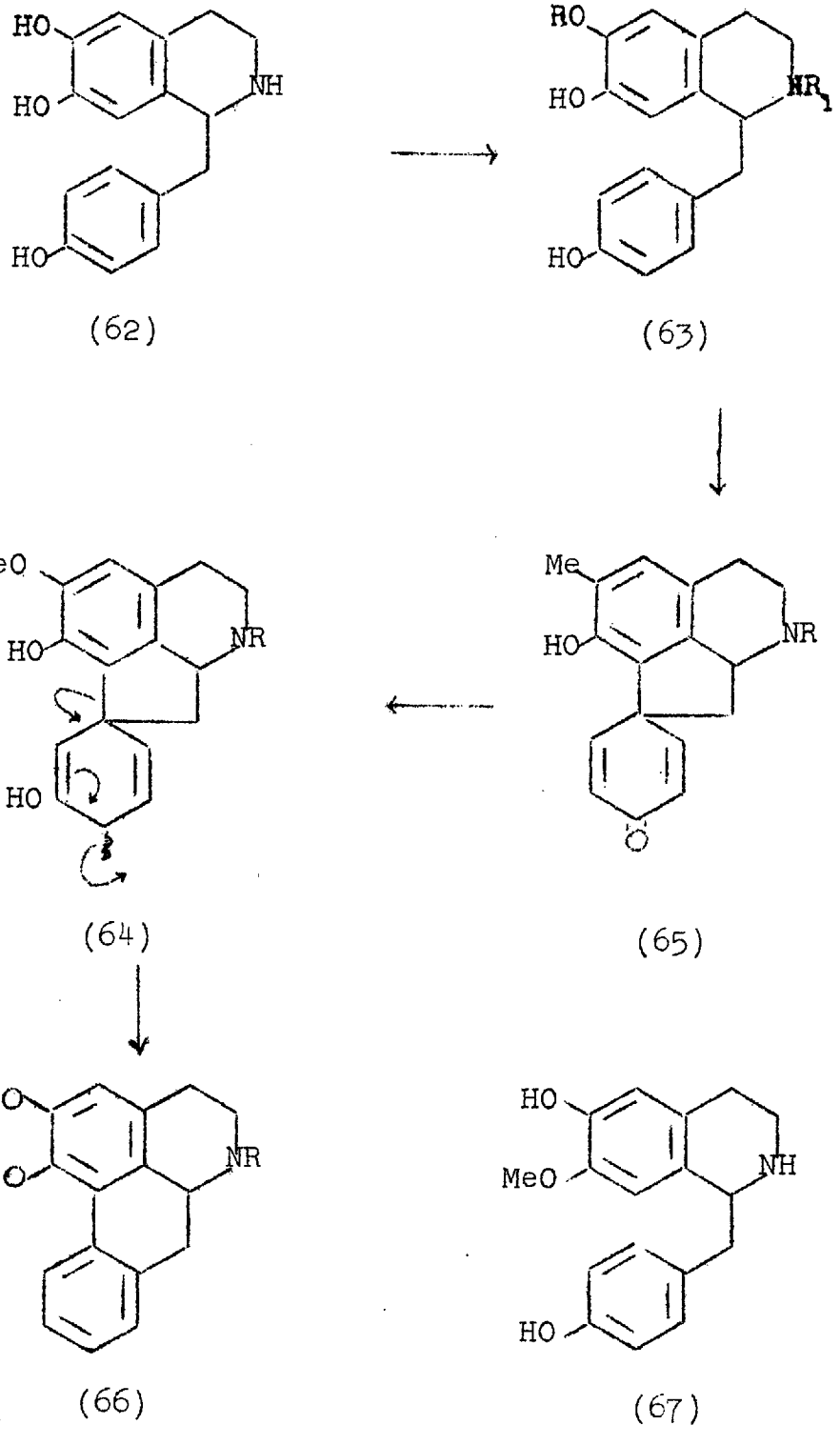
Roemerine is a typical example of an abnormal aporphine which lacks an oxygen function in one of the aromatic rings. The biosynthesis of this group of alkaloids has been mentioned earlier (see Chapter 2). The suggested[6] biosynthetic scheme for roemerine is illustrated on page 104. Oxidative coupling of the phenol (63) gives the dienone (65). Reduction of this to the corresponding dienol (64), followed by dienol-benzene rearrangement, finally affords roemerine (66, R=Me).

The correctness of the biogenetic scheme has gained support from the subsequent isolation from natural sources of a number of dienone alkaloids (see Chapter I) resembling the hypothetical intermediate (65). The reaction sequence from the dienone (65) to the corresponding benzenoid compounds has also been carried out in the laboratory.[21,60]

The real proof for the correctness of a biosynthetic scheme should obviously come from experiments with living plants. The purpose of the present investigation was, therefore, to test the biosynthetic speculation for roemerine *in vivo*.

The biosynthetic studies on Amaryllidaceae alkaloids[9] and also on berberine[37] and protopine[37] have shown that the methylenedioxy group in Nature can be derived from the *O*-methyl group of an *ortho*-methoxyphenol. Further the studies on the morphine[11] and Amaryllidaceae[8] alkaloids have proved that plants use *O*-methyl groups for directing the coupling process. Obviously, coclaurine (63, R=Me, R_1=H) was a plausible precursor for roemerine, while *N*-methylcoclaurine (63, R=R_1=Me) could be the phenol which undergoes oxidative cyclisation. However, to examine the methylation sequence and also to show that the biosynthesis could be blocked by methylation of the phenolic

BIOSYNTHESIS OF ROEMERINE (Barton).



hydroxyl groups involved in the oxidative coupling, feeding experiments with precursors such as norcoclaurine (62) and isococlaurine (67) were also important.

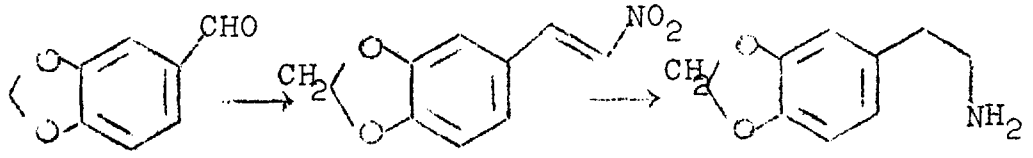
We had already prepared labelled coclaurine, norcoclaurine, and isococlaurine for the biosynthetic studies of crotonosine (see Chapter 2). N-Methylcoclaurine was prepared by N-methylation of the tritium labelled coclaurine.

Papaver dubium, the plant which was selected for feeding experiments, synthesises (+)-roemerine. Supplies of the alkaloid for dilution purposes were not available from natural sources and the synthesis of (±)-roemerine was, therefore, undertaken. Further, the procedure was known for the resolution of the racemate and therefore (+)-roemerine could be made available easily for critical experiments. This part of the work was carried out in collaboration with Mr. G. M. Chapman.

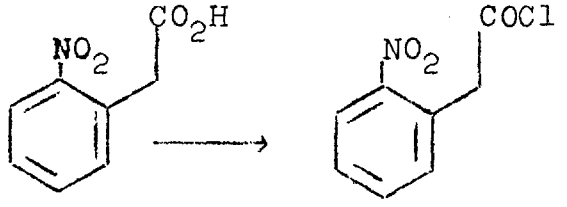
Synthesis of (±)-Roemerine.

(±)-Roemerine (78)[45] has been synthesised from the corresponding amine (79) by Pschorr cyclisation. Certain steps in the reported syntheses needed investigation. In the preparative scale synthesis, we have modified certain steps and the modified synthesis of the (±)-base is outlined on page 106 and is discussed below.

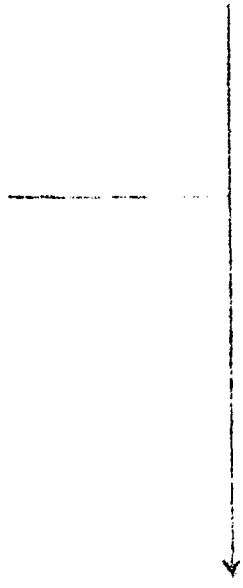
Synthesis of Roemerine.

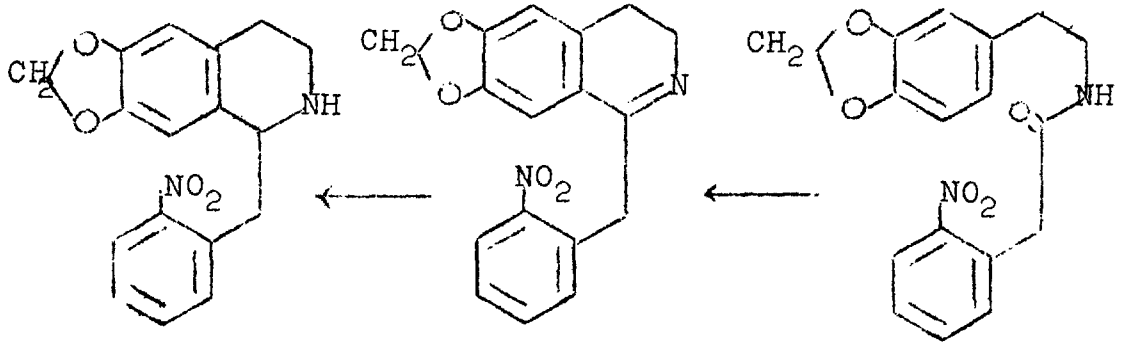


(68) (69) (70)

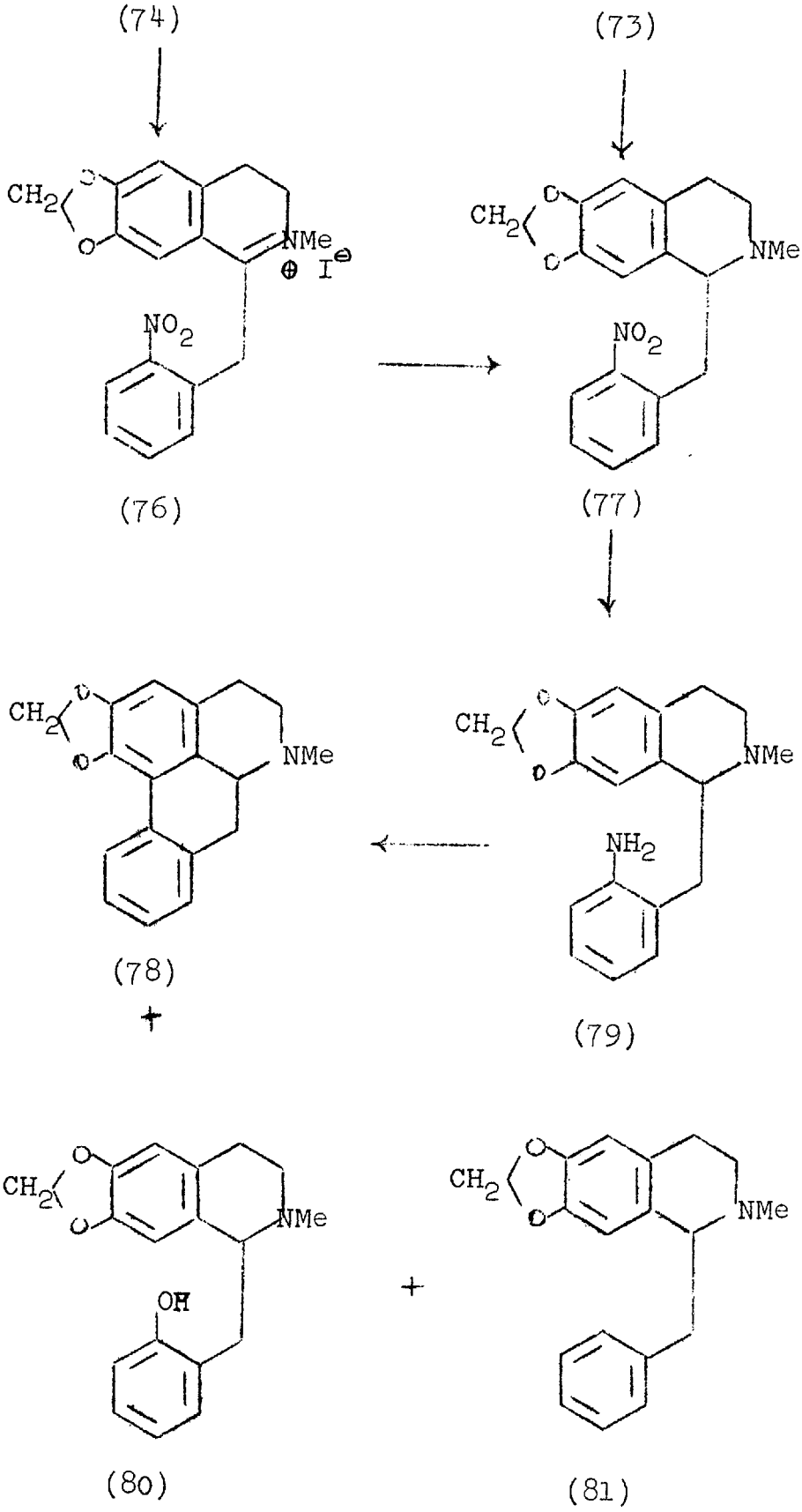


(71) (72)





(73) (74) (75)


(74)
(73)
CH2
NMe
⊕ I⊖
NO2
(76)
(77)
NH2
(78)
+
(79)
OH
(80)
+
(81)

Condensation of piperonaldehyde (68) with nitromethane in the presence of sodium ethoxide in the usual fashion gave the ω-nitrostyrene (69). Excellent yields of the nitrostyrene were obtained when a mixture of methylamine hydrochloride and anhydrous sodium acetate was used in the reaction. Reduction of the nitrostyrene (69) with lithium aluminium hydride in the usual way gave the phenethylamine (70) in fairly good yield. Treatment of O-nitrophenylacetic acid (71) with thionyl chloride afforded the corresponding acid chloride (72). This reaction requires some care in working up. In one experiment when all the solvent was removed *in vacuo*, the residue exploded and a black tar was obtained. However, with care the reaction is not dangerous. Acylation of the phenethylamine (70) with the acid chloride (72) gave the amide (75).

The Bischler-Napieralski reaction of the amide (75) gives variable yields of the desired dihydroisoquinoline (74). In trial experiments when the amide (75), in dry toluene, was refluxed for 1 hour with freshly distilled phosphorus oxychloride in the usual way, polymerisation was the main reaction. The dihydroisoquinoline (74) was obtained in poor yield. However, when the amide (75) in

chloroform was treated with phosphorus oxychloride and left at room temperature for 5 to 6 days, the dihydroisoquinoline (74) was obtained in good yield.

The dihydroisoquinoline (74) was converted to the amine (79) by the two following routes. First, the dihydro compound (74) in benzene was treated with methyl iodide and the corresponding methiodide (76) was obtained in excellent yield. Catalytic reduction of the methiodide to give the amine (79) did not proceed well. An alternative procedure was then adopted. The methiodide (76) was reduced with sodium borohydride to the tetrahydro compound (77). Catalytic reduction of this with palladium on charcoal then afforded the amine (79).

In the second procedure the dihydroisoquinoline (74) was converted into the secondary amine (73) with sodium borohydride in the usual way. This was then treated with a mixture of formaldehyde and formic acid to give the N-methyl compound (77). Hydrogenation in the presence of palladium on charcoal finally afforded the desired amine (79). The overall yield of this amine (79) was much higher by this route than by the first procedure.

The Pschorr cyclisation was the critical step in the synthesis of roemerine. This was then investigated.

It was found that by careful diazotisation of the amine (79) and by decomposition of the diazotised salt at 100° in the presence of copper powder, (±)-roemerine (78) was obtained in 27% yield. A poorer yield of the desired product results when diazotisation is not carried out at a sufficiently low temperature and when enough time is not given for completion of the diazotisation reaction.

Careful chromatography on alumina of the basic fraction of the Pschorr cyclisation products finally afforded (±)-roemerine identical with natural (-)-roemerine (apart from optical rotation.

The phenolic by-product of the cyclisation was presumably the phenol (80). One of the non-phenolic by-products which runs faster than roemerine on alumina plates or columns was identified as 6,7-methylenedioxy-1-benzyl-tetrahydro-2-methylisoquinoline (81).

Tracer Experiments.

The tracer experiments with plants have been carried out in collaboration with Mr. G. M. Chapman in these laboratories. In early experiments (±)-[2-^{14}C]tyrosine was fed to Papaver dubium and the derived roemerine was found to be radioactive. In subsequent experiments tritium labelled (±)-coclaurine, (±)-norcoclaurine and

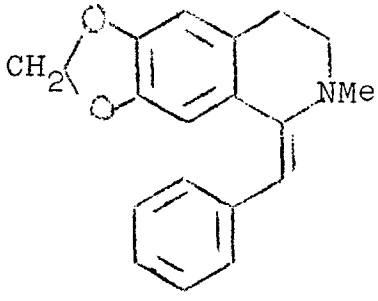
(±)-*N*-methylcoclaurine hydrochlorides were administered to the plants. In each case the derived roemerine was radioactive. When (±)-isococlaurine hydrochloride was fed to the plants the isolated roemerine was essentially inactive. The results of the feeding experiments are given below.

Precursor	Incorporation (%)
Tyrosine	0.17
(±)-Coclaurine	0.06
(±)-Norcoclaurine	0.34
(±)-*N*-Methylcoclaurine	0.48
(±)-Isococlaurine	0.00

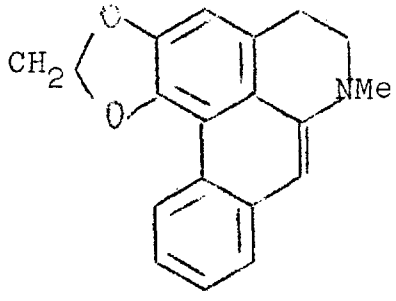
Incorporations are corrected for loss of one tritium. The higher incorporation of *N*-methylcoclaurine than norcoclaurine and coclaurine suggests that the precursor is on the direct biosynthetic path. The lower incorporation of coclaurine than norcoclaurine suggests that *N*-methylation precedes the *O*-methylation. The inability of the plants to utilise isococlaurine is obvious. The present preliminary investigation has proved the basic correctness of the biosynthetic proposal. Further work will be needed to establish the details of roemerine biosynthesis.

An approach to Roemerine Synthesis.

In recent[69,54] years stilbene derivatives have been transformed photochemically to phenanthrenes. An attractive route to the synthesis of roemerine was the photocyclisation of a system such as (82) to (83) as shown below.



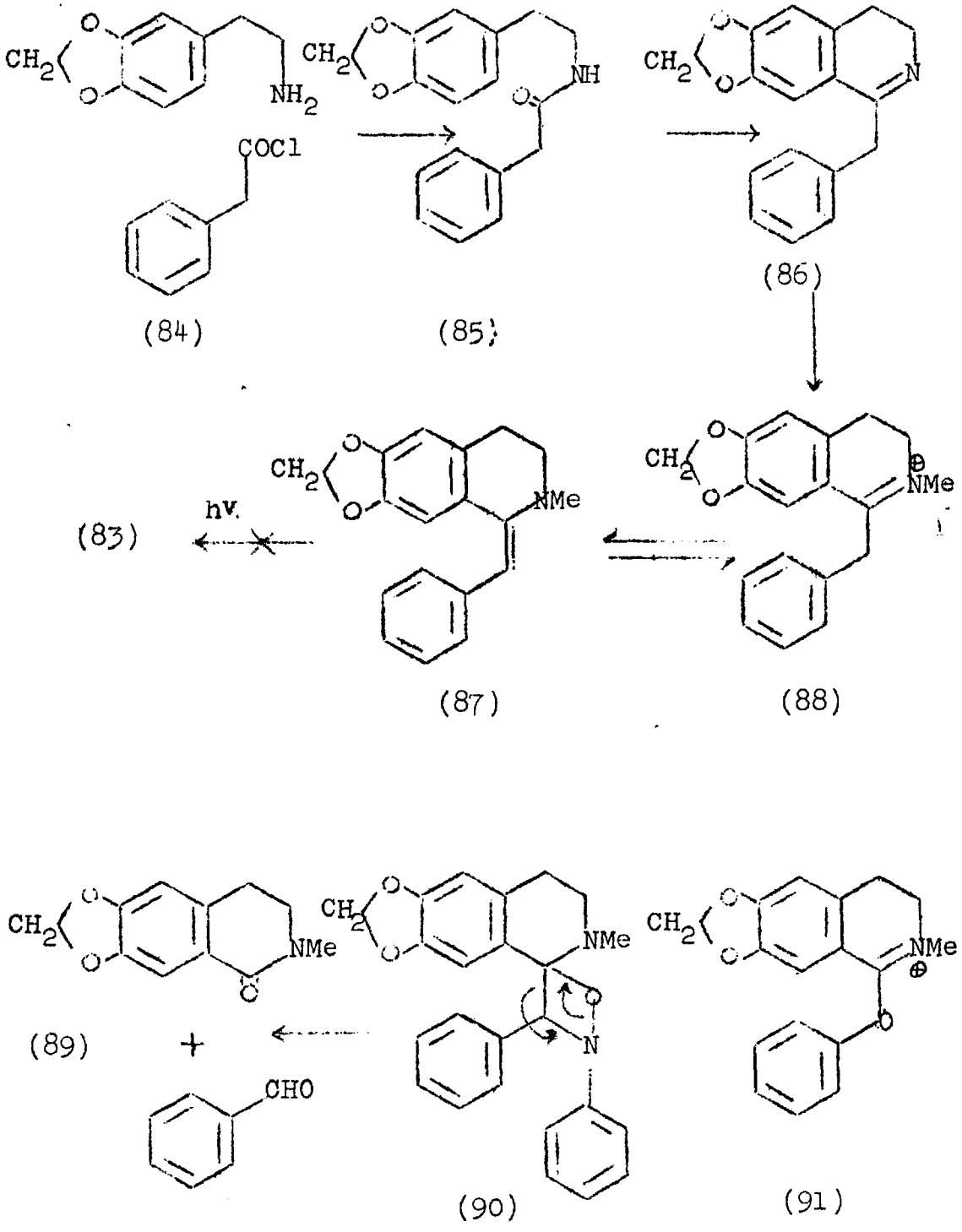
(82)



(83)

The first task was to prepare the compound (82). The dihydroisoquinoline (86) was prepared according to the method of Bills and Noller.[48] This was then treated with methyl iodide in the usual way to give the methiodide (88). The compound (87) needed for the photochemical investigation was obtained easily from the corresponding methiodide (88) by treatment with bases such as sodium hydroxide or potassium tert.-butoxide. Since the compound (87) was found to be very susceptible to aerial oxidation, it was prepared when needed and kept in a nitrogen atmosphere or generated in situ from the methiodide (88).

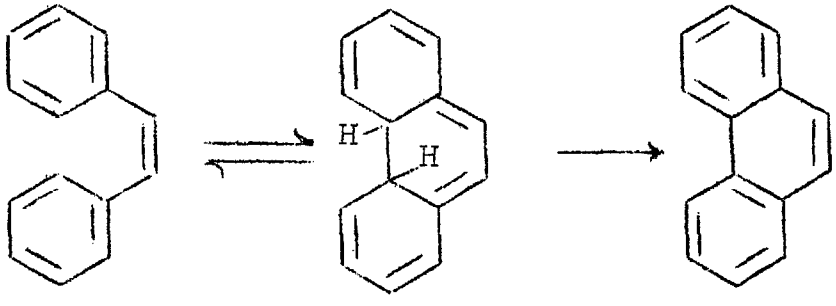
Approach to Roemerine synthesis.



Further, the compound was found to isomerise readily to the iminium form (88) even on dissolving in alcohol. However, in hydrocarbon solvents or in the presence of base the compound exists in the enamine form (87).

It is believed[70,71] that the photochemical transformation of the stilbene derivatives to phenanthrene systems, as shown below proceeds <u>via</u> a dihydro intermediate. Hydrogen abstraction by some suitable oxidant then furnishes the phenanthrene derivatives.

(0)



The photochemical cyclisation of the stilbene (87) was then investigated by using a number of hydrogen abstractors such as iodine, benzophenone, chloranil, selenium dioxide, diphenylpicrylhydrazyl, and nitrosobenzene under different conditions. The compound (87) was dissolved in cyclohexane and the reaction was followed by ultraviolet absorption. Extensive polymerisation occurred when the

compound was irradiated in the presence of iodine. With oxygen the lactam (89) was the main product of the reaction. Irradiation in the presence of benzophenone for 4 to 6 hours showed no change in the ultraviolet region. However, when the compound was exposed to the ultraviolet light for 20 hours and the reaction was worked up the basic fraction showed the characteristic u.v. absorption for a phenanthrene system. No definite compound could be isolated from the complicated mixture.

Irradiation in the presence of chloranil, and the diphenylpicrylhydrazyl radical also did not give any positive results. The reaction was then carried out in the presence of selenium dioxide. Since selenium dioxide is not soluble in cyclohexane, the compound and the reagent were dissolved in ethanol. Exposure to light for a shorter period (6 to 7 hrs.) indicated oxidation at the benzylic position as shown in (91) of the starting material. On exposure for 8 to 10 hours, the lactam (89) was the final product of the light catalysed oxidation. Since in alcohol the equilibrium was shifted to the iminium form (88), alkali was added to force the equilirbium to the enamine form (87). Irradiation under these conditions did not prove effective.

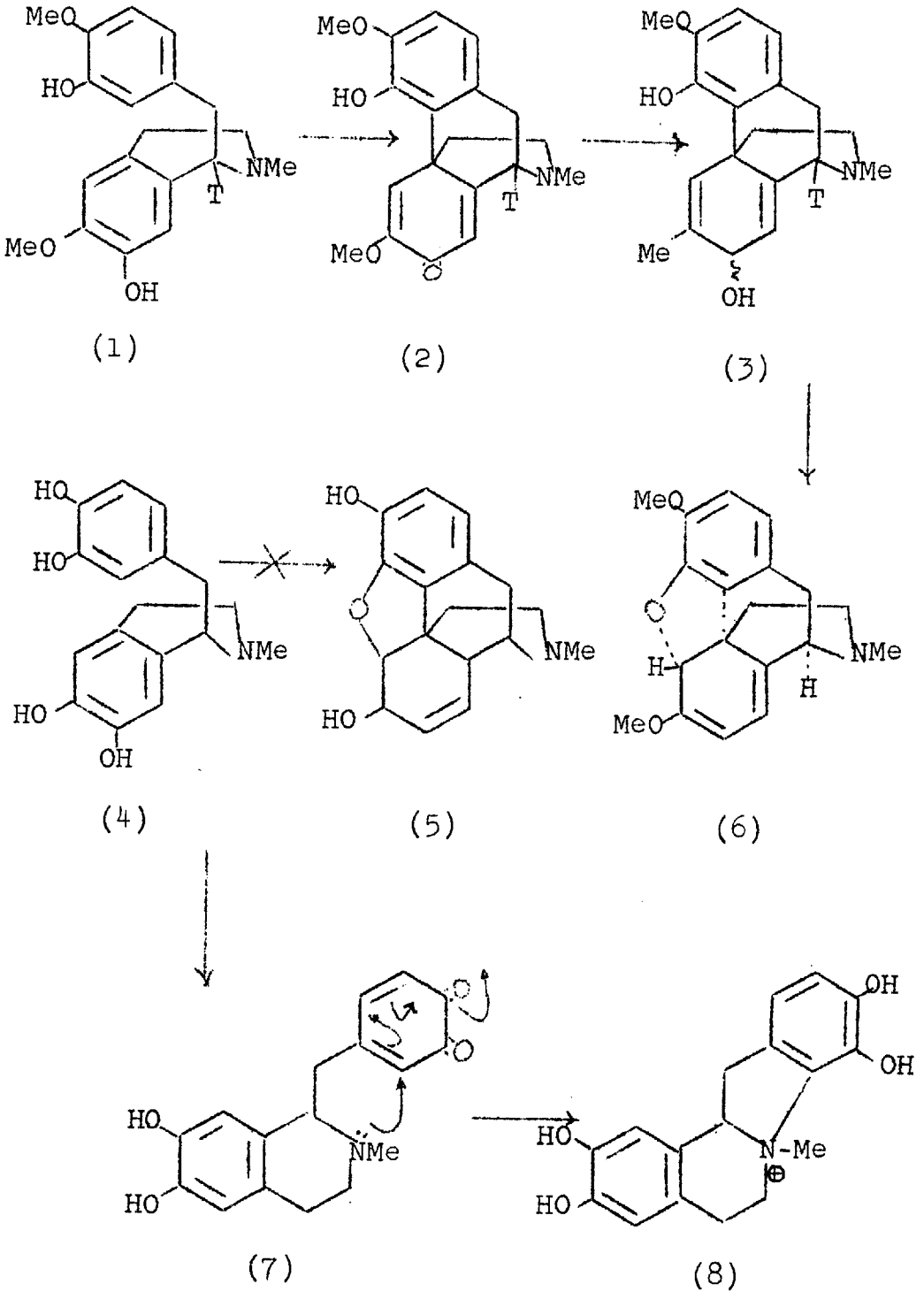
Interesting results were obtained when the compound was treated with nitrosobenzene. The characteristic u.v. absorption at 324 mμ disappeared immediately when the reagent was added to an alcoholic solution of the compound (87). The reaction mixture was worked up and the lactam (89) and benzaldehyde were isolated. The lactam in this reaction is presumably formed by the mechanism shown (90). The desired product could not be obtained in the photochemical cyclisation and this approach was therefore abandoned.

Introduction.

The purpose of the present investigation was to achieve a chemical synthesis of thebaine (6) from reticuline (1) and further to achieve a direct correlation of the absolute configurations of the benzylisoquinoline and morphine alkaloids. Compounds made in the course of this work could also be used for the biosynthetic studies of the alkaloids derived from reticuline.

The morphine alkaloids have presented a fascinating structural problem ever since morphine, the principal alkaloid of this group, was isolated by Sertürner[73] in 1805. The chemistry of this group has been discussed in many authorative reviews.[74-76] Gulland and Robinson[77] in 1925 suggested the correct formula (5) for morphine. In subsequent years this was confirmed by two independent excellent syntheses by Gates,[78] and Ginsberg[79] and their collaborators and by X-ray analysis of morphine hydriodide by Mackay and Hodgkin.[80] The absolute configuration of morphine alkaloids was elucidated chemically by Jeger and his coworkers.[81] The evidence for the relative and absolute configuration of morphine alkaloids as shown in (6) has been surveyed by Stork,[75] and Ginsburg.[76]

Since the recognition by Gulland and Robinson that the morphine skeleton could be formed in Nature from a norlaudanosoline system, there have been many attempts to imitate this process in the laboratory. In 1932 Robinson and Sugasawa[82] and independently Schöpf and Thierfelder[83] attempted to synthesise morphine derivatives from laudanosoline (4). Mild oxidation of the benzylisoquinoline by both groups, instead of giving the desired product afforded the dehydrolaudanosoline (8). This is formed presumably by the mechanism shown in (7). A number of other unsuccessful attempts to prepare morphine derivatives from benzylisoquinolines have also been reported.[84] In most of these cases either dehydrogenation products or aporphine derivatives were obtained. In 1963, Barton and his coworkers[56] completed the long sought synthesis of morphine alkaloids in the following way. (±)-Reticuline labelled with tritium in the position shown (1) was prepared and oxidised with manganese dioxide. The radioactive dienone (2) was produced in very small yield and was isolated after dilution of the reaction mixture with inactive dienone, prepared from thebaine. The dienone was reduced with sodium borohydride to give a mixture of the isomeric alcohols (3). Treatment with mild acid then afforded radioactive thebaine. Since the presence

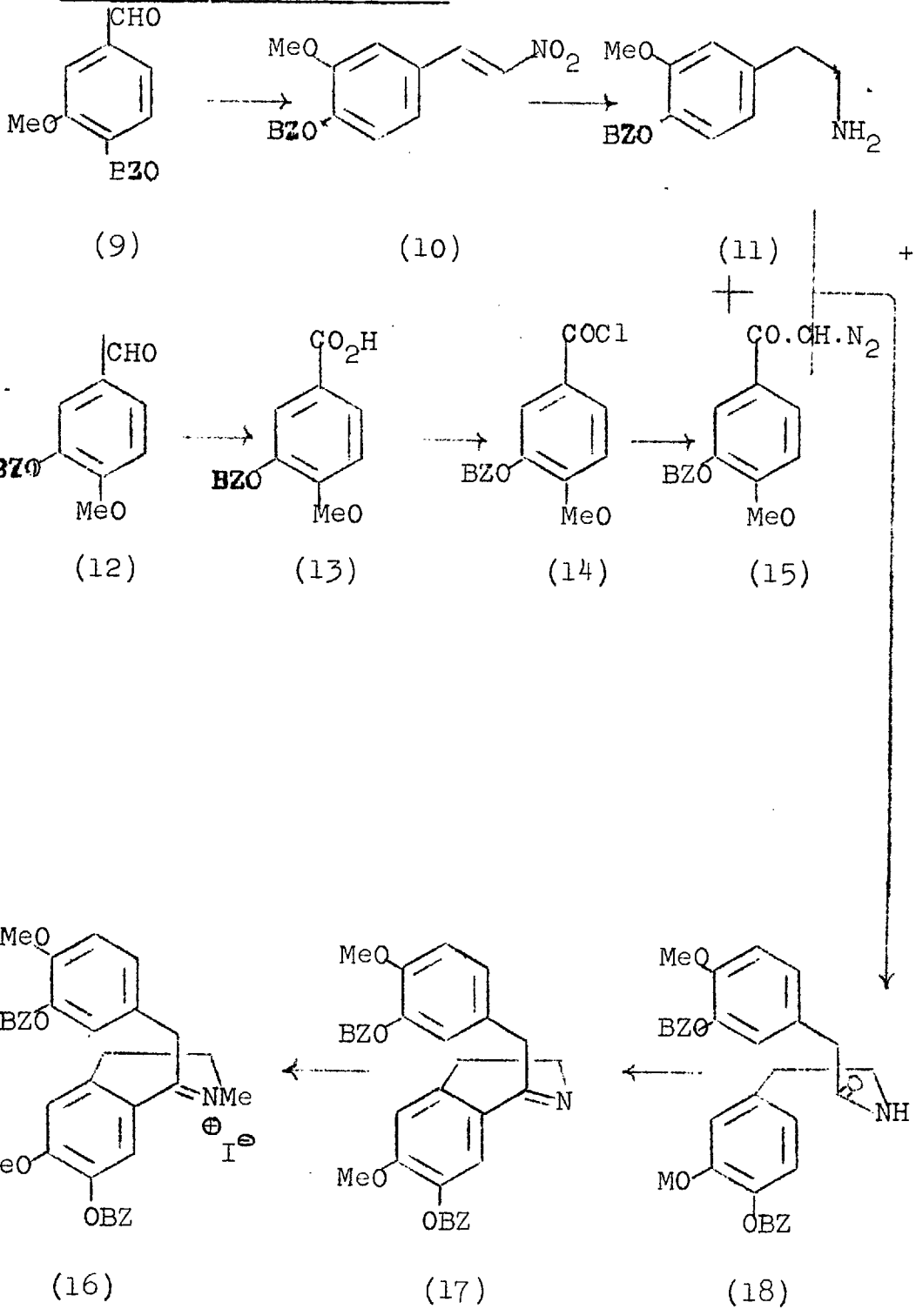

MeO
HO
NMe
T
OH
(1)
(2)
(3)
Me
(4)
(5)
(6)
(7)
(8)
N-Me

of trace radioactive impurities could disclaim the radiochemical synthesis of thebaine, it was important to confirm this finding. The first task in this problem was to procure large supplies of reticuline. (+)-Reticuline is a naturally occurring base and has been isolated from Anona reticulata[85] and more recently from Phylica rogersii.[86] (±)-Reticuline has been synthesised on many occasions.[49-51] A modified synthesis[10] of labelled (±)-reticuline has also recently appeared. Since a large supply of reticuline was required for the present work, some steps in the synthesis needed modification. The procedure adopted by us is given on page 121 and is described in the sequel.

Synthesis of (±)-Reticuline.

3-Benzyloxy-4-methoxyphenylacetic acid has generally been used in the earlier syntheses. The preparation of the acid via the corresponding oxazolone was reported[87] to suffer from the difficulty of separating the desired phenylacetic acid from benzoic acid formed during cleavage of the oxazolone. However, we decided to use the Wolff rearrangement. 3-Benzyloxy-4-methoxybenzaldehyde was oxidised with potassium permanganate in acetone and the resulting acid was converted into the acid chloride (14) in the usual fashion. The crystalline acid chloride, on

Synthesis of Reticuline.



treatment with diazomethane, afforded the diazoketone (15) in high yield.

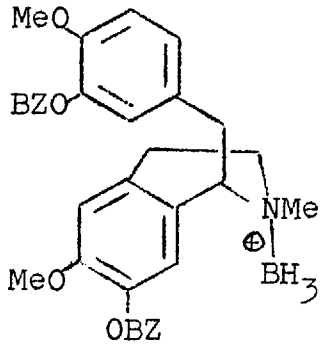
The large scale preparation of 3-methoxy-4-benzyloxyphenethylamine (11) was also equally important. Two routes were available, both from O-benzylvanillin. One proceeded via the aldehyde, alcohol and nitrile, and the other via the ω-nitrostyrene (10). Obviously, the latter was the method of choice. It was known[87] that condensation of O-benzylvanillin with nitromethane in the presence of sodium carbonate gives the desired product in variable yield. An improved yield of the nitrostyrene (10) was obtained when the aldehyde (9) was treated with nitromethane in the presence of sodium ethoxide in the usual way. We investigated the reaction and found that an excellent yield of the pure ω-nitrostyrene (10) was obtained when O-benzylvanillin was condensed with pure nitromethane in the presence of methylamine hydrochloride and anhydrous sodium acetate.

Reduction of the ω-nitrostyrene (10) with lithium aluminium hydride gave a variable yield of the phenethylamine (11). However, the best yield was obtained when the pure nitrostyrene, stabilized with a trace of acetic acid, in dry tetrahydrofuran was slowly added to a large

excess of lithium aluminium hydride. The required amide (18) was prepared by two variants of the Wolff rearrangement.[15] First a mixture of the amine (11) and the diazoketone (15) in benzene was treated with freshly prepared silver oxide in the usual way to get the amide (18). In the second procedure a solution of the diazoketone (15) and the amine (11) in dry benzene was irradiated with u.v. light and the amide (18) was obtained in good yield. More consistent results were obtained by this method and photolysis was performed successfully on a large (ca. 6 g. diazoketone) scale.

Cyclisation of the amide (18) in dry toluene with freshly distilled phosphorus oxychloride in the usual way gave the dihydroisoquinoline (17). In trial experiments, when the amide (18), in chloroform containing phosphorus pentachloride, was left at room temperature for 5 to 6 days it afforded the dihydro compound (17) in relatively poor yield.

The free base (17) corresponding to the dihydroisoquinoline hydrochloride is susceptible to aerial oxidation.[10] In some trial experiments a compound showing strong i.r. carbonyl absorption at 1669 $cm.^{-1}$ was isolated; presumably the compound was a ketone (22). However, the dihydroisoquinoline (17) can safely be converted into



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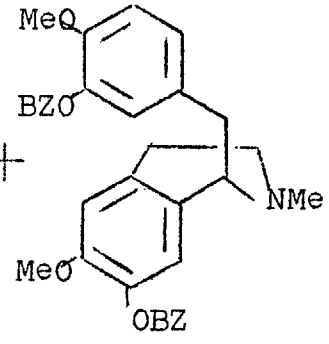

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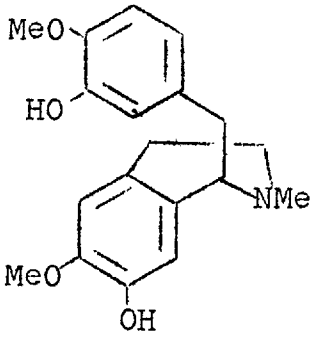

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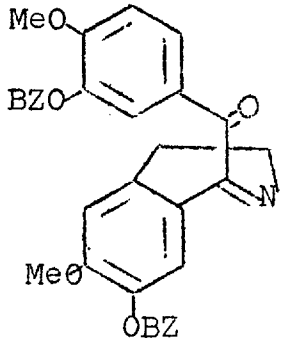

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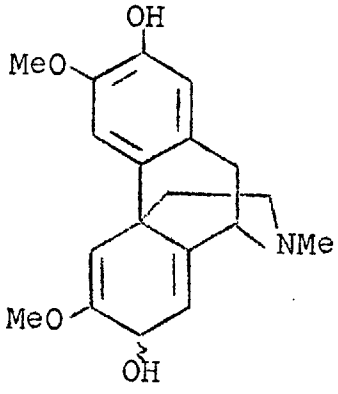

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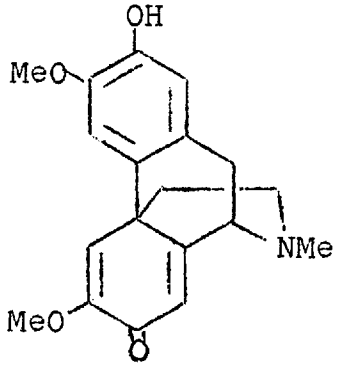

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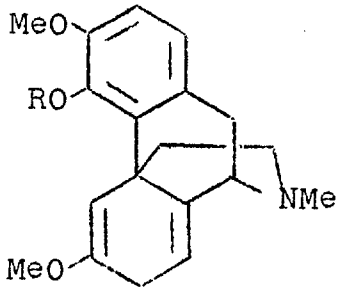

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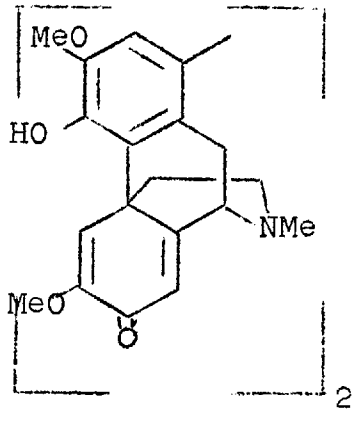


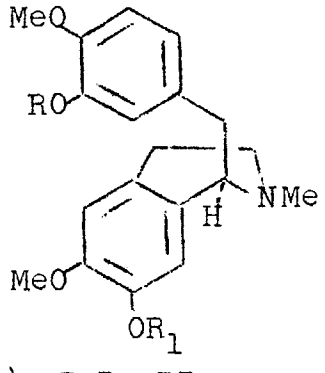
(26)

its methiodide (16) by decomposing the corresponding base hydrochloride under a nitrogen atmosphere with sodium hydrogen carbonate and then treating the free base with methyl iodide in dry benzene. Complications were encountered during the reduction of the methiodide (16) with sodium borohydride. Under the usual conditions the desired product (20) was obtained in a yield of about 50%. The major by-product (ca. 45%) was a non-basic chloroform soluble compound. The infrared spectrum of this product was similar to that of the OO-dibenzyl-reticuline except for weak absorptions at 2400 (B-H) and 710 (B-N) $cm.^{-1}$. Presumably the by-product is an amino-borane derivative (19). Surprisingly, unlike other borane derivatives, it is resistant to acid hydrolysis and attempts to obtain the amine (20) by oxidation of the compound (19) did not succeed. Since the formation of this by-product was undesirable the reaction was further investigated. It was found that high yields of OO-dibenzylreticuline were produced when the reaction was carried out in ice-cold methanol. The final step to complete the synthesis of (±)-reticuline was the removal of the protecting benzyl groups. Hydrogenation[11] with a large quantity 10% palladium on charcoal gave good yields of (±)-reticuline. Hydrogenolysis proceeded smoothly

provided pure dibenzyl ether was used.

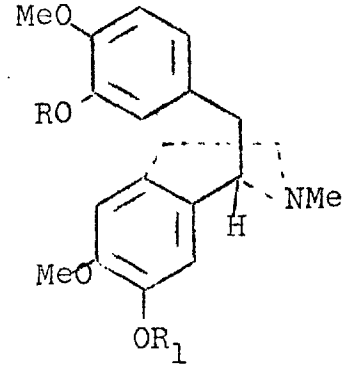
Resolution of Reticuline.

The next task was to resolve (±)-OO-dibenzylreticuline. Battersby and his coworkers[53] meanwhile had resolved the racemate using dibenzoyltartaric acid. Following their method (+)-dibenzylreticuline (28a) was obtained by treatment of the racemate with (-)-dibenzoyltartaric acid whilst the (-)-enantiomer was obtained by the use of (+)-dibenzoyltartaric acid. Hydrogenolysis of these enantiomers in the usual way afforded (+)- and (-)-reticuline. Battersby and his coworkers[53] have also shown that (+)-reticuline (28b) has the same configuration as that of (+)-laudanosine (28, R_1=R=Me).



(27a), $R=R_1$=BZ

(27b), $R=R_1$=H



(28a), $R=R_1$=BZ

(28b), $R=R_1$=H

Exchange Reactions.

With supplies of (±)-, (+)- and (-)-reticuline available, the next task was to introduce radioactive atoms into these compounds. We decided to do this by exchange reactions with tritium oxide. Exploratory exchange experiments were carried out with (±)-reticuline in deuterium oxide both under base and acid catalysed conditions. The reaction was follows by nuclear magnetic resonance measurements. (±)-Reticuline, when heated in deuterium oxide at 100° with potassium tert.-butoxide for 113 hours, gave deuterated (±)-reticuline. N.m.r. measurements of the compound showed specific exchange at positions ortho and para to the hydroxyl groups. In separate experiments, when (±)-reticuline was heated at 100° in deuterium oxide in the presence of deuterium chloride (generated in situ from thionyl chloride and deuterium oxide) for 40 hours, deuterated (±)-reticuline was also obtained. The integrated n.m.r. of this compound showed about 80% exchange of all aromatic protons. Prolonged heating (over 50 hrs.) resulted in complete disappearance of aromatic protons. Clearly, under these conditions, both ortho and meta hydrogens were exchanged.

Two procedures were now available for introducing isotopes of hydrogen into reticuline. Since we were

interested in introducing the maximum number of radioactive atoms into the molecule, we followed the second method. In this way tritium labelled (±)-, (+)- and (-)-reticuline were prepared.

Oxidation of Reticuline.

It was known[56] that, in the synthesis of thebaine, the yield of the desired dienone from the oxidation of reticuline was extremely poor. Obviously, before attempting the chemical synthesis, the critical coupling step had to be investigated. This we decided to do by radio-dilution techniques. For this purpose the dienone (30) was prepared from thebaine, by the method of Barton and his coworkers.[56] The dienone (30) was subsequently isolated from natural sources[88] and is now known as salutaridine. With the supplies of labelled (±)-reticuline for oxidation and non-radioactive (+)-salutaridine for dilution, the coupling step was then investigated.

Tritium labelled (±)-reticuline was oxidised in chloroform by manganese dioxide. Inactive (+)-salutaridine was added to the resulting mixture. Chromatography gave radioactive salutaridine highly contaminated with radioactive impurities. Complete removal of these was not possible by repeated chromatography on alumina. It

appeared that a closely related radioactive compound was following the radioactive salutaridine. Presumably the dienone (24) might have been formed during the oxidative coupling process. The easiest way to separate this compound, if it were present, was to convert the mixture into thebaine. Borohydride reduction of salutaridine (30) and the dienone (24) would give the alcohols (32) and (23) respectively. Treatment of only the alcohol (32) with acid would give thebaine. The phenolic alcohol (23) derived from the dienone (24) could then be easily separated from radioactive thebaine. Indeed, this was the case. The mixture of dienones was reduced with borohydride and then treated with 6N-hydrochloric acid. The products were separated into phenolic and non-phenolic fractions. The latter fraction when chromatographed on alumina afforded radioactive thebaine free from radioactive impurities. Since we had used (±)-reticuline in the oxidation, the activity of the derived thebaine was dropped by half on resolution by fractional crystallisation. The radiochemical yield of thebaine was 0.011%.

The oxidation of reticuline was next studied with potassium ferricyanide. Tritium labelled (±)-reticuline

in aqueous alkaline solution was treated with potassium ferricyanide and inactive (+)-salutaridine was added at the end of the reaction. Work up afforded radioactive salutaridine. The radioactive impurities in this case were easily removed by chromatography on alumina. Repeated crystallisation of the active sample removed the active (-)-salutaridine. The radioactive (+)-enantiomer was converted into thebaine having the same molar activity. The radiochemical yield of thebaine in this experiment was .008%.

The next oxidising reagent tried was ferric chloride. In trial experiments it was found that the reagent reacts extremely slowly with reticuline under acidic conditions. The phenolic base was recovered unchanged even after 24 hours when treated with the reagent in 6N-hydrochloric acid. However, oxidation with aqueous ferric chloride at pH 4 appeared effective. The process was repeated with tritium labelled (±)-reticuline. Determination of the yield in the usual way gave a value of 0.0007% close to the limits of sensitivity of the method. The oxidative coupling reaction was also studied by using Fremy's salt as oxidising agent, however, no better results were obtained.

The repeated low yield of salutaridine in the coupling step cast doubts on the stability of salutaridine

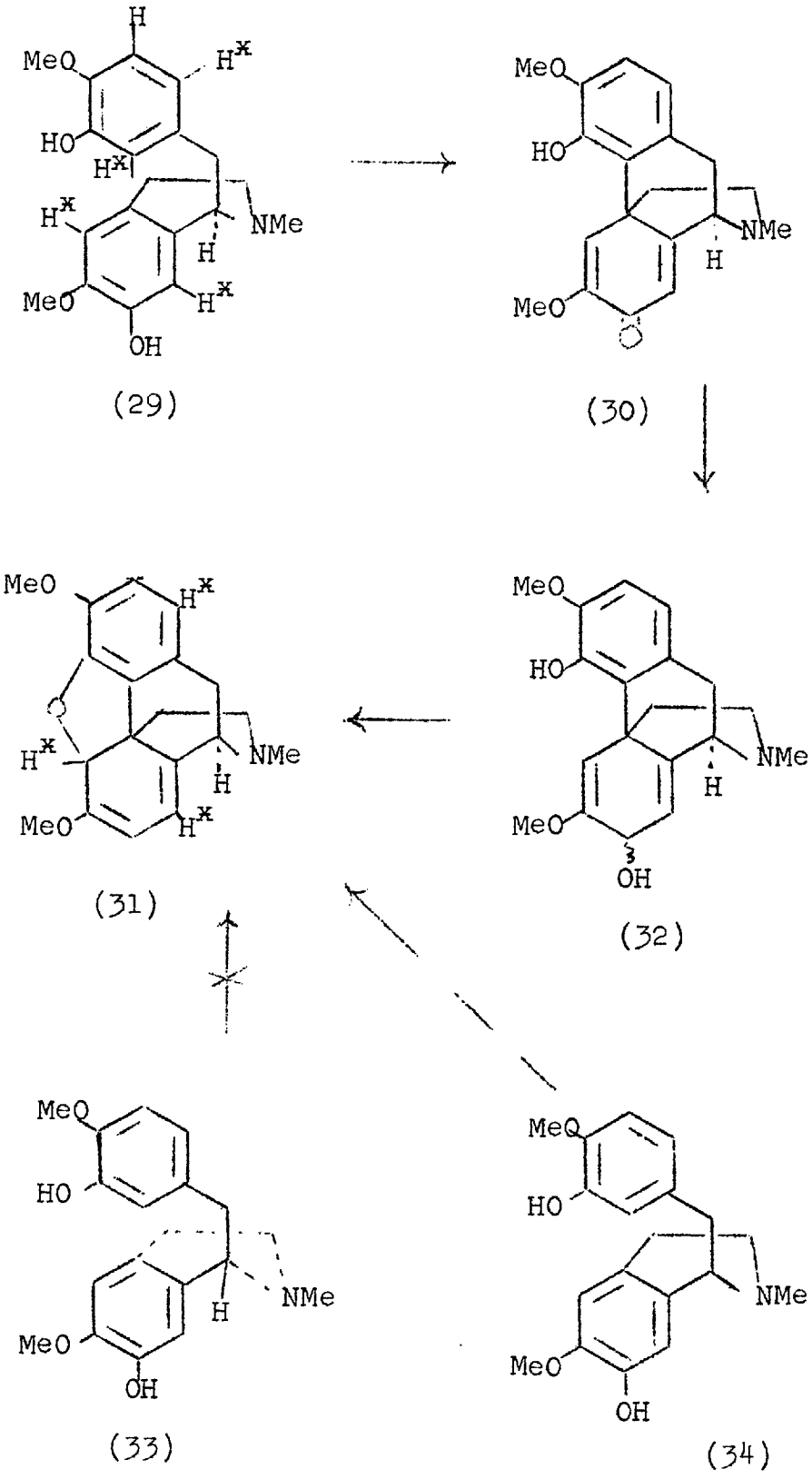
under oxidising conditions. Salutaridine has a phenolic hydroxyl group and is expected to be attacked by phenol oxidising reagents. Indeed, this was the case. Salutaridine was found to be attacked by all the oxidising reagents used for reticuline. In some experiments a crystalline product was isolated from the products of oxidation. The infrared spectrum of this compound was similar to that of salutaridine except that an absorption peak at 1482 cm.$^{-1}$ (present in salutaridine) was absent. The product is presumably a dimer (26) of salutaridine. It was then decided to investigate the relative stability of reticuline and salutaridine. In preliminary experiments, equal amounts of reticuline and salutaridine were separately treated with the equivalent amount of the oxidising reagents. It was found that salutaridine was actually oxidised faster than reticuline. In another experiment, when equal amounts of reticuline and salutaridine were mixed and treated with an equivalent amount (for reticuline) of the oxidising reagents (see Experimental) it was found that salutaridine was oxidised completely whilst reticuline was still left in the reaction mixture. This finding provided one of the basic reasons for the poor yields of salutaridine in the critical coupling step.

Reticuline is a dihydric phenol and is more soluble in aqueous medium than salutaridine. It was thought that oxidation in a heterogeneous medium might protect salutaridine. This was then investigated, in trial experiments, when reticuline and salutaridine were separately treated with aqueous ferricyanide in a mixture of chloroform and water, it was noticed that reticuline was oxidised faster than salutaridine. This appeared encouraging and the process was repeated with radioactive (±)-reticuline. Oxidation with potassium ferricyanide, under these conditions, did improve the yield of the desired product, but again the product was highly contaminated with radiochemical impurities which could not be removed by repeated chromatography. However, after several crystallisations a product with constant activity was obtained. This was then converted into radioactive thebaine having the same molar activity. The radiochemical yield of thebaine in the experiment was 0.015%. Since we had used (±)-reticuline in the oxidation the activity of the derived thebaine might have dropped by half during recrystallisation. However, even after several crystallisations the activity of the sample remained constant. Presumably, the active (+)-salutaridine was

separated from the (-)-enantiomer in the course of earlier purification.

The foregoing experiments thus showed that the yield of salutaridine in the critical coupling step cannot be improved. A preparative synthesis of thebaine from reticuline was therefore impracticable. An alternative procedure was then adopted to confirm our findings. Each of the tritium labelled optical isomers of reticuline was oxidised with potassium ferricyanide (2 mols) in aqueous solution containing sodium hydrogen carbonate. Dilution with inactive (+)-salutaridine and crystallisation to constant activity gave a radiochemical yield 0.0044% for the oxidation of (-)-reticuline (29), and a negligible yield from (+)-reticuline (33). These yields are calculated allowing for a one-fifth loss of tritium in the conversion of (-)-reticuline into (+)-salutaridine (30). The results were confirmed by conversion into thebaine in the usual way. Oxidation of (±)-reticuline (34) under exactly the same conditions afforded (+)-salutaridine in 0.0021% yield. These results thus confirm the "radiochemical synthesis" of thebaine and further provide a direct confirmation of the relative configurations of the benzylisoquinoline and morphine alkaloids. A further

Oxidation of Reticuline.



direct stereochemical correlation of (-)-reticuline with (+)-salutaridine has also been achieved at Imperial College by Mr. R. James.[91] Reduction of (+)-O-methylsalutaridine with sodium and liquid ammonia afforded a mixture of non-ketonic products which on methylation with diazomethane and careful chromatography furnished (-)-laudanosoline (27, $R=R_1=Me$). The identity of the isolated (-)-laudanosine was rigorously established by repetition of the experiment using tritium labelled (+)-O-methylsalutaridine.

Biosynthesis of Alkaloids with enantiomers of Reticuline.

The tritium labelled (+)- and (-)-reticuline, prepared for our investigation have also been used by Dr. R. H. Hesse in his work on berberine and protopine. He has shown[12] that only (+)-reticuline is an efficient precursor for berberine in Hydrastis canadensis and for protopine in both Dicentra spectabilis and Argemone hispida and mexicana. Very recently Battersby and his colleagues have also reported the incorporation of the enantiomers of reticuline into several alkaloids. (+)-Reticuline was found to be a more efficient precursor for narcotine in Papaver somniferum,[89] and for chelidonine, protopine, and (-)-stylopine in chelidonium majus,[90] than the (-)-enantiomer.

EXPERIMENTAL

Melting points were determined on the Kofler block and are uncorrected. Unless otherwise stated, 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. Organic extracts containing basic substances were dried over anhyd. potassium carbonate. Those containing neutral or acidic substances were dried over sodium sulphate. Petroleum ether refers to boiling range 40-60°. Micro-analyses were carried out at Imperial College under the direction of Miss J. Cuckney.

Counting Methods.

Compounds labelled with ^{14}C and tritium were counted in a scintillation counter (Isotope Developments Ltd., Type 6012A), samples being dissolved in dimethylformamide (0.2 ml.) and liquid scintillator (Nuclear Enterprises Ltd., Type N.E. 213) (1.2 ml.) and are uncorrected for self absorption. The counting procedure was calibrated using $[1,2\text{-}^{3}H]$- and $[2\text{-}^{14}C]$-hexadecane standards.

NO-Diacetyltetrahydrocrotonosine.

Crotonosine (100 mg.) in ethyl acetate (6 ml.) was hydrogenated in the presence of platinum oxide (30 mg.) according to the method of Haynes and Stuart.[1] The tetrahydro compound, $\nu_{max.}$ 1708 (C:O) and 3530 (NH) cm.$^{-1}$ was acetylated at room temperature with acetic anhydride (3 ml.) and pyridine (3 ml.). The crude product was chromatographed on alumina (grade V, 30 g.), elution with benzene:chloroform (1:1) gave the diacetate as needles (75 mg.) from ethyl acetate, m.p. 107-108° (lit.[1] 107-108°); $\nu_{max.}$ 1638 (secondary amide), 1720 (C:O), and 1760 and 1230 (phenolic OAc) cm.$^{-1}$; n.m.r. bands in $CDCl_3$ at 6.17 (3H singlet), 3.20 (1H singlet), 7.78 (3H singlet) and 7.63 (3H singlet).

NO-Diacetyldeuterotetrahydrocrotonosine.

Diacetyltetrahydrocrotonosine (70 mg.) in deuterium oxide (0.5 ml.) was heated at 100° for 7 hrs. with potassium tert.-butoxide (140 mg.). The resulting mixture was diluted with water (2 ml.), solid carbon dioxide added, extracted with chloroform (3 x 4 ml.) and the solvent evaporated. The residue was reacetylated as above. The acetylated deuterated product (60 mg.), m.p.

106-108°; $\nu_{max.}$ 1760, 1715 and 1630 cm.$^{-1}$; n.m.r. bands in $CDCl_3$ at τ 6.18 (3H singlet), 7.78 (3H singlet) and 7.64 (3H singlet).

Apocrotonosine (7a).

Crotonosine (100 mg.) was heated on a steam bath with 3N-hydrochloric acid (10 ml.). After 4 hrs. the hydrochloric acid was removed under reduced pressure. The residue was dissolved in water, charcoaled, and crystallised from water by adding a drop of conc. hydrochloric acid as needles (70 mg.), m.p. 218-220°; $[\alpha]_D$ -148° (C, 0.42 in H_2O). (Found: C, 62.47; H, 5.95. $C_{17}H_{18}O_3NCl.\frac{1}{2}H_2O$ requires C, 62.20 and H, 5.79%). N.m.r. bands at τ 2.98 (1H doublet), 3.42 (1H quartet), 2.36 (1H doublet), 6.34 (3H singlet) and 3.52 (1H singlet).

Deuteroapocrotonosine.

Apocrotonosine hydrochloride (40 mg.) in deuterium oxide (0.4 ml.) was treated at 100° with potassium tert.-butoxide for 3 hrs. The resulting mixture was acidified with conc. hydrochloric acid; the precipitated base hydrochloride was centrifuged off, dissolved in water, charcoaled, and crystallised, by adding a drop of conc. hydrochloric acid, as needles (32 mg.), m.p. 217-219°; n.m.r. bands at τ 2.98 (1H singlet) and 6.34 (3H singlet).

Tetrahydroglaziovine.

Glaziovine (54 mg.) in acetic acid (6 ml.) was hydrogenated using platinum oxide (30 mg.) as a catalyst according to the method of Gilbert and his coworkers.[21] The crude product was chromatographed on alumina (grade III, 20 g.), elution with chloroform gave tetrahydroglaziovine as plates (45 mg.) from benzene, m.p. 114-116° (lit.[21], 112-116°); $\nu_{max.}$ 1702 and 3520 cm.$^{-1}$; n.m.r. bands in $CDCl_3$ at τ 3.67 (1H singlet), 6.29 (3H singlet) and 7.62 (3H singlet).

Deuterotetrahydroglaziovine.

A mixture of tetrahydroglaziovine (50 mg.), deuterium oxide (0.4 ml.) and potassium tert.-butoxide (130 mg.) was heated at 100° for 7 hrs. The resulting mixture was diluted with water (1.5 ml.) and treated with solid carbon dioxide. The liberated base was extracted with chloroform (3 x 4 ml.), washed with water, dried and evaporated. The residue was chromatographed on alumina (grade III, 15 g.), elution with benzene:chloroform (1:1) gave the deuterated tetrahydroglaziovine (35 mg.) as plates from benzene, m.p. 113-115°; $\nu_{max.}$ 3530 and 1703 cm.$^{-1}$; n.m.r. bands at τ 3.66 (1H singlet), 6.29 (3H singlet) and 7.62 (3H singlet).

Apoglaziovine (7b).

Glaziovine (58 mg.) in 3N-hydrochloric acid (2.5 ml.) was left at room temperature (24°). After 2 hrs. the acid was removed in vacuo and the product was dissolved in methanol and charcoaled. Crystallisation from methanol-ether afforded apoglaziovine hydrochloride (45 mg.), m.p. 320-324° (decomp.) (lit.,[21] > 300°); n.m.r. bands at τ 3.22 (1H singlet), 5.9 (3H singlet), 1.92 (1H doublet), 2.79 (1H doublet), 3.38 (1H quartet) and 7.2 (3H singlet).

Deuteroapoglaziovine.

Apoglaziovine hydrochloride (45 mg.), deuterium oxide (0.5 ml.) and potassium tert.-butoxide (90 mg.) were heated at 100° for 8 hrs. The resulting mixture was diluted with water (1.5 ml.) and treated with solid carbon dioxide. The liberated base was extracted with chloroform (3 x 4 ml.), washed with water, dried and evaporated. The residue was treated with methanolic hydrogen chloride and the deuterated tetrahydroapoglaziovine crystallised from methanol-ether as needles (39 mg.), m.p. > 300°; n.m.r. bands at τ 3.24 (1H singlet), 7.2 (3H singlet), 5.9 (3H singlet) and 2.79 (1H singlet).

Synthesis of (±)-Coclaurine.[27]

p-Benzyloxybenzaldehyde. (Method of Dr. J.B. Taylor)[22]

p-Hydroxybenzaldehyde (12 g.) in ethanol (50 ml.) was benzylated in the usual manner with benzyl chloride (13 g.) and potassium hydroxide (6 g.). The crude product crystallized from aqueous alcohol to give the benzyl ether (15.2 g., 72.8%), m.p. 71° (lit.,[23] 72°).

p-Benzyloxybenzoic acid.

To a solution of p-benzyloxybenzaldehyde (10 g.) in acetone (100 ml.) and water (50 ml.) was added potassium permanganate (8 g.) during 2 hrs. with stirring. The precipitated manganese dioxide was filtered off and the solvent from the filtrate removed. The alkaline aqueous solution was washed with ether and acidified with conc. hydrochloric acid. The precipitated crude acid was filtered off. Crystallisation from ethanol afforded p-benzyloxybenzoic acid as needles (8 g., 73.4%), m.p. 193-194° (lit.,[24] 189°).

p-Benzyloxybenzoyl chloride.

p-Benzyloxybenzoic acid (3.6 g.) in benzene (30 ml.) was refluxed with thionyl chloride (5 ml.). After 3 hrs.

the solvent and excess of thionyl chloride were removed under reduced pressure in the usual manner. The residue on being treated with dry ether-petroleum ether gave the acid chloride (3.8 g.), m.p. 90-92°; $\nu_{max.}$ 1772 and 1737 cm.$^{-1}$. A much purer product, m.p. 102-104° was obtained by using oxalyl chloride.

p-Benzyloxy-ω-diazo-acetophenone (30).

p-Benzyloxybenzoylchloride (1.2 g.) was treated with an ethereal solution of diazomethane (from 8 g. of nitrosomethylurea) according to the method of Jeffrey's[25] to give the diazoketone (1 g., 81.6%), crystallized from benzene-petroleum ether as pale yellow plates, m.p. 116-117° (lit.,[25] 117°), $\nu_{max.}$ 2160 cm.$^{-1}$.

p--Benzyloxyphenylacetic acid. (Method of Barton and Kirby)[26]

p-Hydroxyphenylacetic acid (5 g.) in ethanol (45 ml.) was benzylated with benzyl chloride (5 g.) and potassium hydroxide (5 g.) in the usual way. The p-benzyloxyphenylacetic acid crystallised from aqueous ethanol as plates (5.2 g., 61%) m.p. 120-121° (lit.,[26] 120-122°).

N-(3-methoxy-4-benzyloxyphenethyl)-p-benzyloxyphenyl-acetamide (31b).

(A) A mixture of p-benzyloxy-ω-diazo-acetophenone (30) (4 g.) and 3-methoxy-4-benzyloxyphenethylamine (25b) (4.2 g.) in dry benzene (250 ml.) in an atmosphere of nitrogen was irradiated with U.V. light (high pressure mercury lamp) in a pyrex flask and the reaction was followed by infrared absorption. After 12 hrs., when the absorption at 2160 $cm.^{-1}$ had disappeared, the resulting solution was washed with 3N-hydrochloric acid, water, dried and the solvent was evaporated. The crude product was chromatographed on alumina (grade III, 250 g.), elution with benzene:chloroform (1:4) gave the amide which crystallised from ethyl acetate as needles (4.8 g., 62.8% based on amine) m.p. 117-118° (lit.,[27] 118°).

(B) To a mixture of 3-methoxy-4-benzyloxyphenethylamine (6.4 g.), benzene (50 ml.), and 4N-sodium hydroxide (15 ml.) was added with stirring a solution of p-benzyloxyphenyl-acetyl chloride (6.4 g.) in dry benzene (50 ml.) during $\frac{1}{2}$ hr. After 1 hr. the reaction mixture was worked up in the usual manner. Crystallisation of the product from ethyl acetate gave the amide (8.0 g., 82.8% based on amine) m.p. 118°.

6-Methoxy-7-benzyloxy-1-(p-benzyloxybenzyl)-3,4-dihydroisoquinoline (32b).

To a refluxing solution of the amide (31b) (6 g.) in dry toluene (20 ml.) was added freshly distilled phosphorus oxychloride (6 ml.) with stirring. After 2 hrs. the solvent and excess of phosphorus oxychloride were removed under reduced pressure. The residue was triturated with dry ether, dissolved in ethanol and charcoaled. Crystallisation of the product from ethanol-ether gave the dihydroisoquinoline hydrochloride as needles (5.2 g., 83.3%) m.p. 166-167° (lit.,[27] 164-165°).

6-Methoxy-7-benzyloxy-1-(p-benzyloxybenzyl)-1,2,3,4-tetrahydroisoquinoline (33b).

The dihydroisoquinoline (32b) hydrochloride (3.6 g.) was treated with 4N-sodium hydrogen carbonate under nitrogen. The free base was extracted with chloroform (30 x 5 ml.), washed with water and the solvent evaporated. The residue was dissolved in methanol (25 ml.), cooled to 0° and sodium borohydride (1 g.) added with stirring during 45 min. After leaving at room temperature for 2 hrs., the solvent was removed, 4N-sodium hydroxide (20 ml.) was added and the base extracted with ether (25 x 5 ml.). The ether extract was washed with water,

dried, evaporated and an oily residue (2.9 g.) was obtained. This was chromatographed on alumina (grade III, 90 g.). Elution with benzene gave the product which crsytallised from ethanol as plates (2.7 g., 80.0%) m.p. 87°. (Found: C, 79.64; H, 6.68; N, 3.05. $C_{31}H_{31}O_3N$ requires C, 80.00; H, 6.68 and N, 3.01%.)

6-Methoxy-7-hydroxy-1-(p-hydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline. (34b).

OO-Dibenzylcoclaurine (33b) (1 g.) in ethanol (100 ml.) and conc. hydrochloric acid (1 ml.) was hydrogenolysed in the presence of 10% palladised charcoal (400 mg.). After 1 hr. the catalyst was filtered off, the solvent from the filtrate removed under reduced pressure and (±)-coclaurine hydrochloride crystallised frommethanol-ether as plates (658 mg., 93.8%) m.p. 256-258° after drying over P_2O_5 in vacuo at 100° for 20 hrs. (lit.,[27] 255-256°).

Synthesis of (±)-isococlaurine[28]/3-Benzyloxy-4-methoxy-ω-nitrostyrene (23c).

A mixture of O-benzylisovanillin (10 g.), nitromethane (12 ml.) anhyd. sodium acetate (700 mg.) and methylamine hydrochloride (700 mg.) was shaken at room temperature (23°). After 12 hrs. the yellow crystalline product was

filtered off and washed with ether and water. Crystallisation of the product from a mixture of ethanol-glacial acetic acid (10:1) gave the nitrostyrene as yellow needles (10.5 g., 89.0%) m.p. 129° (lit.,[29] 127-128°).

3-Benzyloxy-4-methoxyphenethylamine (25c).

To a well stirred refluxing suspension of lithium aluminium hydride (9 g.) in dry tetrahydrofuran (150 ml.) was added a solution of the nitrostyrene (23c) (13 g.) in dry tetrahydrofuran (250 ml.) during 2 hrs. The resulting mixture was stirred for another 2 hrs. at room temperature, the excess of lithium aluminium hydride was decomposed with ethyl acetate and 10N-sodium hydroxide (50 ml.) added. The separated solvent layer was decanted off, and the pasty residue was extracted with tetrahydrofuran (100 x 3 ml.) and ether (100 x 3ml.). The solvent from the combined extracts was removed, the residue was treated with 6N-hydrochloric acid (100 ml.) and washed with ether (50 x 3 ml.). The aqueous acidic solution was basified with 6N-sodium hydroxide, the liberated base was extracted with chloroform (50 x 3 ml.) washed with water, dried and evaporated. The residue was treated with ethanolic hydrogen chloride to give the amine hydrochloride which was crystallised from ethanol-ether as needles (9.1 g.,

68%) m.p. 168° (lit.,[29] 166°).

N-(3-benzyloxy-4-methoxyphenethyl)-p-benzyloxyphenyl-acetamide (31c).

The amide was prepared by irradiation of a mixture of p-benzyloxy-ω-diazo-acetophenone (30) (2.9 g.), the amine (25c) (liberated from its hydrochloride 2.98 g.), and dry benzene (300 ml.) under exactly the same conditions as earlier. The crude product was chromatographed on alumina (grade III, 80 g.), elution with chloroform:benzene (9:1) gave the amide, crystallised from methanol as needles (3.5 g., 70%) based on amine hydrochloride) m.p. 121° (lit.,[28] 119.5°).

6-Benzyloxy-7-methoxy-1-(p-benzyloxy benzyl)-3,4-dihydroisoquinoline (32c).

To a refluxing solution of the amide (31c) (2.2g.) in dry toluene (30 ml.) was added freshly distilled phosphorus oxychloride (3 ml.). After 2 hrs. the resulting mixture was worked up in the usual way. Crystallisation of the crude product from ethanol-ether gave the dihydroisoquinoline hydrochloride as needles (1.6 g., 69.9%) m.p. 202-203° (lit.,[28] 203°).

6-Benzyloxy-7-methoxy-1-(p-benzyloxybenzyl)-1,2,3,4-tetrahydroisoquinoline (33c).

The dihydroisoquinoline (32c) hydrochloride (1.2 g.) in methanol at 0° was treated with 2N-sodium hydroxide (4 ml.) in an atmosphere of nitrogen and reduced with sodium borohydride (400 mg.). The resulting mixture was worked up in the usual way. The product was chromatographed on alumina (grade III, 30 g.), elution with benzene:chloroform (9:1) gave OO-dibenzylisococlaurine, crystallised from methanol as plates (800 mg., 71.6%) m.p.55°. The compound was reported[28] as an oil.

6-Hydroxy-7-methoxy-1-(p-hydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline (34c).

OO-Dibenzylisococlaurine (230 ml.) in ethanol (50 ml.) and conc. hydrochloric acid (0.3 ml.) was hydrogenolysed with 10% palladium on charcoal and worked up as earlier. The product was crystallised from ethanol-ether to give (±)-isococlaurine hydrochloride as needles (140 mg., 88.0%) m.p. 214-215° after drying in vacuo at 100° for 18 hrs. (lit.,[28] 215°).

Synthesis of (±)-Norcoclaurine

Isovanillin (10 g.) in methylene chloride (100 ml.)

was demethylated with anhyd. aluminium chloride (9.7 g.) and dry pyridine (25 ml.) according to the method of Lange[30] to give protocatechuic aldehyde (7.6 g., 83.6%) m.p. 153-154°.

3,4-Dibenzyloxybenzaldehyde. (Method of Dr. J.B. Taylor)[22]

Protocatechuic aldehyde (8 g.), benzyl chloride (20 ml.) and anhyd. potassium carbonate (16 g.) in acetone (80 ml.) were refluxed for 30 hrs. The resulting mixture was worked up in the usual manner. Crystallisation of the product from methanol gave the dibenzyl ether as plates (16 g., 86.4%) m.p. 90-91° (lit.,[31] 91°).

3,4-Dibenzyloxy-ω-nitrostyrene (22a).

3,4-Dibenzyloxybenzaldehyde in ethanol was treated with nitromethane in the presence of potassium hydroxide, sodium ethoxide, and methylamine hydrochloride and ammonium acetate. In most of the experiments a yellowish brown polymerised product was obtained. In some experiments a light yellow compound which on treatment with 6N-sulphuric acid gave the nitrostyrene, was also obtained. Temperature variation did not improve the yield of the desired nitrostyrene.

3,4-Dibenzyloxybenzaldehyde (5 g.) dissolved in

distilled nitromethane (15 ml.) was treated with methylamine hydrochloride (600 mg.) and anhyd. sodium acetate (600 mg.) and shaken at room temperature (24°). After 5 hrs. the deposited yellow crystalline product was filtered off, washed with water to give the sufficiently pure nitrostyrene (2.4 g.). The excess of nitromethane from the filtrate was removed in vacuo at low temperature, the residue washed with ether and water and crystallised from glacial acetic acid as yellow needles (3.3 g.). The combined crystalline product was recrystallised from ethanol-glacial acetic acid (10:1) to give the nitrostyrene as yellow rods (5.2 g., 91.6%) m.p. 120° (lit.,[32] 118-119°).

3,4-Dibenzyloxyphenethylamine (25a).

The nitrostyrene (23a) (6.8 g.) in dry tetrahydrofuran (200 ml.) was reduced with lithium aluminium hydride (5 g.) and worked up in the usual way. The crude base was converted into its hydrochloride and crystallised from ethanol-ether to give the amine hydrochloride (4.5 g., 65%) m.p. 134° (lit.,[33] 133°).

N-(3,4-Dibenzyloxyphenethyl)-p-benzyloxyphenylacetamide (31a).

p-Benzyloxy-ω-diazo-acetophenone (30) (1 g.) and the

phenethylamine (25a) (1.7 g.) in dry benzene (150 ml.) were irradiated with U.V. light and worked up in the usual way. The crude product was chromatographed on alumina (grade III, 40 g.). Elution with benzene:chloroform (1:1) gave the amide, crystallised from methanol as needles (1.1 g., 83.2% based on diazoketone), m.p.125°; $\nu_{max.}$ 1660 cm.$^{-1}$. (Found: C, 79.89; H, 6.26; N, 2.70. $C_{37}H_{35}NO_4$ requires C, 79.71; H, 6.28; and N, 2.51%).

6,7-Dibenzyloxy-1-(p-benzyloxybenzyl)-1,2-dihydroisoquinoline (32a).

To a refluxing solution of the amide (31a) (1 g.) in dry toluene (20 ml.) was added freshly distilled phosphorus oxychloride (3 ml.). After 1 hr. the resulting mixture was worked up as earlier. The dihydroisoquinoline hydrochloride (800 mg., 75.7%) m.p. 167-168°, showed a strong tendency to form a solvate and, therefore, suitable analytical data could not be obtained. It was, however, characterised as its tetrahydro derivative.

6,7-Dibenzyloxy-1-(p-benzyloxybenzyl)-1,2,3,4-tetrahydroisoquinoline (34a).

The dihydroisoquinoline (32a) hydrochloride (800 mg.) was treated with 6N-sodium hydrogen carbonate under nitrogen. The liberated base was extracted with chloroform

(25 x 5 ml.) and the solvent removed. The residue was dissolved in methanol (20 ml.), cooled to 0°, sodium borohydride (500 mg.) was added during 1 hr. and left overnight. The resulting mixture was worked up as usual, and the product chromatographed on alumina (grade III, 25 g.). Elution with benzene:chloroform (9:1) gave an oil which crystallised from benzene-ether, and methanol to give the tetrahydro compound as plates (520 mg., 70.0%) m.p. 89°. (Found: C, 82.24; H, 6.62; N, 2.62. $C_{37}H_{33}NO_3$ requires C, 82.07; H, 6.46 and N, 2.58%.)

6,7-Dihydroxy-1-(p-hydroxybenzyl)-1,2,3,4-tetrahydro-isoquinoline (34a).

The tetrahydro compound (33a) (1.2 g.) in ethanol (50 ml.) and conc. hydrochloric acid (0.8 ml.) was hydrogenolysed with 10% palladium on charcoal. After 3 hrs. the reaction mixture was worked up in the usual way. The product crystallised from methanol-ether as plates (650 mg., 95%) m.p. 265-266°. (Found: C, 61.72; H, 5.81; N, 4.45. $C_{16}H_{18}NO_3Cl$ requires C, 62.11; H, 5.85 and N, 4.55%.) The free base m.p. 210° (lit.,[34] 208-210°).

Deuterium Exchange Experiments.

The exchange experiments described below and elsewhere in the thesis have been carried out by heating the reaction mixture in a sealed tube under nitrogen over a steam bath. The progress of the reaction has been followed by n.m.r. measurements. After completion of the reaction, the deuterated compounds have been isolated and characterised. The n.m.r. spectra of the phenolic compounds have been taken in deuterium oxide-potassium tert.-butoxide, unless otherwise stated.

Deuteration of (±)-Coclaurine.

(±)-Coclaurine hydrochloride gave in its n.m.r. bands at τ 3.44 (2H doublet), 2.97 (2H doublet), 3.41 (1H singlet), 3.40 (1H singlet) and 6.32 (3H singlet).

(A) (±)-Coclaurine hydrochloride (100 mg.) in deuterium oxide (.5 ml.) was heated with potassium tert.-butoxide (150 mg., ca. 3 mol. equ.). After 100 hrs. the resulting mixture was diluted with water (1.3 ml.), treated with solid carbon dioxide and the liberated base was extracted with chloroform (3 x 4 ml.), washed with water, dried and evaporated. The residue was treated with methanolic hydrogen chloride and charcoaled. The deuterated (±)-coclaurine hydrochloride (60 mg.) mp. and mixed m.p. 254-256°;

n.m.r. bands at τ 2.99 (ca. 2H singlet), 3.41 (1H singlet) and 6.32 (3H singlet).

(B) (±)-Coclaurine hydrochloride (50 mg.) in deuterium oxide (0.5 ml.) was heated with potassium tert.-butoxide (62 mg. ca. 3 mol. equ.) for 80 hrs. The resulting deuterated (±)-coclaurine hydrochloride showed in its n.m.r. bands at τ 3.45 (ca. 2H doublet), 2.98 (2H doublet) 3.42 (1H singlet). In this experiment an old sample of potassium tert.-butoxide was used and it was found to contain potassium carbonate.

(C) (±)-Coclaurine hydrochloride (60 mg.) was treated with deuterium oxide (0·5 ml.) in the presence of deuterium chloride (ca. 3 mol.equ., generated in situ from deuterium oxide and thionyl chloride). Dimethylformamide (0.1 ml.) was added to dissolve the hydrochloride. After heating for 18 hrs. the n.m.r. measurements showed no exchange of aromatic hydrogens with deuterium.

Deuteration of (±)-Isococlaurine.

(±)-Isococlaurine hydrochloride showed in its n.m.r. bands at τ 2.96 (2H singlet), 3.54 (2H doublet), 3.32 (2H singlet) and 6.29 (3H singlet). The base hydrochloride

(86 mg.), potassium tert.-butoxide (120 mg., ca. 3.5 mol. equ.) and deuterium oxide (0·5 ml.) were heated at 100° for 6 days. The resulting mixture was worked up in the usual way to give the deuterated (±)-isococlaurine hydrochloride (41 mg.) m.p. and mixed m.p. 213-215°, n.m.r. bands at τ 2.90 (ca. 2H singlet) and 3.29 (1H singlet).

Deuteration of (±)-Norcoclaurine.

(±)-Norcoclaurine hydrochloride gave signals in its n.m.r. spectrum at τ 3.13 (2H doublet), 3.55 (2H doublet), 3.7 (1H singlet) and 3.55 (1H singlet). A mixture of the base hydrochloride (76 mg.), potassium tert.-butoxide (130 mg.) and deuterium oxide (0.6 ml.) was heated at 100° for 100 hrs. The resulting mixture was worked up in the usual way to give the deuterated (±)-norcoclaurine hydrochloride m.p. and mixed m.p. 264-266°, n.m.r. band at τ 3.02 (2H singlet).

Tritium Labelled Precursors.

Labelled (±)-Coclaurine Hydrochloride.

(±)-Coclaurine hydrochloride (110 mg.) was heated with potassium tert.-butoxide (150 mg.) in tritiated water (0·5 ml., 0.1 curie) for 80 hrs. The resulting mixture was diluted with water (1.5 ml.), treated with

solid carbon dioxide, the precipitated base centrifuged off, washed with water (0.5 x 2 ml.) and extracted with chloroform (3 x 4 ml.). The solvent from the chloroform extract was removed. The residue was treated with methanolic hydrogen chloride to give the tritiated (±)-coclaurine hydrochloride which was crystallised from methanol-ether as plates (55 mg.) m.p. 257-259°. The sample was crystallised to a constant activity.

(±)-Coclaurine-HCl	4.30×10^5 d.p.s./mg.

The active sample (1.1 mg.) diluted with inactive material (253 mg.)

Cryst.	4.40 d.p.s./mg.
Recryst.	4.32 "

Labelled (±)-Isococlaurine Hydrochloride.

(±)-Isococlaurine hydrochloride (110 mg.) was heated with potassium tert.-butoxide (146 mg.) in tritiated water (0·5 ml., 0.1 curie) for 98 hrs. The resulting mixture was worked up as above to give the tritiated (±)-isococlaurine hydrochloride (45 mg.) m.p. 213-215°. The sample was crystallised from ethanol-ether to a constant activity.

(±)-Isococlaurine·HCl	1.09×10^6 d.p.s./mg.

Labelled (±)-Norcoclaurine Hydrochloride.

(±)-Norcoclaurine hydrochloride (100 mg.) was heated with potassium tert.-butoxide (170 mg.) in tritiated water (0.5 ml., 0.1 curie) for 150 hrs. The resulting mixture was worked up in the usual way to give the tritium labelled (±)-norcoclaurine hydrochloride (55 mg.) which was crystallised from ethanol-ether as plates m.p. 263-265°.

(±)-Norcoclaurine·HCl 1.15×10^6 d.p.s./mg.

The active sample (0.47 mg.) diluted with inactive material (148 mg.).

Cryst.	1.18×10^6 d.p.s./mg.
Recryst.	1.15×10^6 "

Degradation of (±)-Coclaurine.

OO-Dimethylcoclaurine methiodide (44).

(A) Coclaurine hydrochloride (100 mg.) in 5N-methanolic potassium hydroxide (4 ml.) under nitrogen was refluxed with an excess of methyl iodide (2 ml.). After 3 hrs. the solvent and excess of methyl iodide were removed, the residue extracted with chloroform (3 x 4 ml.) and the solvent evaporated. The quaternary salt was crystallised frommethanol-ether as yellowish needles, (70 mg.) m.p.

112-114° (lit.,[35] 110-112°).

(B) To a solution of (±)-coclaurine hydrochloride (120 mg.) in dry dimethylformamide (5 ml.) under nitrogen was added sodium hydride (60 mg.). To this was added methyl iodide (1 ml.) and the resulting mixture was left overnight. The excess of methyl iodide and the solvent were then removed in vacuo. The residue was worked up as above to give the methiodide (90 mg.) m.p.111-113°.

Hofmann Degradation.

The methiodide (44) (100 mg.) was dissolved in 20% aqueous potassium hydroxide (7 ml.) and gently refluxed for 20 hrs. The resulting mixture was extracted with ether (6 x 5 ml.), washed with water, dried and evaporated. The crude product was chromatographed on alumina (grade III, 25 g.). Elution with benzene:chloroform (5:1) gave an oil, crystallised from ether-petroleum ether to give the methine (46) as needles (30 mg.) m.p.83-84° (lit.,[35] 82-85°).

Oxidation of the Methine.

To a solution of the methine (46) (30 mg.) in aqueous acetone (20 ml.) and 6N-sulphuric acid (1 ml.) at 0° was added 5% aqueous solution of potassium permanganate

(20 ml.) during $\frac{1}{2}$hr. The resulting solution was stirred for 2 hrs., the solvent removed and sulphur dioxide was passed into the aqueous solution. The acidic solution was extracted with ether (5 x 5 ml.), washed with water, dried and evaporated. The crude acid was sublimed and then crystallised from aqueous ethanol to give anisic acid (5 mg.).

Ozonolysis and Oxidation of the Ozonides.

Styrene (200 mg.) in methylene chloride (10 ml.) at 0° was ozonolysed in the usual way. The ozonide was treated with 2N-sodium hydroxide (10 ml.) and hydrogen peroxide (2 ml.) to give benzoic acid (25%).

Stilbene (220 mg.) in methylene chloride (10 ml.) was ozonolysed in the usual way. The ozonide was treated with 1N-sodium hydroxide (10 ml.) and hydrogen peroxide (2 ml.) to give benzoic acid (30%). Treatment of the ozonide with silver oxide and sodium hydroxide as described below gave benzoic acid (70%).

To a solution of the inactive methine (46) (50 mg.) in methylene chloride (5 ml.) at 0° was bubbled dry ozone, the reaction being followed by the u.v. absorption. After 45 min. the absorption at 295 mμ disappeared. The

solvent from the resulting mixture was then removed. The ozonide in ether (60 ml.) was added into a suspension of silver oxide (100 mg.) in 10% aqueous sodium hydroxide (10 ml.) during $\frac{1}{2}$ hr. with stirring. After heating at 100° for 2 hrs., the silver oxide was filtered off. The alkaline filtrate was acidified with 6N-hydrochloric acid, extracted with ether (6 x 4 ml.), washed with water, dried and evaporated. The crude product was crystallised from aqueous ethanol to give anisic acid (12 mg., 67%).

Degradation of Radioactive (±)-Coclaurine.

Radioactive (±)-coclaurine hydrochloride (200 mg.) in dimethylformamide (7 ml.) and sodium hydride (100 mg.) was treated with methyl iodide (2 ml.). The resulting mixture under nitrogen, after keeping at room temperature for 18 hrs., was worked up as above. The quaternary salt was refluxed with 20% aqueous potassium hydroxide (7 ml.) for 20 hrs. and worked up as usual. The product was chromatographed on alumina (grade III, 25 g.), elution with benzene:chloroform (5:1) gave the radioactive methine, crystallised from ether-petroleum ether as needles, m.p. 82-85°.

The radioactive methine (50 mg.) was dissolved in methylene chloride (8 ml.), cooled to 0° and ozonolysed. After 1 hr. the resulting mixture was worked up in the usual way. Oxidation of the ozonide with alkaline silver oxide as above gave radioactive anisic acid (11 mg.). The acid was crystallised from ethanol to a constant activity.

Active Methine.

Cryst.	1.59×10^3 d.p.s./mg.
Recryst.	1.63×10^3 "
Recryst.	1.60×10^3 "

Anisic Acid.

Cryst.	2.35×10^3 d.p.s./mg.

Active anisic acid (4.2 mg.) was diluted with inactive material (56 mg.).

Cryst.	2.41×10^3 d.p.s./mg.
Recryst.	2.37×10^3 "

Results.

(±)-Coclaurine·HCl	5.45×10^5 d.p.s./mMole
Methine	5.50×10^5 "
Anisic acid	3.62×10^5 "

Synthesis of [O-methyl-^{14}C] Coclaurine.

3-Hydroxy-4-benzyloxybenzaldehyde.

To a refluxing mixture of protocatechuic aldehyde (4.6 g.), ethanol (50 ml.) and benzyl chloride (4.5 ml.) was added a solution of potassium hydroxide (1.9 g.) in methanol (40 ml.) during $1\frac{1}{2}$ hrs. with stirring. The solvent from the resulting mixture was then removed, the concentrate diluted with water, 4N-sodium hydroxide (20 ml.) added and ether extracted (50 x 4 ml.). The aqueous alkaline layer was acidified with 6N-hydrochloric acid, extracted with ether (100 x 4 ml.), washed with water, dried and evaporated. The chloroform soluble portion of the residue was chromatographed on alumina (grade III, 50 g.). Elution with chloroform gave a product which was crystallised from methanol-ether to give 3-hydroxy-4-benzyloxybenzaldehyde (1.85 g., 17.6%) as rods m.p. 121° (lit.,[36] 122°).

3-Methoxy [^{14}C] -4-benzyloxybenzaldehyde.

3-Hydroxy-4-benzyloxybenzaldehyde (80 mg.) in dry dimethylformamide (1.2 ml.) under nitrogen was treated with sodium hydride (20 mg.). Inactive methyl iodide (4 mg.) was added to it and the mixture was frozen with liquid nitrogen. To it was distilled in vacuo [^{14}C] methyl

iodide (0.96 mg., .1 mc). The residual radioactive methyl iodide from the vacuum line was washed into the reaction mixture by distilling inactive methyl iodide (15 mg.) in dimethylformamide (1.2 ml.). The resulting mixture under vacuum was stirred for 2 days at room temperature. Inactive methyl iodide (1 ml.) was then added and left for 1 hr. at room temperature and then extracted with ether (6 x 5 ml.), washed with water, dried and the solvent was evaporated. The yellow oily residue was chromatographed on alumina (grade III, 20 g.), elution with benzene gave the radioactive O-benzylvanillin (72 mg., .078 mc.) as rods from aqueous ethanol, m.p. 64-65°.

O-Benzylvanillin	4.04×10^4 d.p.s./mg.

The active O-benzylvanillin (1.5 mg.) diluted with inactive material (74 mg.).

Cryst.	4.10×10^4 d.p.s./mg.
Recryst.	4.08×10^4 d.p.s./mg.
Radiochemical yield	76.4%.

3-Methoxy[^{14}C]-4-benzyloxy-ω-nitrostyrene.

A mixture of O-benzylvanillin (70 mg.), nitromethane (3 ml.), anhyd. sodium acetate (20 mg.), methylamine

hydrochloride (20 mg.) and glacial acetic acid (.1 ml.) was shaken at room temperature. After 3 hrs. the excess of nitromethane was removed under reduced pressure, the residue was washed with ether (1.2 x 2 ml.) and water (1.5 x 2 ml.) to give the radioactive nitrostyrene (71 mg., 85%) m.p. 119-122°.

3-Methoxy [^{14}C] -4-benzyloxyphenethylamine.

To a refluxing solution of lithium aluminium hydride (200 mg.) in dry tetrahydrofuran (20 ml.) was added a solution of the radioactive nitrostyrene (70 mg.) in dry tetrahydrofuran (10 ml.) during 20 min. After 2 hrs. the excess of lithium aluminium hydride was decomposed and 10N-sodium hydroxide (5 ml.) was added. The separated solvent layer was taken out and was mixed with the ether extract (25 x 4 ml.) of the pasty mass. The solvent from the combined extracts was removed, the residue dissolved in ether (30 ml.), washed with water, dried and evaporated to give radioactive amine (47 mg., 70%). The amine hydrochloride m.p. 176-177°.

N-(3-Methoxy [^{14}C] -4-benzyloxyphenethyl)-p-benzyloxyphenylacetamide.

The radioactive amine hydrochloride (43 mg.) was

treated with p-benzyloxyphenyl acetyl chloride (prepared from 60 mg. of the corresponding acid by treatment with oxalyl chloride) in the presence of 2N-sodium hydroxide (3 ml.) and worked up under the same conditions as earlier (p. 143) to give the radioactive amide (60 mg., 85%) m.p. 114-116°.

The synthesis of [O-methyl-^{14}C]-(±)-coclaurine was completed, using the radioactive amide, by following exactly the same procedure as in case of inactive (±)-coclaurine. Radioactive (±)-coclaurine hydrochloride (12 mg.), crystallised from ethanol as needles m.p.256-257°, was obtained.

Coclaurine.HCl	2.08×10^6 d.p.s./mMole

The radioactive base hydrochloride (.66 mg.) diluted with inactive material (50 mg.).

Cryst.	2.16×10^6 d.p.s./mMole
Recryst.	2.12×10^6 "

Synthesis of [O-methyl-^{3}H]-(±)-coclaurine.

3-Hydroxy-4-benzyloxybenzaldehyde (125 mg.) was methylated by the method described earlier with [^{3}H]methyl iodide (kindly supplied by Dr. E. McK. Wilson) to give

3- methoxy-[^{3}H]-4-benzyloxybenzaldehyde (115 mg., .12 mc.). The synthesis of [O-methyl-^{3}H]-(±)-coclaurine was completed by using the **radioactive** O-benzylvanillin. Exactly the same procedure was followed in the synthesis as in the case of [O-methyl-^{14}C]-(±)-coclaurine.

(±)-Coclaurine.HCl 6.20 x 10^{6} d.p.s./mMole

Degradation of Crotonosine.

Crotonosine isolated from the feeding of (±)-coclaurine hydrochloride labelled with ^{14}C in the methoxyl group and tritium in the ortho positions, was converted into apocrotonosine hydrochloride by the method described earlier.

The methoxyl group in the radioactive crotonosine and apocrotonosine was cleaved by the Ziesel method by using samples (ca. 18 mg.). The methyl iodide evolved was collected in ethanolic triethylamine to give triethylmethylammonium iodide.

Radioactive crotonosine, apocrotonisine hydrochloride, diacetylcrotonosine and triethylmethylammonium iodide were assayed for the ^{14}C activity in the scintillation counter at ^{14}C optimum settings. Sample (ca. 12 mg.) were dissolved in dimethylformamide (2 ml.) and liquid scintillator (11 ml.). Quenching was corrected either

by extrapolating the log. of the specific activity-versus-concentration or by using the efficiency of the counter at ^{14}C optimum settings, measured by [1-^{14}C]-hexadecane standard in the presence of equal amount of inactive material.

Results.

Compound	Activity (d.p.m./mMole)
Diacetylcrotonosine	4.60×10^3
Crotonosine	4.61×10^3
$(Et)_3\overset{+}{N}Me\bar{I}$	4.11×10^3
Apocrotonosine.HCl	4.50×10^3
$(Et)_3\overset{+}{N}Me\bar{I}$	4.13×10^3

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

Each of [1-^{14}C]-hexadecane and [1-^3H]-hexadecane (ca. 5 mg.) was exactly weighed and dissolved in dimethylformamide (2 ml.) and liquid scintillator (11 ml.). Each of these was then counted at the voltage settings for the maximum efficiency of tritium (setting I, 1540 V/1700 V) and that of ^{14}C (setting II, 1300 V/1400 V).

[^3H] Standard (Sp.activity 4.00×10^3 d.p.m./mg.)

Sp. activity at setting I = 5.08×10^2 c.p.m./mg.

Efficiency of the counter = 12.7%.

Sp. activity at setting II = 2.34×10^2 c.p.m./mg.

Efficiency of the counter = 5.9%.

[^{14}C] Standard (Sp.activity 2.15×10^3 d.p.m./mg.)

Sp.activity at setting I = 9.33×10^2 c.p.m./mg.

Efficiency of the counter = 43.4%.

Sp. activity at setting II = 1.50×10^3 c.p.m./mg.

Efficiency of the counter = 70%.

Resolution.

(-)-OO-Dibenzylcoclaurine (48a)

(±)-OO-Dibenzylcoclaurine (1.74 g.) was treated with (+)-dibenzoyltartaric acid (1.34 g.). The resulting salt was fractionally crystallised successively from benzene-ether, benzene, ethanol-ether, ethanol and methanol finally to give needles (0.95 g.) in polymorphic forms, m.p. 142-143°, $[\alpha]_D$ + 72° (C 0.94 in MeOH); m.p. 162-165°, $[\alpha]_D$ + 70° (C 0.8 in MeOH) and m.p. 113°, $[\alpha]_D$ + 71° (C 1.0 in MeOH).

The salt (380 mg.) $[\alpha]_D$ + 72° was treated with 2N-sodium hydroxide (3 ml.). The liberated base was extracted with chloroform (10 x 3 ml.), washed with water, dried and evaporated. The residue was chromatographed on alumina (grade III, 15 g.), elution with benzene:chloroform

(1:1) gave OO-dibenzylcoclaurine (190 mg.) crystallised from ethanol as plates m.p. 86-87°, $[\alpha]_D$ + 15° (C 0.5 in MeOH) and -26.5° (C 0.8 in $CHCl_3$). The base hydrochloride crystallised from ethanol as needles m.p. 170-171°, dried *in vacuo* over P_2O_5, $[\alpha]_D$ + 47° (C 0.1 in MeOH).

(-)-Coclaurine (48b) Hydrochloride.

The above (+)-OO-dibenzylcoclaurine hydrochloride (230 mg.) in ethanol (2 ml.) was hydrogenolysed in the presence of 10% palladium on charcoal (115 mg.) and the resulting mixture was worked up in the usual way to give (-)-coclaurine hydrochloride (110 mg.). Crystallisation from ethanol gave needles m.p. 165-166°. When dried at 100° *in vacuo* for 25 hrs. the base hydrochloride m.p. 247-248° and $[\alpha]_D$ -14° (C 1.1 MeOH). Decomposition of the hydrochloride with 6N-sodium hydrogen carbonate and extraction with chloroform (5 x 5 ml.) gave (-)-coclaurine $[\alpha]_D^{23}$ -16° (C 1.0 in MeOH), identical with (±)-coclaurine (I.R. and T.L.C.).

(+)-N-Methylcoclaurine (48c).

(-)-Coclaurine hydrochloride (100 mg.) was dissolved in water (1 ml.), treated with 2N-sodium hydroxide (1 ml.) under nitrogen and to it was added formic acid (1.2 ml,

98%), pH *ca.* 5 and formaldehyde (1.2 ml., 37-41%). The resulting mixture was heated on a water bath (100°) for 15 min. The excess formaldehyde and formic acid were removed under reduced pressure, the residue treated with 6N-sodium hydrogen carbonate and the liberated base was extracted with chloroform (5 x 4 ml.), washed with water, dried and evaporated. The residue was chromatographed on alumina (grade III, 25 g.), elution with chloroform:ethanol (94:6) gave (+)-N-methylcoclaurine (60 mg.) m.p. 178°, $[\alpha]_D^{23}$ + 123° (C 0.5 in MeOH) (lit.,[38] 178-179° and + 124° in MeOH).

(+)-NOO-Trimethylcoclaurine (48d).

(+)-N-Methylcoclaurine (50 mg.) in methanol (0.3 ml.) was treated with an ethereal solution of diazomethane (from 2 g. of nitrosomethylurea). After 3 days the solvent was removed, the residue was dissolved in ether (25 ml.), washed with 4N-sodium hydroxide (10 ml.), water, dried and evaporated. The residue was chromatographed on alumina (grade III, 10g.), elution with benzene:chloroform (3:2) gave NOO-trimethylcoclaurine (25 mg.) m.p. 60°, $[\alpha]_D^{23}$ + 83° (C 0.3 in ethanol) (lit.,[40] + 86° in ethanol, m.p. 61-62°. M.p. 91° has also been reported and it has been shown that the compound exists in polymorphic forms).

(+)-OO-Dibenzylcoclaurine (47a).

The mother liquors, obtained after separating the crystalline salt of (+)-dibenzoyltartaric acid and (±)-OO-dibenzylcoclaurine were combined, treated with 2N-sodium hydroxide (25 ml.), washed with water, dried (anhyd. K_2CO_3) and evaporated. The residue was chromatographed on alumina (grade III, 55 g.), elution with benzene:chloroform (1:1) gave the partially resolved OO-dibenzylcoclaurine crystallised from ethanol as plates m.p. 86-87°.

The above dibenzyl ether (842 mg.) was treated with (-)-dibenzoyltartaric acid (651 mg.). The resulting salt was fractionally crystallised successively from benzene-ether, benzene, ethanol-ether, ethanol and methanol to give needles (660 mg.) in polymorphic forms, m.p. 142-144°, $[\alpha]_D^{24}$ -72° (C 0.8 in MeOH) and m.p. 138-140°, $[\alpha]_D^{24}$ -72.8° (C 0.7 in MeOH).

The (-)-salt (390 mg.) $[\alpha]_D$ -72° was decomposed with 2N-sodium hydroxide (4 ml.). The free base was extracted with chloroform (20 x 3 ml.), washed with water, dried (anhyd. K_2CO_3), evaporated and the residue was chromatographed on alumina (grade III, 20 g.). Elution with benzene:chloroform (1:1) gave (+)-OO-dibenzylcoclaurine

(200 mg.), crystallised from ethanol as cubes m.p.88-89°, $[\alpha]_D^{22}$ -16° (C 0.6 in MeOH) and + 24.5° (C 0.7 in $CHCl_3$). The base hydrochloride crystallised from ethanol as needles. After drying in vacuo at 50° over P_2O_5 for 10 hrs., m.p. 171-172°, $[\alpha]_D^{22}$ -48° (C 1.0 in MeOH).

(+)-Coclaurine (47b) Hydrochloride.

The above (+)-OO-dibenzylcoclaurine hydrochloride (150 mg.) was hydrogenolysed with 10% palladium on charcoal (150 mg.) in the usual way to give (+)-coclaurine hydrochloride (98 mg.). Crystallisation from ethanol gave the hydrochloride as plates m.p. 168-170°, when dried in vacuo over P_2O_5 at 100° for 24 hrs., m.p. 246-248° and $[\alpha]_D^{22}$ + 13° (C 0.8 in MeOH). The free base from the hydrochloride was identical with (-)- and (±)-coclaurine (I.R. and T.L.C.).

(-)-N-Methylcoclaurine (47c).

(+)-Coclaurine hydrochloride (90 mg.) was methylated with formaldehyde (1.3 ml.) and formic acid (1.2 ml.), under similar conditions as earlier to give (-)-N-methylcoclaurine (45 mg.) m.p. 177-178° and $[\alpha]_D^{23}$ -120° (C 1.0 in MeOH) (lit.,[38] 178° and -121.8° in MeOH).

(-)-NOO-Trimethylcoclaurine (47d).

(-)-N-Methylcoclaurine (40 mg.) was methylated with diazomethane (from 2 g. of nitrosomethylurea) under exactly the same conditions as earlier, to give (-)-NOO - trimethylcoclaurine (20 mg.) m.p. 59-60° and $[\alpha]_D^{22}$ -83° (C 0.76 in ethanol) (lit.,[40] 61-62° and -85.7° in ethanol.). The compound exists in polymorphic forms.[39]

Labelled (-)-Coclaurine Hydrochloride.

(-)-Coclaurine hydrochloride (82 mg.), tritiated water (0.4 ml.) and potassium tert.-butoxide (145 mg.) was heated for 96 hrs. The resulting mixture was worked up in the usual way to give tritium labelled (-)-coclaurine hydrochloride (58 mg.) as needles, hydrated form m.p. 169-171°, anhyd. salt m.p. 248-250°.

(-)-Coclaurine.HCl	4.63×10^5 d.p.s./mg.
Cryst.	4.42×10^5 "
Recryst.	4.54×10^5 "

Labelled (+)-Coclaurine Hydrochloride.

(+)-Coclaurine hydrochloride (83 mg.) tritiated water (0.4 ml.) and potassium tert.-butoxide (154 mg.) were heated for 100 hrs. The resulting mixture, on working up in the usual way, gave tritium labelled (+)-coclaurine

hydrochloride (52 mg.), m.p.248-250°.

(+)-Coclaurine.HCl	4.32×10^5 d.p.s./mg.
Cryst.	4.22×10^5 "
Recryst.	4.41×10^5 "

Attempted Synthesis of 7-Hydroxy-1-(3-hydroxy-5-methoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (1).

4-Benzyloxy-ω-nitrostyrene. (Method of Lange and Hambourger).[42]

p-Benzyloxybenzaldehyde (20 g.) in ethanol (300 ml.) was treated with nitromethane (12 g.) in the presence of sodium methoxide (.05 mole). Decomposition of the precipitated sodium salt with 6N-hydrochloric acid afforded the nitrostyrene, crystallised from ethanol as yellow plates (11.8 g., 50%) m.p. 120° (lit.,[41] 120°).

4-Benzyloxyphenethylamine. (Method of Dr. M.N. Afzal).[43]

4-Benzyloxy-ω-nitrostyrene (10 g.) was reduced with lithium aluminium hydride (4 g.) in dry ether (200 ml.) in the usual way to give the amine. The amine was treated with methanolic hydrogen chloride to give the base hydrochloride which was crystallised from ethanol as needles m.p. 202-203°. The corresponding free base m.p. 51° (lit.,[9] 50°).

3-Hydroxy-5-methoxybenzoic acid.

The method of Späth and Kromp[44] was followed with some modification. 3,5-Dihydroxybenzoic acid methyl ester (14 g.) in methanol (50 ml.) was treated with dimethyl sulphate (8 g.) in the presence of sodium methoxide (from 1.5 g. of sodium). The resulting mixture was kept at 20° for 14 hrs. and then refluxed for 2 hrs. The solvent was removed, water added, ether extracted (50 x 4 ml.), dried and evaporated. The benzene soluble portion of the residue was chromatographed on alumina (grade V, 500 g.), elution with benzene:ethyl acetate (7:3) gave 3-hydroxy-5-methoxybenzoic acid methyl ester (5 g., 35%) crystallised from benzene as needles m.p.97°. Alkaline hydrolysis of the ester in the usual manner gave 3-hydroxy-5-methoxybenzoic acid m.p. 203-204° (lit.,[44] 203-204°).

3-Benzyloxy-5-methoxybenzoic acid.

A mixture of 3-hydroxy-4-methoxybenzoic acid methyl ester (1 g.), ethanol (30 ml.), potassium hydroxide (.36 g.) and benzyl chloride (.8 g.) was refluxed under nitrogen for 5 hrs. Potassium hydroxide (.4 g.) in methanol (20 ml.) was again added to it and refluxing was continued for another $1\frac{1}{2}$ hrs. The solvent was then evaporated, the concentrate diluted with water and washed

with ether (25 x 4 ml.). Acidification of the aqueous alkaline layer with 6N-hydrochloric acid gave 3-benzyloxy-5-methoxybenzoic acid (1.2 g., 89%), crystallised from ethyl acetate as needles m.p. 140°, $\nu_{max.}$ 1669 cm.$^{-1}$. (Found: C, 70.06; H, 5.42. $C_{15}H_{14}O_4$ requires C, 69.76; H, 5.42%).

3-Benzyloxy-5-methoxy-ω-diazoacetophenone.

3-Benzyloxy-5-methoxybenzoic acid (1 g.) in dry benzene (10 ml.) was treated with oxalyl chloride (1.2 ml.). After 3 hrs. the resulting mixture was worked up in the usual manner and the corresponding acid chloride was obtained. A solution of the acid chloride in dry benzene was added into an ethereal solution of diazomethane (from 6 g. of nitrosomethylurea) at 0°. After keeping for 4 hrs. at 0° the resulting mixture on working up in the usual manner gave the diazoketone (.9 g.), $\nu_{max.}$ 2110 cm.$^{-1}$ as a yellow solid m.p. 55-58°.

N-(p-benzyloxyphenethyl)-3-benzyloxy-5-methoxyphenylacetamide.

To a refluxing mixture of 3-benzyloxy-5-methoxydiazoacetophenone (1 g.) and p-benzyloxyphenethylamine (1 g.) in dry benzene (40 ml.) was added freshly prepared silver

oxide (.15 x 3 g.). After 3 hrs. the resulting mixture was filtered, the filtrate washed with 4N-sodium hydroxide, 2N-hydrochloric acid, water, dried and the solvent was removed. The crude product was crystallised from ethanol to give the amide as needles (1.15 g., 70%) m.p. 122-123°, $\nu_{max.}$ 3448 and 1658 cm.$^{-1}$. (Found: C, 77.29; H, 6.53; N, 2.96. $C_{31}H_{31}NO_4$ requires C, 77.33; H, 6.44; N, 2.91%).

Approach to the preparation of 7-benzyloxy-1-(5-methoxy-3-benzyloxybenzyl)-3,4-dihydroisoquinoline.

N-(p-Benzyloxyphenethyl)-3-benzyloxy-5-methoxyphenylacetamide was treated with phosphorus oxychloride, phosphorus pentoxide, a mixture of phosphorus oxychloride and pentoxide, in dry benzene, toluene, xylene, and chloroform under Bischler-Napieralski reaction conditions. At higher temperature polymerisation was the main reaction, whereas at lower temperature the starting material was recovered unchanged. The basic fraction which was obtained in very poor yield (3-8%) in some cases, was a mixture and no definite compound could be isolated from it.

To study the reaction conditions, the preparation of N-(p-benzyloxyphenethyl)-phenylacetamide was undertaken. Phenylacetyl chloride (2.1 g.) was treated with p-benzyloxyphenethylamine (2 g.) in benzene (50 ml.) in the

presence of 4N-sodium hydroxide (10 ml.) in the usual way. After 2 hrs. the benzene layer was separated, washed with 2N-hydrochloric acid, water, dried and evaporated. The product crystallised from ethanol to give needles m.p. 122°, $\nu_{max.}$ 3436 and 1656 cm.$^{-1}$. (Found: C, 80.26; H, 6.43; N, 4.24. $C_{23}H_{23}NO_2$ requires C, 80.00; H, 6.66; N, 4.06%).

N-(p-Benzyloxyphenethyl)-phenylacetamide, when treated with the usual dehydrating reagents, under the Bischler-Napieralski reaction conditions, behaved in the same manner as the amide referred to above and no cyclodehydration product was obtained.

Since the cyclodehydration of the amides referred to above, could not be successful under the usual reaction conditions, presumably due to the unactivated benzenoid ring of the substituted β-phenethylamine, it was then decided to activate the position involved in the cyclisation by introduction of a removable electron releasing group such as -SR in the proper position in the benzenoid ring of the amide.

Toluene-p-thiosulphonic acid benzyl ester was selected for the thioalkylation of the phenolic compounds. In the preparation of the reagent when toluene-p-sulphonyl

chloride was treated with benzylmercaptan, dibenzyl disulphide was the main product of the reaction. Toluene-p-sulphonyl chloride when treated with silver-benzyl mercaptide, toluene-p-thiosulphonic acid benzyl ester was obtained in a yield of 35%. However, treatment of the toluene-p-sulphonyl iodide with silverbenzyl mercaptide gave the desired thioester (yield, 70%), crystallised from ethanol as plates m.p. 56-57°, $\nu_{max.}$ 1311 and 1133 cm.$^{-1}$. (Found: C, 60.59; H, 5.08; S, 22.46. $C_{14}H_{14}O_2S_2$ requires C, 60.43; H, 5.03; S, 23.02%).

1-S-Benzyl-β-naphthol.

A solution of β-naphthol in ethanol when treated with toluene-p-thiosulphonic acid benzylester (1.5 mol.) in the presence of sodium ethoxide, the desired 1-S-benzyl-β-naphthol could not be obtained.

To a refluxing mixture of β-naphthol (1 g.), sodium hydride (60 mg.), dry benzene (50 ml.), and ethanol (0.2 ml.) under an atmosphere of nitrogen, was added a solution of toluene-p-thiosulphonic acid benzyl ester (207 mg.) in dry benzene (4 ml.). After 4 hrs. the resulting mixture was acidified with 3N-hydrochloric acid, the benzene

layer was separated and the aqueous layer extracted with benzene (15 x 3 ml.). The combined benzene extracts were washed with 10% sodium hydrogen carbonate, water, dried and evaporated. The residue was chromatographed on alumina (grade V, 40 g.), elution with petroleum ether gave dibenzyl disulphide. Subsequent elution with petroleum ether:benzene (3:2) gave 1-S-benzyl-β-naphthol (.56 g., 30%), crystallised from benzene-petroleum ether as plates m.p. 40°; $\nu_{max.}$ 3367, 1453, 1381, 1344 and 1129 cm.$^{-1}$. (Found: C, 76.40; H, 5.24; S, 11.96. $C_{17}H_{14}OS$ requires C, 76.60; H, 5.26; S, 12.03%).

Thioalkylation of p-cresol and p-hydroxybenzaldehyde under the condition as above did not succeed. Even further modification of the conditions did not yield the S-substituted products. The approach was, therefore, abandoned.

Synthesis of (±)-Roemerine[45]

3,4-Methylenedioxy-ω-nitrostyrene (69).

A mixture of piperonaldehyde (12.5 g.), nitromethane (30 ml.), methylamine hydrochloride (2 g.) and anhyd. sodium acetate (2 g.) was shaken at room temperature. After 20 hrs. the yellow crystalline product was filtered off, washed with ether and water. Crystallisation of the product from glacial acetic acid gave yellow needles (14.6 g., 91%) m.p. 162-163° (lit.,[42] 161.5°).

3,4-Methylenedioxyphenethylamine (70).

A solution of the nitrostyrene (69) (28 g.) in dry tetrahydrofuran (750 ml.) was added to a suspension of lithium aluminium hydride (27 g.) in dry ether (200 ml.) during 4 hrs. It was then refluxed for 3 hrs. and left overnight. The excess lithium aluminium hydride was decomposed, sodium hydroxide (10 g.) added and the pasty mass was extracted with ether (200 x 3 ml.). The combined ether extract was washed with water and dried. Evaporation of the solvent gave the amine (18 g., 71%). The amine hydrochloride m.p. 210° (lit.,[46] 210-211°).

1-Nitrophenylacetyl chloride.

1-Nitrophenyl acetic acid (9 g.) in dry benzene (20 ml.) was refluxed for 6 hrs. with thionyl chloride (30 ml.). The excess thionyl chloride was removed very carefully *in vacuo* with benzene. The acid chloride without further purification was used in the subsequent step.

N-(3,4-Methylenedioxyphenethyl)-1-nitrophenylacetamide (75).

The acid chloride (72), (from 9 g. of the corresponding acid), in dry benzene (100 ml.) was added to a mixture of the amine (70) hydrochloride (10 g.), benzene (100 ml.) and 4N-sodium hydroxide (40 ml.) during $\frac{1}{2}$ hr. with stirring. The resulting mixture after 1 hr. was worked up in the usual way. Crystallisation of the product from methanol gave the amide (14 g., 85%) as needles m.p. 121° (lit.,[45] 120°).

6,7-Methylenedioxy-1-(1-nitrobenzyl)-3,4-dihydroisoquinoline (74).

(A) A mixture of the amide (75) (6 g.), phosphorus oxychloride (8 ml.) and chloroform (30 ml.) was kept in a stoppered flask at room temperature. After 6 days the solvent and excess phosphorus oxychloride were removed

in vacuo, the residue dissolved in acetone (20 ml.) and poured into 6N-hydrochloric acid (120 ml.) The process was repeated with the insoluble material. The combined aqueous acidic solution was basified with 4N-sodium hydroxide, the liberated base was extracted with chloroform and the solvent evaporated. The residue was taken up in methanol and charcoaled. Crystallisation from methanol gave the dihydroisoquinoline (3.4 g., 60%) as needles m.p. 164° (lit.,[45] 164.5°).

(B) To a refluxing solution of the amide (75) (.5 g.) in dry toluene (15 ml.) was added freshly distilled phosphorus oxychloride (3 ml.). After 45 min. the reaction mixture was worked up as above. Crystallisation of the product from methanol gave the dihydroisoquinoline (47 mg., 10%).

6,7-Methylenedioxy-1-(1-nitrobenzyl)-1,2,3,4-tetrahydroisoquinoline (73). (Method of Mr. G.M. Chapman).[47]

The nitrodihydroisoquinoline (74) (1.5 g.) in methanol (100 ml.) was reduced with sodium borohydride (.5 g.). After $1\frac{1}{2}$ hrs. the solvent was removed, the residue treated with 4N-sodium hydroxide (40 ml.), the free base extracted with ether (50 x 3 ml.) washed with water, dried and the

solvent was evaporated. The residue was treated with methanolic hydrogen chloride to give the amine hydrochloride (1.5 g., 89%) m.p. 228-229°.

6,7-Methylenedioxy-1-(1-nitrobenzyl-1,2,3,4-tetrahydro-2-methylisoquinoline (77).

The secondary base (73)(liberated from the corresponding base hydrochloride 1.5 g.) was treated with formic acid (20 ml.) and formaldehyde (20 ml.). The resulting mixture was heated on a steam bath. After 45 min. the excess of formic acid and formaldehyde was removed in vacuo. The residue was treated with 6N-hydrochloric acid (30 ml.), washed with ether (30 x 3 ml.) and then treated with 4N-sodium hydroxide. The free base was extracted with ether (40 x 3 ml.), washed with water, dried and evaporated. The residue treated with methanolic hydrogen chloride to give the amine hydrochloride (1.15 g., 90%) m.p. 203-204°.

6,7-Methylenedioxy-1-(1-nitrobenzyl)-3,4-dihydroisoquinoline methiodide (76).

To a solution of the dihydroisoquinoline (74) (600 mg.) in benzene (20 ml.) was added an excess of methyl iodide (1.5 ml.). The resulting mixture was kept at room temperature overnight to give the methiodide (640 mg.,

94%), crystallised as yellow needles m.p.260-261° (lit.,[45] 262°).

6,7-Methylenedioxy-1-(1-nitrobenzyl)-1,2,3,4-tetrahydro-2-methylisoquinoline (77).

To a suspension of the methiodide (76) (500 mg.) in ice cold methanol (25 ml.) was added sodium borohydride (300 mg.) during $\frac{1}{2}$ hr. After 1 hr. the reaction mixture was worked up in the usual manner. The free base was treated with ethanolic hydrogen chloride to give the tetrahydroisoquinoline hydrochloride (216 mg., 60%) m.p. 203-204°.

6,7-Methylenedioxy-1-(1-aminobenzyl)-1,2,3,4-tetrahydro-2-methylisoquinoline (79).

The nitrotetrahydroisoquinoline (77) hydrochloride (1.4 g.) in ethanol (200 ml.) was hydrogenated with 10% palladium on charcoal (400 mg.). The uptake of hydrogen was very rapid. After 3 hrs. the catalyst was filtered off. The filtrate was concentrated (ca. 50 ml.) and treated with ethanolic hydrogen chloride to give the amine hydrochloride (1.2 g., 89%) which crystallised from ethanol as needles m.p. 282-283° (lit.,[45] 283-284°).

(±)-Roemerine (78).

The amine (79) hydrochloride (400 mg.) in 2N-sulphuric acid (20 ml.) was cooled to 0° (ice and salt) for ½ hr. To it was added sodium nitrite (14 mg.) during ½ hr. The resulting mixture after keeping at 0° for 4 hrs. was heated on a steam bath in the presence of copper powder for 2 min. The resulting mixture was basified with 6N-ammonium hydroxide, extracted with chloroform (15 x 3 ml.), washed with water, dried and evaporated. The residue was extracted with ether and washed with 4N-sodium hydroxide (5 x 2 ml.). Acidification of the aqueous alkaline layer with 6N-hydrochloric acid gave a phenolic compound. The solvent from the ether layer was removed, the residue carefully chromatographed on alumina (grade V, 50 g.) and the column was eluted with carbon tetrachloride: benzene (1:1). The solvent from the fractions (6 to 24) which contained mainly roemerine (T.L.C.), was removed. The residue was treated with ethanolic hydrogen chloride to give (±)-roemerine hydrochloride (93 mg., 27%), crystallised from ethanol as needles m.p. 272° (lit.,[45] 274°). The free base liberated from the hydrochloride was identical with natural (-)-roemerine (I.R. and T.L.C.)

From the earlier carbon tetrachloride:benzene (1:1) fractions (1-5) a non-phenolic compound was isolated. The infrared spectrum of this compound was superimposable with that of 6,7-methylenedioxy-1-(benzyl)-1,2,3,4-tetrahydro-2-methylisoquinoline. The behaviour of both the compounds on alumina plate was exactly the same.

N-(3,4-Methylenedioxyphenethyl)-phenylacetamide (85).

A mixture of phenylacetyl chloride (3.2 g.), dry benzene (20 ml.) was added to a mixture of the phenethylamine (84) hydrochloride (3.1 g.), benzene (50 ml.) and 4N-sodium hydroxide (20 ml.) during $\frac{1}{2}$ hr. After 1 hr. the reaction mixture was worked up in the usual manner to give the amide, crystallised from ethanol as plates (3.75 g., 87%) m.p. 100° (lit.,[48] 96-97°).

6,7-Methylenedioxy-1-(benzyl)-3,4-dihydroisoquinoline (86).

To a refluxing solution of the amide (85) (3.7 g.) in dry toluene (25 ml.) was added freshly distilled phosphorus oxychloride (4 ml.). After 1 hr. the resulting mixture was worked up as usual to give the dihydroisoquinoline hydrochloride, crystallised from ethanol as needles (3.37 g., 85%) m.p. 214-215° (lit.,[48] 215-216°).

6,7-Methylenedioxy-1-(benzyl)-3,4-dihydroisoquinoline methiodide (88).

The dihydroisoquinoline (86) (3 g.) in benzene (15 ml.) was treated with excess methyl iodide (2 ml.). The resulting mixture when kept at room temperature for 20 hrs. gave the methiodide as yellow needles (3.4 g., 90%) m.p. 243-244°.

6,7-Methylenedioxy-1-(benzyl)-1,2,3,4-tetrahydro-2-methylisoquinoline (81).

To a suspension of the methiodide (88) (500 mg.) in methanol (25 ml.) was added sodium borohydride (200 mg.). After 1 hr. the solvent was removed, the residue treated with 4N-sodium hydroxide (5 ml.), extracted with ether (20 x 3 ml.) washed with water, dried and evaporated. The residue was chromatographed on alumina (grade III, 50g.), elution with benzene:chloroform (1:1) gave the product which was treated with ethanolic hydrogen chloride. Crystallisation of the product from ethanol gave the amine hydrochloride as plates (320 mg.) m.p. 181°. (Found: C, 61.50; H, 6.68; N, 3.63. $C_{18}H_{20}NO_2Cl.2H_2O$ requires C, 61.10; H, 6.78; N, 3.96%). N.m.r. bands in carbon tetrachloride τ 7.64 (3H singlet), 4.18 (2H singlet), 3.58 (1H singlet), 3.77 (1H singlet) and 2.9 (5H singlet).

6,7-Methylenedioxy-1-(benzylidene)-1,2,3,4-tetrahydro-2-methylisoquinoline (87).

A suspension of the methiodide (88) (700 mg.) in cyclohexane (150 ml.) under nitrogen, was treated with 4N-sodium hydroxide (10 ml.). The resulting mixture was stirred at room temperature for 2 hrs. The cyclohexane layer was separated, washed with water, dried and evaporated. The product showed $\lambda_{max.}$ at 347 and 289 mμ in cyclohexane; $\lambda_{max.}$ at 350 and 290 mμ in alcoholic N-sodium hydroxide and $\lambda_{max.}$ at 360, 309 and 250 mμ in 1.5N-alcoholic hydrochloride (lit.,[48] $\lambda_{max.}$ at 360, 310 and 250 mμ in 1.5N-hydrochloric acid).

6,7-Methylenedioxy-1-oxotetrahydroisoquinoline (89).

To a mixture of 1-benzylidene-2-methyl-6,7-methylene dioxytetrahydroisoquinoline (87) (300 mg.), ethanol (25 ml.) and potassium tert.-butoxide (50 mg.) under nitrogen was added a solution of nitrosobenzene (80 mg.) in ethanol (30 ml.) at room temperature. After 10 min. the solvent was removed in vacuo and the residue showed strong carbonyl absorption at 1640 $cm.^{-1}$ in the infrared region. It was treated with 6N-hydrochloric acid. Steam distillation gave benzaldehyde, characterized as its 2:4-dinitrophenyl-hydrazone. The product which was left behind in the

distillation flask was extracted with chloroform (15 x 4 ml.), washed with water, dried and the solvent was evaporated. The residue was chromatographed on alumina (grade V, 20 g.), elution with benzene gave the lactam, crystallised from benzene-petroleum ether as plates m.p. 97° (lit.,[72] 97-98°).

Synthesis of (±)-Reticuline.[49-51,10]

3-Benzyloxy-4-methoxybenzoic acid.

To a mixture of 3-benzyloxy-4-methoxybenzaldehyde (10 g.), acetone (120 ml.), 4N-sodium hydroxide (30 ml.) was added a solution of potassium permanganate (12 g.) in water (150 ml.) during 2 hrs. The resulting mixture was stirred for another 2 hrs., the precipitated manganese dioxide was filtered off and the alkaline filtrate was washed with ether. Acidification with 6N-hydrochloric acid gave the acid (9.3 g., 87%), crystallised from ethanol as plates m.p. 178-179° (lit.,[49] 180°).

3-Benzyloxy-4-methoxybenzoyl chloride.

A mixture of 3-benzyloxy-4-methoxybenzoic acid (9 g.), dry benzene (50 ml.) and thionyl chloride (10 ml.) was refluxed for 6 hrs. The excess thionyl chloride was removed in the usual way. The residue when treated with

benzene-petroleum ether gave the crystalline acid chloride (7.5 g., 77.7%) m.p. 77-79° (lit.,[49] 68°), $\nu_{max.}$ 1748 cm.$^{-1}$.

3-Benzyloxy-4-methoxy-ω-diazo-acetophenone (15).

To an ethereal solution of diazomethane (from nitrosomethylurea 19.2 g.) at 0° was added a solution of the acid chloride (14) (7.2 g.) in dry benzene (30 ml.) during $\frac{1}{2}$ hr. with stirring. The resulting mixture was kept at 0° for 16 hrs. and the precipitated yellow crystalline product (5.6 g.) was filtered off. The solvent from the filtrate was removed and the residue gave a second crop of yellow crystals (1.4 g.) from benzene-petroleum ether. The combined product was recrystallised from benzene-petroleum ether to give the diazoketone as yellow plates (6 g., 81%) m.p. 113-114° (lit.,[49] 114-115°), $\nu_{max.}$ 2096 cm.$^{-1}$

4-Benzyloxy-3-methoxy-ω-nitrostyrene (10).

(A) (Method of Dr. G. M. Thomas).[52] To a solution of O-benzylvanillin (4 g.) in ethanol (120 ml.) cooled to 5° was added nitromethane (4 ml.). A solution of sodium hydroxide (2 g.) in ethanol (40 ml.) was then added to it. The precipitated salt was filtered off and decomposed with 6N-hydrochloric acid to give the nitrostyrene (2.9 g.,

61%) m.p.115-117° (lit.,[42] 122-3°).

(B) A mixture of O-benzylvanillin (20 g.,), distilled nitromethane (20 ml.), anhyd. sodium acetate (600 mg.), methylamine hydrochloride (600 mg.) was shaken at room temperature (24°). After 15 hrs. glacial acetic acid (3 ml.) was added to the mixture, the depositied yellow crystalline product was filtered off, washed with ether and water. Crystallisation of the product from ethanol-glacial acetic acid (10:1) gave the nitrostyrene (19 g., 90%) m.p. 123-124° (lit.,[42] 122-123°).

3-Methoxy-4-benzyloxyphenethylamine (11).

To a suspension of lithium aluminium hydride (24 g.) in refluxing tetrahydrofuran (300 ml.) was added a solution of the nitrostyrene (10)(43 g.) in dry tetrahydrofuran (450 ml.) during 3 hrs. with stirring. After 3 hrs. the resulting mixture was worked up in the usual way to give the amine (34 g., 87%) as an oil. The amine oxalate m.p. 162-163° (lit.,[50] 162-163°). The amine hydrochloride m.p. 176-177°.

N-(3-methoxy-4-benzyloxyphenethyl)-3-benzyloxy-4-methoxyphenylacetamide (18).

(A) (Method of Dr. G. M. Thomas).[52] The diazoketone

(15) (200 mg.) and the phenethylamine (11) (210 mg.) in benzene (10 ml.) were treated with freshly prepared silver oxide (30 mg.) to give the amide, crystallised from ethanol as needles (220 mg., 58% based on diazoketone) m.p. 139-41°.

(B) A mixture of the diazoketone (15)(4.6 g.), the amine (11) (5.2 g.) and dry benzene (200 ml.) under an atmosphere of nitrogen was irradiated with U.V. light in a pyrex flask. After 12 hrs. when the absorption at 2096 $cm.^{-1}$ in the infrared region had disappeared, the resulting mixture was washed with 6N-hydrochloric acid, water and dried. The residue was dissolved in ethanol, charcoaled and concentrated (ca. 12 ml.) to give the amide (6.5 g., 75% based on diazoketone), crystallised from ethanol as needles m.p. 141-142° (lit.,[49] 140-141°).

6-Methoxy-7-benzyloxy-1-(4-methoxy-3-benzyloxybenzyl)-3,4-dihydroisoquinoline (17).

(A) To a refluxing solution of the amide (18) (1.8 g.) in dry toluene (250 ml.) was added freshly distilled phosphorus oxychloride (5 ml.) with stirring. After $\frac{1}{2}$ hr. phosphorus oxychloride (7 ml.) was again added to the

reaction mixture and refluxing continued for 2 hrs. The solvent and excess of phosphorus oxychloride was removed under reduced pressure, the viscous residue triturated with ether-petroleum ether, dissolved in ethanol, charcoaled and concentrated (ca. 25 ml.). The crude crystalline product was recrystallised from ethanol-ether to give dihydroisoquinoline hydrochloride (13 g., 76%) as needles m.p. 201-202° (lit.,[49] 203-204°).

(B) A mixture of the amide (18) (1 g.), chloroform (20 ml.) and phosphorus pentachloride (1 g.) was left at room temperature. After 6 days the resulting mixture was worked up as above. Crystallisation of the product from ethanol gave the dihydroisoquinoline hydrochloride (0.4 g., 39%) m.p. 198-200°.

6-Methoxy-7-benzyloxy-1-(4-methoxy-3-benzyloxybenzyl)-3,4-dihydroisoquinoline methiodide (16). (Method of Jain).[50]

The dihydroisoquinoline (17) (liberated from the corresponding hydrochloride 12 g., under nitrogen) in dry benzene (25 ml.) was treated with methyl iodide (8 ml.). The resulting mixture when left at room temperature for 20 hrs. gave the crystalline methiodide (13 g., 90.4%) m.p. 197-198° (lit.,[50] 196-198°).

6-Methoxy-7-benzyloxy-1-(4-methoxy-3-benzyloxybenzyl)-1,2,3,4-tetrahydro-2-methylisoquinoline (20).

(A) (Method of Jain).[50] To a suspension of the methiodide (16) (4.7 g.) in methanol (150 ml.) at room temperature (24°) was added sodium borohydride (1 g.) during 1 hr. with stirring. After 2 hrs. the solvent was removed, the residue treated with 4N-sodium hydroxide (15 ml.), extracted with ether (40 x 3 ml.), and chloroform (40 x 2 ml.). The ether extract was washed with water, dried and the solvent was evaporated. The residue was chromatographed on alumina (grade III, 30 g.). Elution with benzene gave the tetrahydro compound, crystallised from benzene-petroleum ether as needles m.p. 90-91° (lit.,[49,50] 108° and 89°).

The chloroform extract gave a compound (1.89 g.) m.p. 174-176°, $\nu_{max.}$ 2400 (B-H) and 710 (B-N) $cm.^{-1}$, n.m.r. bands in $CDCl_3$ at τ 6.65 (3H), 6.24 (3H), 6.20 (3H), 4.9, 4.1, 3.34 and 2.68. This compound did not form a hydrochloride on treatment with ethanolic hydrogen chloride and could not be hydrolysed with conc. hydrochloric acid. Attempted oxidation of the boron containing compound with manganese dioxide and chromic acid did not afford the desired amine.

(±)-Reticuline (21).

(A) A mixture of OO-dibenzylreticuline (250 mg.), ethanol (1 ml.) and conc. hydrochloric acid (1.5 ml.) was heated on a steam bath. After 2 hrs. the resulting mixture was treated with 4N-sodium hydroxide (6 ml.), shaken with ether (5 x 3 ml.) and solid carbon dioxide added. The liberated base was extracted with chloroform (5 x 4 ml.), washed with water and dried. Evaporation of the solvent gave (±)-reticuline (210 mg., 56%) as a colourless foam. The picrate m.p. 191-193° (lit.,[50] 190-192°).

(B) (±)-OO-Dibenzylreticuline (150 mg.) in ethanol (8 ml.) was treated with 10% palladium on charcoal (120 mg.). The hydrogen was taken up immediately (2 mole equi. in 30 min.). After 2 hrs. the catalyst was filtered off and the solvent was evaporated to give (±)-reticuline (92 mg., 92%). The picrate m.p. 190-193°.

Resolution of (±)-OO-Dibenzylreticuline.

(±)-OO-Dibenzylreticuline (2 g.) was treated with (+)-dibenzoyltartaric acid (1 g.). The resulting salt was fractionally crystallised from benzene-ether, benzene, and ethanol. The crystalline salt m.p. 134°, $[\alpha]_D$ -20° (C 1.0 in chloroform) was decomposed with 4N-sodium

hydroxide. The liberated base was extracted with chloroform (5 x 4 ml.) washed with water, dried and the solvent evaporated. The residue chromatographed on alumina (grade III, 25 g.) and eluted with benzene. The product on fractional crystallisation from ethanol gave (-)-OO-dibenzylreticuline (200 mg.) as needles m.p. 94-95°, $[\alpha]_D$ -46° (C 1.0 in chloroform) (lit.,[53] -44° in chloroform).

(-)-Reticuline (29).

(-)-OO-Dibenzylreticuline (160 mg.) in ethanol (8 ml.) was hydrogenolysed with 10% palladium on charcoal (100 mg.) in the usual way to give (-)-reticuline (85 mg.). The base hydrochloride $[\alpha]_D$ -72° (C 0.5 in water) (lit.,[53] -73° in water).

(+)-OO-Dibenzylreticuline.

The partially resolved OO-dibenzylreticuline was treated with (-)-dibenzoyltartaric acid (600 mg.). The resulting salt was fractionally crystallised from benzene-ether, benzene, and ethanol to give a crystalline salt m.p. 136°, $[\alpha]_D$ +25° (C 1.1 in chloroform). This was decomposed with 4N-sodium hydroxide and the liberated base was extracted with chloroform (5 x 4 ml.) washed with water, dried and the solvent evaporated. The residue was

chromatographed on alumina (grade III, 20 g.) and eluted with benzene. The product on fractional crystallisation from ethanol gave (+)-OO-dibenzylreticuline (190 mg.) m.p. 94-95°, $[\alpha]_D$ +46° (C, 1.0 in chloroform) (lit.,[53] +44° in chloroform).

(+)-Reticuline (33).

(+)-OO-Dibenzylreticuline (150 mg.) in ethanol (8 ml.) was hydrogenolysed with 10% palladium on charcoal (100 mg.) and was worked up in the usual way to give (+)-reticuline (83 mg.). The base hydrochloride $[\alpha]_D$ +72° (C 0.5 in water) (lit.,[53] +73° in water).

Exchange Experiments.

Exchange experiments with (±)-reticuline were carried out in the same manner as described earlier (p.153).

The n.m.r. spectrum of (±)-reticuline in deutero-chloroform gave bands at τ 7.53 (3H singlet), 6.14 (6H singlet), 3.65 (1H singlet), 3.45 (1H singlet), 3.35 (1H singlet) and doublets at τ 3.23 and 3.37.

(A) A mixture of (±)-reticuline (100 mg.), potassium tert.-butoxide (90 mg.) and deuterium oxide (0.6 ml.) was heated at 100° for 113 hrs. The resulting mixture

was diluted with water (1.2 ml.), solid carbon dioxide added, the liberated base extracted with chloroform (5 x 3 ml.), washed with water, dried and the solvent was evaporated. The residue was chromatographed on alumina (grade V, 12 g.). Elution with chloroform:ethanol (100:4) gave the deuterated (±)-reticuline (87 mg.). The n.m.r. spectrum of this compound in deuterochloroform showed bands at τ 7.54 (3H singlet), 6.15 (6H singlet), 3.46 (1H singlet) and 3.28 (1H singlet).

(B) (±)-Reticuline (60 mg.) was heated at 100° with deuterium oxide (.5 ml.) containing thionyl chloride (32 mg.). After 40 hrs. the resulting solution was treated with sodium hydrogen carbonate and buffered with solid carbon dioxide. The liberated base was extracted with chloroform (5 x 4 ml.) and worked up as above. The product was chromatographed on alumina (grade V, 20 g.), elution with chloroform:ethanol (100:4) gave the deuterated (±)-reticuline. The n.m.r. spectrum of the compound gave signals only at τ 7.55 (3H singlet), 6.16 (6H singlet).

Tritium labelled Reticuline.

Tritiated (±)-reticuline. (±)-Reticuline (100 mg.) in tritiated water (0.3 ml., 0.5 c.) containing thionyl

chloride (48 mg.) was heated for 96 hrs. The resulting mixture was worked up as above to give tritium labelled (±)-reticuline (90 mg.).

Tritiated (-)-reticuline. A mixture of (-)-reticuline (66 mg.), tritiated water (0.4 ml.) and thionyl chloride (40 mg.) was heated for 116 hrs. The resulting mixture on working up in the usual way gave tritium labelled (-)-reticuline (54 mg.). The base hydrochloride $[\alpha]_D$ -72^o (C 0.5 in water).

Tritiated (+)-reticuline. A mixture of (+)-reticuline (80 mg.), tritiated water (0.5 ml.) and thionyl chloride (48 mg.) was heated for 120 hrs. The resulting mixture was worked up in the usual way to give tritium labelled (+)-reticuline (60 mg.). The base hydrochloride $[\alpha]_D$ $+72^o$ (C 0.6 in water).

Compound	Activity (d.p.s./mg.)
(±)-Reticuline	7.80×10^5
(-)-Reticuline	9.08×10^5
(+)-Reticuline	1.13×10^6

'Phenolic'-dihydrothebaine.

Thebaine (15 g.) was reduced with sodium (2.9 g.) and liquid ammonia (160 ml.) at -25° according to the method of Bentley and Robinson[55] to give the 'phenolic'-dihydrothebaine (13.4 g., 88.7%) as needles m.p. 154° (lit.,[55] 154°).

O-Acetyl-'phenolic'-dihydrothebaine.

The 'phenolic'-dihydrothebaine (12.9 g.) was acetylated with pyridine (40 ml.) and acetic anhydride (8 ml.) at room temperature in the usual way. The crude product was chromatographed on alumina (grade III, 450 mg.). Elution with benzene:ethyl acetate (8:2) (fractions 1-30) gave the desired acetate (12 g., 81.8%), $\nu_{max.}$ 1761 (OAc), 1663 cm.$^{-1}$.

O-Acetylsalutaridine.

O-Acetyl-'phenolic'-dihydrothebaine (12 g.) in dry dioxane (200 ml.) was oxidised with selenium dioxide (4.4 g.), and manganese dioxide (48 g.) according to the method of Barton *et al.*[11] The crude product (10.8 g.) was chromatographed on alumina (grade III, 500 g.). Elution with benzene:chloroform (8:2) gave O-acetylsalutaridine (7.2 g., 57.7%) as needles m.p. 171° (lit.,[56] 171°), $\nu_{max.}$ 1761, 1702 and 1663 cm.$^{-1}$.

Salutaridine (30)

O-Acetylsalutaridine (5.5 g.) in ethanol (100 ml.) was hydrolysed with 2-N-sodium hydroxide (40 ml.) according to the method of Barton et al.[11] The crude product (2.9 g.) was chromatographed on alumina (grade III, 200 g.). Elution with ethyl acetate:chloroform (1:1) gave salutaridine which was crystallised from ethyl acetate as needles (2.1 g., 42.8%) m.p. 198° (lit.,[56] 197°), $\upsilon_{max.}$ 3550, 1670, 1640, 1620 and 1484 cm.$^{-1}$.

Oxidation of (±)-Reticuline.

With Manganese dioxide.

The tritium labelled (±)-reticuline (10 mg., 0.2 mc.) diluted with inactive material (58 mg.) was dissolved in chloroform (5 ml.) and shaken with active manganese dioxide (236 mg.). After 3 hrs. the inactive salutaridine (58 mg.) was added to the resulting mixture and the manganese dioxide was filtered off. The solvent was removed, the residue was chromatographed on alumina (grade III, 25 g.) and elution with benzene:chloroform (1:1) gave the radioactive salutaridine (54 mg.).

Salutaridine	Activity (d.p.m./mMole)
1st chrom.	1.96×10^6
2nd chrom.	1.62×10^6
3rd chrom.	1.50×10^6

The product after 3rd chromatography was converted into thebaine.

Conversion into Thebaine.

The radioactive salutaridine (52 mg.) in methanol (2 ml.) was treated with sodium borohydride (70 mg.). After 45 min. the methanol was removed under reduced pressure. The residue was extracted with chloroform (3 x 3 ml.), washed with water and the solvent was evaporated. The mixture of the dienols was treated with 1N-hydrochloric acid (1 ml.) and left at room temperature. After 1 hr. the resulting mixture was basified with 4N-sodium hydroxide (2.5 ml.) and the free base was extracted with chloroform (4 x 4 ml.), washed with water, dried and evaporated. The residue (16 mg.) was chromatographed on alumina (grade III, 25 g.). Elution with benzene: chloroform (1:1) gave radioactive thebaine (10 mg.)

Thebaine	Activity (d.p.m./mMole)
After chrom.	1.53×10^5
1st cryst.	1.38×10^5
2nd cryst.	6.80×10^4
3rd cryst.	6.77×10^4

Radiochemical yield 0.011%,

on the basis of thebaine counts.

With potassium ferricyanide.

(A) To a mixture of tritium labelled (±)-reticuline (5 mg., 0.1 mc.), inactive (±)-reticuline (51 mg.), N-sodium hydrogen carbonate (10 ml.) and water (80 ml.), in an atmosphere of nitrogen and cooled to 0°, was added a solution of potassium ferricyanide (114 mg.) in water (50 ml.) during 1 hr. with vigorous stirring. After 1 hr. inactive (+)-salutaridine (50 mg.), was added and the resulting mixture was extracted with chloroform (25 x 4 ml.), washed with water, dried and evaporated. The residue was chromatographed on alumina (grade III, 30 g.). Elution with benzene:chloroform (1:1) gave radioactive salutaridine (38 mg.).

Salutaridine	Activity (d.p.m./mMole)
After 3rd chrom.	9.25×10^4
1st cryst.	5.29×10^4
2nd cryst.	4.53×10^4
3rd cryst.	4.63×10^4

The alumina column, after separating the salutaridine, when eluted with chloroform gave a product (8 mg.) which was crystallised from chloroform-ether as needles m.p. 243-245°, $\nu_{max.}$ 3560, 1670, 1642 and 1622 $cm.^{-1}$.

Conversion into Thebaine.

The radioactive salutaridine (30 mg., 4.6×10^4 d.p.m./mMole) was reduced with sodium borohydride (50 mg.) in methanol. The mixture of dienols was converted into thebaine (6 mg.) as above.

Thebaine	Activity (d.p.m./mMole)
cryst.	4.57×10^4
recryst.	4.42×10^4
Radiochemical yield	0.008%.

(B) To a mixture of radioactive (±)-reticuline (6.4 mg., 0.12 mc.), inactive (±)-reticuline (46 mg.), chloroform (50 ml.), water (50 ml.) and sodium hydrogen carbonate

(200 mg.) under nitrogen at room temperature, was added a solution of potassium ferricyanide (110 mg.) in water (50 ml.) during $\frac{1}{2}$ hr. with stirring. After 1 hr. the inactive (+)-salutaridine (50 mg.) was added. The chloroform layer was separated off. The aqueous layer was extracted with chloroform (15 x 3 ml.). The combined chloroform solution was washed with water, dried and the solvent was evaporated. The residue was chromatographed on alumina (grade III, 25 g.). Elution with chloroform: benzene (1:1) gave the radioactive salutaridine (45 mg.) which was crystallised from ethyl acetate to a constant activity.

Salutaridine 1.66×10^5 d.p.m./mMole.

Radioactive salutaridine (40 mg.) was converted into radioactive thebaine (9 mg.) as earlier

Thebaine 1.69×10^5 d.p.m./mMole.

yield 0.015% .

With ferric chloride.

To a solution of radioactive (±)-reticuline (4.2 mg., 0.08 mc.) diluted with inactive material (50 mg.) in water (100 ml.) was added a solution of ferric chloride

(130 mg.) in water (50 ml.). The resulting mixture under nitrogen was stirred for 20 hrs. at room temperature (23°) and inactive (+)-salutaridine (51 mg.) was added. The acidic solution was neutralised with sodium hydrogen carbonate, extracted with chloroform (12 x 5 ml.), washed with water, dried and evaporated. The residue was chromatographed on alumina (grade III, 25 g.). Elution with chloroform:benzene (1:1) gave a product which was contaminated with radiochemical impurities. This product was crystallised from ethyl acetate to a constant activity.

(+)-Salutaridine	1.4×10^4 d.p.m./mMole.
Radiochemical yield	0.0007%.

Oxidation of Reticuline and Salutaridine.

To a mixture of (±)-reticuline (55 mg.), (+)-salutaridine (50 mg.), water (80 ml.) and N-sodium hydrogen carbonate (10 ml.) at 0° under nitrogen was added a solution of potassium ferricyanide (114 mg.) in water (50 ml.) with vigorous stirring during $\frac{1}{2}$ hr. After 1 hr. the resulting solution was extracted with chloroform (15 x 5 ml.), washed with water, dried and the solvent was evaporated. The residue was chromatographed on alumina (grade III, 30 g.). Elution with benzene:chloroform

(1:1) did not give salutaridine. The subsequent chloroform eluate gave a compound which was crystallised from chloroform-ether as needles (30 mg.) m.p. 242-245°, $\nu_{max.}$ 3560, 1670, 1642 and 1622 cm.$^{-1}$. The absorption peak at 1482 cm.$^{-1}$ present in salutaridine was absent.

The chloroform:ethanol (100:4) eluate gave reticuline (40 mg.).

Oxidation with Fremy's salt.

To a mixture of radioactive (±)-reticuline (4.4 mg., 0.088 mc.), inactive (±) reticuline (24 mg.), (+)-salutaridine (40 mg.), sodium acetate (70 mg.), chloroform (50 ml.) and water (50 ml.) at 0° under nitrogen was added a solution of potassium nitrosodisulphonate (53 mg.) in water (50 ml.) during $\frac{1}{2}$ hr. with stirring. After 1 hr. the chloroform layer was separated and combined with the chloroform extract (20 x 2 ml.) of the aqueous layer. The combined extract was washed with water, dried and evaporated. The residue was chromatographed on alumina (grade III, 30 g.), elution with benzene:chloroform (1:1) gave crude salutaridine which was crystallised from ethyl acetate to a constant activity.

(+)-salutaridine	7.66×10^4 d.p.m./mMole
Radiochemical yield	.0054%

Oxidation of (-)-Reticuline.

To a mixture of tritium labelled (-)-reticuline (10 mg., 0.245 mc.), inactive (±)-reticuline (43 mg.), water (50 ml.) and 2N-sodium hydrogen carbonate (25 ml.) under nitrogen and cooled to 0° was added a solution of potassium ferricyanide (114 mg.) in water (50 ml.) during 45 min. with stirring. After 2 hrs. inactive (+)-salutaridine (90 mg.) was added. The resulting mixture was extracted with chloroform (30 x 4 ml.), washed with water, dried and evaporated. The residue was chromatographed on alumina (grade III, 25 g.), elution with benzene: chloroform (1:1) gave the radioactive salutaridine (70 mg.) which was crystallised successively from ethyl acetate, chloroform-ethyl acetate, and ethanol-ether.

1st cryst.	210 d.p.m./mg.
2nd cryst.	210 "
3rd cryst.	206 "
(+)-Salutaridine	6.8×10^4 d.p.m./mMole.
Radiochemical yield	0.044%.

The radioactive salutaridine (55 mg., 208 d.p.m./mg.) was converted into thebaine in the usual way.

The derived thebaine was crystallised from ethanol to a constant activity.

Thebaine	6.6×10^4 d.p.m./mMole.

Oxidation of (+)-Reticuline.

To a mixture of tritium labelled (+)-reticuline (12 mg., 0.366 mc.), inactive (±)-reticuline (27 mg.), water (50 ml.) and 2N-sodium hydrogen carbonate (25 ml.) at 0° under nitrogen was added a solution of potassium ferricyanide (78 mg.) in water (50 ml.) during 45 min. After 2 hrs. inactive (+)-salutaridine (80 mg.) was added, the resulting mixture extracted with chloroform (30 x 3 ml.) washed with water, dried and evaporated. The residue was chromatographed on alumina (grade III, 25 g.), elution with benzene:chloroform (1:1) gave the product (72 mg.).

After chrom.	3276 d.p.m./mg.

The sample was crystallised from ethyl acetate, and chloroform-ethyl acetate.

2nd cryst.	60 d.p.m./mg.
4th cryst.	19 d.p.m./mg.

Oxidation of (±)-Reticuline.

Tritium labelled (±)-reticuline (8.6 mg., 0.164 mc.) diluted with inactive (±)-reticuline (45 mg.) was oxidised with potassium ferricyanide (115 mg.) exactly under similar conditions as above. After 2 hrs. inactive (+)-salutaridine (73 mg.) was added and the resulting mixture was worked up in the usual way. The product was chromatographed on alumina (grade III, 30 g.), elution with benzene:chloroform (1:1) gave the radioactive salutaridine (68 mg.).

1st chrom.	1389 d.p.m./mg.

The crude salutaridine was rechromatographed on alumina (grade III, 40 g.) elution with benzene:chloroform (3:2) gave salutaridine (52 mg.).

2nd chrom.	332 d.p.m./mg.

The product was crystallised from ethyl acetate, and chloroform-ethyl acetate.

1st cryst.	139 d.p.m./mg.
2nd cryst.	122 "
3rd cryst.	116 "
(+)-Salutaridine	3.8×10^4 d.p.m./mMole.

Radiochemical yield 0.021%.

The radioactive (+)-salutaridine (40 mg.) was converted into thebaine in the usual way.

Thebaine 3.7×10^4 d.p.m./mMole.

The radiochemical yields of (+)-salutaridine in the above oxidations are corrected for loss of tritium.

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