STUDIES IN ALKALOID BIOSYNTHESIS

A thesis submitted by

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ABSTRACT.

The biosynthesis of alkaloids derived from the 1-benzylisoquinoline framework by intramolecular phenol coupling, and attempts to simulate the process in the laboratory, are reviewed.

Various Erythrina, aporphine and pro-aporphine alkaloids have been obtained from natural and synthetic sources. Hypothetic phenolic precursors have been prepared and feeding experiments, to elucidate the biosynthetic pathways to certain aporphine and pro-aporphine alkaloids, have been carried out.

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Alkaloid Biogensis and Phenol Coupling

Studies of alkaloid biosynthesis serve many purposes. They satisfy the interest in the means by which many complicated molecules are synthesised in nature, and help correlate and organise apparently unrelated compounds. Alkaloid biogenesis has a bearing on reaction mechanisms and aids the search for new and better chemical syntheses. New natural products may be predicted and unknown structures elucidated. The formation of alkaloids also has a relationship with enzymology and plant physiology, which is not yet understood.

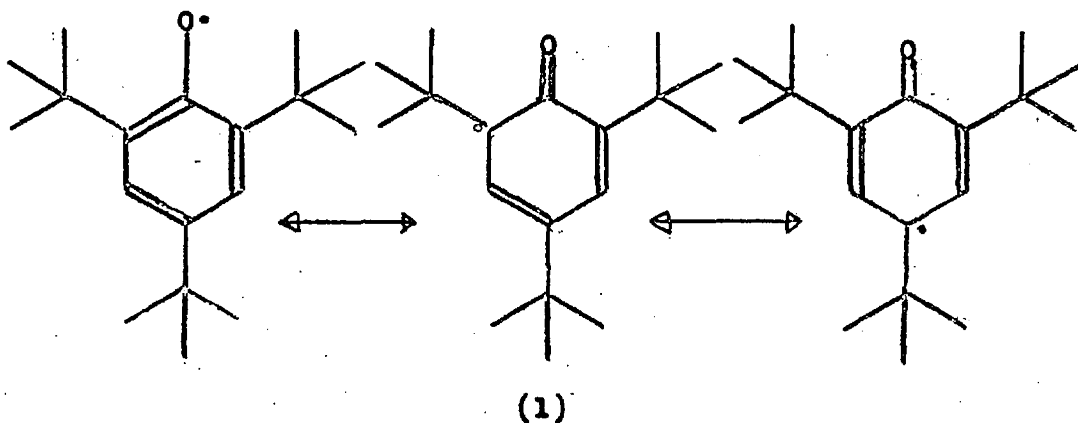
Many interesting theories of alkaloid biosynthesis have been contributed by, for example Winterstein and Trier [1], Robinson [2], Schöpf [3], Barton and Cohen [4], Woodward [5,6], Wenkert [7,8,9] and Thomas [10].

Evidence for biosynthetic pathways has come from biogenetic type synthesis at "physiological pH", normal organic syntheses and enzymatic syntheses. Co-occurrence of alkaloids and the existence of predicted intermediates has also been used as evidence but more convincing confirmation is derived from radioisotope (or in certain cases non-radioactive isotope) incorporations, particularly with specifically doubly-labelled precursors, although even then there is the possibility of forming artefacts [11] and a chance that the main biosynthetic pathway may not be the one observed.

There have been many reviews of alkaloid biosynthesis - Robinson [2], Woodward [5], Marion [12], Mothes [13], Battersby [14,15], Mothes and Schutte [16], Leete [17], Barton [18] and Ramstead and Agurell [19] - but only the later ones contain evidence from radioisotope incorporations.

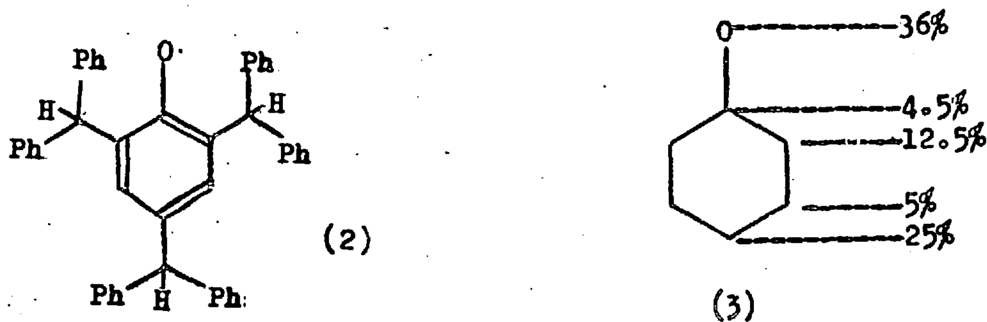
One idea that has been particularly fruitful in suggesting biosynthetic pathways is that of phenol oxidation [4,20], and recent progress in the investigation of phenol oxidation with particular reference to biogenetic schemes and biogenetic type syntheses has been reviewed [21,22,23].

Phenoxyl radicals can be produced by a one electron transfer oxidant, such as ferricyanide. The subsequent fate of the radical depends on the substitution pattern. Certain radicals, having <u>ortho</u> and <u>para</u> substituents carrying no α-hydrogen atoms (such as that derived from 2,4,6 - tri-t-butylphenol (1)), are stable and their e.s.r. spectra can be simply observed [24].



(1)

By investigating the radical (2) it has been shown that the free electron density is as depicted in the diagram (3)[25].



The radicals can be intercepted by radical trapping agents [26], reduced back to the original phenol or can undergo self-coupling to give dimers. Oxygen-oxygen coupling is not observed [27], possibly for thermodynamic reasons. Carbon-carbon coupling, however, is well-known, and it can occur either <u>ortho-ortho</u>, <u>para-para</u> or <u>ortho-para</u>, examples of each being found [26]. Carbon-oxygen coupling is also observed and the oxygen can couple with either the <u>ortho</u> or <u>para</u> positions. The actual coupling process could occur in one of three ways [21] - homolytic coupling, radical insertion or heterolytic coupling - and although evidence suggests that the chemical process involves simple homolytic coupling, each of the three possibilities could account for the coupling products; the mechanism of the biological process is not yet understood.

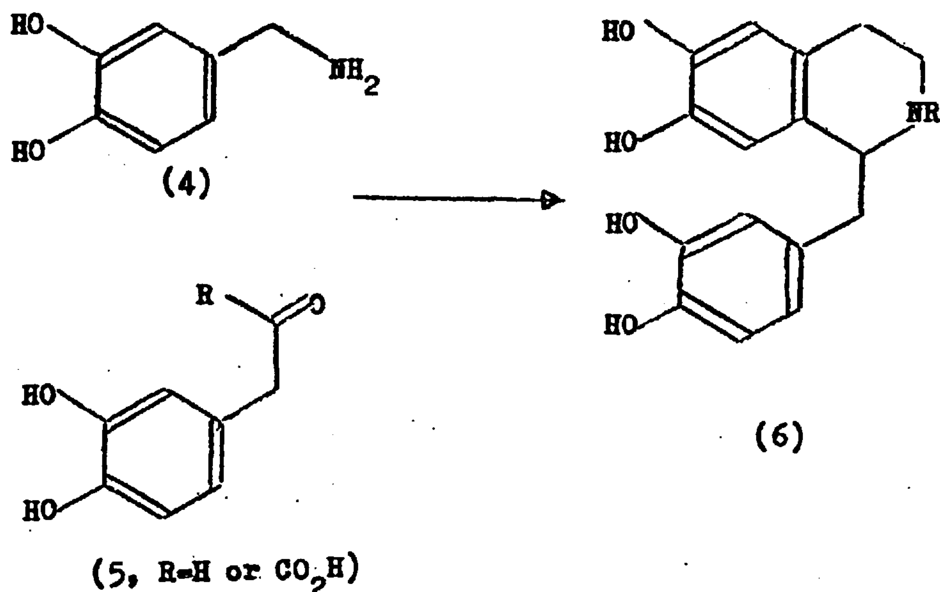
Several enzymes capable of performing phenol oxidations are known, the ractions usually being carried out using a peroxidase with hydrogen peroxide and a phosphate buffer [28]. However, these systems do not give optically active products [29], and several metabolites formed by phenol oxidation in nature are optically inactive (for example geodoxin [30]).

Barton and Cohen [4] recognised the possibility of the formation of a vast range of natural products using this reaction. By selectively protecting phenolic precursors with suitable groups (methyl or enzyme sites) the coupling could be directed to give a variety of compounds. In alkaloid biogenesis they suggested biosynthetic pathways to numerous alkaloids such as the morphine,

sinomenine, aporphine, cularine, Amaryllidaceae, Erythrina and bisbenzylisoquinoline groups, suggesting the participation of many intermediates which have subsequently been found in nature.

Origin of the Benzylisoquinoline Framework

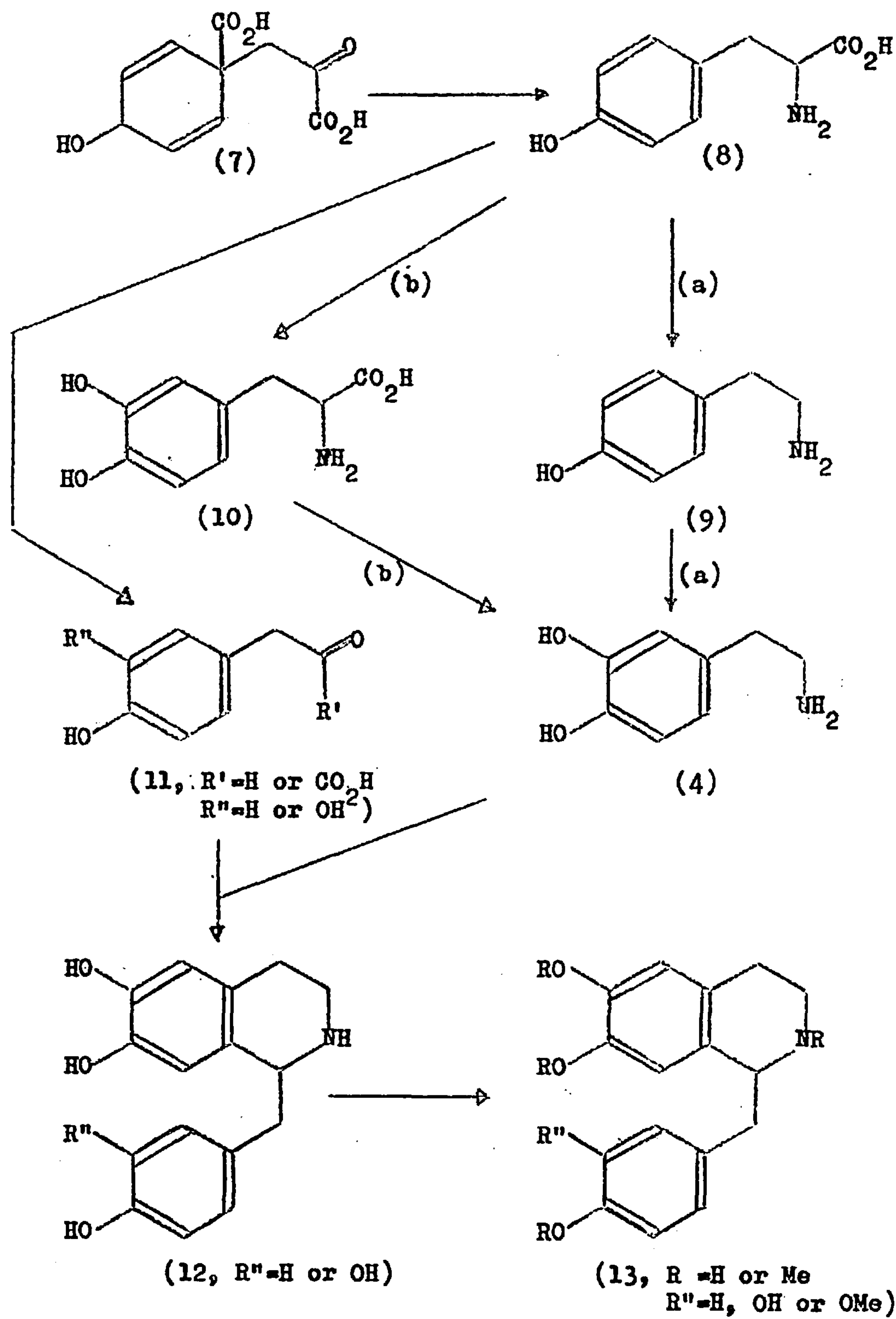
As early as 1910 Winterstein and Trier [1] suggested that the benzylisoquinoline skeleton (that is 1-benzyl - 1,2,3,4 - tetrahydroisoquinoline) is derived from two molecules of 3,4 - dihydroxyphenylalanine (10). This scheme was elaborated by Robinson [2], who suggested that 3,4 - dihydroxyphenethylamine (4) condensed with 3,4 - dihydroxyphenylacetaldehyde (5,R=H) or 3,4 - dihydroxyphenylpyruvic acid (5,R = CO_2H) to form the benzylisoquinoline framework (for example nor-laudanosoline, 6, R=H).



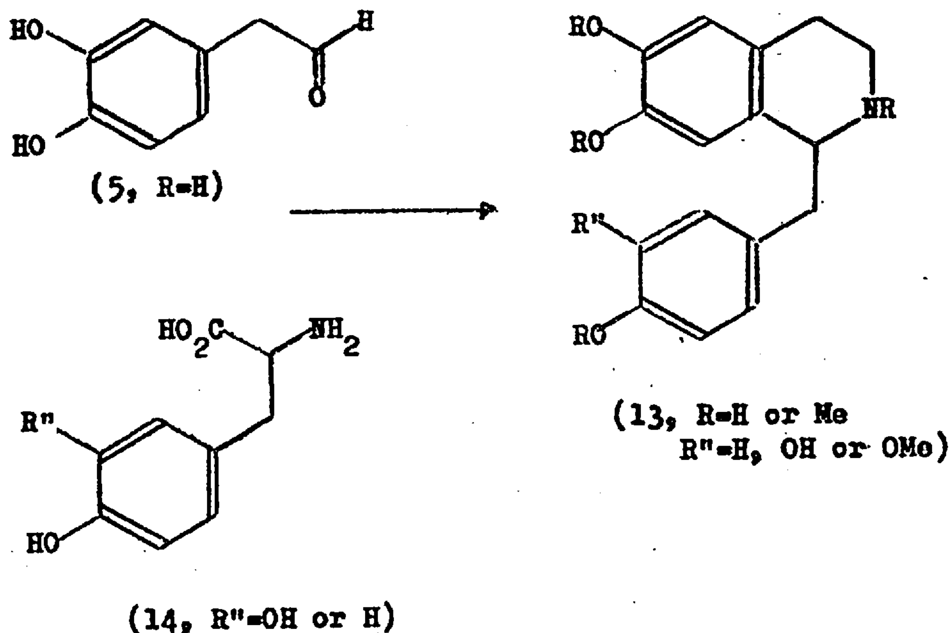
There have been several "model syntheses" of benzylisoquinolines, using either the aldehyde (5,R=H) or pyruvic acid (5,R = CO_2H), generally carried out under mild conditions (for example pH 7 at room temperature)[31].

Another proposal has been made by Wenkert[8], based on prephenic acid (7) as the precursor and not using tyrosine (8), but this has been disproved in some specific cases[32,33,34] and is probably not the main pathway for any benzylisoquinolines.

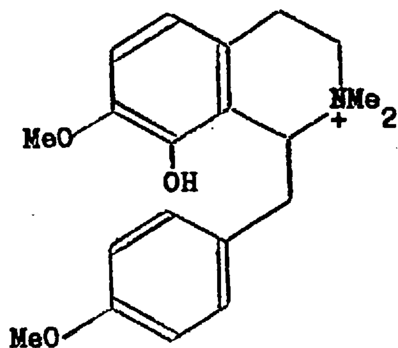
The most likely route is depicted in Scheme I. The evidence for this scheme is not conclusive and in particular it is not known from which of the two "halves" the _N_-atom is derived, for it is possible that the link is made by joining the aldehyde (5,R=H) to the amino-acid (14) to form the benzylisoquinoline (13), but the _in vitro_ experiments suggest it as in Scheme I.



SCHEME I



Most of the evidence so far obtained has been with tetraoxygenated benzylisoquinolines (13,R'' = OH or OMe), but it is likely the route is very similar for the trioxygenated compounds (13,R'' = H). It is also assumed that the route is the same for all plants. No evidence is yet available for the method by which benzylisoquinolines such as petaline (15)[35] and thalifendlerine (16)[36] obtain their "odd" oxygen substitution patterns; this also applies to the derived compounds such as cularine (17)[37] and atherospermidine (18)[38]. However for compounds such as berberastine (20), it appears the side chain hydroxylation occurs at an early stage, since 3,4-dihydroxyphenylethylamine and noradrenaline (19) are specifically incorporated[39].



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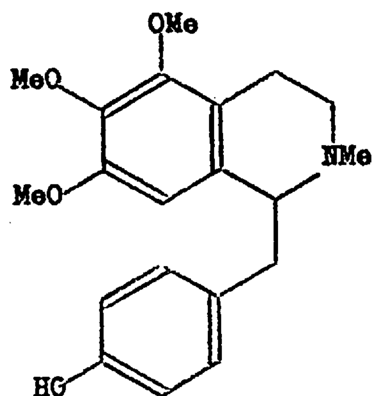

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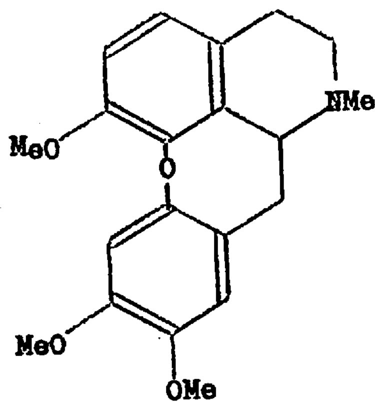

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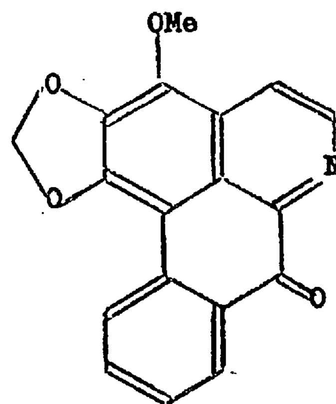

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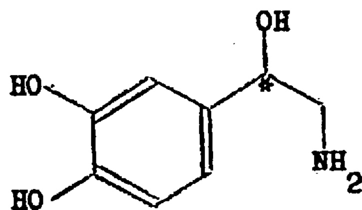

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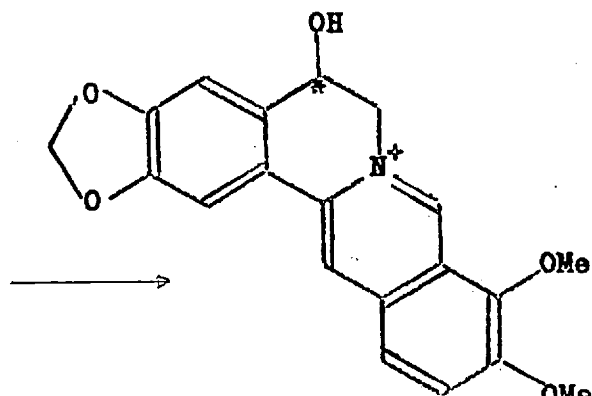
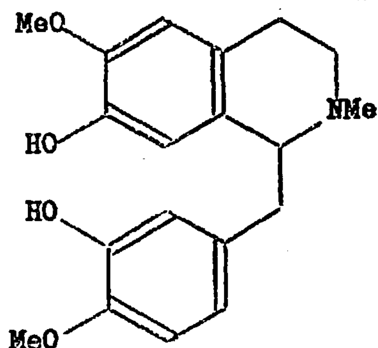
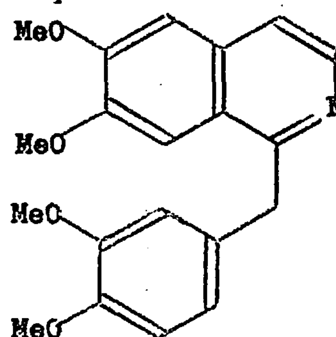


(20)

Most of the evidence for the steps in Scheme I is indirect, since the only tetrahydrobenzylisoquinoline whose biogenesis has been investigated is reticuline (21)[40]; the isoquinoline papaverine (22) has also been studied [41]. However, generally, evidence has been obtained with alkaloids more biogenetically remote, such as the berberine and morphine alkaloids, where the benzylisoquinoline skeleton is still recognisable, and which have been shown to be derived from benzylisoquinolines.



(21)

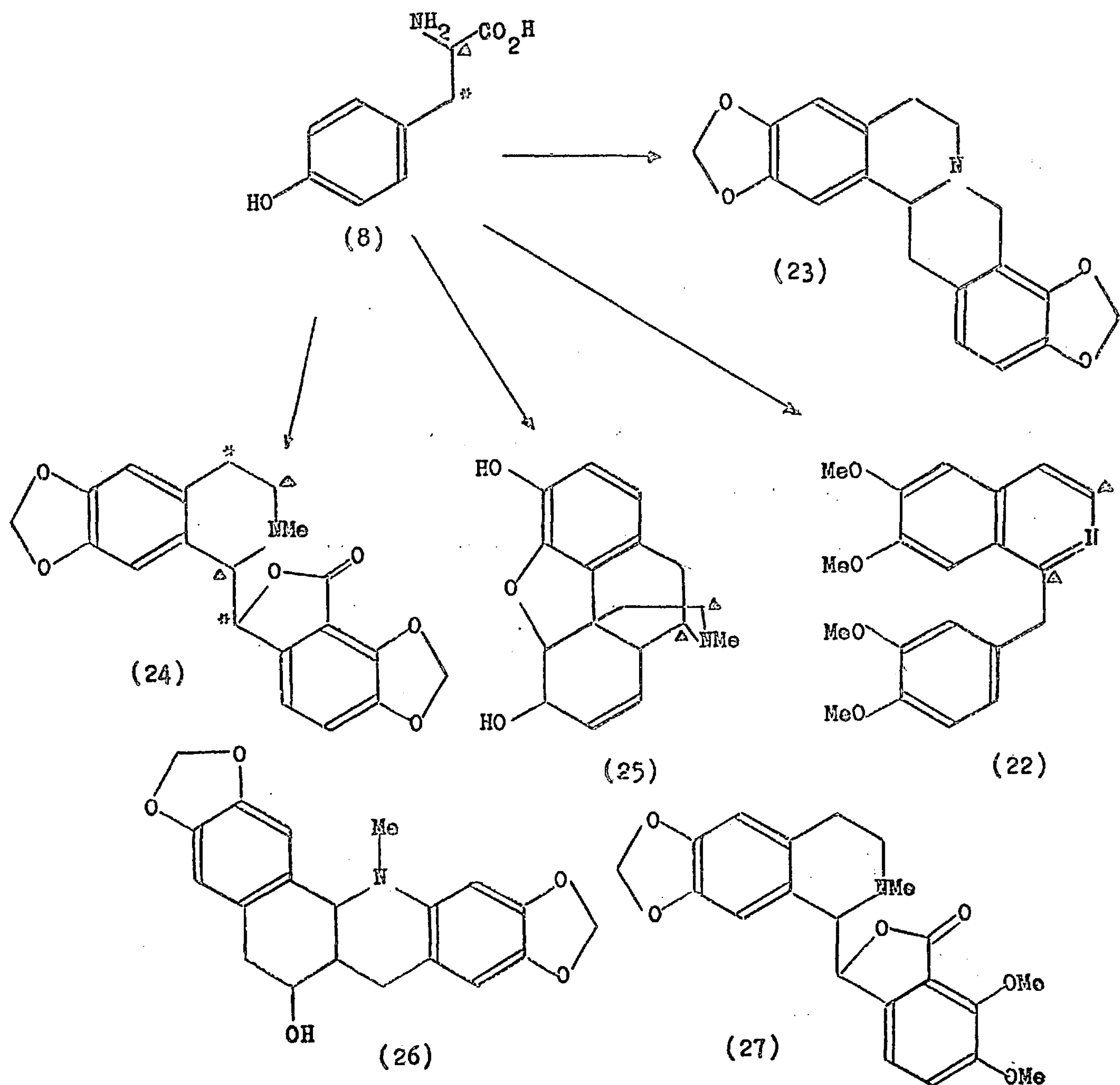


(22)

Phenylaline is often incorporated into alkaloids derived from isoquinolines, but generally less efficiently than tyrosine; Spenser and Gear[34] found that tyrosine is a better precursor for berberine (23) and hydrastine (24) and Leete[42] and Battersby[43] found tyrosine more efficiently incorporated into the morphine alkaloids than phenylalanine. The conversion of phenylalanine to tyrosine is known in mammals[44], but it has been shown not to occur in some plants[19,45].

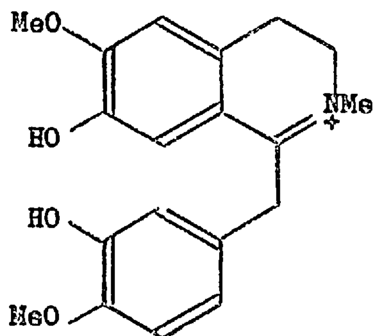
The first stage in Scheme I, the conversion of prephenic acid (7) into tyrosine (8), is well established[46], but for the

biosynthesis of 3,4-dihydroxyphenylethylamine two routes are possible, (a) or (b). The conversion of tyrosine into 3,4-dihydroxyphenylalanine is known [47] and it has been shown to occur in plants [48], but some experiments suggest route (a) [49] whilst others suggest route (b)[50].



Other evidence bearing on the biogenetic route is that tyrosine is not equally incorporated into both "halves" of hydrastine (24)[34], chelidonine (26)[51], or morphine (25) when fed over a comparatively short time with $^{14}CO_2$ [52]. It has also been observed that 3,4-dihydroxyphenylethylamine (4) is incorporated into only one "half" (the upper "half") of hydrastine (24) [33], berberine (23) [33], morphine (25) [53,54] and chelidonine (26) [54], this confirming that one of the condensing units is 3,4-dihydroxyphenylethylamine (4) or something closely derived from it, such as the aldehyde (5,R=H), and the other is a compound that cannot be derived from 3,4-dihydroxyphenylethylamine, such as 3,4-dihydroxyphenylalanine or the pyruvic acid.

It is likely that O and N-methylation occur at the benzylisoquinoline stage and Battersby's feeding experiments with nor-laudanosoline (6,R=H) to give papaverine (20)[55], reticuline (21)[40], morphine (25)[55] and narcotine (27)[56], and with laudanosoline (6,R=Me) to give berberine (23)[57] support this idea. It is also likely that the conversion of nor-laudanosoline (6,R=H) to reticuline (21) proceeds via 1,2-dehydroreticuline (28)[58]. It has also been shown that tetrahydropapaverine (29)[55] and the reticuline isomers protosinomenine (30) orientaline (31) and the benzylisoquinoline (32)[58] are not incorporated into the morphine alkaloids.



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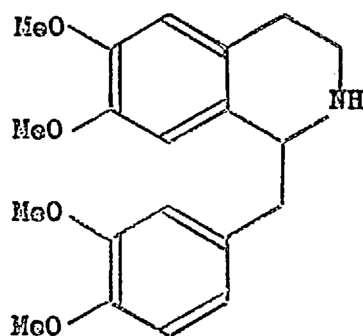
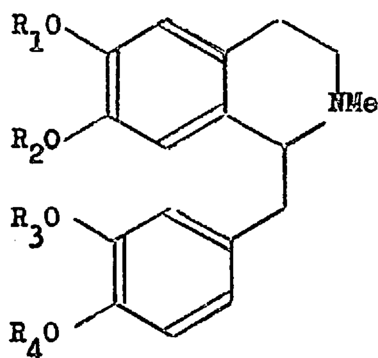


(29)



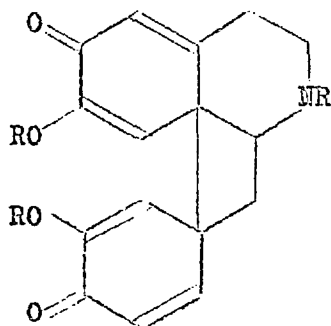
	R_1	R_2	R_3	R_4
(21)	Me	H	H	Me
(30)	H	Me	H	Me
(31)	Me	H	Me	H
(32)	H	Me	Me	H

Our and previous[59] experiments with the trioxygenated system agree, since the nor-benzylisoquinolines are incorporated into crotonosine, anonaine and roemerine as are the N-methyl and O-methyl compounds.

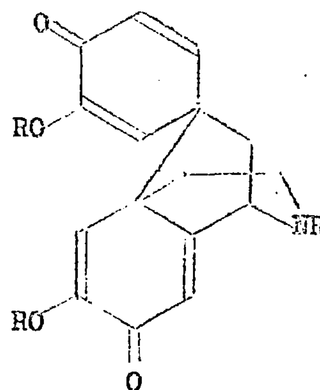
Alkaloids Derived from Benzylisoquinolines by Intramolecular Phenol Coupling.

Various intramolecular modes of coupling of relatively few benzylisoquinolines theoretically can give rise to a large number of alkaloids. The possibilities are summarised in Scheme II.

It must be stressed that for the most part this Scheme is speculative and in certain cases duplication occurs, in the sense that one pathway is obviously very much more likely than another. Other possibilities of coupling can exist, but these involve unknown alkaloids. Furthermore unlikely couplings to give di-dienones (33 and 34) are ignored.



(33)

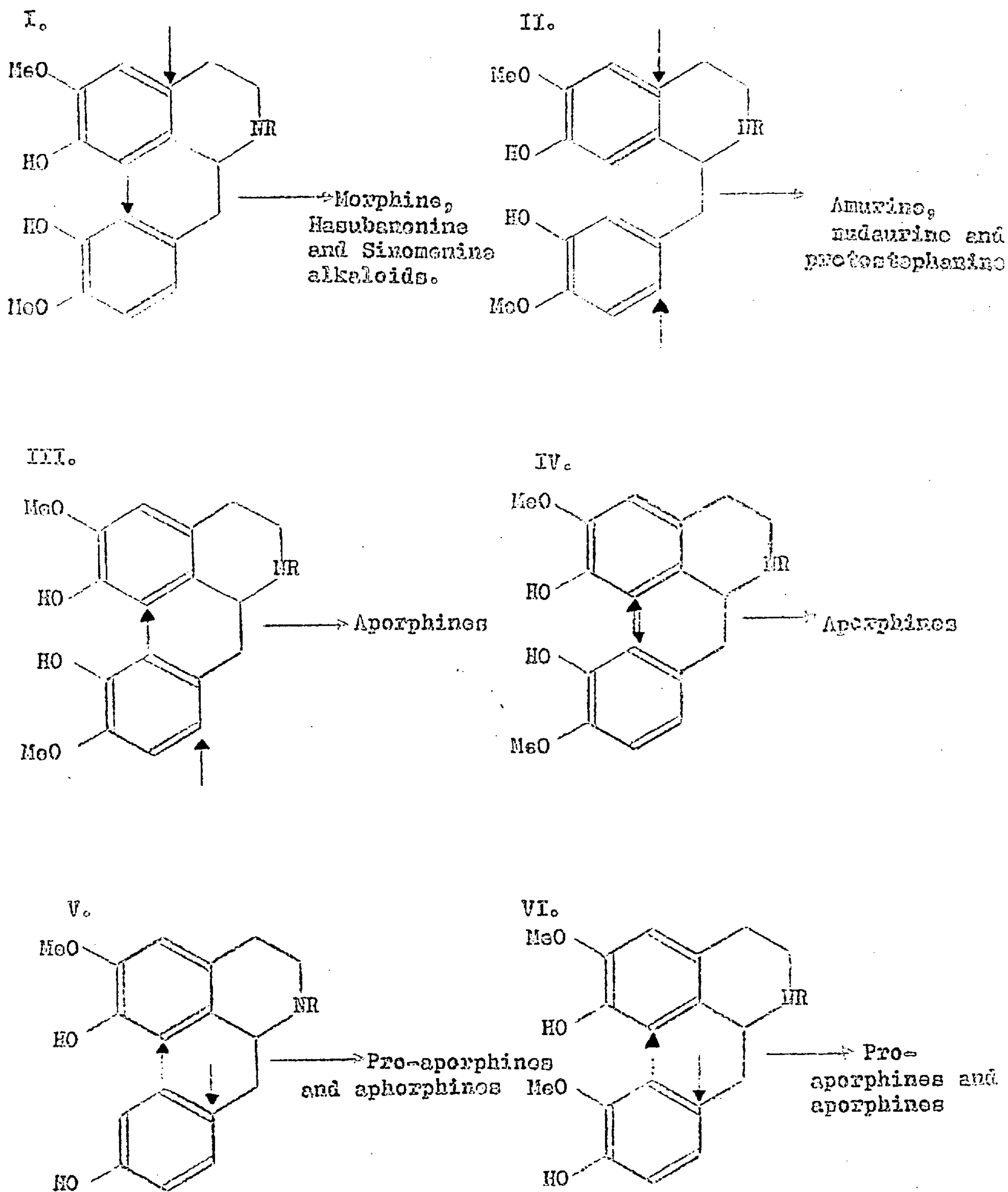


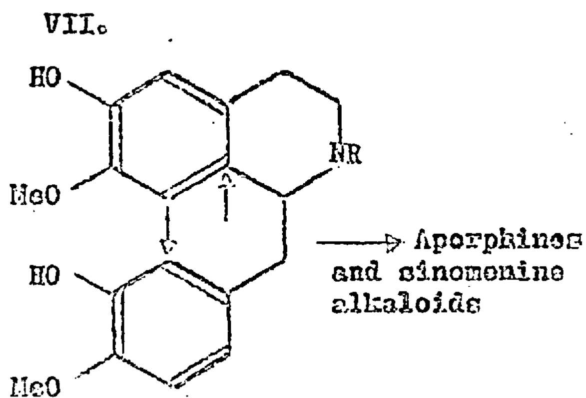
(34)

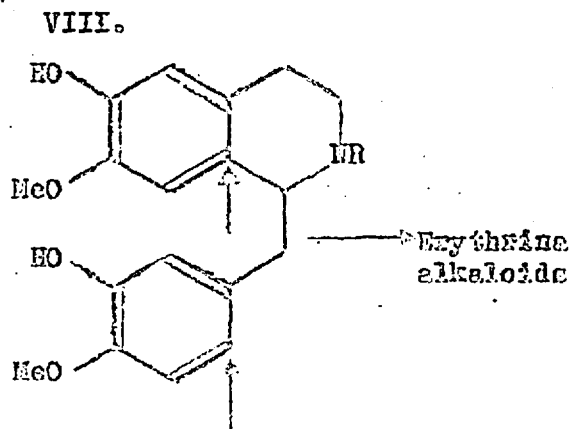
The particular modes of coupling will be discussed in detail in the light of attempts to simulate the process *in vitro* and tracer experiments with plants.

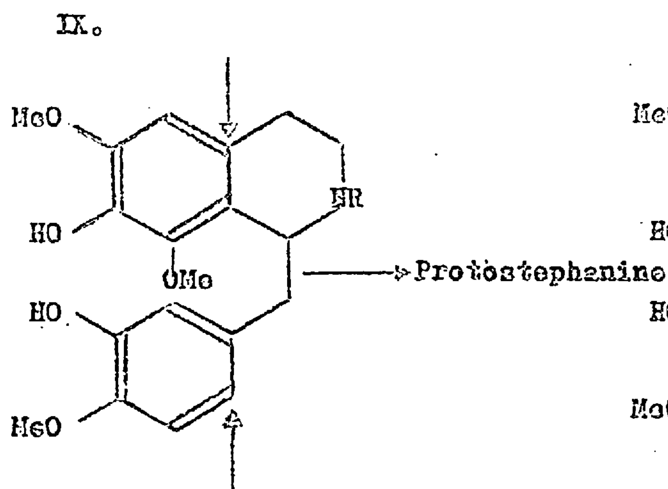
SCHEME II

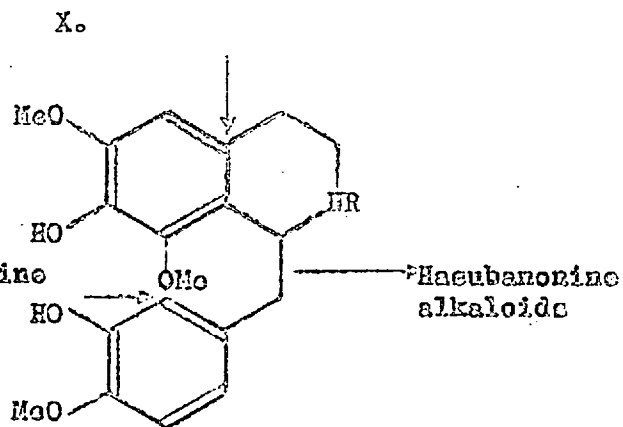
R=H, Me, or Me_2 with quaternary nitrogen.

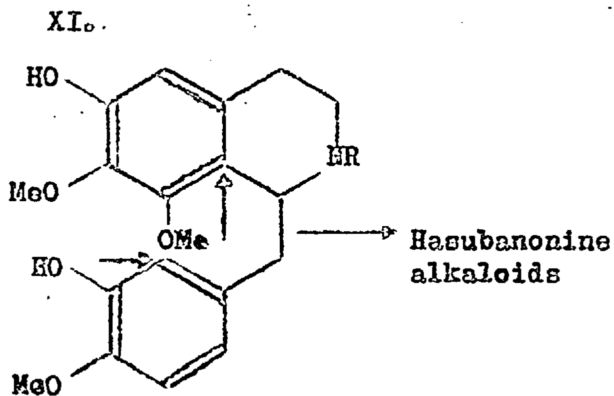


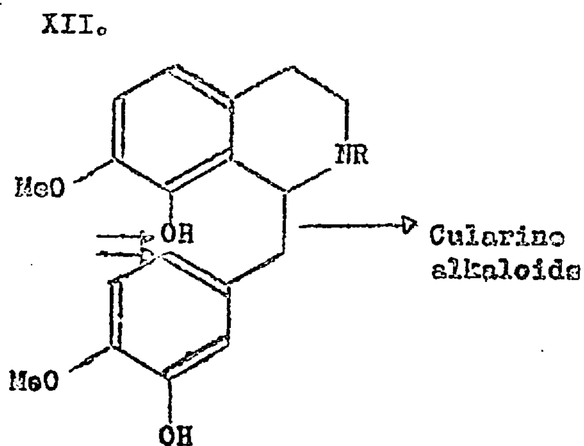

VII.
HO
MeO
NR
HO
MeO
Aporphines and sinomenine alkaloids


VIII.
HO
MeO
NR
HO
MeO
Erythrina alkaloids

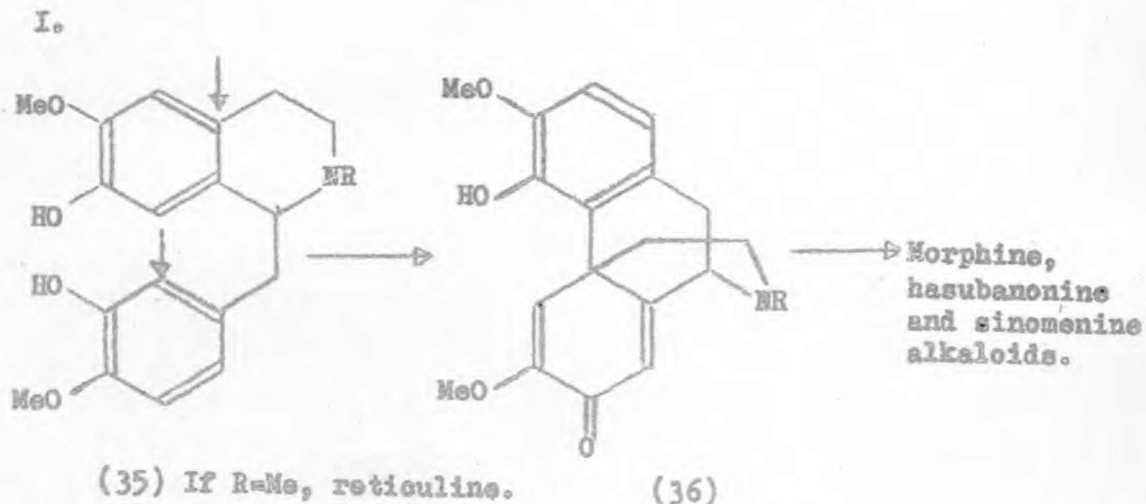

IX.
MeO
HO
NR
OMe
HO
MeO
Protostephanine


X.
MeO
HO
NR
OMe
HO
MeO
Hasubanonine alkaloids


XI.
HO
MeO
NR
OMe
HO
MeO
Hasubanonine alkaloids

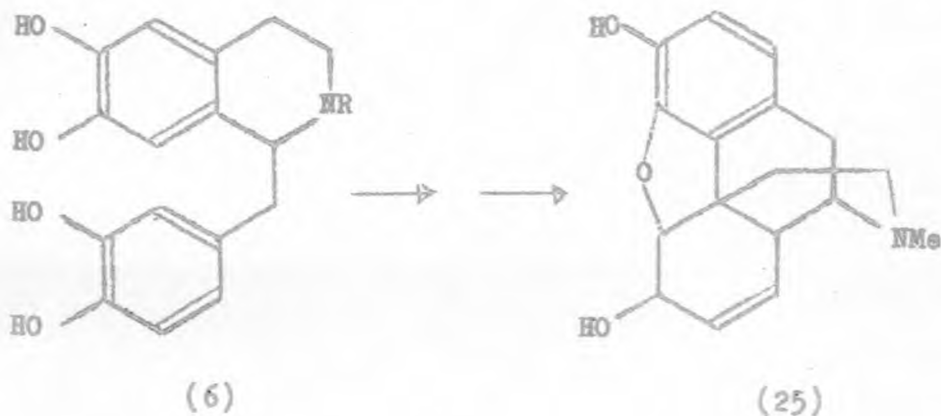

XII.
MeO
NR
OH
MeO
OH
Cularine alkaloids

I.



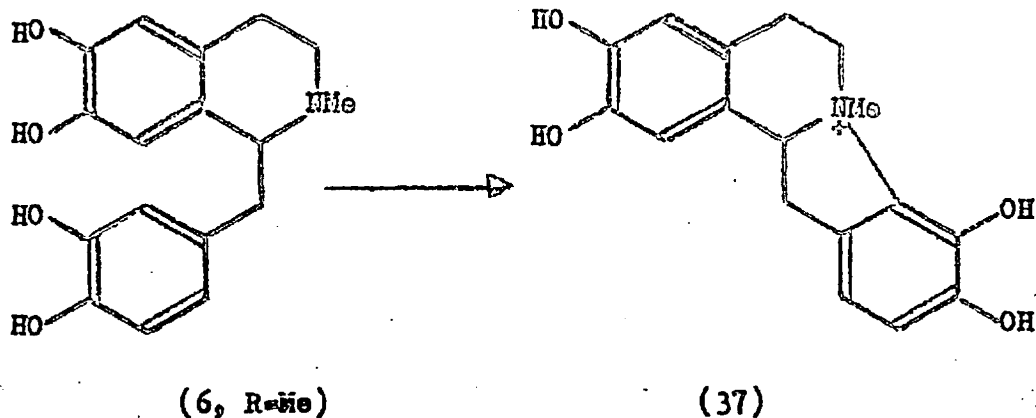
(35) If R=Me, reticuline. (36)

In 1925 is was suggested by Gulland and Robinson[60] that morphine (25) was derived from a benzylisoquinoline and Robinson[2,61] elaborated the scheme suggesting laudanosoline (6,R=Me) was oxidised to give the morphine alkaloids (for example 6,R=Me → 25).



(6) (25)

Attempts to synthesise morphine and aporphine derivatives by the oxidation of laudanosoline (6, R=Me)[62] succeeded in producing only a dibenzotetrahydropyrrocoline derivative (37); subsequently a group of alkaloids corresponding to this structure was found in nature [63].



(6, R=Me) (37)

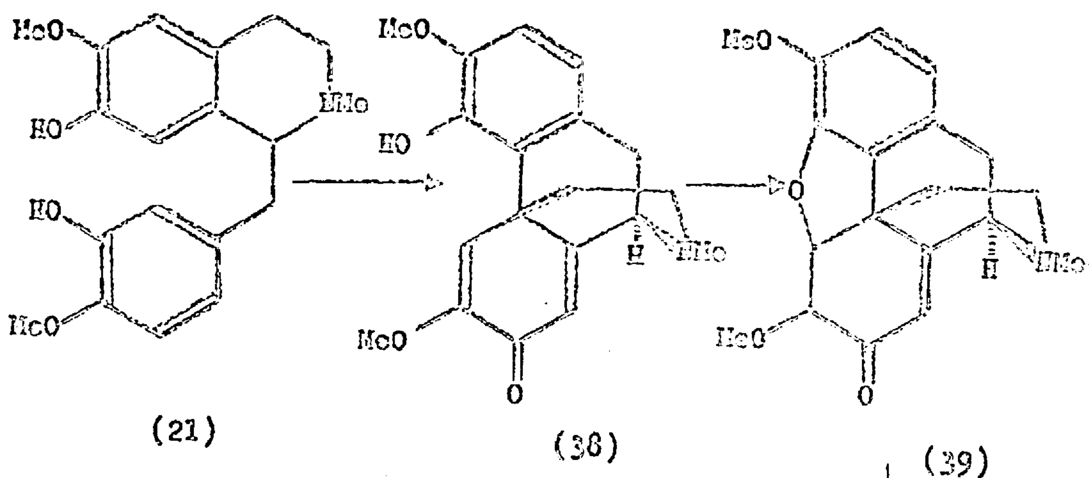
In 1956 Barton and Cohen[4], basing their suggestion on the formation of Pummerer's ketone, the correct structure of which had been elucidated[64], proposed a biosynthetic scheme by which reticuline (21) (at that time an unknown alkaloid), or nor-laudanosoline (6, R=H) suitably protected with enzyme sites, undergoes phenol coupling to give the dienone (38). This dienone can cyclise to give the enone (39),

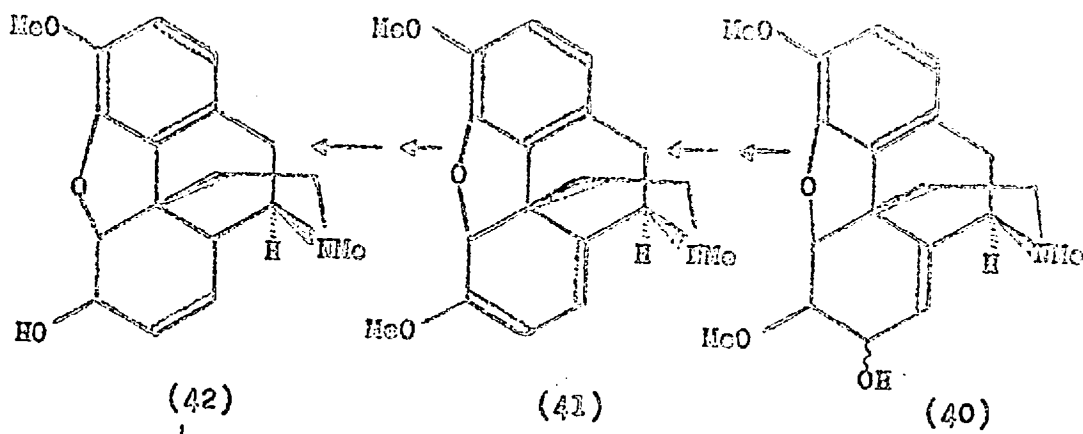
which after reduction to the allylic alcohol (40) and simple transformations can form thebaine (41), codeine (42) and morphine (25).

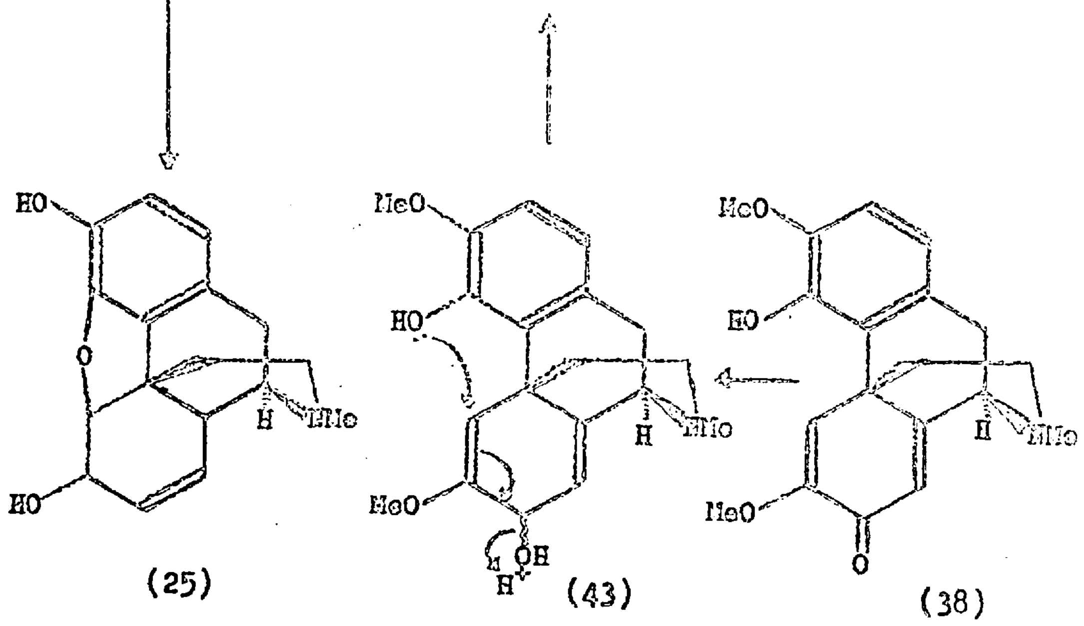
An alternative theory was proposed[15,65] by which the dienone (38) is reduced to a dienol (43), which undergoes allylic elimination, possibly as the phosphate ester, to give thebaine (41).

Subsequent to Barton and Cohen's proposal the dienone (38) was found in nature and named salutaridine[66]; it has been shown to be present in _Papaver somniferum_, the poppy which produces the morphine alkaloids, by radio-dilution[67], and has since been isolated from _Papaver orientale_[68] and from _Croton balsamifera_[69].

Attempts at simulating the biogenesis of the morphine alkaloids in the laboratory have been disappointing. Using manganese dioxide as the oxidising agent the yield of the dienone (38) from reticuline (21) was only 0.012%[68], but considering the many competing side-reactions and the instability of the product to the oxidising conditions this is not surprising. Reduction of salutaridine (38) to the two enantiomeric dienols (43) and treatment with acid gave thebaine (41)[70].


MeO
HO
NMe
HO
MeO
MeO
HO
H
NMe
MeO
O
MeO
O
H
NMe
MeO
O
(21)
(38)
(39)


MeO
O
H
NMe
HO
MeO
O
H
NMe
MeO
MeO
O
H
NMe
MeO
OH
(42)
(41)
(40)

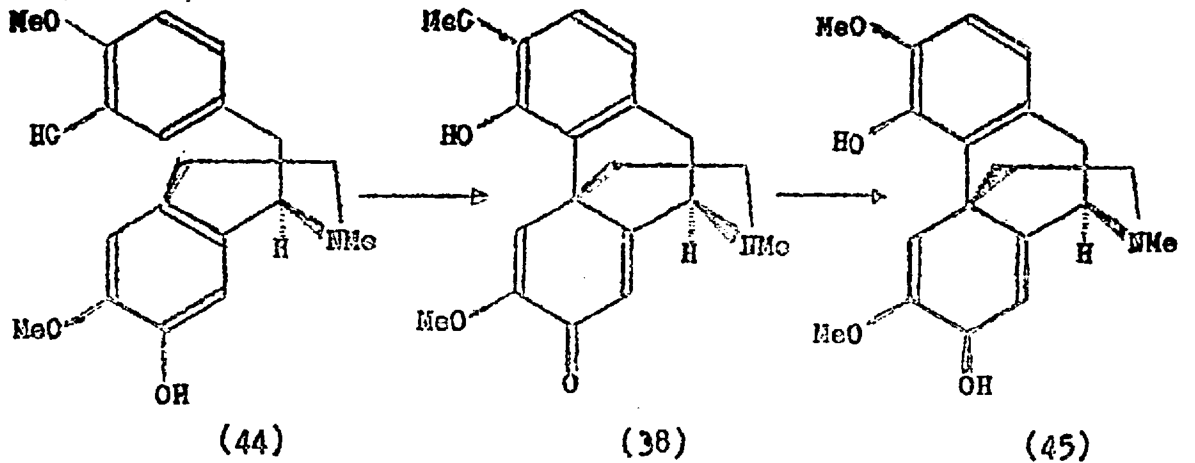

HO
O
H
NMe
HO
MeO
HO
H
NMe
MeO
OH
H
MeO
HO
H
NMe
MeO
O
(25)
(43)
(38)

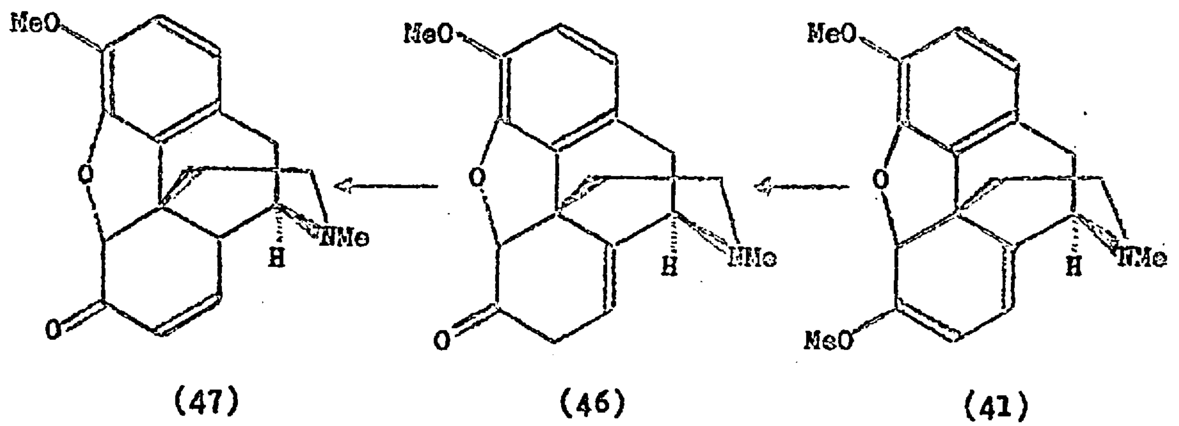
The course of morphine biogenesis in Papaver somniferum has been studied in considerable detail by various groups, in particular those of Barton, Battersby, Leete and Rapoport. The results show that the biogenetic scheme is that proposed by Barton and Cohen[4] with the suggested modification[15,65].

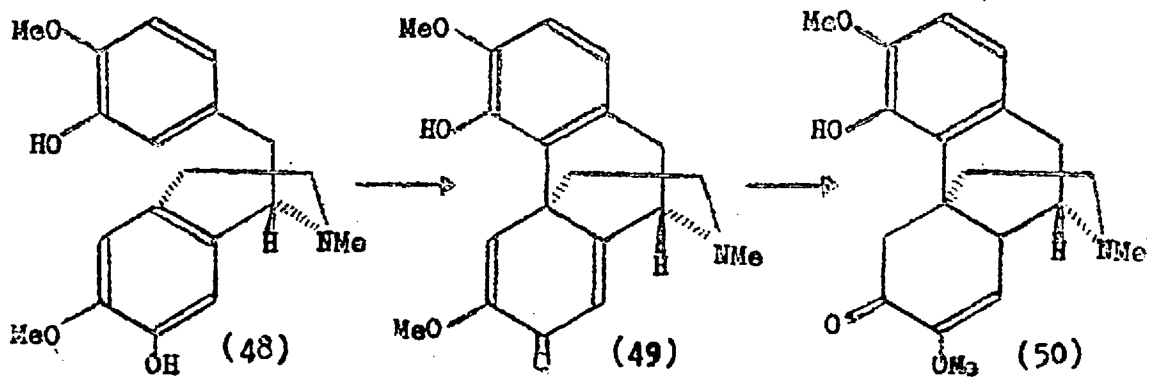
By numerous multiply-labelled compound incorporations it has been shown that (-)-reticuline (44) is converted into salutaridine (38) which is reduced to the dienol (45) and this is converted to thebaine (41)[55,67,70,71,72].

Amongst the morphine alkaloids it has been shown[73,74] that thebaine (41) is produced first and this is converted into codeine (42) which is demethylated to morphine (25). The conversion of thebaine (41) into codeine (42) probably proceeds via neopinone (46) and codeinone (47)[67].

(+)-Reticuline (48) (The enantiomer of that used in morphine biogenesis) can also serve as the precursor for sinomenine (50) by cyclising in a manner similar to that involved in the formation of salutardine, but in this case forming the enantiomer (49)[4,75]. Subsequent to this proposal the dienone (49) was found in Sinomenicum acutum[76], a plant which produces sinomenine, and named sinoacutine.


MeO
HO
NMe
H
MeO
OH
O
(44)
(38)
(45)

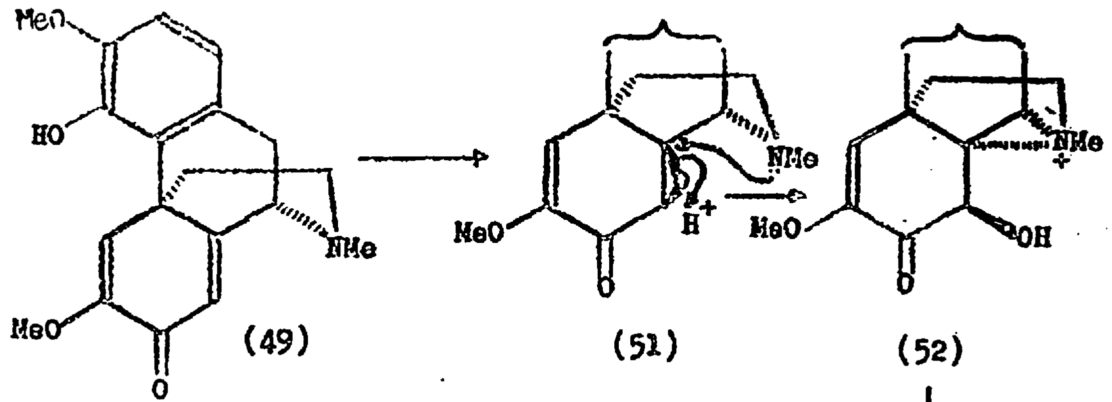

MeO
O
NMe
H
MeO
(47)
(46)
(41)
(42)


MeO
HO
NMe
H
OH
O
(48)
(49)
(50)

N-norsinoacutine has recently been isolated from *Croton balsamifera*[69].

Labelled reticuline (21) and sinoacutine (49) were both incorporated into sinomenine[77], confirming Barton's biosynthetic sequence.

Barton's original proposal[75] for the biogenesis of hasubanonine (53) and metaphanine (56) was based on the wrong structures for these alkaloids. The new structures of these alkaloids[78] could theoretically be derived from sinoacutine (49). For this conversion a ring contraction to form the hasubanan skeleton is required with the introduction of another oxygen function. A possible method for this[79] involves the epoxidation of the dienone (49) to give the epoxide (51). Attack on the epoxide by the nitrogen would give the aziridinium compound (52) which could lead to hasubanonine (53). Probably this alkaloid is formed first; demethylation gives homostephanoline (54); reduction, demethylation with allylic elimination and hydroxylation at C-10 leads to prometaphanine (55), which can simply give metaphanine (56).



MeO
MeO
OH
NMe
O
OMe
(55)

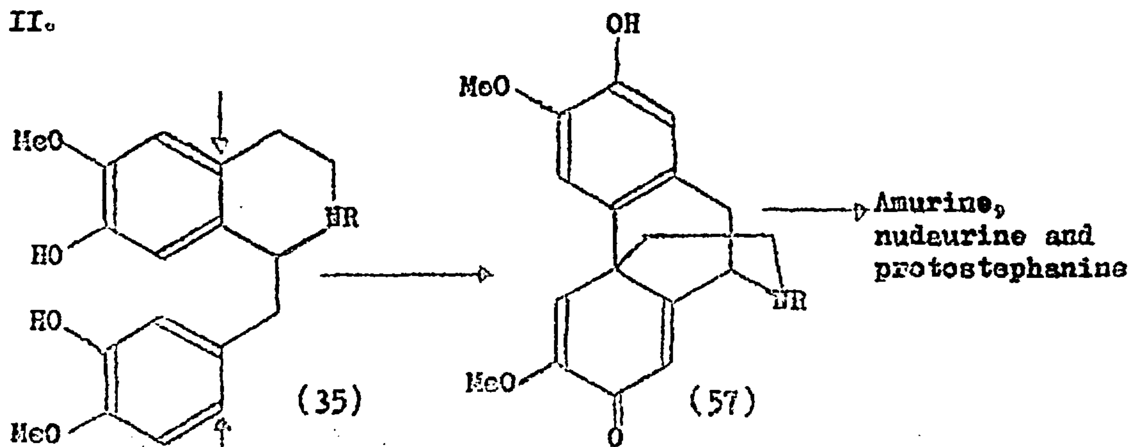
MeO
MeO
NMe
O
OMe
OMe
(53)

MeO
MeO
NMe
O
OH
O

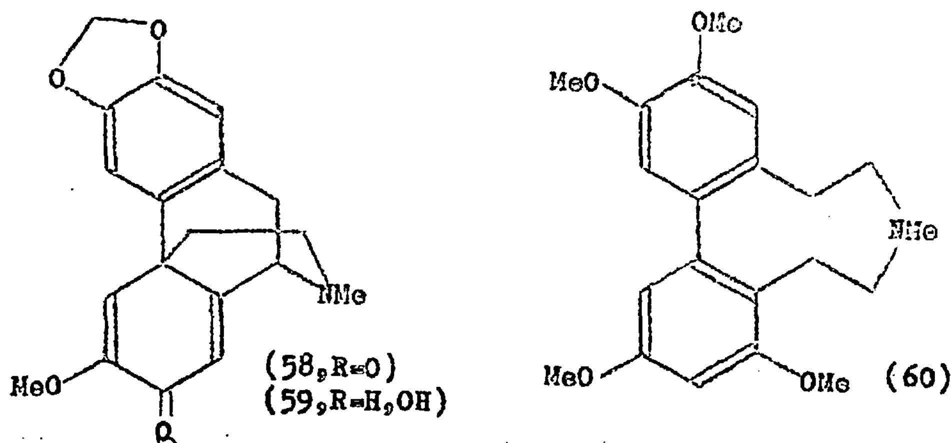
MeO
MeO
NMe
O
OH
OMe

HO
MeO
NMe
O
OMe
OMe
(54)

II.



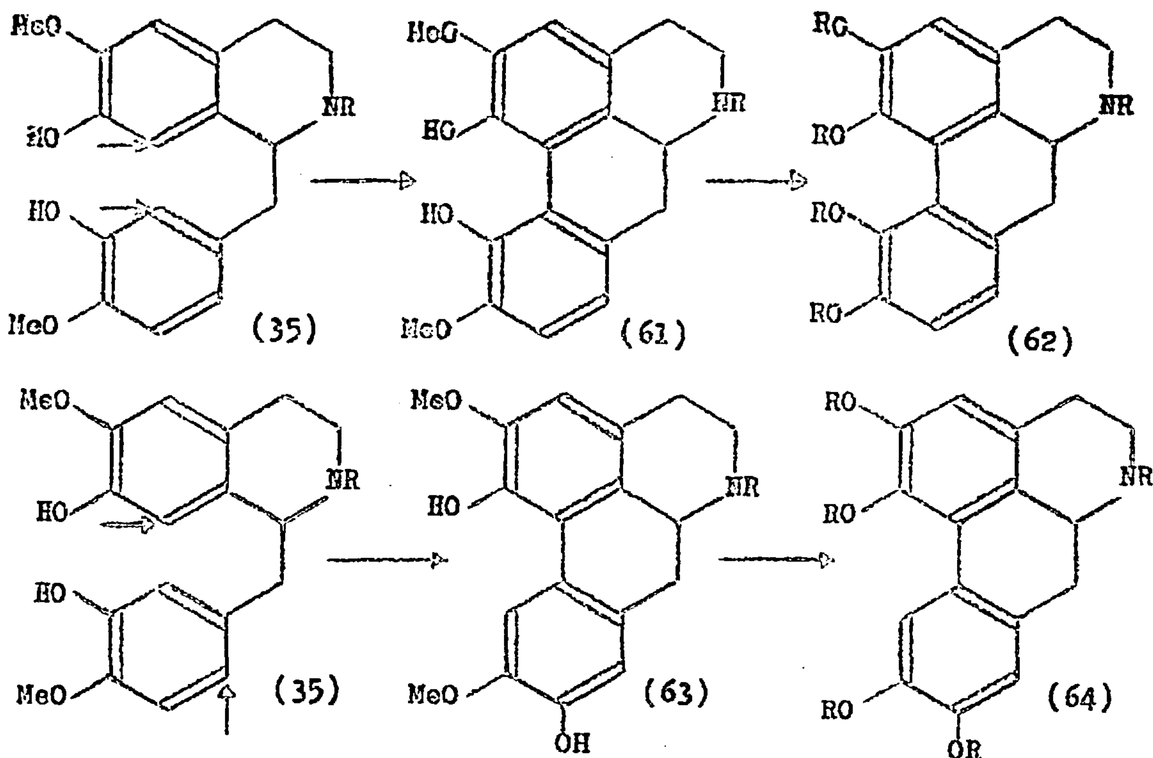
If reticuline (35, R=Me) coupled in the manner indicated the product would be a dienone (57, R=Me). An alkaloid which corresponds to this dienone has recently been discovered in nature and named amurine (58); with it the dienol (59) was also isolated[80].



The dienone (57) could also lead to protostephanine (60) by steps including hydroxylation and ones analogous to those involved in Barton's original scheme[75].

Feeding experiments[79], however, indicate that reticuline (21) is not the precursor of protostephanine and although negative experiments can be inconclusive it is unlikely that this is the route taken in the plant.

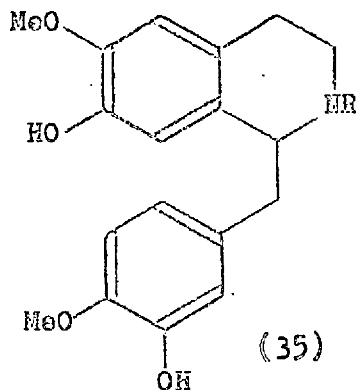
III. and IV.



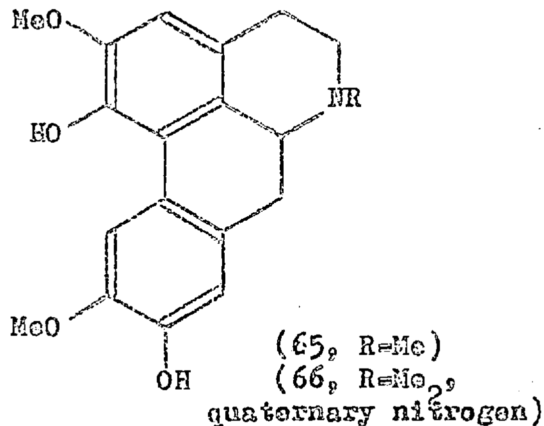
The formation of the aporphine alkaloids by phenol coupling is complicated by the number of possibilities that can occur, and in only a few cases have the biosynthetic routes been evaluated[58,81,82] (vide infra). Many routes appear more attractive than others and in vitro experiments are beginning to give indications of more likely biosynthetic pathways, although, until feeding experiments have been conducted on plants, no decision can be made.

That aporphines are formed by oxidation of benzylisoquinolines has been suggested by Robinson[2,61] and by Manske[83]. However, rigorous application of the principles of phenol oxidation provides a more detailed insight into the modes of coupling[4].

Reticuline (35, R=Me), *N*-norreticuline (35, R=H) or *N*-methylreticuline (35, R=Me_2, quaternary nitrogen) theoretically could be the precursor of two groups of aporphine alkaloids, the 1,2,10,11 (62) and the 1,2,9,10 tetra-oxygenated (64) bases, by the couplings shown. Indeed the conversion of reticuline (35, R=Me) into isoboldine (65) has been achieved in the laboratory[84] as has the analogous conversion of (+)-tembetarine (35, R=Me_2 quaternary nitrogen) into (+)-laurifoline (66) [85].

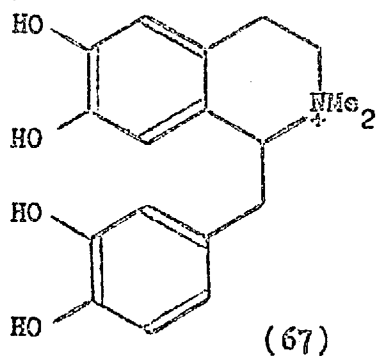


(35)

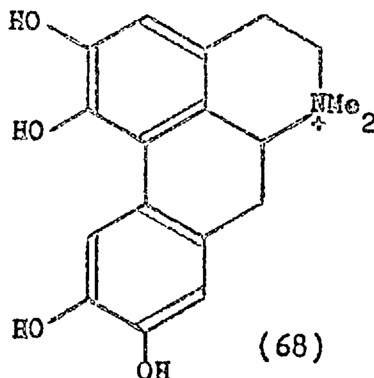


(65, R=Me)
(66, R=Me_2, quaternary nitrogen)

Franck has also succeeded in oxidising the *O*-nor compound (67) to the aporphine (68) in good yield (60%)[86], but this is unlikely



(67)



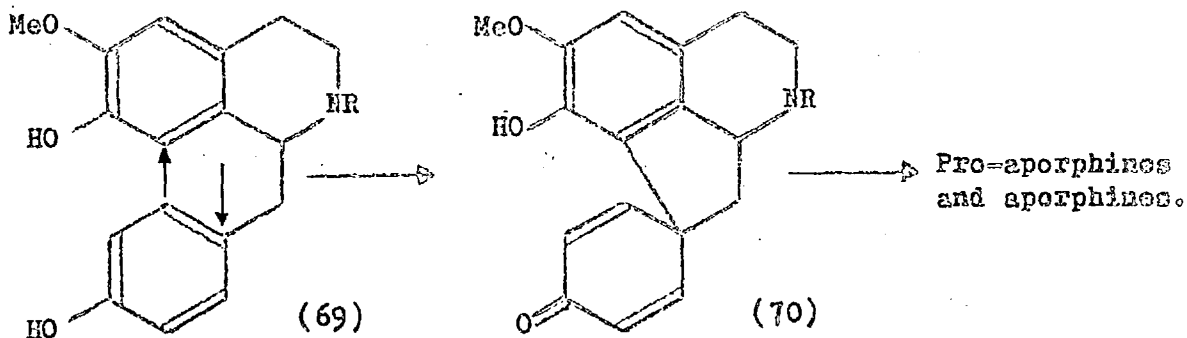
(68)

to be the mode of biogenesis. It is interesting that the yield is so good. Franck ascribes this to the protection of the

nitrogen atom as the quaternary salt, thus preventing oxidation to a "true" isoquinoline[23]. Certainly it is found that coupling is aided if there is a similar oxygenation pattern in both rings[23,87] as occurs here. It may also be possible that N-methylation has a conformational affect forcing ring C closer to ring A[88], but N-acylation[23] and N-formylation[87] do not help, although their conformational affects will not necessarily be similar.

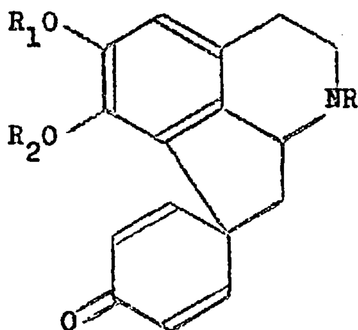
With the *in vitro* oxidative cyclisations as yet no 1,2,10,11 tetra-oxygenated aporphines (62) have been isolated, and it is possible, as has been suggested[84], that these bases are formed by a dienone-phenol rearrangement from a suitable dienone (*vide infra*). The direct cyclisation is difficult presumably because of the interaction between the C-1 and C-11 substituents[89,90,91,92,93] and the route *via* the dienone may be the *in vivo* as well as the *in vitro* method of overcoming this difficulty.

V.

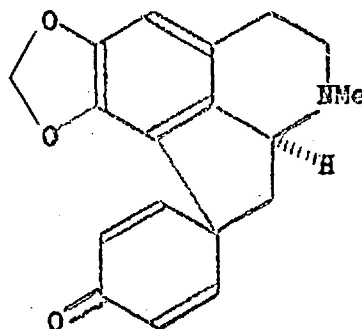


Coclaurine (69,R=H) or N-methylcoclaurine (69,R=Me), on phenol oxidation, could yield the dienone (70). The existence of these dienones was predicted by Barton and Cohen[4] to account for the biogenesis of aporphines carrying no oxygen substituent in ring B. Since this proposal many of these compounds, for which the name "pro-aporphines" has been suggested[94], have been discovered in nature. Most known pro-aporphines theoretically can be derived from coclaurine or N-methylcoclaurine.

The immediate product of the phenol coupling of N-methylcoclaurine (69,R=Me) was isolated from Ocotea glaziovii and named glaziovine (71)[95].



	R	R_1	R_2
(71)	Me	Me	H
(72)	Me	Me	Me
(73)	H	Me	Me
(74)	H	H	Me

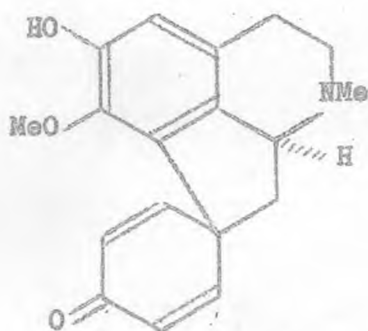


(75)

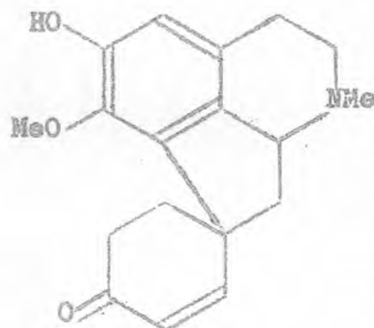
O-Methylation of glaziovine produces pro-nuciferine (72), which has been isolated from the Asiatic lotus Nelumbo nucifera[96] and from Croton linearis[97]. It has also been isolated from

Stephania glabra and is identical with *N*-methylstepharine, stepharine (73) also being isolated[94].

Conversion of the methyl group to a methylenedioxy group would give mecambrine (75) in the enantiomeric series[98]. Transfer of the methyl group is also known forming crotonosine (74)[97,99] and the biosynthesis of this compound from coclaurine (69,R=H) has been confirmed[59]. In *Croton linearis*, besides crotonosine (74) and pro-nuciferine (72), "homolinearisine" (76) and its dihydro derivative linearisine (77) have been discovered[97,99].



(76)



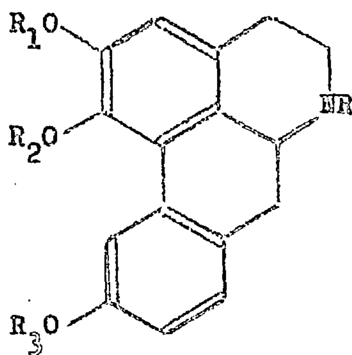
(77)

(±)-Pro-nuciferine has been synthesised along classical lines[100] and we have synthesised (±)-glaziovine by ferricyanide oxidation of *N*-methylcoclaurine in 1.1% yield.

The conversions of pro-aporphines into aporphines, either by reduction and acid treatment to give the unsubstituted ring D compound,

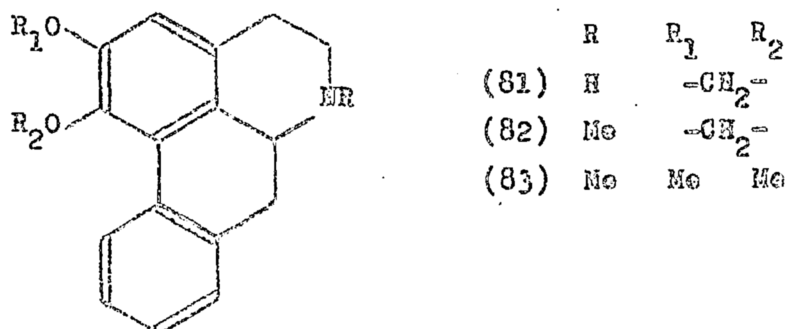
or by acid treatment to give the phenol, have been carried out in several cases and they provide models for the biosynthesis of these compounds as outlined by Barton and Cohen[4].

By dienone-phenol rearrangement aporphines such as mecambroline (78), laureline (79) and tuduranine (80) can be formed, but it is unlikely to be the mode of biogenesis of the tri-oxygenated aporphines with an oxygen substitutent at C-9, since at least _in vitro_ only products derived from aryl migration are isolated; these are more probably derived from orientalinone.

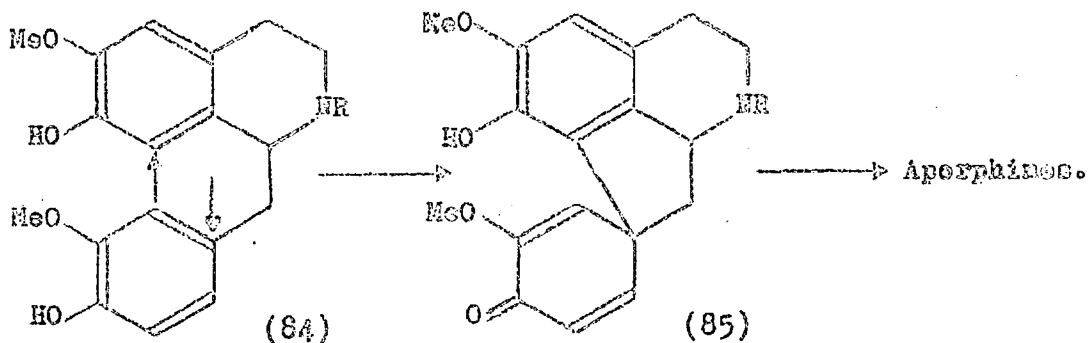


	R	R_1	R_2	R_3
(78)	Me	$-CH_2-$		H
(79)	Me	$-CH_2-$		Me
(80)	H	Me	Me	H

Reduction followed by dienol-benzene rearrangement leads to compounds with no oxygen substituent in ring D, such as anonaine (81), roemerine (82) and nuciferine (83).



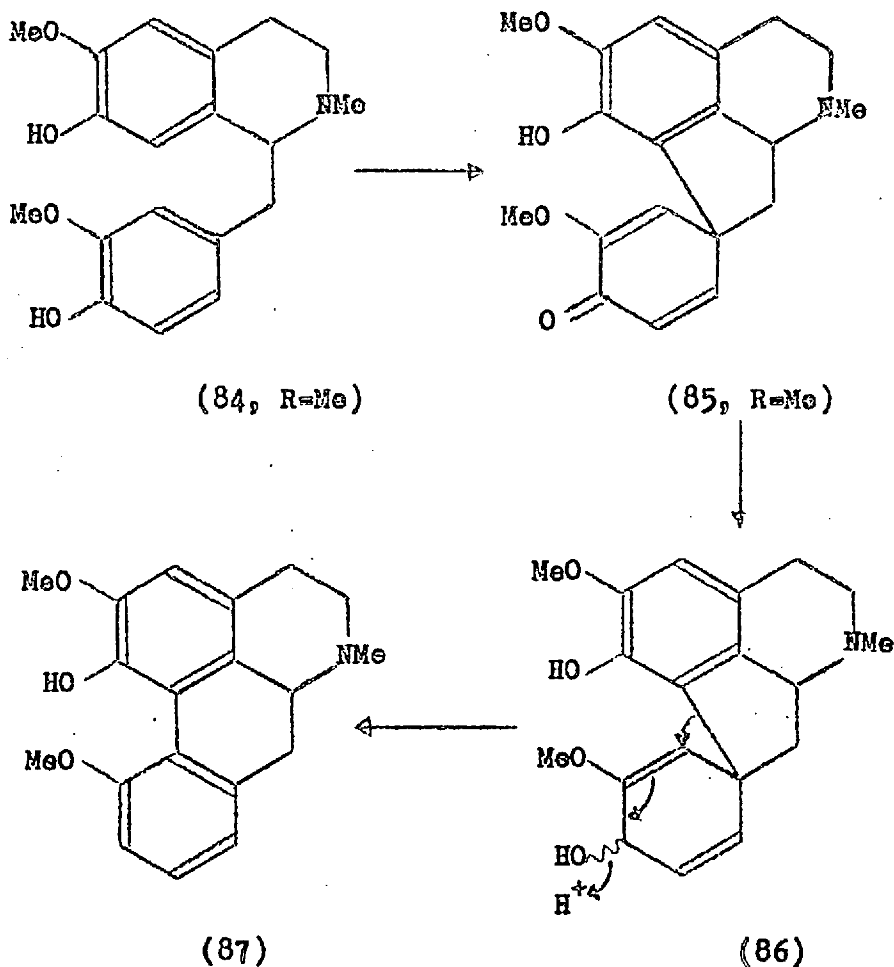
VI.



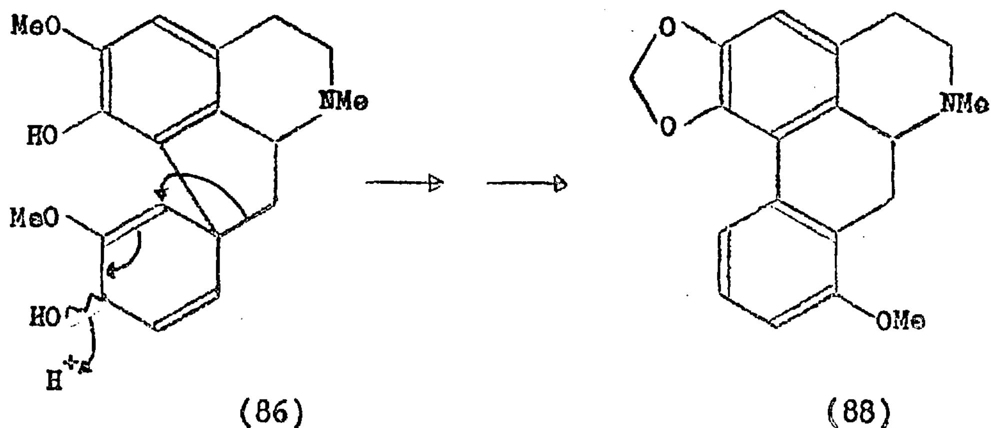
Orientaline (84,R=Me) on phenol coupling _in vitro_ or _in vivo_ yields the dienone orientalinone (85,R=Me)[68,81,101]. This coupling and subsequent transformations were suggested by Battersby to account for the formation of the "abnormal" aporphines,

isothebaine (87) and stephanine (88)[15].

These transformations were carried out <u>in vitro</u> to give isothebaine by reduction of orientalinone (85, R=Me) to the dienol (86) and acid treatment. Orientalinone was formed by phenol oxidation of orientaline (84, R=Me) in 2.5 - 3.5% yield[101]. This transformation requires the relatively facile aryl migration.

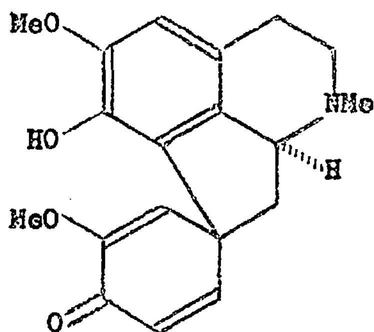


However, to form stephanine (88), the dienone-phenol migration must be "abnormal" (86⟶88).



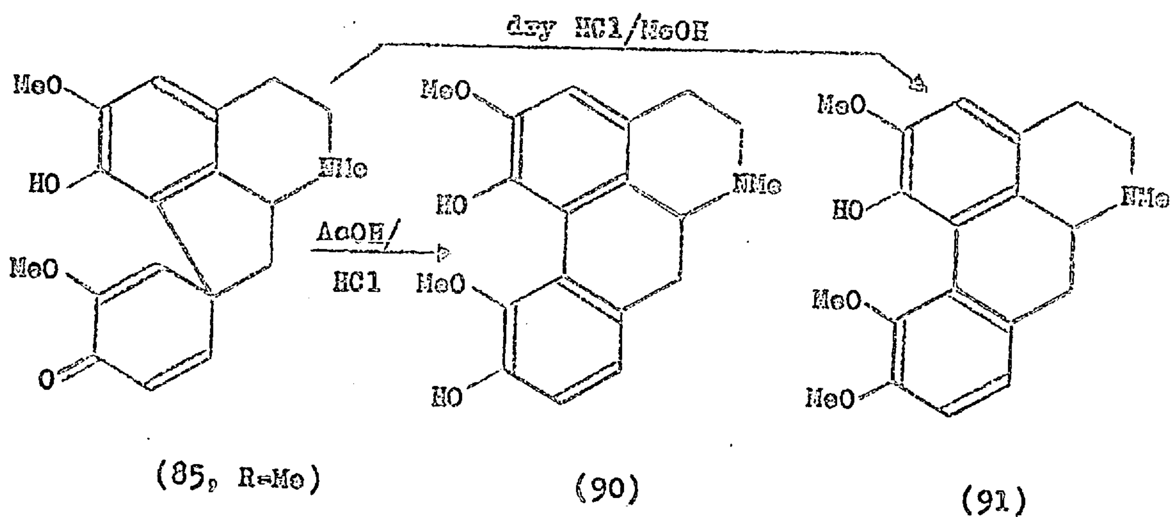
(86) (88)

The biosynthesis of isothebaine (87) in Papaver orientale has been confirmed[68,81] to proceed from orientaline (84, R=Me) via orientalinone (85, R=Me). Orientalinone (absolute configuration as in 89) has been shown to be present in Papaver orientale, where it occurs with its dihydro derivative[68].

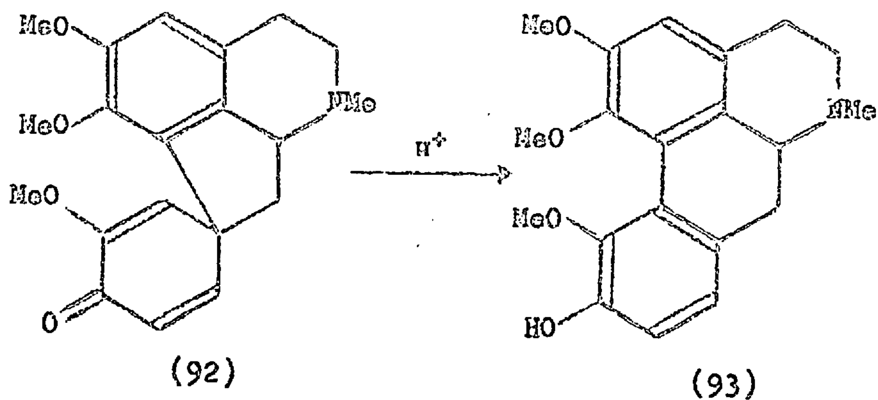


(89)

Besides isothebaine and stephanine, orientalinone can serve as the precursor for numerous other aporphine alkaloids. It has been shown[84,102] that orientalinone (85, R=Me) (not however prepared as above) on treatment with aqueous acid gives the 1,2,10,11 tetra-oxygenated aporphine isocorydine (90) or with dry methanolic acid corydine (91), aryl migration again being observed. Similarly O-methyl orientalinone (92) on acid treatment yields pseudocorydine (93)[103].



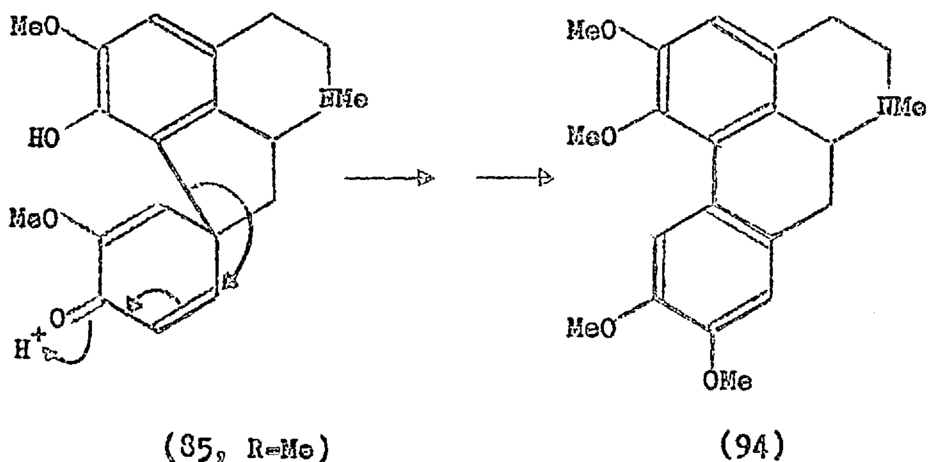
(85, R=Me) (90) (91)



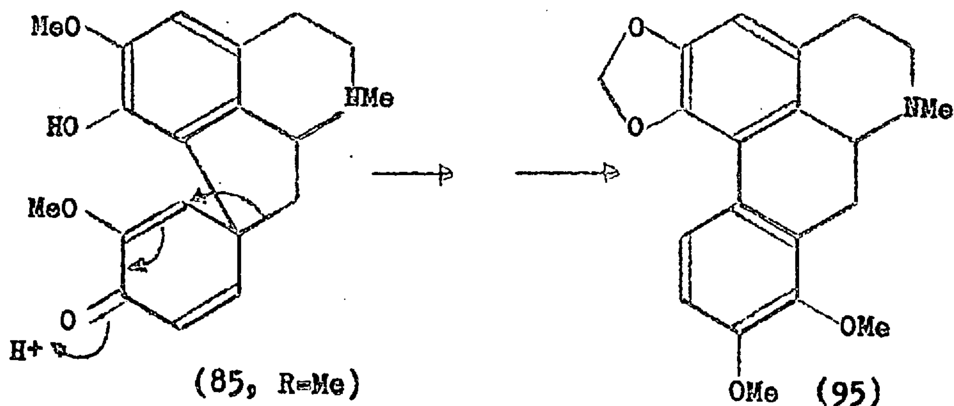
(92) (93)

This is very possibly the method by which plants overcome the difficulty, encountered at least with in vitro experiments, of cyclising to give aporphines with C-1 and C-11 oxygen substituents[82].

It is also possible, although less likely, that aporphines with an oxygen pattern as in glaucine (94) are formed from a dienone such as orientalinone (85,R=Me) by aryl migration to the alternative carbon atom. Alkyl migration could account for the biogenesis of the "abnormal" aporphine crebanine (95).

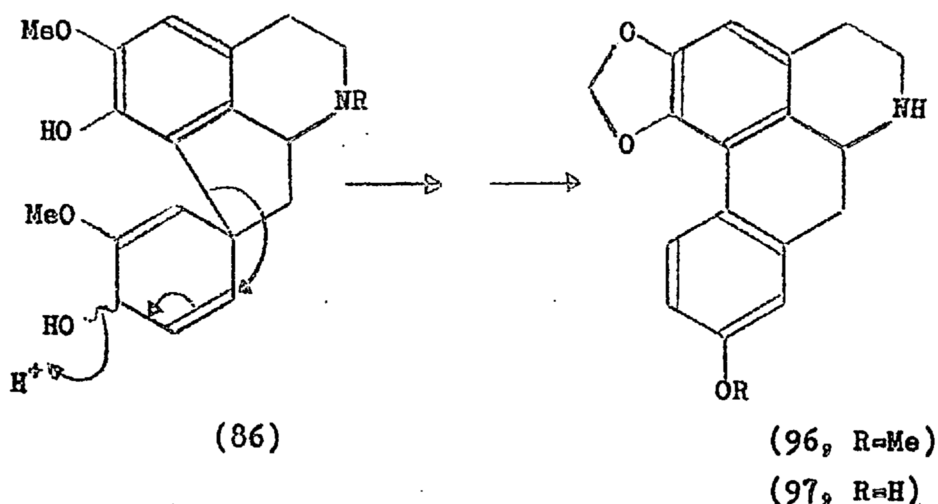


(85, R=Me) (94)



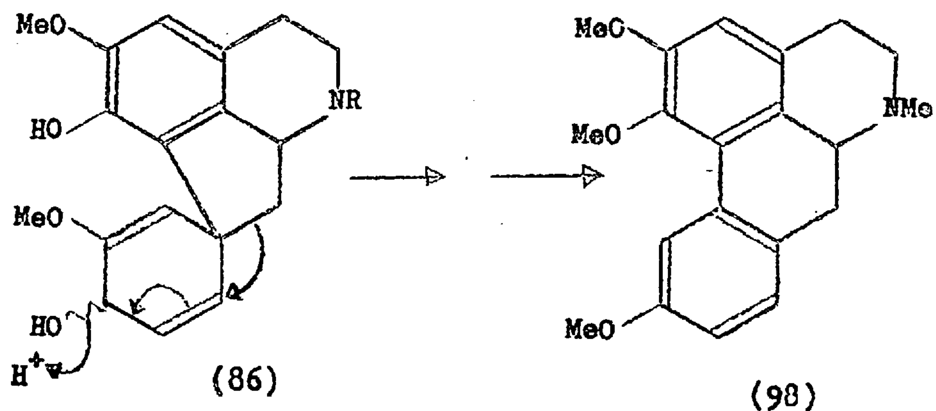
(85, R=Me) (95)

After reduction to the dienol (86) acid treatment can yield isothebaine and pukateine as has been described, but if the aryl group migrated in the opposite sense xylopine (96) and anolobine (97) could be formed. Alkyl migration can give stephanine, or, but it is unlikely, alkaloids such as laureline (98). Generally it is found the migration is to the double bond bearing the methoxyl group, the stability of the rearranged carbonium ion apparently governing the direction of migration.



(86)

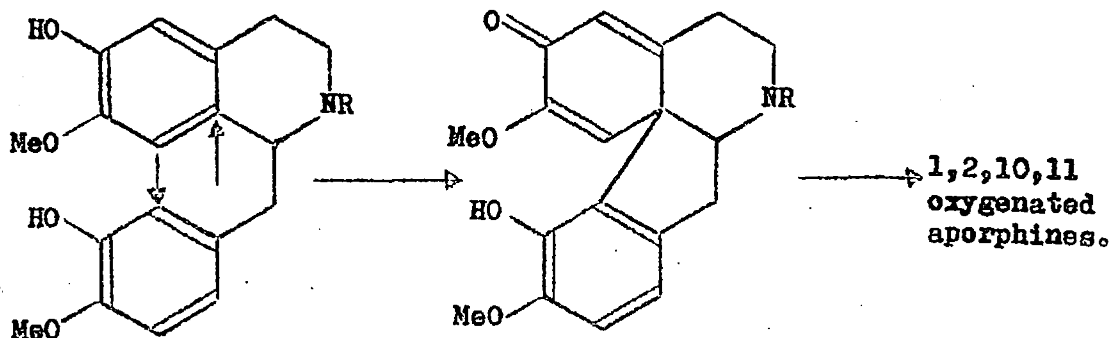
(96, R=Me)
(97, R=H)



(86)

(98)

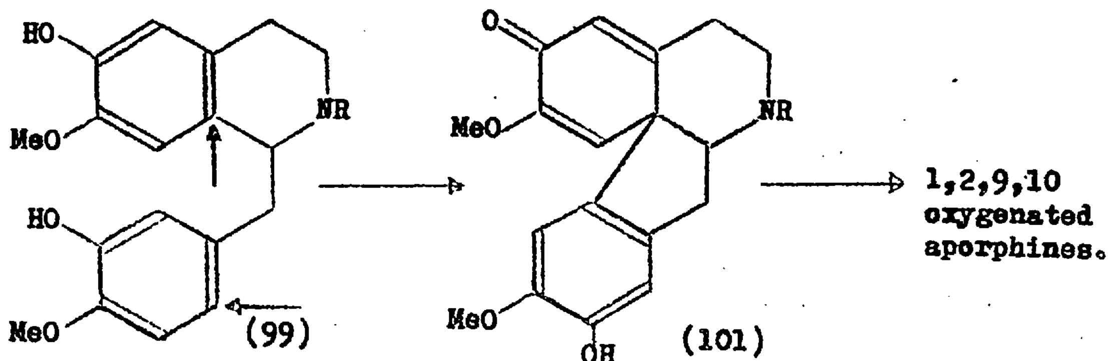
VII.



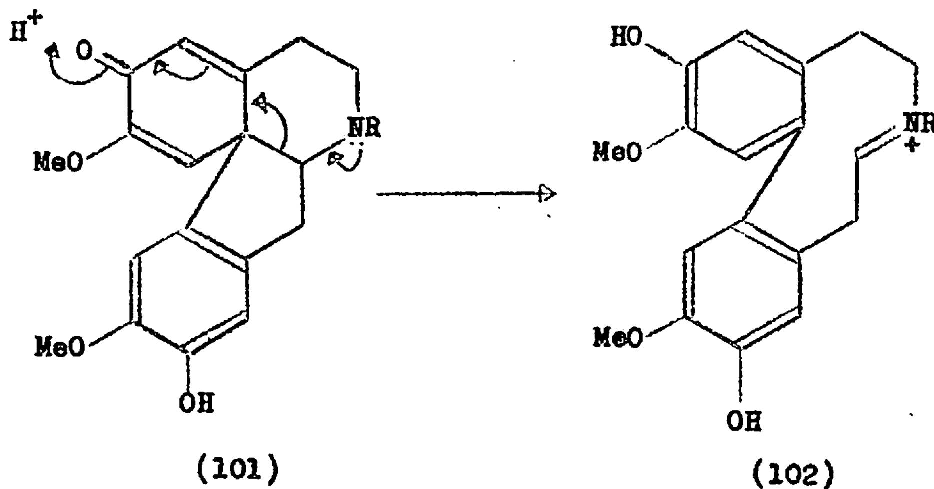
(99) If R=Me, protosinomenine (100)

Protosinomenine (99,R=Me) could on phenol oxidation yield a dienone (100,R=Me). If this were sufficiently stable it could be re-arranged to a 1,2,10,11 aporphine (62), or reduction and re-arrangement could give a 1,10,11 oxygenated aporphine. Neither this type of dienone nor the 1,10,11 oxygenated aporphines have been found in nature. This dienone (100,R=Me) was postulated as an intermediate[75] if protosinomenine (99,R=Me) were the precursor of sinomenine (50), but it was subsequently shown that sinomenine was biosynthesised from reticuline[77].

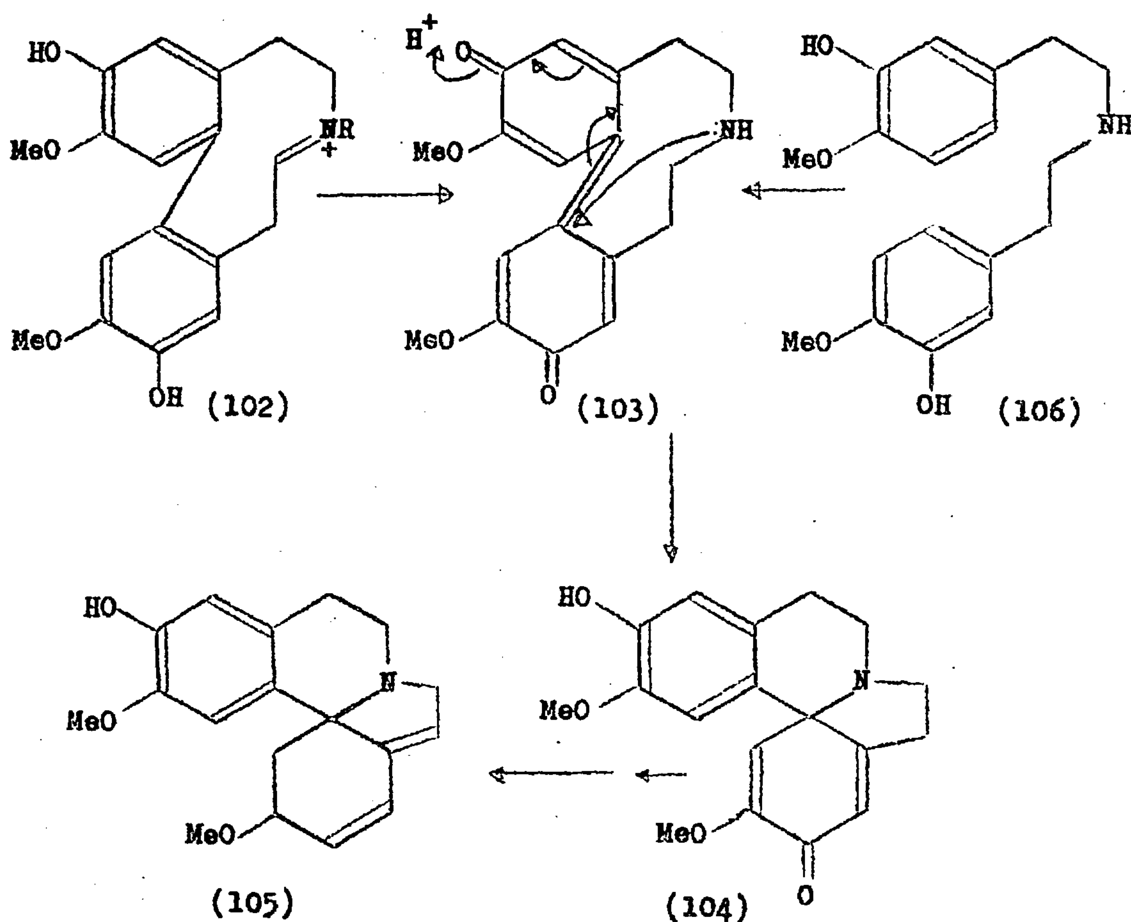
VIII.



If protosinomenine (99,R=Me) coupled in the alternative sense to give the dienone (101), this could provide an unlikely mode of biogenesis for 1,2,9,10 (64) or 1,9,10 - oxygenated aporphines. However, no dienones of this type (100 or 101) have been discovered, and this may be due to their immediate opening to give the 9-membered ring compound (102). This could serve as a precursor for the Erythrina alkaloids[104].

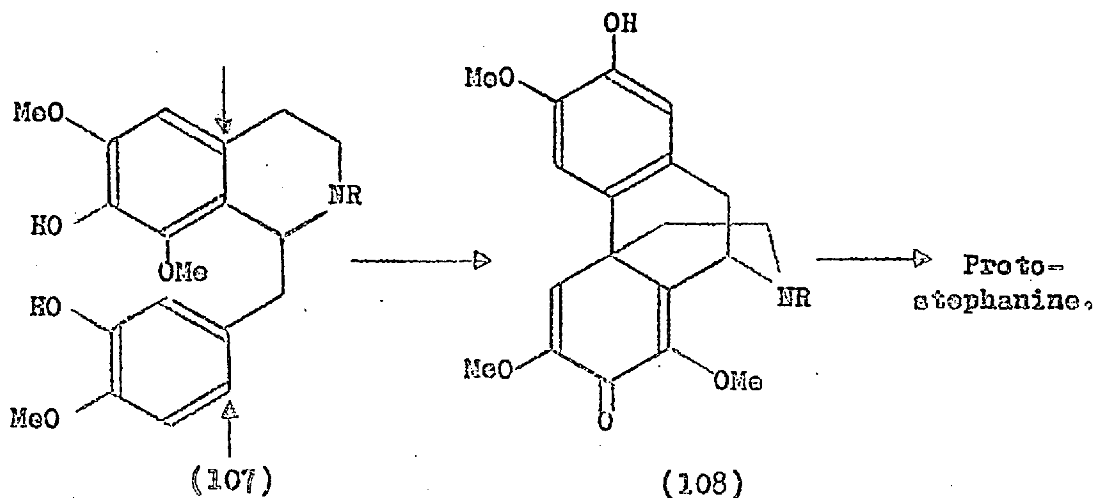


After reduction of the immonium bond and oxidation to the di-dienone (103), cyclisation gives the dienone (104) which is the likely precursor of the Erythrina alkaloids such as erysodine (105). This contrasts with Barton and Cohen's original proposal[4] in which phenol oxidation of the open-chain phenol (106) gives the di-dienone (103).

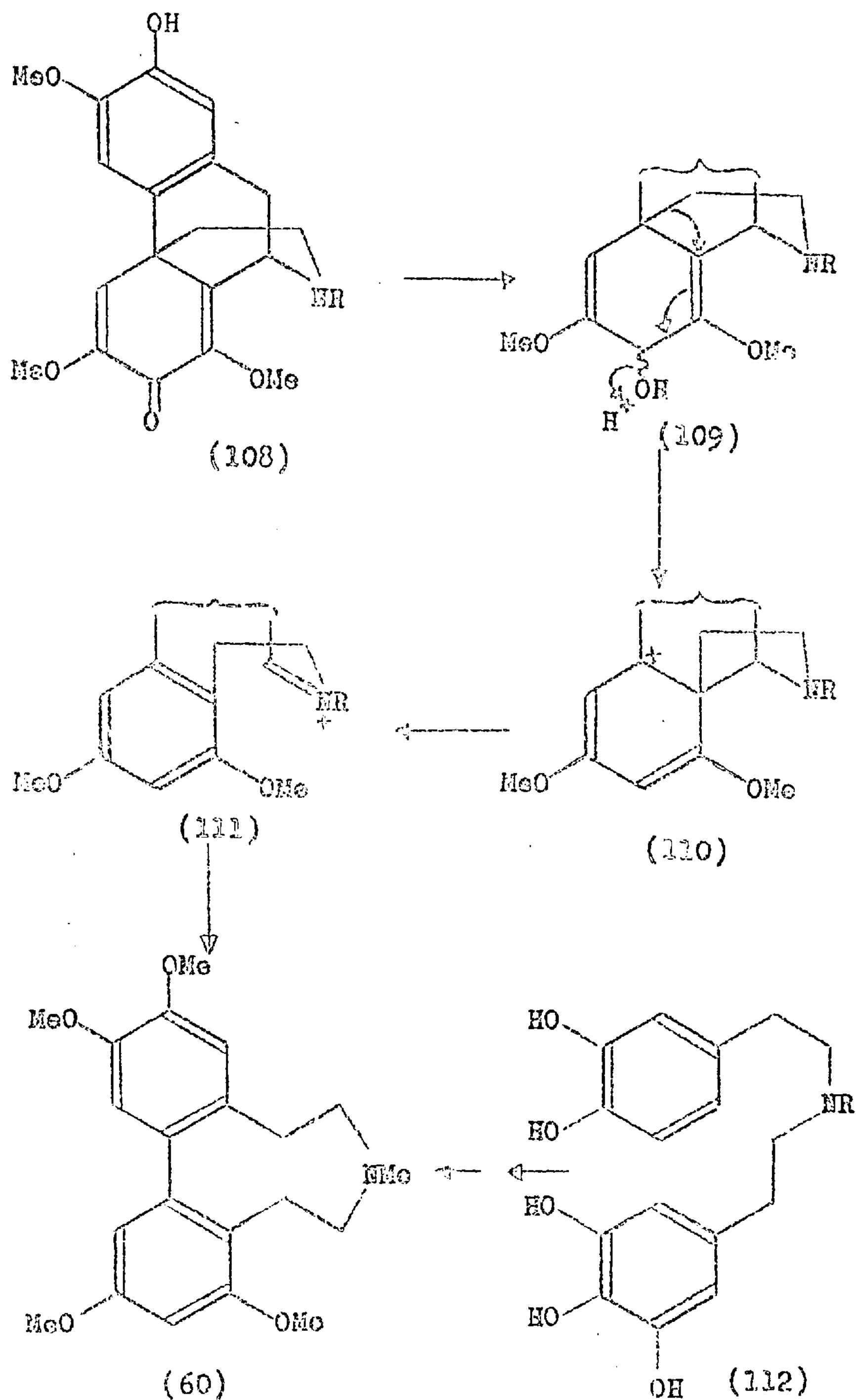


Experiments[105] with cyclising the phenol (106) in vitro, which proceeds in surprisingly high yield (35%), to give the dienone (104) directly, suggest the biosynthesis is as in Barton and Cohen's original proposal. Feeding experiments with plants are awaited.

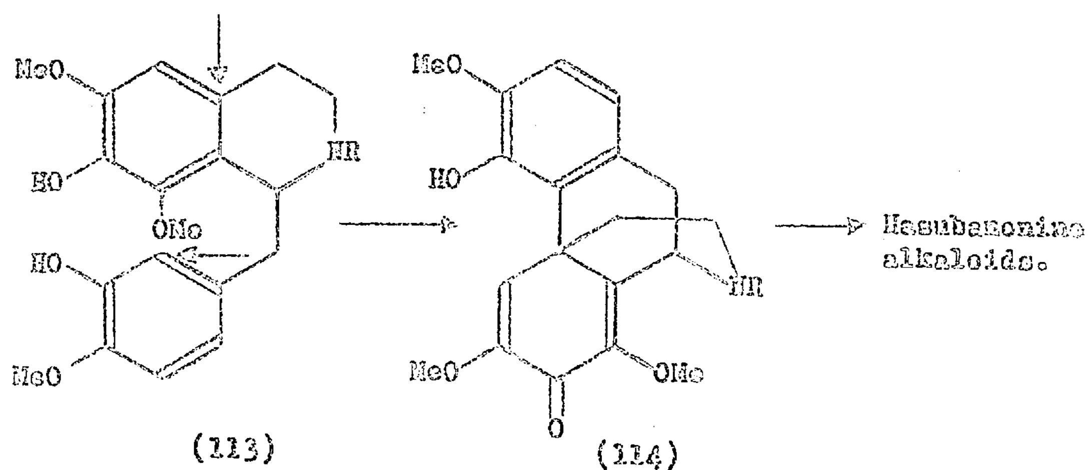
IX.



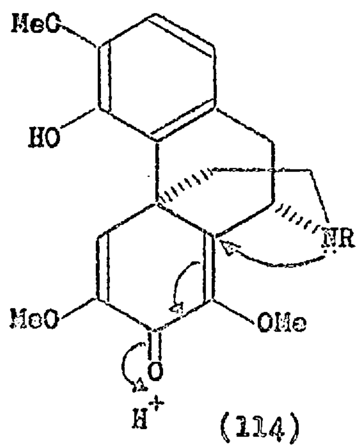
If the penta-oxygenated benzylisoquine (107) coupled to the dienone (108), this could serve as a precursor for protostephanine (60). This is Barton's scheme[75] in which the dienone (108) is reduced to the dienol (109); migration affords the carbonium ion (110) which opens to the immonium ion (111) which is reduced to protostephanine (60). An alternative has been suggested by Boit[106] in which the open-chain phenol (112) couples to give a dienone.


OH
MeO
MeO
OMe
O
NR
(108)
MeO
OMe
OH
H
NR
(109)
MeO
OMe
NR
(110)
MeO
OMe
NR
(111)
OMe
MeO
MeO
OMe
NMe
(60)
HO
HO
HO
HO
OH
NR
(112)

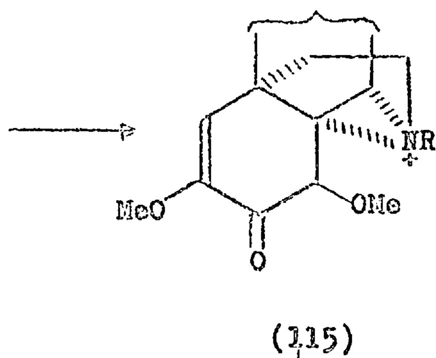
X.



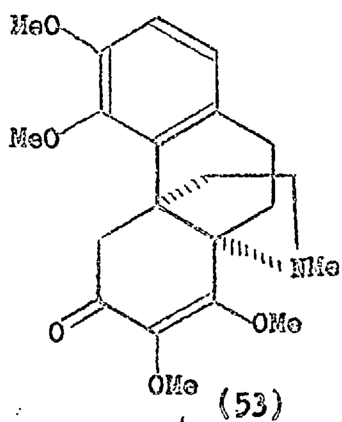
If the penta-oxygenated benzylisoquinoline (113) coupled to give the dienone (114), this could serve as a precursor for the hasubanonine alkaloids. The ring contraction is achieved by attack of the nitrogen on the dienone to give the aziridinium ion (115); reduction to the enone (116) and simple transformations to give hasubanonine (53).



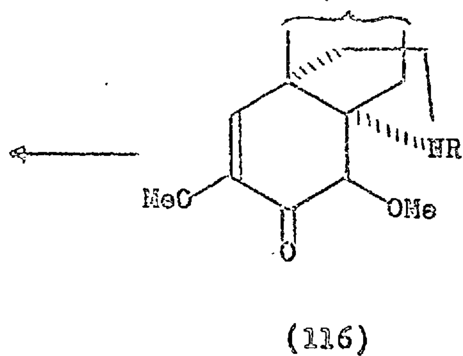
(114)



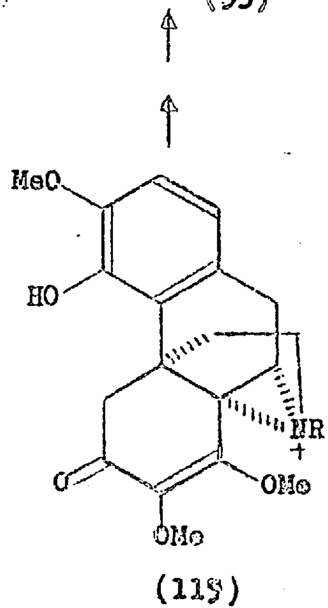
(115)



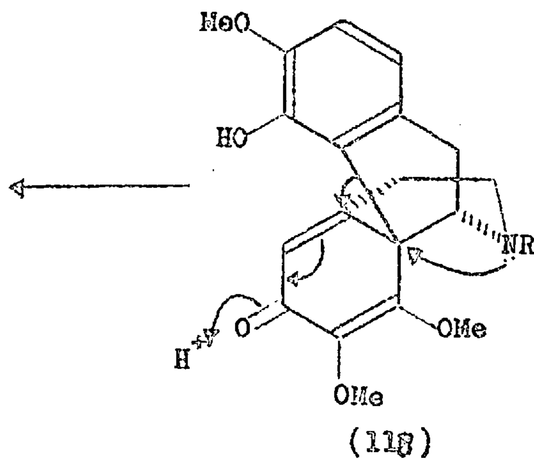
(53)



(116)

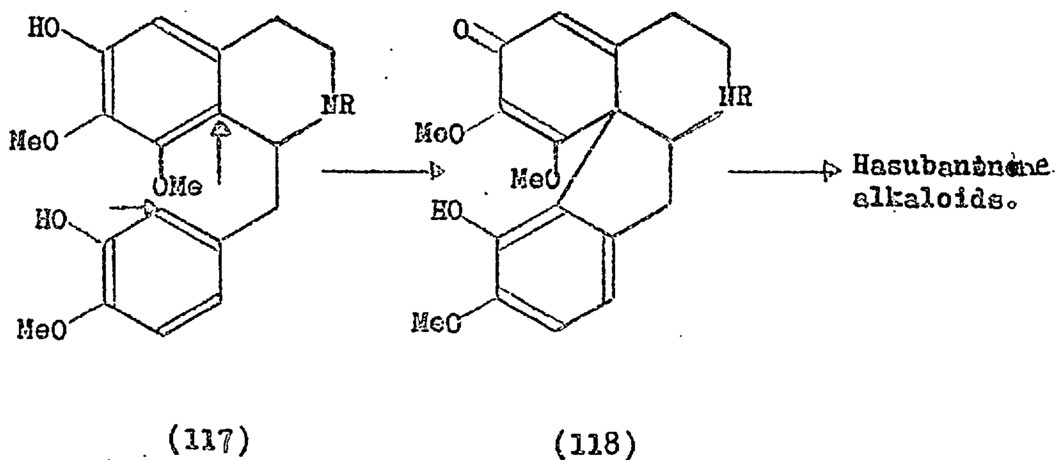


(119)



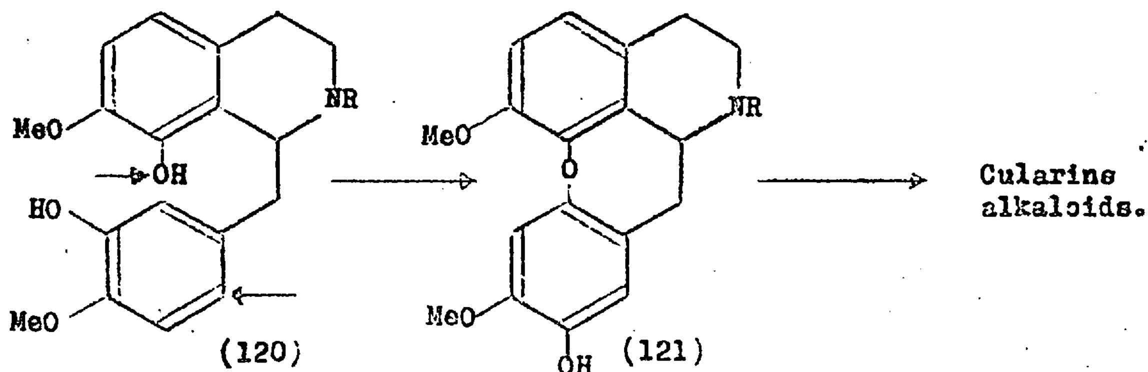
(118)

XI.

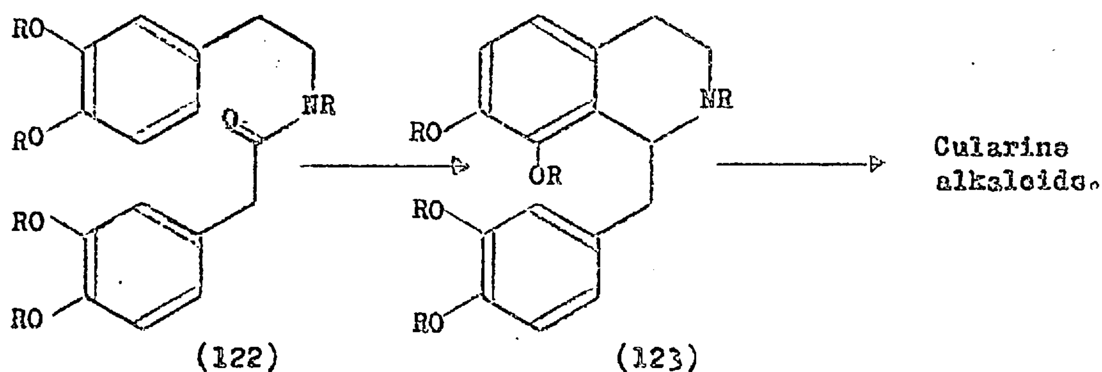


Hasubanonine (53) could also be derived from the penta-oxygenated benzylisoquinoline (117), which on oxidative coupling yields the dienone (118). Rearrangement to the aziridinium ion (119) and other reactions could lead to hasubanonine (53).

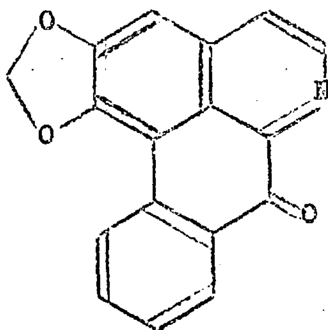
XII.



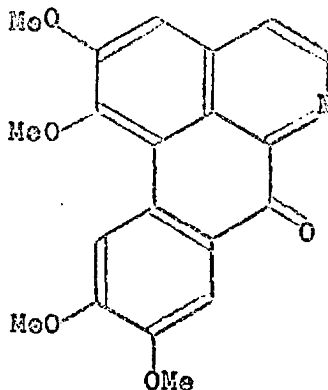
The idea that cularine (17) is derived from a benzylisoquinoline was suggested by Manske[83] and Robinson[2]. The tetraoxygenated benzylisoquinoline (120) could couple to give the cularine skeleton (121). Manske[83] suggested that the biological cyclisation to form the benzylisoquinoline had proceeded in an unusual sense (122 → 123), but it has also been proposed that the cularine skeleton is derived from a pentaoxygenated benzylisoquinoline with subsequent loss of an oxygen function[107].



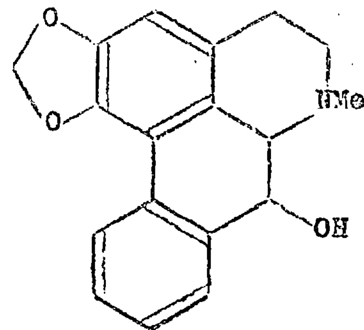
The possible modes of biogenesis of aporphines have been discussed, however, there are many compounds derived from further transformations of aporphines. Oxidation products of aporphines are widespread, such as liriodenine[108] (124), the base (125)[109,110] and ushinsunine (126)[111]. The oxidations to compounds of the type (124 and 125) have been performed <u>in vitro</u> using chromium **trioxide** and pyridine [112] and it is possible, as has been suggested[108, 109, 113], that these compounds are artefacts. In support of this theory it is pointed out that glaucine co-occurs with the compound (125), and anonaine (81) on exposure to aerial oxidation is converted into liriodenine (124)[113].



(124)

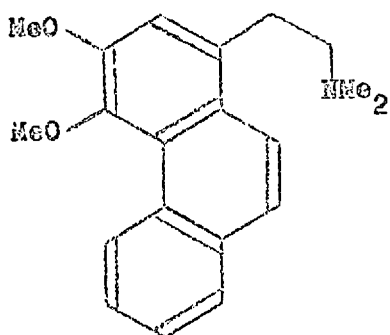


(125)

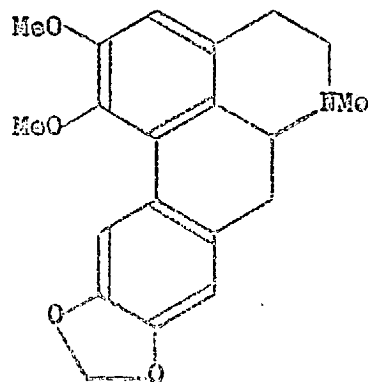


(126)

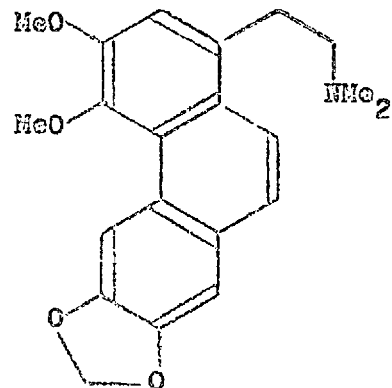
Compounds that are probably biogenetically derived by a process analogous to the Hofmann degradation have been found in plants. The base (127) derived from nuciferine (83)[114] and that from nantenine (128), thalictuberine (129)[115] exemplify this.



(127)

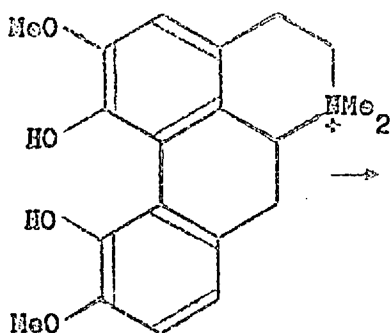


(128)

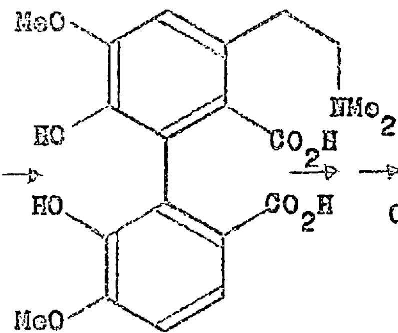


(129)

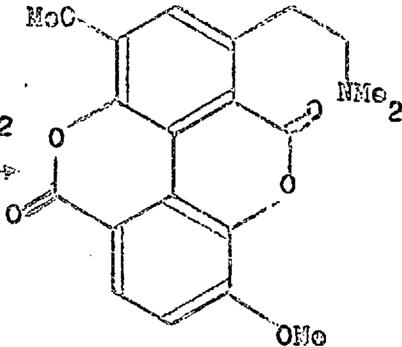
A further degradation of the methine base from magniflorine (130), after oxidation to the acid (131), could give compounds such as taspine (132)[116].



(130)

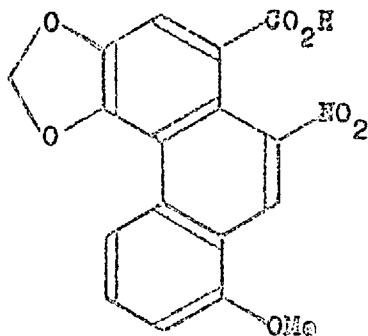


(131)

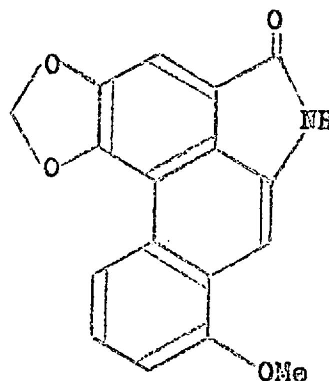


(132)

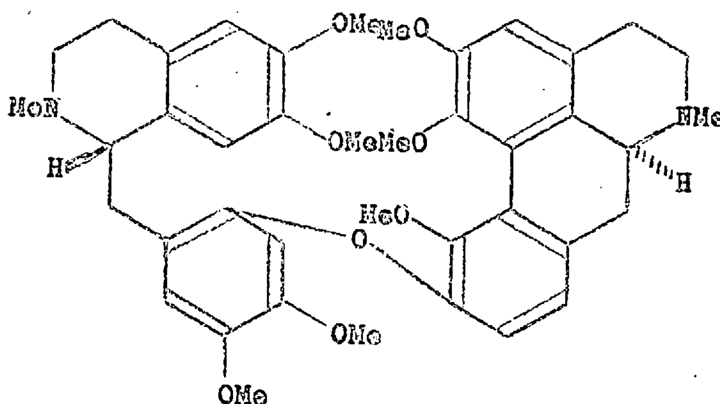
A different degradation of an aporphine could lead to the aristolochic acids (for example 133) which co-occurs with the lactam (134)[117]. Indeed experiments suggest this is how they are derived[118]. Since nor-adrenaline is incorporated it is possible that a hydroxyl group at the C-4 in the benzylisoquinoline is carried through in the biosynthesis and predisposes the aporphine intermediate to the subsequent oxidation [118].



(133)



(134)



(135)

In certain cases further coupling occurs to form compounds analogous to bisbenzylisoquinolines, such as thalicarpine (135)[119].

Isolation of Erythrina Alkaloids.

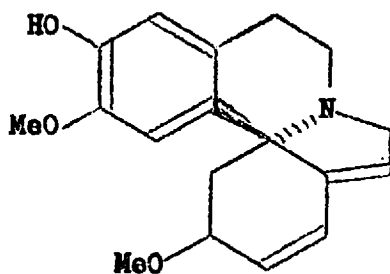
In 1947 Deulofeu[120] showed that *Erythrina crista-galli* contained erythramine, erythraline, erythratine, erysodine, erysovine, erysopine and hypaphorine, although at that time the structures were not known. After the Erythrina alkaloid skeleton had been correctly determined[121], the structures of the alkaloids were deduced, although the positions of the methoxyl and hydroxyl groups in ring D of erysodine and erysovine were undecided and the position of the double bond in erythratine was assumed to be 6-7[122].

The alkaloids were isolated by the "preferred" method of Folkers[123] according to the description of Deulofeu[120]. From the amount of seed extracted the only alkaloid completely identified was erysodine (105), although erythraline (136) was obtained pure and what was apparently erysotrine (137) was isolated, crystallized but could not be recrystallized. A small amount of crystalline erythratine (138) was obtained.

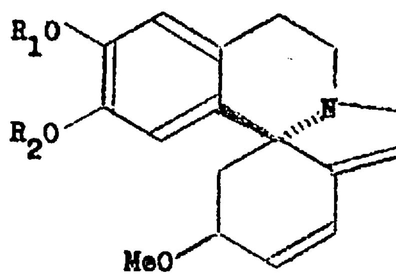
Seasonal variations in the alkaloid content of the plants were observed; no erysotrine (137) could be isolated in summer, but in late autumn it predominated over erythraline (136).

It has subsequently been shown[124] that erysodine has the

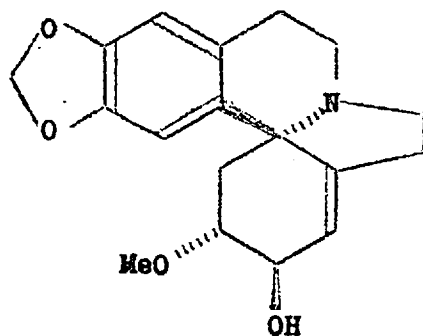
structure (105) and erythratine is an allylic alcohol (138).



(105)



(136, R_1+R_2 = $-CH_2-$)
(137, R_1 = R_2 = CH_3)



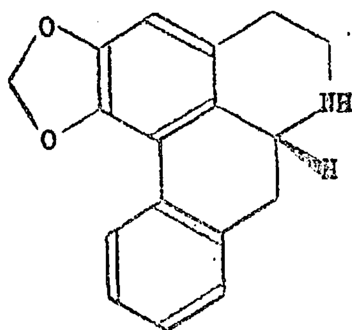
(138)

Isolation of Anonaine

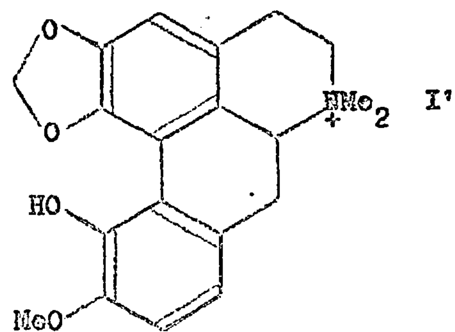
Anonaine had been isolated from Anona reticulata plants in 1930 by Santos[125] and the extraction was repeated by Govindachari in 1959[126] when besides anonaine (139) reticuline (21) was found. Anonaine had originally been isolated from Anona squamosa by Trimurti[127]. Its forumla was determined by Barger and Weitnauer[128] and confirmed by synthesis[128,129].

The anonaine was obtained from Anona reticulata bark by the method of Govindachari[126] and was shown to be identical (except for optical rotation) with synthetic material. The nmr spectrum of anonaine (139) showed the characteristic double-doublet of aporphines having a 1,2-methylenedioxy group. This is due to the two methylenedioxy group hydrogens being in different environments because of the twist in the biphenyl system. The two possible shapes of the aporphines molecule are depicted in the formulae (140 and 141) and the angle of twist in bulbocapnine methiodide (142) has been found by X-ray crystallography to be 29.9° [130].

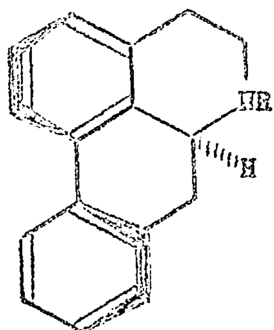
The absolute configurations of aporphines were first indicated by Faltis and Adler who converted (-)-laudanosine of known absolute configuration into (-)-glaucine[131]. It was also noted[132] that (-)-morphothebaine and (+)-glaucine are enantiomeric at C-6a. Since the configuration of (-)-morphothebaine is known (+)-glaucine must be as in the formula (140). Because of the chemical interconversions of



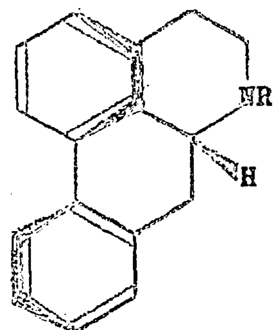
(139)



(142)



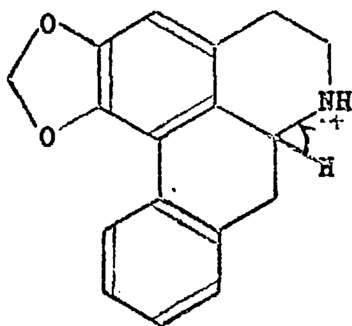
(140)



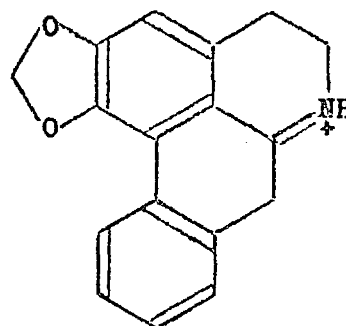
(141)

(+)-glaucine, (+)-dicentrine, (+)-laurotetanine, (+)-actinodaphnine and (+)-boldine, it was assumed[132] that all (+)- aporphines are represented by the absolute configuration (140). Optical rotationary dispersion measurements[133] support Bentley's assumption that the sign of rotation of the sodium-D line is adequate to predict the absolute configuration. Anonaine, with $[\alpha]_D^{20°}$ of -52^0, must have the absolute configuration depicted in the formula (139).

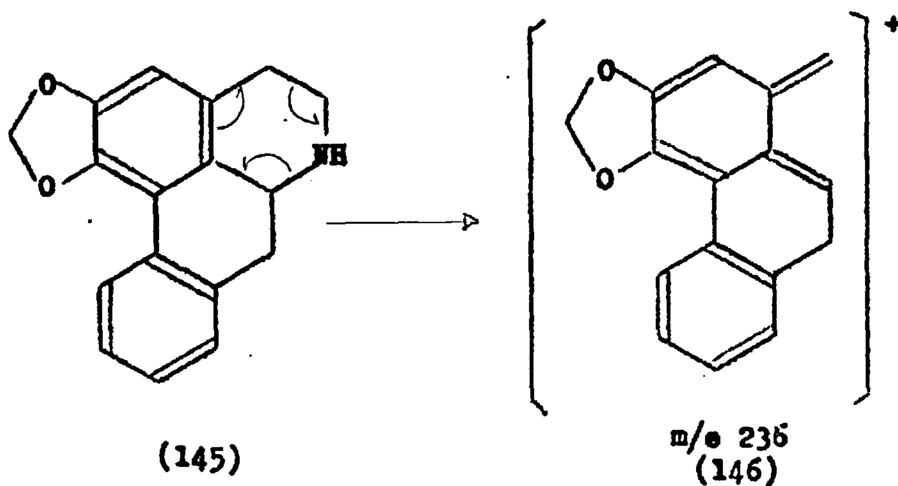
The mass spectra of aporphine alkaloids have been reviewed[134]. For anonaine with no methoxyl groups there are few recognisable abundant peaks. The base peak is at M-1 due to the loss of the benzylic hydrogen (143 → 144), and the other large peak is due to the retero-Diels-Adler reaction (145 → 146) occurring at M-29.



m/e 265
(143)



m/e 264
(144)



The U.V. spectrum of anonaine shows the typical three maxima of an aporphine with no substituent at C-10 or C-11[89,90].

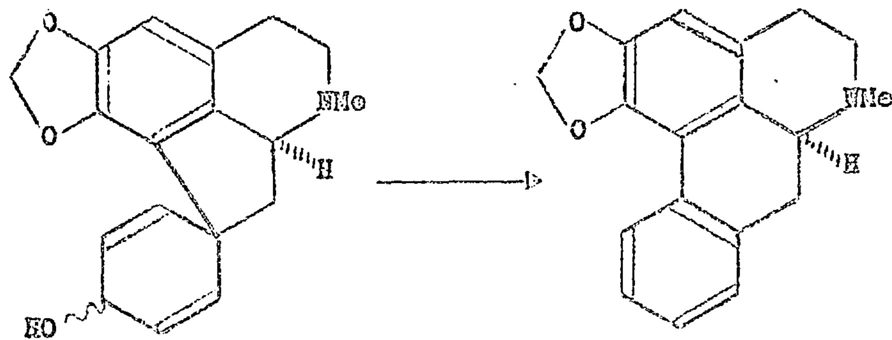
Isolation of Roemerine

(-)- Roemerine was first isolated from Roemeria refracta[135], and on Hofmann degradation of the methiodide it yielded a series of compounds, which Barger and Weitnauer identified as those derived from anonaine[128]. They synthesised (±)-roemerine from (±)-anonaine by N-methylation. (-)-Roemerine has also been isolated from Neolitsea sericiea[136], Nelumbo nucifera[137] and Cryptocarya angulata[114].

In 1963 Slavik isolated (+)-roemerine (147) from Papaver dubium[138] along with mecambrine and it was with this plant that the biosynthesis of roemerine was investigated. (+)-Roemerine has also been obtained from Papaver fugax along with mecambrine (fugapavine)[139].

We obtained (+)-roemerine from Papaver dubium by a simplified procedure. It was not, however, known whether roemerine (147) was an artefact, since any of the previous procedures (using dilute acid) would have converted the dienol (148) to roemerine. When the isolation was carried out without using acid strong enough to cause the isomerisation, none of the dienol (148) was observed. It is likely that the alkaloid is present in the plant as the aporphine, although its production from the dienol need

not necessarily be enzymatic.

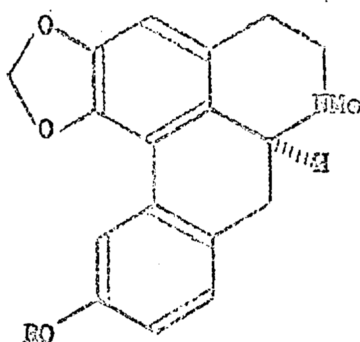


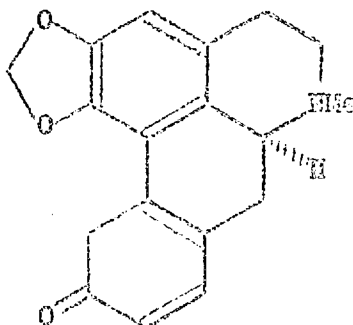
(148) (147)

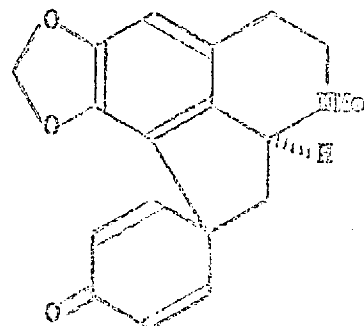
Since the rotation of roemerine isolated from *Papaver dubium* is positive, its absolute configuration is as in the diagram (147).

Isolation of Mecambrine

Mecambrine was first isolated from Papaver fugax[139] and from Meconopsis cambrica (the Welsh poppy)[140]. On the basis of its conversion into (+)-laureline (149) after treatment with acid followed by diazomethane, the formation of (+)- roemerine after reduction and dehydration, functional group tests and physical evidence the Russians proposed the structure (150)[141].







(149, R=Me) (150) (75)
(151, R=H)

It was argued[95,142] that the Russians' evidence could also be explained by the more likely structure (75), although it was pointed out[90] that the uv spectra of mecambrine and fugapavine were not identical. Slavik confirmed the structure (75)

as that of mecambrine[98], and supported the suggestion[91] that mecambrine and fugapavine and also mecambroline and isofugapavine (151), the product of acid treatment of the dienone, were identical.

The absolute configurations of pro-aporphines have been determined by conversion into benzylisoquinolines or aporphines of known configuration. Thus (+)-pro-nuciferine (152) has been cleaved[94] to (-)- armepavine (153) of known configuration[143] showing that (+)-pro-nuciferine belongs to the D (or R) configuration.

(152) (153)

Pro-nuciferine can also be converted into (-)- nuciferine, indicating the absolute configuration of the latter. In general it appears that (+)- pro-aporphines belong to the D (or R) configuration.

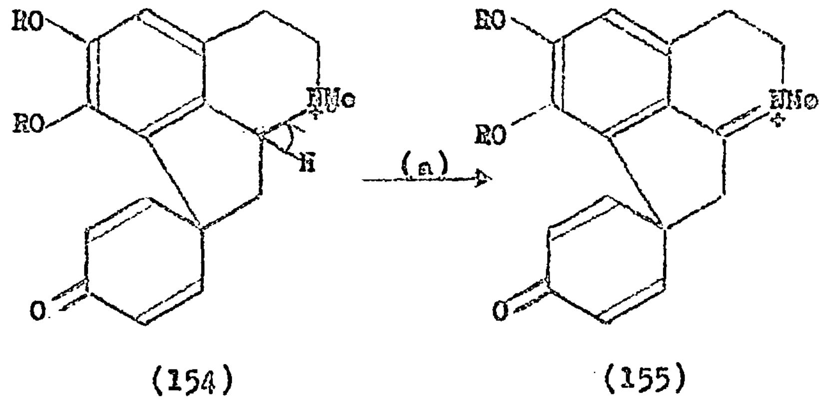
(-)-Mecambrine after acid treatment and methylation gives (+)-laureline[141], and after reduction and acid treatment (+)- roemerine confirming that it has the L (or S) configuration as in the diagram (75).

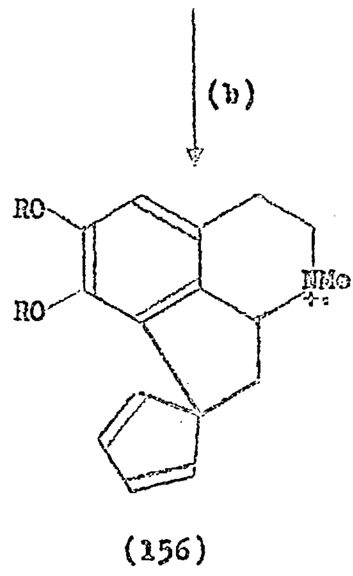
Slavik has also isolated the product of acid rearrangement of mecambrine, mecambroline (151), from _Meconopsis cambrica_[98].

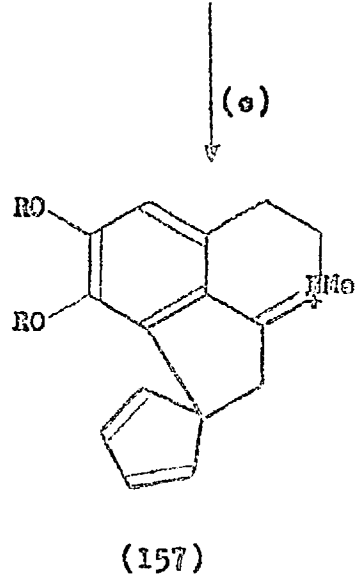
Mecambrine has also been shown to occur, with (+)-armepavine (153, enantiomer) in _P. caucasicum_, _P. triniaefolium_, _P. armeniacum_, _P. Persicum_[98,144,145], with (+)-roemerine in _P. dubium_[138], and in _P. polychaetum_[145,146].

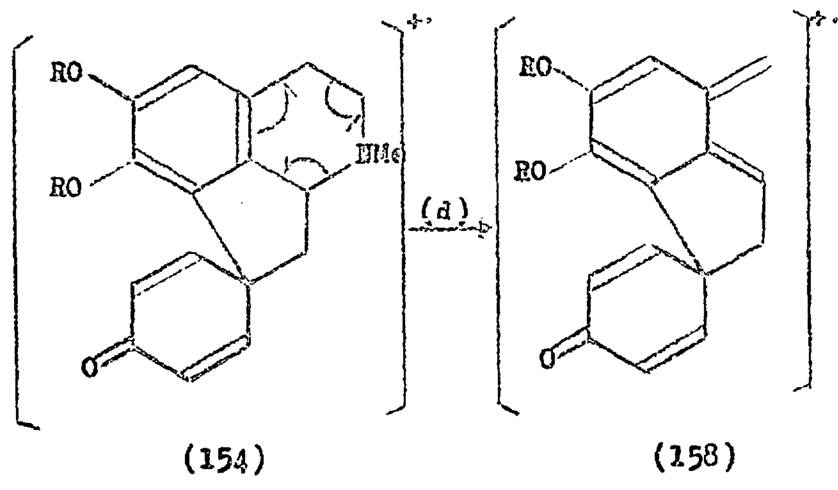
We isolated it from _Meconopsis cambrica_ roots. Its mass spectrum was very similar to that published for pro-nuciferine[147] and had similar peaks to those reported for glaziovine[95] (_vide infra_).

		m/e for mecambrine	m/e for pro-nuciferine	Process
M^+	(154)	295	311	
M-1	(155)	294	310	(a) loss of benzylic H
M-28	(156)	267	283	(b) loss of CO
M-29	(157)	266	282	(c) loss of H & CO
M-43	(158)	252	268	(d) loss of $CH_2 = NMe$


RO
RO
NMe
H
(a)
O
RO
RO
NMe
O
(154)
(155)


(b)
RO
RO
NMe
(156)

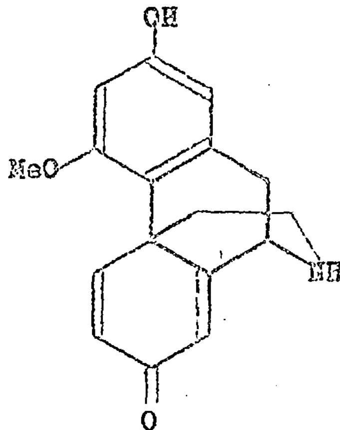

(c)
RO
RO
NMe
(157)


RO
RO
NMe
O
(d)
RO
RO
O
(154)
(158)

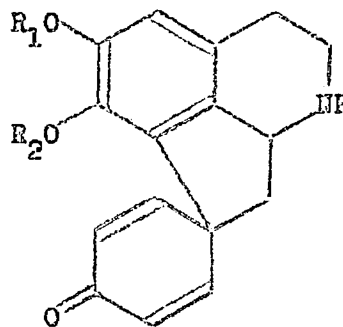
Isolation of Crotonosine

Crotosine, the major alkaloid of *Croton linearis*, was isolated in 1963[97] with linearisine, homolinearisine and base A. Subsequnet work has shown the identity of base A with pronuciferine and *N*-methylstepharine.

On the basis of functional group determination, physical evidence and the rearrangement to apocrotonosine, the structure (159) was assigned to crotonosine[97]. However on the basis of a thorough examination of the n.m.r. spectrum the formulae (160 and 161) were proposed[99]. Finally from an examination of the alkaline deuterium oxide exchange products of



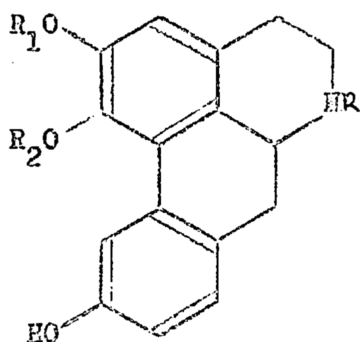
(159)



	R_1	R_2
(160)	Me	H
(161)	H	Me

apocrotonosine and apoglaziovine and a comparison of crotonosine and glaziovine (the formula of the latter having been determined[95])

it was shown that crotonosine had the less biogenetically probable formula (161)[99]. Apocrotonosine (162) exchanged three protons whereas apoglaziovine (163) exchanged only two.



	R	R_1	R_2
(162)	H	H	Me
(163)	Me	Me	H

It was with _Croton linearis_ that the feeding experiments were carried out in the West Indies by Professor Haynes and Dr. Stuart, and from this plant that further crotonosine was isolated.

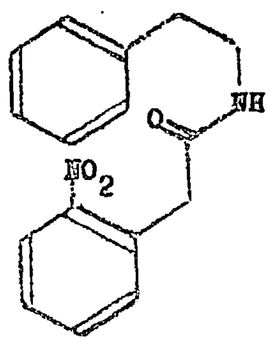
Synthesis of Alkaloids

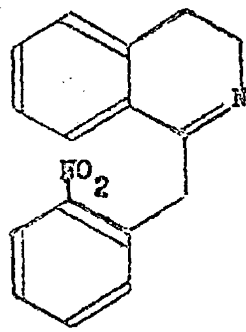
Ever since Gadamer[148] synthesised glaucine by the route previously worked out by Pschorr[149], many aporphine alkaloids have been synthesised using the Pschorr phenanthrene synthesis in which an amine (166) is diazotised and the diazonium compound decomposed to give an aporphine (167).

Several methods have been used for preparing the amine (166), the most common involving cyclodehydration of the simply prepared amide (164) to give the dihydroisoquinoline (165) - the Bischler - Napieralski reaction[150]. Reduction of the dihydroisoquinoline gives the amine required for cyclisation.

In certain cases the amine (166) can be synthesised from a readily available isoquinoline as in Pschorr's original attempt at the synthesis of glaucine[149].

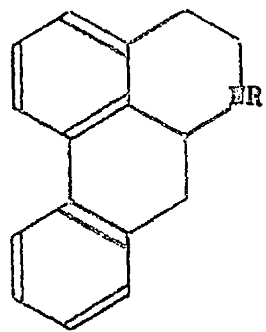
Recently, in the synthesis of laureline (79), the amine (170) was synthesised via a benzyne intermediate (169), generated from the bromo-compound (168) by potassamide [151].

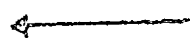


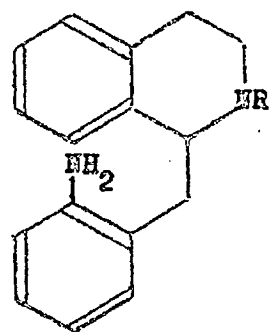


(164)

(165)

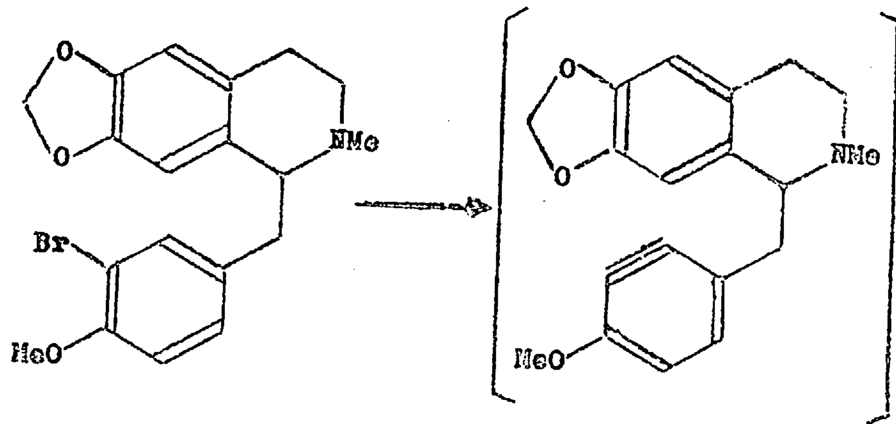


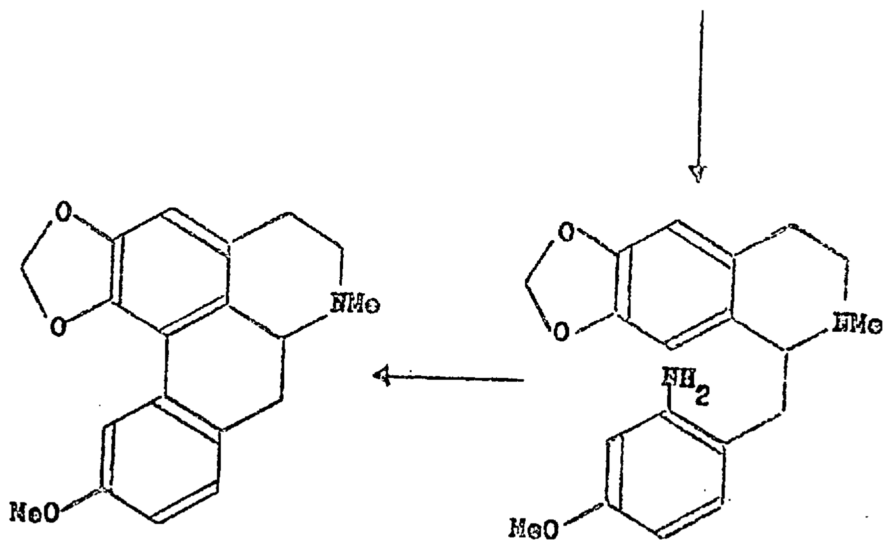




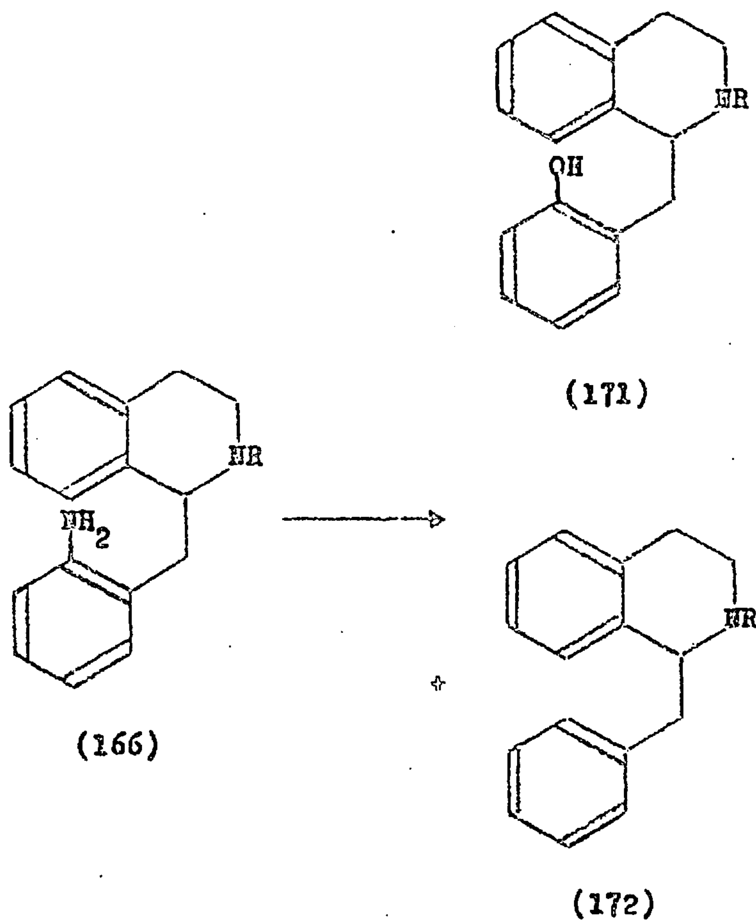
(167)

(166)


O
O
NMe
Br
MeO
(168)
O
O
NMe
MeO
(169)


O
O
NMe
MeO
(79)
O
O
NMe
NH_2
MeO
(170)

All these syntheses suffer from the disadvantage that the Pschorr cyclisation to form aporphines generally proceeds in poor yield[152]. The best yield so far recorded[153] is 40%, but usually the yields are about 20%. The main by-products of the cyclisation are the phenol (171) and the benzylisoquinoline (172), and in several cases these have been isolated.

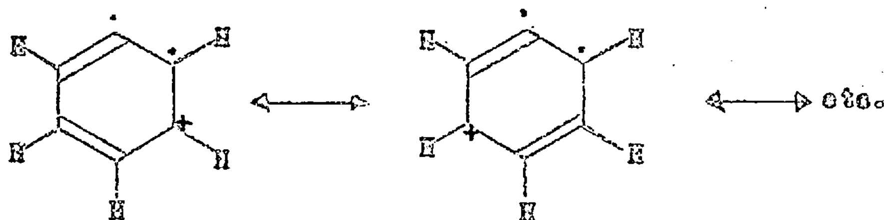


+ Aporphine (167)

With N-noraporphines the yields are often very much lower, for example 2.5% for the synthesis of (±) anonaine[129] and 3.5% for the synthesis of (±)- N - nornuciferine[154], possibly because of the side-reactions that occur with N-hydrogen, such as the formation of the N-nitroso group which is subsequently reduced off. Another possible cause of better yields with N-methyl and other N-substituated compounds is the conformational effect, whereby ring C is forced closer to ring A [88,155,156,157] (*vide infra*) encouraging cyclisation. Recently methods have been described for the protection of the N-H during cyclisation [113,154], but the increase in yield during the cyclisation does not necessarily warrant the extra steps involved.

Even now, over sixty years since the discovery of the Pschorr phenanthrene synthesis, the mechanism is not fully understood. For the uncatalysed reaction there is evidence of an S_N1 mode of decomposition of the diazonium compound [158,159], but the copper catalysed reaction has been regarded as a radical process analogous to the Gomberg reaction [160,161]. The suggestion has also been made that both processes occur simultaneously [158,160].

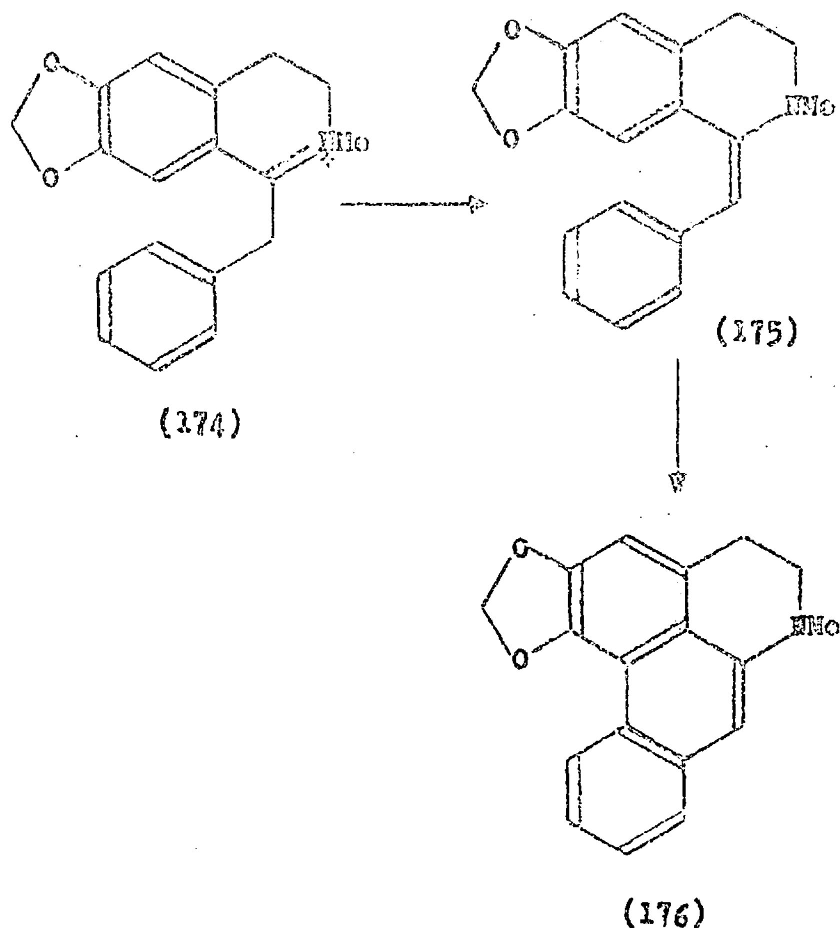
The existence of a diradical cation (173) has also been postulated[162,163] to account for the participation of a radical in the uncatalysed reaction[164] and the improbability of a straightforward electrophilic attack[165].



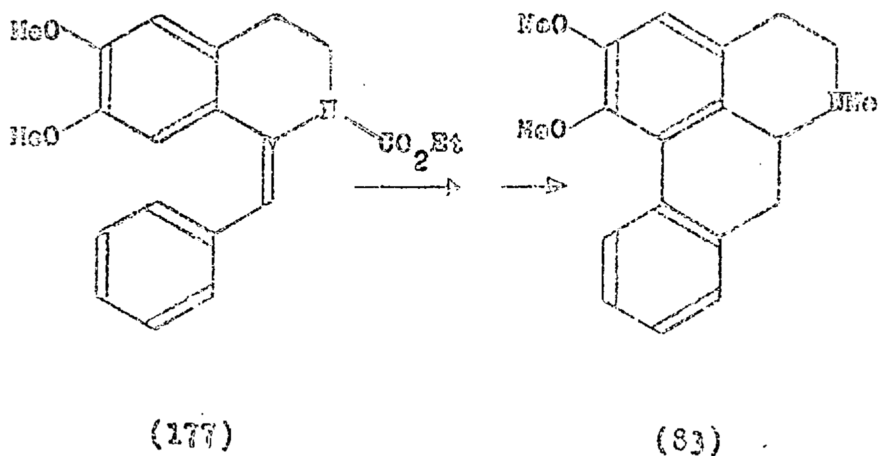
(173)

It has also been shown[163] that u.v. irradiation can increase the yield of the cyclised compound, and with a similar system variations in temperature have been shown to have little effect, although steric factors appear to be very important[158].

Because of the low yields in the last stage other methods of aporphine synthesis have been attempted. By analogy with the photocyclisation of stilbene derivatives to phenanthrenes [166] it was thought that the substituted stilbene (175), generated from the methiodide (174) could be cyclised, in the presence of a hydrogen abstractor, to a dehydroaporphine (176). When this was attempted in the presence of various oxidising agents no aporphine could be obtained [167].



However, the photocyclisation has been achieved in cases where the influence of the nitrogen lone pair electrons is removed, as in the cyclisation of the substituted stilbene (177) to give, after reduction, (±)- nuciferine (83)[168].



(177) (83)

Aporphines have also been synthesised by methods simulating the biogenetic processes, but these have already been described.

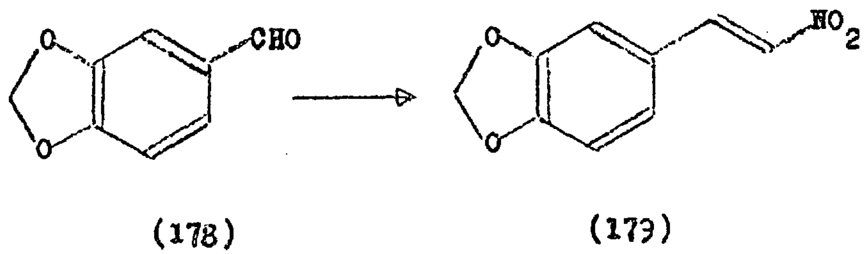
(±)-Anonaine (81) has been synthesised twice previously[128,129] and, besides being formed by the methylation of (±)-anonaine[128,129].

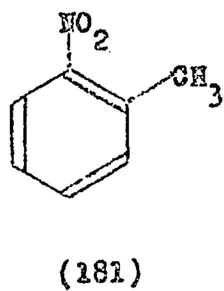
($\pm$)-roemerine (82) has been synthesised independently[169].
Recently, using the benzyl group to protect the _N_-H during
the ring closure ($\pm$)-anonaine has been re-synthesised[113].

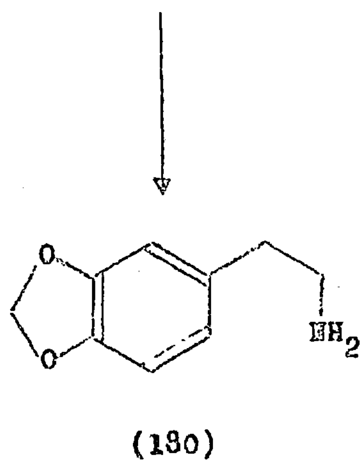
Our synthesis of ($\pm$)-anonaine, which was required for radio-dilution, was essentially similar to the procedure of Barger and Weitnauer[128]. Piperonaldehyde (178) was converted into the nitrostyrene (179) using a successful modified method and the nitrostyrene was reduced to the amine (180). The acid (183) was prepared from o-nitrotoluene (181) _via_ the pyruvic acid (182). The amide (184), prepared from the acid chloride of the acid (183) and the amine (180), was dehydrated to the dihydroisoquinoline (185).

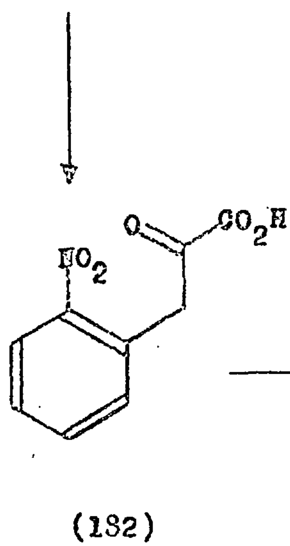
At this point the routes to anonaine and roemerine diverged. For anonaine the dihydroisoquinoline (185) was reduced to the diamine (186).

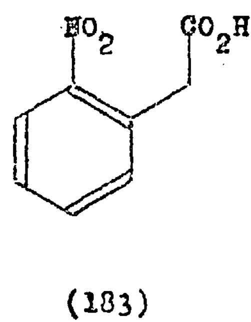
The diamine was diazotised for five hours (shorter reaction times resulted in incomplete diazotisation) and the diazonium compound was decomposed quickly. No methanol, which could act as a reducing agent, was added to help prevent formation of the reduced compound. Photolysis of the diazonium compound did not increase the yield. After cyclisation the _N_-nitroso group was reduced off. The best yield obtained (of solid hydrochloride) was 20%.


CHO
NO_2
(178)
(179)

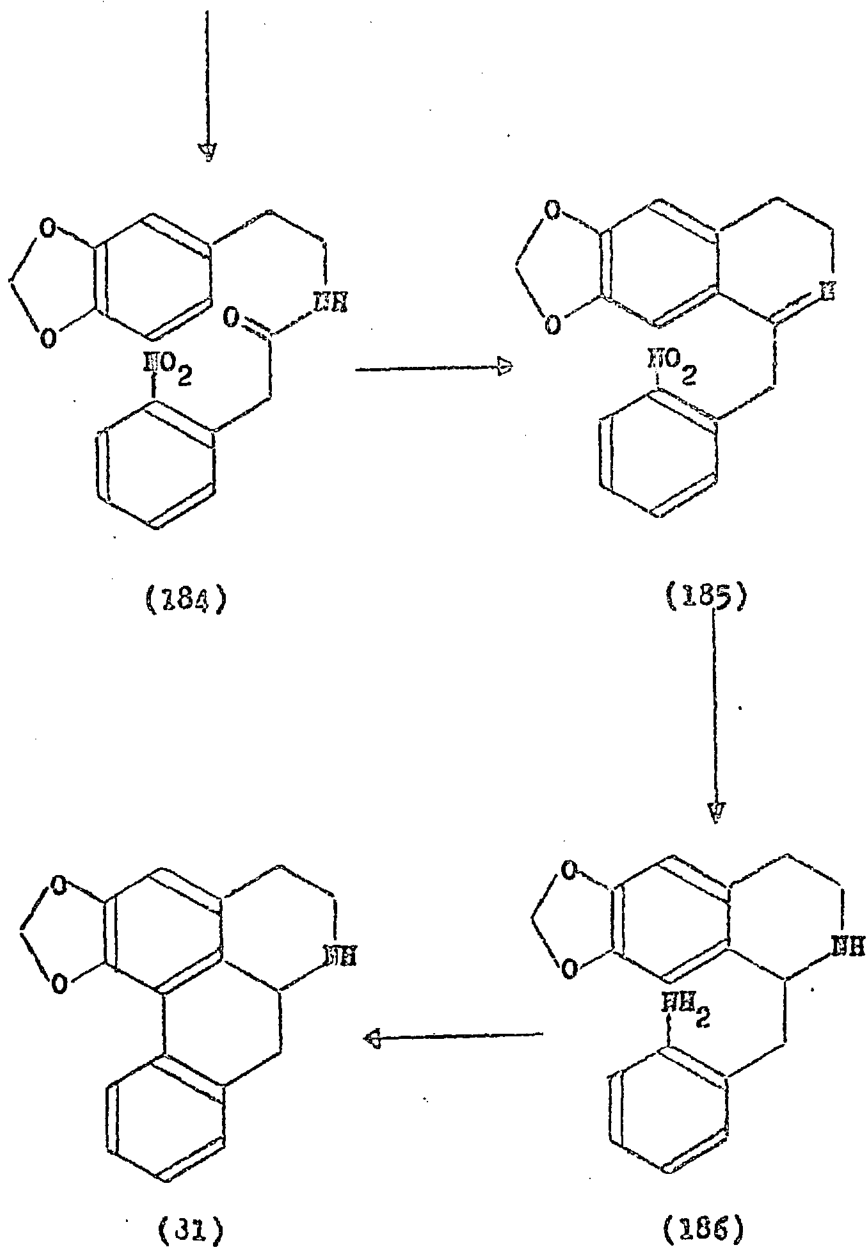

NO_2
CH_3
(181)


NH_2
(180)

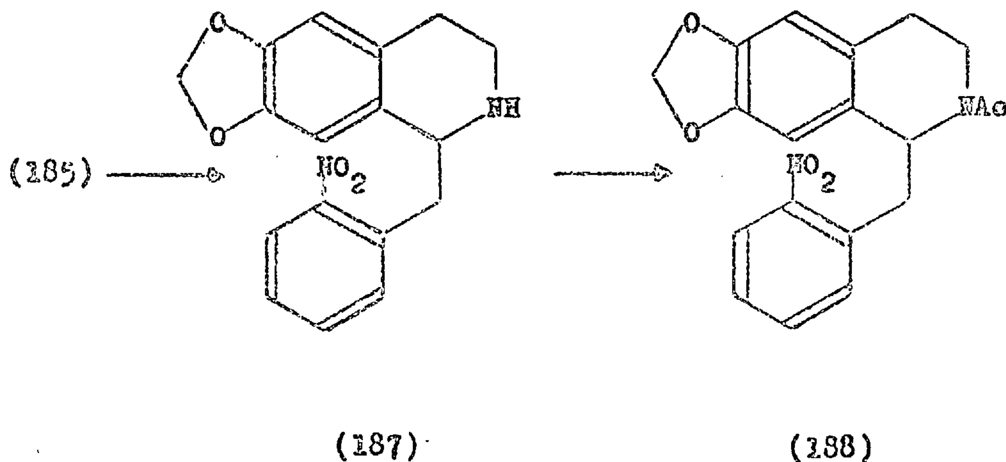

O
CO_2H
NO_2
(182)


NO_2
CO_2H
(183)

(180) + (183)



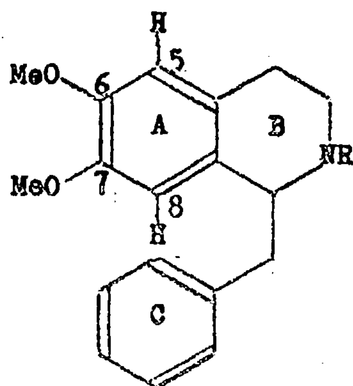
It was suggested that the nitrogen might be protected in order to prevent side reactions. The imine (185) was reduced to give the tetrahydroisoquinoline (187), which was acetylated to give the N-acetyl compound (188).



(187) (188)

Although this synthesis did not proceed further, since satisfactory amounts of (±)-anonaine had been obtained, the N-acetyl compound showed unusual properties. It exists at room temperature as two relatively stable rotational isomers.

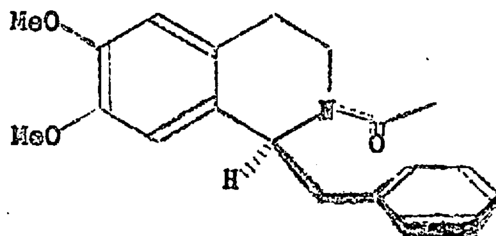
This effect has also been observed by Cava[88]. In his series (189, 190, 191) the n.m.r. resonances were observed at:



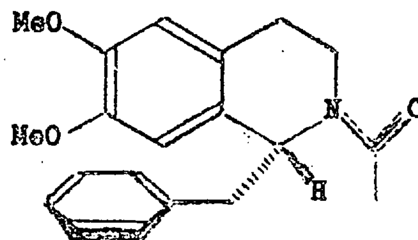
	R
(189)	H
(190)	Me
(191)	CH_3CO

	Aromatic protons		O-Methyl protons		Substituent on nitrogen
	C - 5,	C - 8	C - 6,	C - 7	
(189)	3.39	3.39	6.16	6.22	8.06 (H)
(190)	3.43	4.01	6.18	6.48	7.47 (CH_3)
(191) (a)	3.37	3.41	6.15	6.22	8.40 (CH_3CO)
(b)	3.52	3.39	6.18	6.45	7.89 (CH_3CO)

With the N-H compound (189) the two aromatic protons at C-5 and C-8 absorb at the same position, but if ring C is forced towards ring A by an N-methyl group the proton at C-8 becomes shielded and absorbs at τ 4.01; this is a well known effect [88,155,156,157]. However, on N-acetylation the barrier is sufficient to cause two isomers to exist (191a and 191 b).



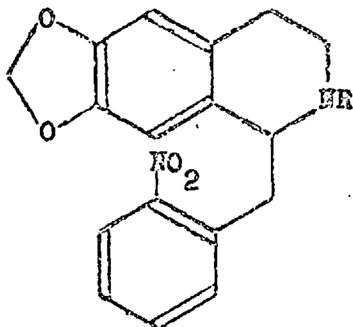
(191a)



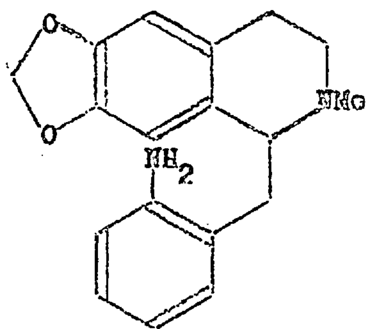
(191b)

In one isomer (191a, acetyl methyl up) the acetyl methyl group offers no repulsion to ring C, which can exist away from ring A. Thus the aromatic protons in ring A occur at the relatively normal positions (τ 3.37 and 3.41), but the *N*-acetyl group suffers considerable shielding and the methyl group occurs at τ 8.40. In the other isomer (191 b, acetyl methyl down) ring C is forced close to ring A and the aromatic protons occur further upfield at τ 3.52 and 3.89; the protons of the methyl group of the acetyl portion absorb at the more normal position at τ 7.89. Cava's explanation may be an over-simplification, since it does not take account of the conformational effects in ring B.

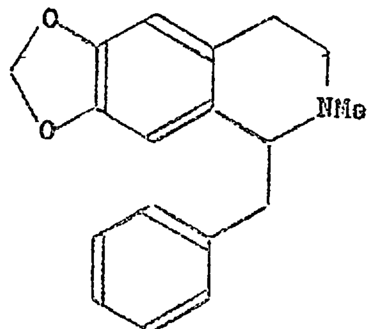
With our compounds the resonances for the compounds (187, 192, 188) occur at



	R
(187)	H
(192)	Me
(188)	CH_3CO



(193)

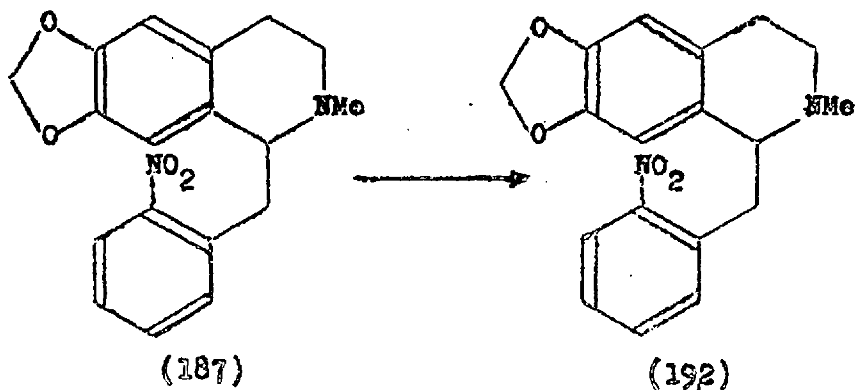


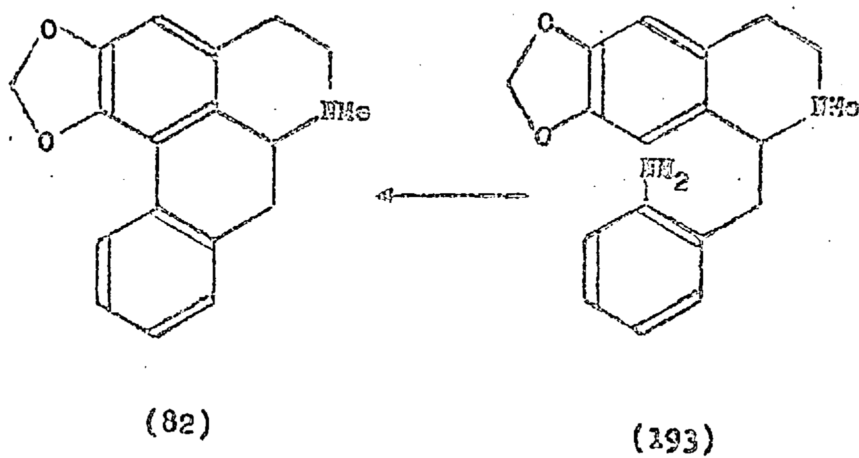
(194)

	Aromatic protons (probable assignments) at C-5	C-8	Methylenedioxy protons	Substituent on nitrogen
(187)	3.30	3.50	4.15	7.73 (H)
(192)	3.50	3.61	4.17	7.73 (CH_3)
(188)	3.30	3.58	4.22	8.60 (CH_3CO)
	3.53	3.58		3.05
(193)	3.51	3.57	4.16	7.55 (CH_3)
(194)	3.58	3.77	4.18	7.64 (CH_3)

With these compounds the assignments are complicated by the presence of the nitro group. In the benzylisoquinoline (194) the ring A aromatic protons can be assigned thus: C-5, τ3.58 and C-8, τ3.77. With the nitro compounds the effect of N-methylation shows a general shift upfield of the ring A protons, but they cannot confidently be assigned, since the effect of the nitro group may be to shield or deshield. With the N-acetyl compound in one case ring C shields the methyl protons of the acetyl group sufficiently for their positions to be at τ8.60.

For the synthesis of roemerine the tetrahydroisoquinoline (187) was methylated to give the N-methyl compound (192). Reduction of this gave the diamine compound (193). This was diazotised as before and heated to give ($\pm$)-roemerine (82). The yield was generally about 29%, and could be improved by the addition of cuprous iodide to 35%.

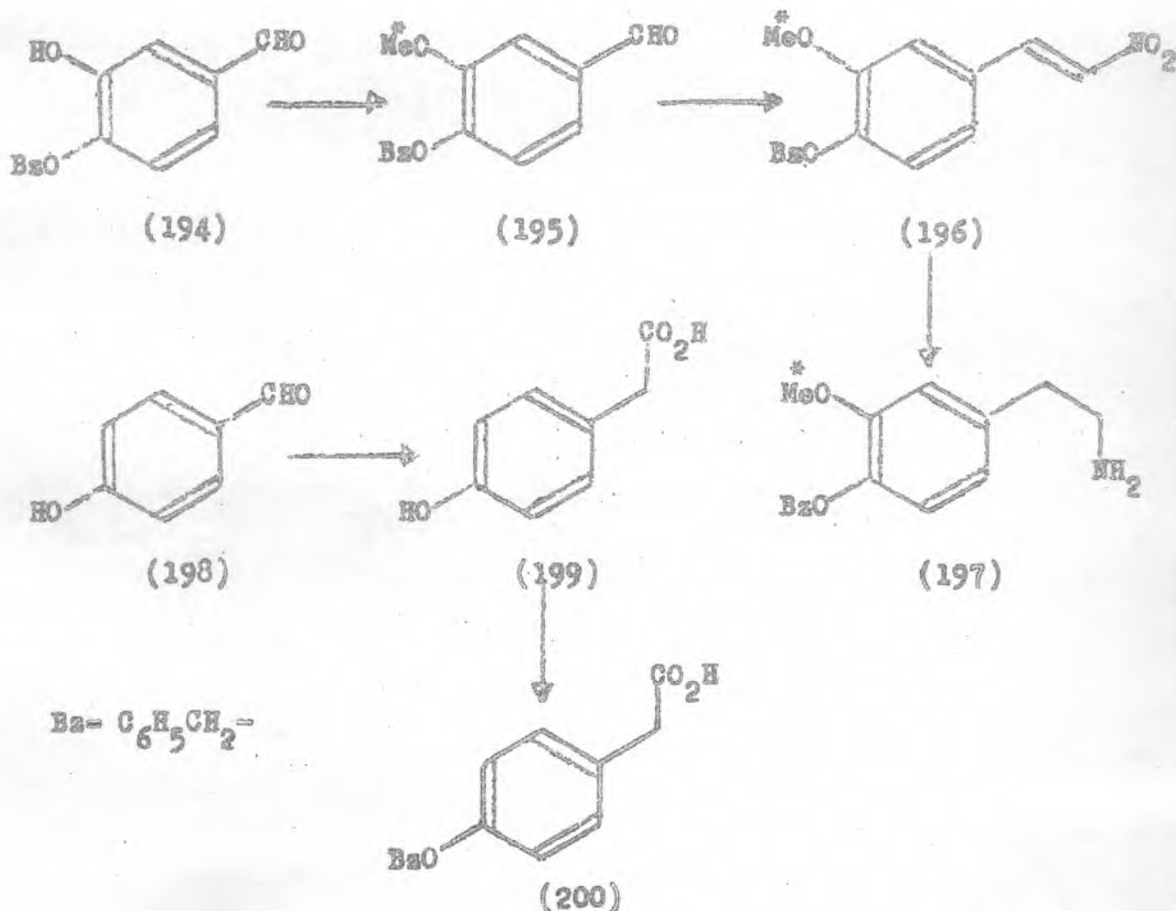

NMe
NO2
(187)
NMe
NO2
(192)


NMe
(82)
NMe
NH2
(193)

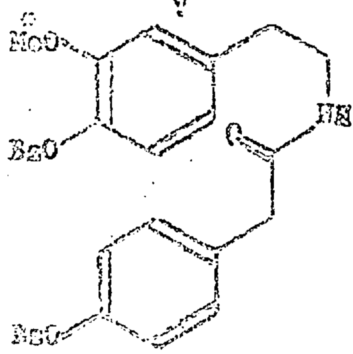
Synthesis of Precursors

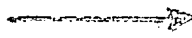
($\pm$)-Coclaurine (69,R=H) was synthesised according to the general method for substituted benzylisoquinolines. It had previously been synthesised several times [167,170], but the method used was essentially that of Dr. Bhakuni.

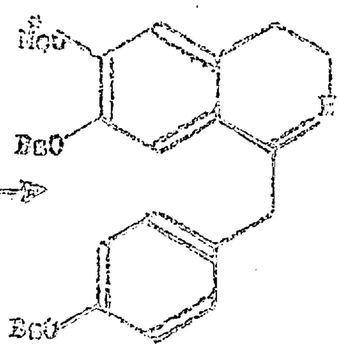
For [O-methyl - ^{14}C] coclaurine (204) the label was introduced at an early stage of the synthesis using [^{14}C] methyl iodide (194 → 195 → 196 → 197. 198 → 199 → 200. 197 + 200 → 201 → 202 → 203 → 204)



(197) + (200)

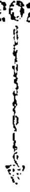


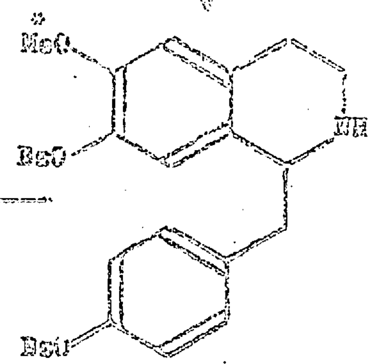


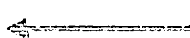


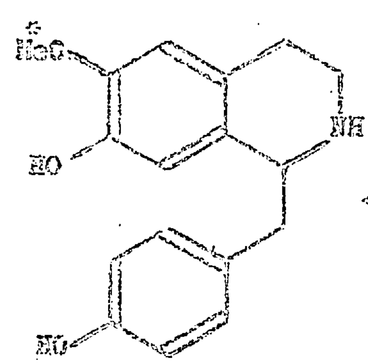
(201)

(202)





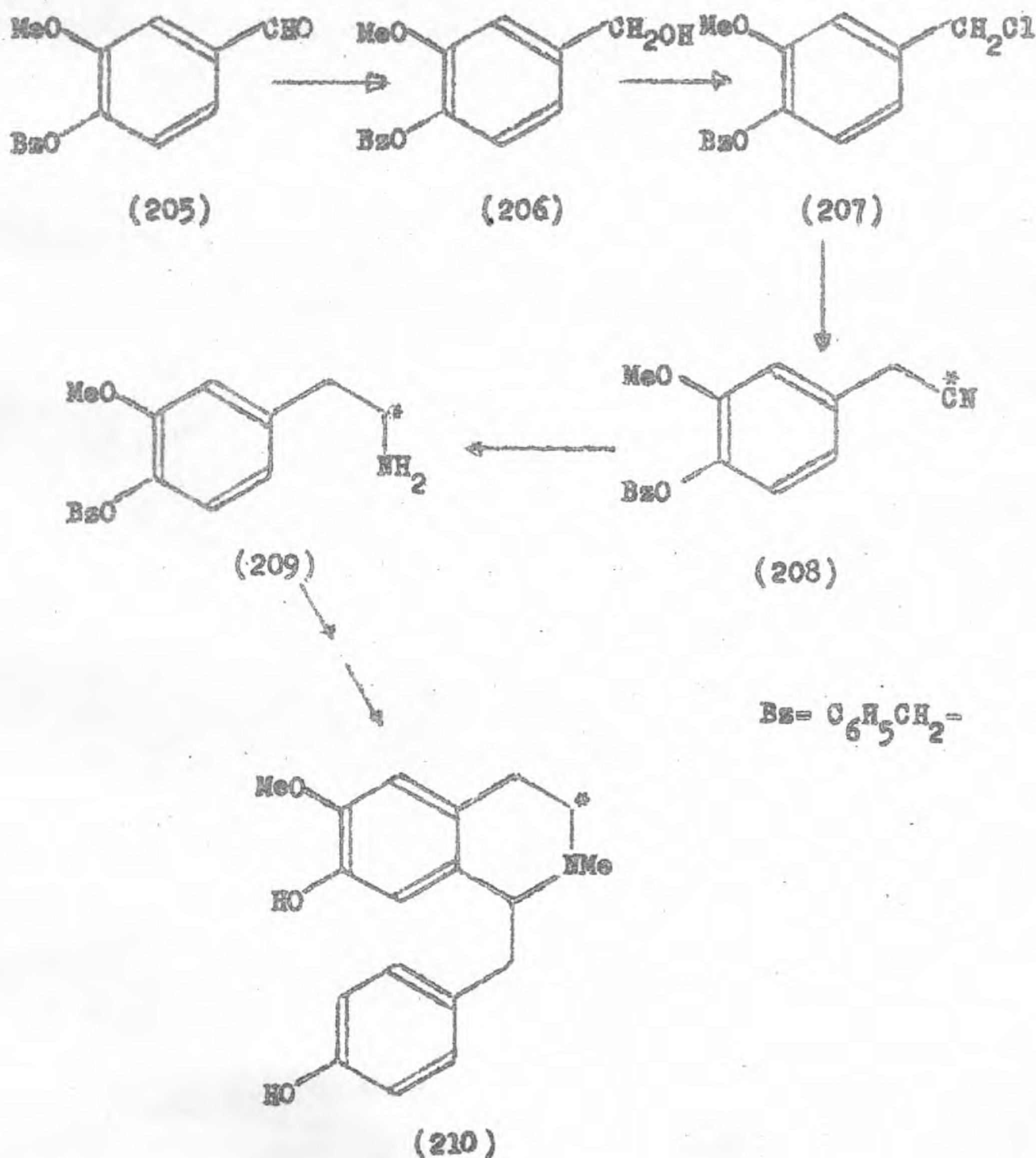




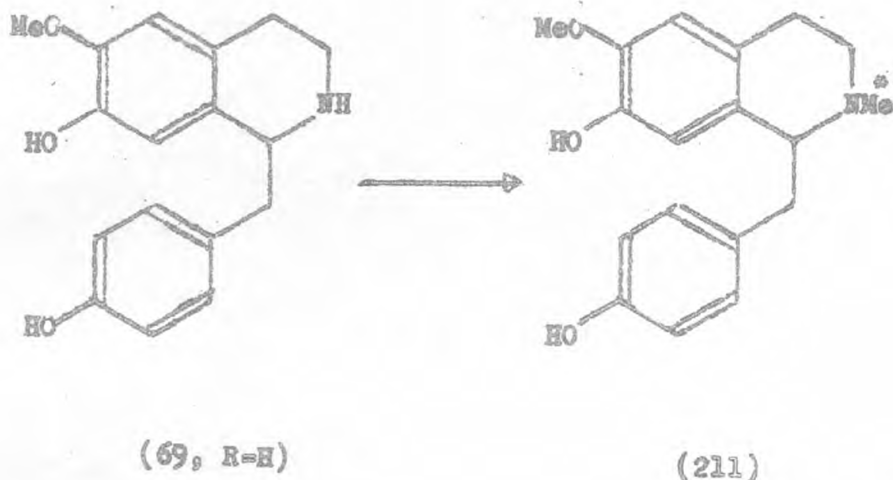
(204)

(203)

For N-methyl - [3-^{14}C] coclaurine (210) the label was introduced by treating the benzyl chloride (207) with sodium [^{14}C] cyanide [55,171]. The benzyl chloride (207) was prepared from O-benzylvanillin (205) *via* the alcohol (206). Reduction of the benzyl cyanide (208) gave the amine (209) required for the coclaurine synthesis.

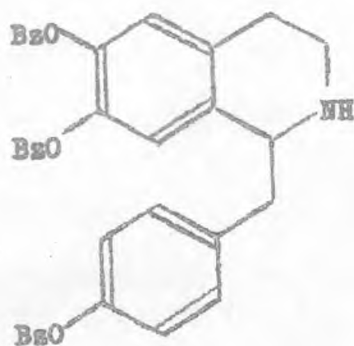


[N-methyl - ^{14}C] Coclaurine was prepared from coclaurine by the Eschweiler-Clark method using [^{14}C] paraformaldehyde (69,R=H ⟶ 211).

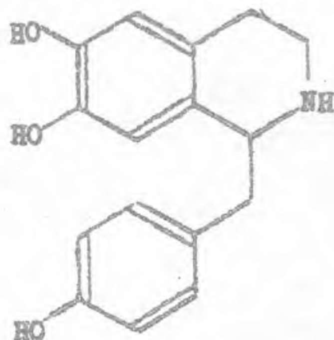


(69, R=H) (211)

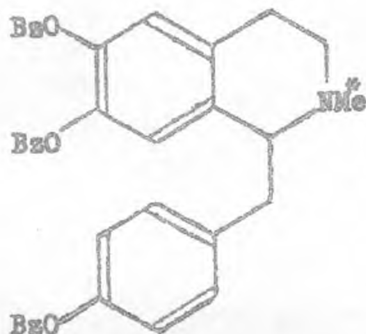
Norcoclaurine (213) was synthesised according to the standard benzylisoquinoline synthesis using the procedure of Dr. Bhakuni [167] (194 ⟶ 204, Bz replaces $\overset{*}{M}e$). However, N-methylation of norcoclaurine did not proceed satisfactorily. Instead the tri-O-benzyl compound (212) was methylated using radioactive paraformaldehyde to give the N-methyl compound (214), which was debenzylated to N-methylnorcoclaurine (215).



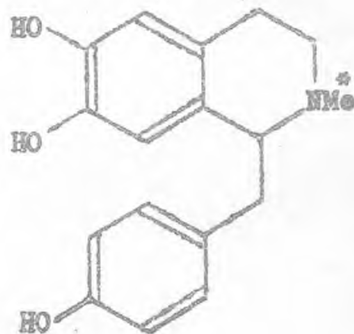
(212)



(213)

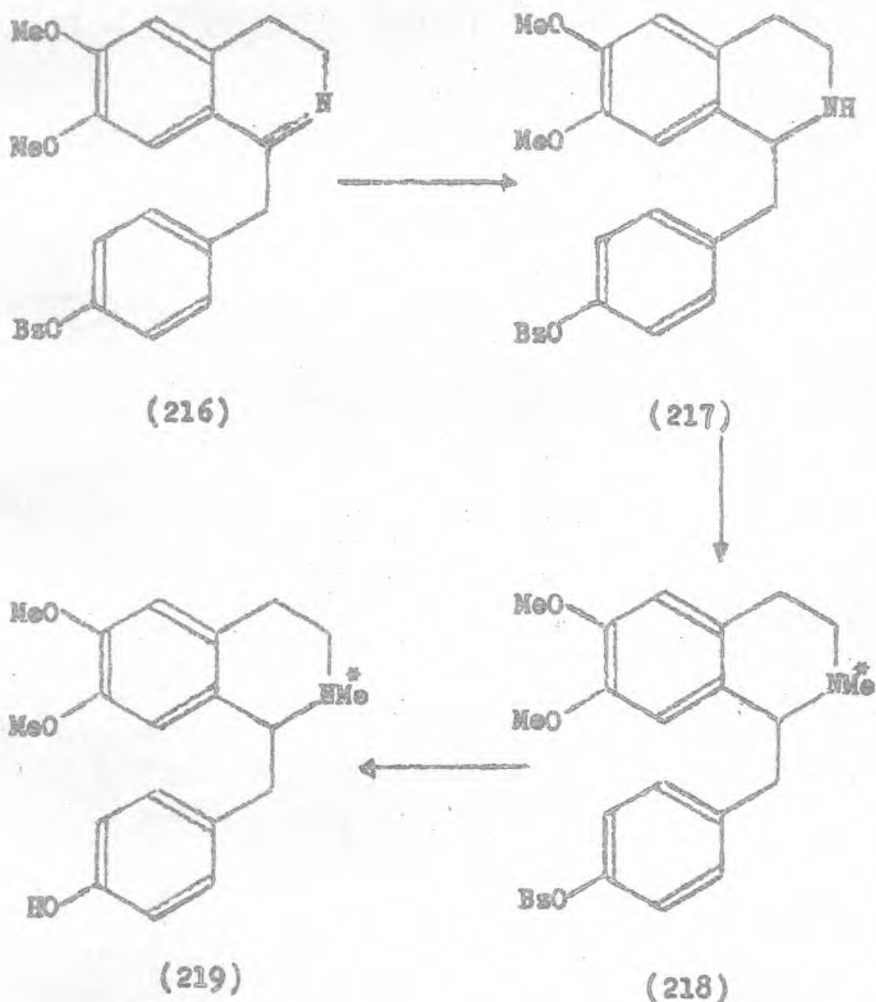


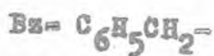
(214)



(215)

O-Benzyl-N-norarmepavine (219) was synthesised from the dihydroisoquinoline (216). N-Methylation using radioactive paraformaldehyde and subsequent debenzylation gave [N-methyl - ^{14}C] armepavine (219).





Results of Feedings

The theory of the biogenesis of roemerine and anonaine as outlined by Barton and Cohen[4] has been reviewed. The discovery of mecambrine, a probable (although not obligatory) intermediate in the biosynthesis of roemerine, supported their hypothesis.

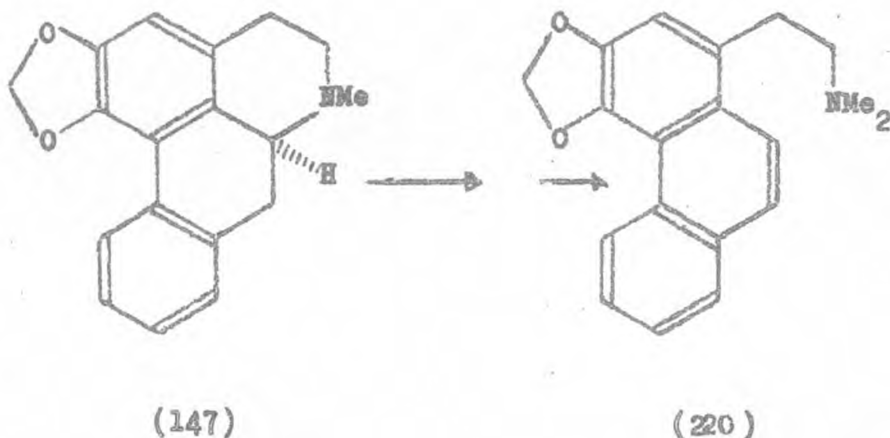
Besides these three alkaloids the biosynthesis of mecambroline, which occurs with mecambrine in Meconopsis cambrica, and of crotonosine another pro-aporphine dienone was investigated.

The first season's experiments were conducted on Papaver dubium plants and were designed to test the feasibility of the project as well as to give indications of the biosynthetic route to (+)-roemerine (147).

The poppies (P. dubium and M. cambrica) were fed by injecting the precursors except tyrosine, as their hydrochlorides in water, into the seed-pods after the petals had dropped. The plants were left for ten days to metabolise the precursors and were then harvested.

($\pm$)-$\left[2 - {}^{14}C\right]$ Tyrosine, in aqueous solution at pH6, was fed first, both to give indications of the biosynthetic pathway and to establish that the plant was synthesising the alkaloid. The (+)-roemerine, obtained from the plant, was diluted with ($\pm$)-roemerine. Recrystallization of this mixture of (+)- and

($\pm$)-roemerine did not give constant activity, so the centre of optical activity of the roemerine was removed by converting it to its methine base (220) by Hofmann degradation of its methiodide.



The methine base (220) was crystallized to constant activity as its hydrochloride and the activity was checked by making the methine base methiodide. In all cases in which (+)-roemerine of the plant was diluted with ($\pm$)-roemerine this procedure was used.

The incorporation of tyrosine was 0.17% confirming the biosynthesis of roemerine from this amino-acid and that the plant was active in synthesising the aporphine.

The other compounds fed during the 1964 season were (±)-coclaurine (221), (±)-isococlaurine (222), (±)-norcoclaurine (223) and (±)-*N*-methylcoclaurine (224). All these compounds were labelled with tritium *ortho* to the phenolic hydroxyl groups[172] and were prepared by Dr. Bhakuni. The results of these feedings are summarised in Table I.

Compound		R	R_1	R_2	Incorporation %
(±)-Coclaurine	(221)	H	Me	H	0.062
(±)-Isococlaurine	(222)	H	H	Me	0.00
(±)-Norcoclaurine	(223)	H	H	H	0.34
(±)-*N*-methylcoclaurine	(224)	Me	Me	H	0.48
(±)-Tyrosine	(8)				0.17

Incorporations allow for tritium loss from C-8 where appropriate.

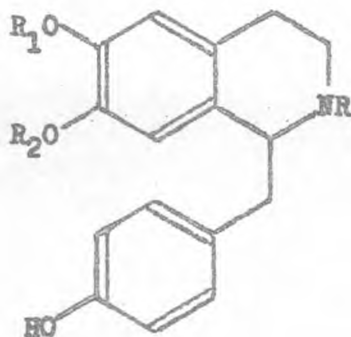


TABLE I

As expected, (±)-isococlaurine (222), having the wrong methylation pattern for the phenol coupling was not incorporated. This is analogous to Battersby's experiments with the tetraoxygenated system[58] (see page 17). Furthermore, since (±)-norcoclaurine was incorporated, demethylation of (±) isococlaurine to give the nor-compound had not occurred.

(±)-Coclaurine was incorporated less efficiently than either (±)-norcoclaurine or (±)-N-methylcoclaurine, suggesting that N-methylation occurs at the norcoclaurine stage and precedes O-methylation. Since N-methylcoclaurine was incorporated more efficiently than coclaurine, the N-methyl derivate is probably the compound that undergoes the phenol coupling.

The next season's feeding experiments with P. dubium were designed to confirm the biosynthesis of roemerine from N-methylcoclaurine using a doubly-labelled precursor. The methylation sequence at the benzylisoquinoline stage and the stereospecificity of the biosynthesis were also investigated.

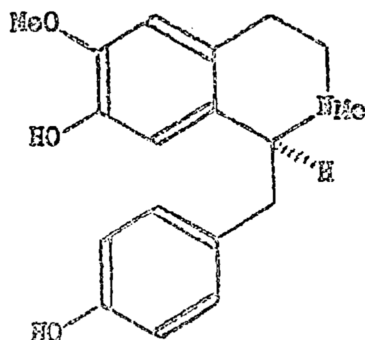
N-Methyl labelled (±)-N-methylnorcoclaurine (226) was fed in parallel with (±)-coclaurine but was not incorporated as efficiently. It seems probable that the biogenetic methylation of norcoclaurine can proceed in either way to give coclaurine or N-methylnorcoclaurine which are subsequently methylated to form

N-methylcoclaurine. All the activity of the derived (+)-roemerine was located in the N-methyl group.

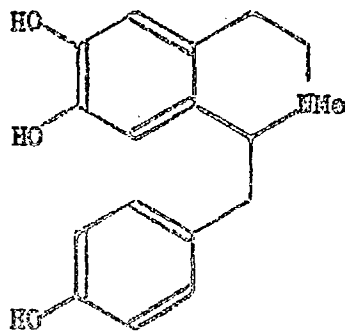
(+)-N-Methylcoclaurine (225) and (-)-N-methylcoclaurine (enantiomer of 225) were incorporated as expected (0.11% and 0.000% respectively) confirming the stereospecificity of the biosynthetic process. With these feedings (±)-roemerine was used for dilution, since, if the plant was able to produce (-)-roemerine from (-)-N-methylcoclaurine, activity would still be retained in the purified (±)-roemerine. However this was not observed showing that at least one stage between the benzylisoquinoline and aporphine is stereospecific.

The doubly-labelled (±)-N-methylcoclaurine contained 81% of the activity in the N-methyl group and 19% in the O-methyl, the ratio being checked by a selective Herzig-Meyer determination. It was expected that the ratio would be the same in the roemerine obtained, since conversion of an o-methoxyphenol to a methylenedioxy group is well known [155,173] and generally not accompanied by demethylation. However, in the biosynthetic roemerine the N-methyl group contained 87% of the activity and the O-methyl group 11%. Thus a significant proportion of the O-methyl label was lost. The N-methyl activity was determined by the Herzig-Meyer method, and the methylenedioxy group activity was obtained by making the dimedone derivative of the formaldehyde liberated by acid hydrolysis.

The 1965 season's feedings to *P. dubium* are summarised in Table II.



(225)



(226)

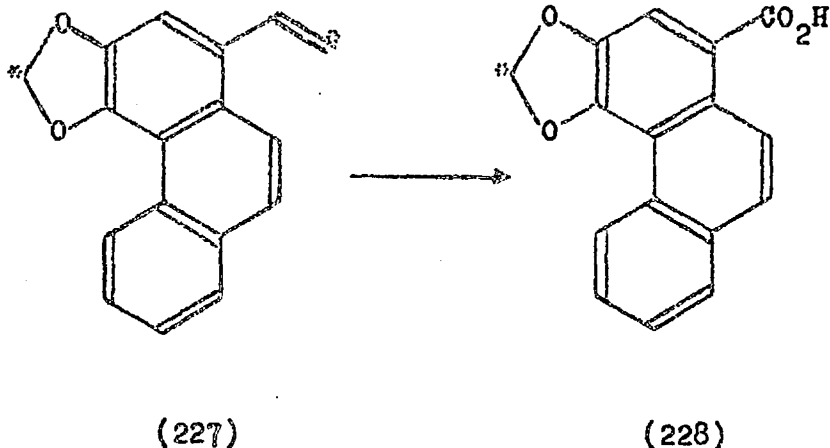
Precursor		Labelling Pattern	Roemerine used for dilution	Incorporation %
(±)-Coclaurine	(221)	[8,3',5', - $^{3}H_{3}$]	(+)	0.15
(±)-*N*-methylcoclaurine	(224)	[*N*,*O*-Methyl - ^{14}C]	(+)	0.19
(+)-*N*-methylcoclaurine	(225)	[8,3',5' - $^{3}H_{3}$]	(±)	0.11
(-)-*N*-methylcoclaurine		[8,3',5' - $^{3}H_{3}$]	(±)	0.000
(±)-*N*-methylnorcoclaurine	(226)	[*N*-Me - ^{14}C]	(+)	0.10

TABLE II

Feeding experiments were also conducted on Meconopsis cambrica plants. (±)- [8,3',5' - $^{3}H_{3}$] Coclaurine (221) was incorporated into mecambrine (75) in 0.066% yield. Acid treatment of mecambrine gave mecambroline (78), which on treatment with aqueous alkali lost all its activity, indicating the tritium was located at C-9 and C-11 as expected. (±)-N-Methylcoclaurine was also incorporated into mecambrine (0.03%).

For the 1966 feeding season triply ^{14}C labelled (±)-N-methyl-coclaurine was prepared to confirm its incorporation into roemerine and mecambrine and to determine if the loss of O-methyl activity was proportionally the same. The precursor labelling pattern was: N-methyl (61.6%), O-methyl (13.0%) and C-3 (25.4%). The incorporation into roemerine was 0.19% and into mecambrine 0.089%.

The activity at the C-3 position was determined by Hofmann degradation of the methine base methiodide to give the vinyl phenanthrene (227). The difference between the activity of the vinyl phenanthrene and the phenanthrene carboxylic acid (228), obtained by oxidation of the former, indicated the activity at C-3.



(227) (228)

The activity of the N-methyl and methylenedixoy groups was determined as before. The roemerine labelling pattern was: N-methyl (72.0%), O-methyl (1.2%), and C-3 (29.4%). For mecambrine the corresponding activities were 72.1%, 1.6% and32.0%. Thus, although O-methyl activity was lost as before, the ratio between the N-methyl activity and that at the C-3 position remained essentially constant.

Labelled mecambrine was prepared by exchange with tritiated aqueous sodium hydroxide at room temperature. It was well incorporated into roemerine (2.34%) and mecambroline (2.76%). This confirms that the formation of the methylenedioxy group occurs at the dienone stage.

In agreement with Battersby's experiments [55,59] with the tetraoxygenated system (see page 17), (±)-[N-methyl-^{14}C] armepavine was not incorporated into roemerine.

These experiments have shown the correctness of Barton and Cohen's original scheme[4]. The loss of methoxyl activity during the biosynthesis has not been adequately explained and it is not known at which stage it occurs. It is possibly due to the reversibility of the methylation of N-methylnorcoclaurine. The proven sequence is summarised in Scheme III.

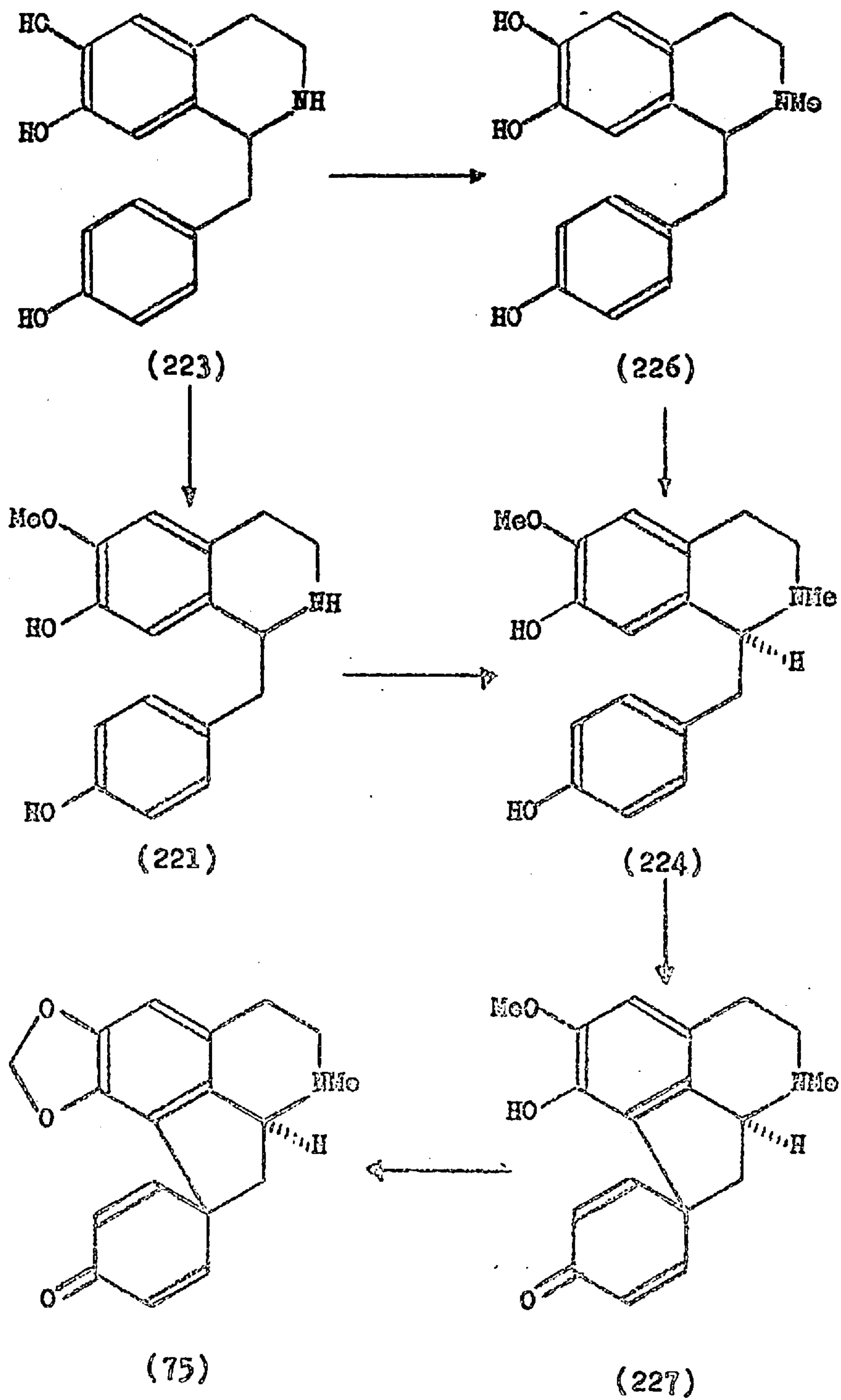
The phenol coupling step (224→227) was attempted in the laboratory using ferricyanide and a two-phase system. The product, (±)-glaziovine, was isolated in 1.1% yield.

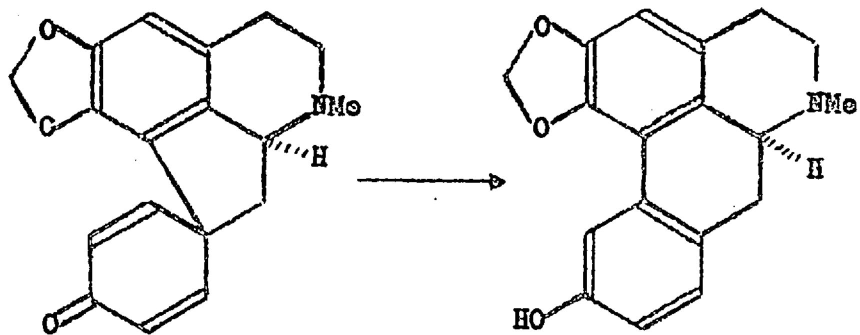
The biosynthesis of anonaine (81) was investigated with *Anona reticulata* plants. (±)-[8,3',5' $^{3}H_{3}$] Coclaurine and (±)-[5,8,3',5' $^{3}H_{4}$] norcoclaurine were both incorporated into anonaine (0.44% and 0.49% incorporations respectively).

Earlier experiments[59] had indicated that crotonosine (161) was derived from coclaurine (221), and in order to determine whether methyl migration occurred during the biosynthesis doubly-labelled coclaurine was prepared from [O-methyl-^{14}C] coclaurine and [8,3',5' - $^{3}H_{3}$] coclaurine.

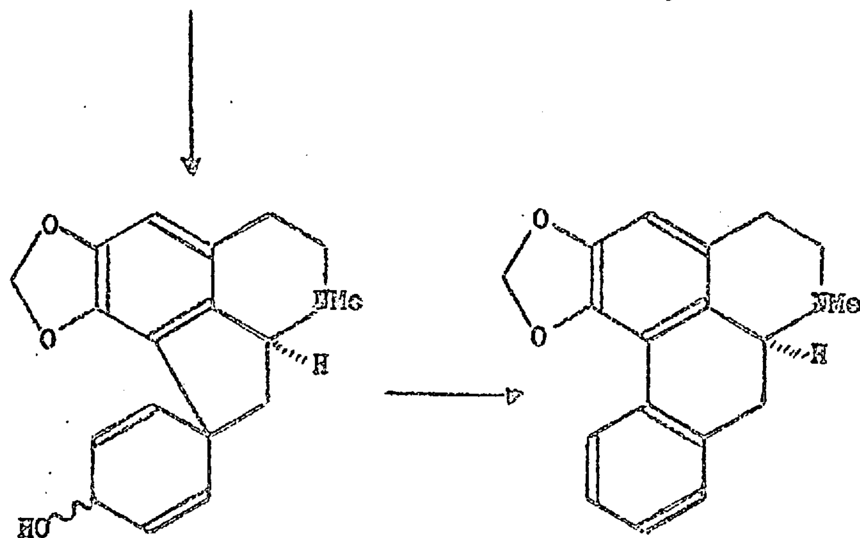
The ^{14}C: ^{3}H ratio in the precursor was 13.0:1 and although "theory" suggests the ratio in the derived crotonosine (0.034 % incorporation) should be 8.7:1 (loss of one tritium during cyclisation), it was found to be 21.5:1. Again methyl loss had occurred in the biosynthesis of a pro-aporphine.

SCHEME III





(75) (78)



(148) (147)

EXPERIMENTAL

All melting points were determined on a micro Kofler block and are uncorrected. Unless otherwise stated the ultraviolet absorption spectra refer to ethanol, infra-red absorption spectra to chloroform and n.m.r. spectra to deuterochloroform solutions. The n.m.r. spectra were recorded on a Varian A-60 spectrometer, and the multiplicities were designated by the abbreviations: s. (singlet), d. (doublet) and m. (multiplet). The mass spectra were recorded on an A.E.I. MS.9 double-focusing mass spectrometer, the samples being run using direct probe insertion with an electron beam of 70 eV. Micro-analyses were carried out at Imperial College initially under the direction of Miss J. Cuckney and thereafter under Mr. K.I. Jones. With benzene, ether, chloroform, ethanol and methanol solutions, unless otherwise stated, the solvents were removed under reduced pressure on a steam-bath. Chromatography, unless specified to the contrary, was carried out using neutral alumina of Brockmann activity III. Petroleum-ether refers to the fraction b.p. 40-60°.

Counting methods.

All ^{14}C and tritium labelled compounds were counted in a scintillation counter (Isotope Developments Ltd. Type 6012 A), the samples being dissolved in dimethylformamide (0.2 ml.) and liquid scintillator (1.2 ml., Nuclear Enterprises Ltd., Type NE.213) and are uncorrected for self-absorption except where stated. The respective efficiences were obtained by counting [1,2-^{3}H] and [2-^{14}C] - hexadecane standards.

The percentage incorporations were calculated by multiplying the total activity obtained by 100 and dividing by the total activity fed.

Isolation of *Erythrina* Alkaloids (Method of Folkers and Doulofeu 120, 123)

Ground *Erythrina crista galli* seeds (363 g.) were extracted in a Soxhlet first with petroleum-ether for 6 hrs. then with methanol for 36 hrs. After removal of the methanol the crude gum (75 g.) remaining was dissolved in N/50 hydrochloric acid (500 ml.). The acid solution was filtered and extracted with petroleum-ether (100 ml.) then with chlorofrom (2 x 50 ml.). The acid solution was neutralised with saturated aqueous sodium bicarbonate and extracted with chloroform (3 x 70 ml.), from which, after drying (K_2CO_3) and removal of the solvent, a gum (2.4 g.) was obtained.

The neutral aqueous solution was re-acidified with hydrochloric acid (conc. 50 ml.) and refluxed for 1½ hrs. The alkaloids from this acid solution were obtained as before, but on evaporating the chloroform erysodine (50 mg.) crystallized.

The mixture of alkaloids obtained from the earlier gum was chromatographed over alumina (70 g.) and the following fractions were obtained (TLC control).

Eluent	Amount and Compound	
Benzene-chloroform(1:1)	160 mg. Erythraline (136)	n.m.r. 3.40 (1H,s), 3.53 (1H,s) in CCl_4 4.20 (2H,s), 6.78 (3H,s) u.v. $\lambda_{max.}$ 290, 233mμ lit.[120] u.v. $\lambda_{max.}$ 292, 238mμ
Benzene-chloroform(1:1)	170 mg. Erythraline-Erysotrine	
Chloroform	80 mg. Erysotrine (137)	m.p. 96-98° from petroleum-ether (60-80°), (lit.[174] 97-98°). n.m.r. 3.15 (1H,s), 3.35 (1H,s) 6.15 (3H,s), 6.23 (3H,s), 6.68 (3H,s).
Chloroform-ethanol (19:1)	83 mg. Erysodine (105)	m.p. 202° (lit.[123] 202-205°) n.m.r. at 60° 3.11 (1H,s), 3.24 11H,s), 6.20 (3H,s) 6.68 (3H,s) $[\alpha]_D^{29°}$ +243° in $CHCl_3$ (lit. $[\alpha]_D^{29°}$ +248°)
Chloroform-ethanol (9:1)	7.3 mg. Erythratine (138)	m.p. 170° from ethyl acetate (lit.[174] 171-172°)

The erysodine was identical (m.p. and mixed m.p.) with an authentic specimen[176].

Isolation of Anonaine. (Method of Govindachari[126])

Anona reticulata bark (406 g.), dried and powdered, was first extracted with petroleum-ether in a Soxhlet, then with successive portions of 1% hydrochloric acid in ethanol (4l. total) at room temperature for two days. The ethanol solution was filtered, and after removal of the ethanol the resulting red gum (41.5 g.) was dissolved in N hydrochloric acid (400 ml.). The acid solution was filtered, extracted with ether (100 ml.), basified with saturated aqueous sodium bicarbonate, and extracted with chloroform (3x50 ml.). After extracting this chloroform solution with water, the phenolic alkaloids were extracted into N sodium hydroxide (100 ml.). After drying (Na_2SO_4) and removal of the chloroform a crude gum (418 mg.) of non-phenolic alkaloids was obtained.

After washing the sodium hydroxide solution with ether (50 ml.), the phenols were precipitated with carbon dioxide and extracted with chloroform (3x50 ml.). After drying (Na_2SO_4) and removal of the chloroform, a gum (469 mg.) of the crude phenolic alkaloids was obtained.

The crude non-phenolic alkaloids were chromatographed over alumina (50 g.), the elution being followed by TLC. Anonaine (139) (54 mg.) was removed with benzene-chloroform (1:1), its presence being shown on TLC by chromotropic acid in 50% sulphuric acid, a violet spot developing on heating with compounds having a methylene-dioxy group.

Anonaine hydrochloride, m.p. > 250° (decomp. lit.[126] 273-274°,[177] 287-288°,[128] 270-275°) was formed by dissolving the free base in ethanolic hydrogen chloride and adding ether.
n.m.r. 5.49 (1H,s), a double doublet (J=0.15) centred at 6.04 (2H)
i.r. closely similar to that published .
u.v. of hydrochloride $\lambda_{max.}$ 318, 282, 242 mμ.
lit.[178] $\lambda_{max.}$ 325, 277, 237 mμ.

mass. spec. M^{+} (m/e 265) 54.4%, $(M-1)^{+}$ (m/e 264) 100%
$(M-29)^{+}$ (m/e 236) 14.4%

N-acetyl anonaine was made by dissolving anonaine (15 mg.) in pyridine (0.5 ml.) and acetic anhydride (0.5 ml.) and leaving at room temperature for 16 hrs. After removal of the solvent the N-acetyl anonaine (14 mg.) was crystallized from ethanol, m.p. 229-230° (lit.[128] 229-230°). The i.r. spectrum was closely similar to that published[178] .

<u>Isolation of Roemerine.</u>

<u>Papaver dubium</u> plants (2.0 kg.), harvested in June, were blended with ethanol (4 l.) and left to soak for 2 days. After removal of the ethanol the resulting gum was dissolved in <u>N</u> hydrochloric acid (100 ml.); the solution was filtered, extracted with ether and basified with aqueous 4<u>N</u> sodium hydroxide; the basic solution was extracted with ether (3x50 ml.). After drying (K_2CO_3) the ether was removed to give the crude bases (430 mg.). The crude bases were chromatographed over alumina (30 g.) and the roemerine was eluted with carbontetrachloride-benzene (1:1) as shown by TLC. Roemerine (147) was crystallized as its hydrochloride (152 mg.) from ethanolic hydrogen chloride, m.p. 265 - 270° (decomp., lit.[197] 262-263°, [198] 266-267°,[136] 271-272°). It had an R_F, i.r. and m.p. identical with an authentic specimen of (-)-roemerine hydrochloride.

A small specimen of the crude ethanol extract was dissolved in chloroform, shaken with 1% aqueous tartaric acid, which was immediately basified with 4<u>N</u> sodium hydroxide, and the alkaloids were re-extracted into chloroform. No spot on TLC could be seen which corresponded with the dienol (148), which was prepared by borohydride reduction of mecambrine (75). [It had been shown that the dienol (148) was stable in 1% aqueous tartaric acid for a short time.]

Isolation of Mecambrine

Meconopsis cambrica roots (1.37 kg.) were blended in ethanol (3 l.) and left to soak for 3 days. After removal of the ethanol the crude gum (52.4 g.) was dissolved in 0.1 N hydrochloric acid (200 ml.) and filtered. The acid solution was extracted with ether (50 ml.), basified with ammonia and extracted with ether (3x50 ml.). After drying (K_2CO_3) the ether was removed to give the crude bases (260 mg.). The crude bases were chromatographed over alumina (30 g.) and the mecambrine (75) was eluted with benzene-chloroform (9:1). The free base (600 mg.) was crystallized from ether, m.p. 178° (lit.[138] 178°). The R_F, i.r. and m.p. were identical with an authentic specimen[180].

mass. spec. M^+ (m/e 295) 100%, $(M-1)^+$ (m/e 294) 36.4%, $(M-28)^+$ (m/e 267) 9.0%, $(M-29)^+$ (m/e 266) 50%, $(M-43)^+$ (m/e 252) 29.5%

SYNTHESIS OF ANONAINE AND ROEMERINE

3,4 - Methylenedioxy -ω- nitrostyrene (179)

To piperonaldehyde (25 g.), dissolved in redistilled nitromethane (60 ml.), were added methylamine hydrochloride (5 g.) and anhydrous sodium acetate (5 g.). After the mixture had been shaken at room temperature for 20 hours, the crystals of the nitrostyrene were filtered off and washed with ether and water. Recrystallization of the product from glacial acetic acid gave yellow needles (30.5 g., 95%), m.p. 161° (lit.[181] 159 - 160°).

3,4 - Methylenedioxyphenethylamine (180, Method of Tomita and Kikuchi[182])

The nitrostyrene (15g.) in dry tetrahydrofuran (400 ml.) was added dropwise to a suspension of lithium aluminium hydride (15 g.) in refluxing tetrahydrofuran (100 ml.). It was refluxed for a further hour, cooled and the excess lithium aluminium hydride was destroyed by ethyl acetate and water. Sodium hydroxide was added and the tetrahydrofuran solution was decanted off. The precipitate was washed twice with ether (2 x 100 ml.), and the combined ether and tetrahydrofuran were removed. The residue was dissolved in ethanol and ethanolic hydrogen chloride was added. The precipitated hydrochloride was recrystallised from ethanol to give needles (10.2 g., 65% m.p. 210° (lit.[181] 208°), (m.p. of picrate 175°, lit.[181] 175°).

Synthesis of o-nitro-phenylpyruvic acid.(182)

Sodium (5.7 g.) was dissolved in methanol (100 ml.) and the excess methanol was removed. o-Nitritoluene (34.2 g., 29.5 ml.) diethyl oxalate (36.5 g., 34 ml.) and the sodium methoxide were dissolved in absolute alcohol (75 ml.) and stirred for half an hour at room temperature. After being heated on a steam bath for a further half hour, the solution was cooled and water (100 ml.) was added. After refluxing for a further hour the excess o-nitrotoluene was steam-distilled out of the solution, which was then treated with charcoal. Hydrochloric acid was added, and after removal of some of the water *in vacuo*, the o-nitrophenylpyruvic acid crystallized. The acid was recrystallized from water to give needles (21.4 g., 41%), m.p. 118°- 119° (lit.[183] 119-120°)

o-Nitrophenylacetic acid.(183)

To a purple solution of o-nitrophenylpyruvic acid (33.2 g.) in 2N sodium hydroxide (370 ml.) hydrogen peroxide (20 vol., 42.7 ml.) was added, soon discharging the colour; the solution was stirred for a further half hour. After filtration, the filtrate was acidified and concentrated to give o-nitrophenylacetic acid as needles (18.2 g., 63%), m.p. 138 - 140° (lit.[169] 139-140°).

N - (3,4 - methylenedioxyphenethyl) -1-nitrophenylacetamide (184)

o-Nitrophenylacetyl chloride was prepared by refluxing o-nitrophenylacetic acid (5 g.) in repurified thionyl chloride (60 ml.). The excess thionyl chloride was partly removed *in vacuo*, but the solution of the acid chloride was not evaporated to dryness, since explosions could then occur [184]. To remove the remaining thionyl chloride, dry benzene was added and removed under reduced pressure; this was repeated.

The acid chloride in benzene was added dropwise to a vigorously stirred mixture of the amine hydrochloride (10g.), N sodium hydroxide (250 ml.) and benzene (50 ml.). Stirring continued for half an hour after the addition was complete and the amide was filtered off. The benzene layer was separated from the filtrate; after removal of the benzene the combined amide portions were recrystallized from methanol to give needles (11.6 g., 71% based on amine) m.p. 122° (lit. [128] 119°, [169] 120°).

1 - (1-Nitrobenzyl) - 3,4 - dihydro - 6,7 - methylenedioxyisoquinoline. (185 Method of Barger and Weitnauer [128]).

Redistilled phosphorus oxychloride (6.5 ml.) was added to the amide (2.0 g.) dissolved in chloroform (8.0 ml.) and the solution was allowed to stand at room temperature.

After four days the chloroform and phosphorus oxychloride were removed under reduced pressure, the residual gum was dissolved in the minimum volume of acetone and poured into hydrochloric acid (1 part conc. hydrochloric acid: 1 part water, 40 ml.). The insoluble gum was filtered off, redissolved in acetone and poured into the same volume of hydrochloric acid; the black precipitate was filtered off. After treatment with charcoal the combined acidic filtrates were made alkaline with 4N sodium hydroxide and the fine precipitate was filtered off with Whatman No. 20 filter paper. The dihydroisoquinoline was recrystallised from methanol to give buff needles (1.15 g., 61%) m.p. 165° (lit.[128] 165°).

1 - (1 - Aminobenzyl) - 1,2,3,4 - tetrahydro - 6,7 - methylenedioxy isoquinoline (106)

To a stirred solution of the dihydroisoquinoline (4.0 g.) in warm hydrochloric acid (1 part conc. hydrochloric acid: two parts water, 175 ml.) was added zinc dust (17.5 g.) during half an hour.

The mixture was cooled, filtered, made alkaline with ammonia (0.880) and extracted with ether (3 x 50 ml.). After drying the ether over potassium hydroxide the solvent was removed and the residual base was dissolved in methanol-ether. The base was precipitated by passing dry hydrogen chloride through and purified by reprecipitation from methanol-acetone

giving a colourless dihydrochloride (3.5 g., 76%).

<u>(±) - Anonaine (81)</u>

The diamino dihydrochloride (2 g.) was dissolved in 2N sulphuric acid (62.5 ml.) and cooled to 0°. After sodium nitrite (0.76 g.) had been added during half an hour, the solution was allowed to stand at 0° for five hours.

When the diazotisation was complete, the solution was heated at 100° until the evolution of nitrogen ceased (approximately 2 mins.). Concentrated hydrochloric acid (10 ml.) and zinc (10 g.) were added, and the mixture was warmed until it became clear. After filtering off the zinc, the solution was made alkaline with ammonia (0.880) and the bases were extracted into ether (3 x 50 ml.).

The ether was removed and the residue was chromatographed over alumina. The anonaine was eluted with chloroform, dissolved in ether and precipitated by passing hydrogen chloride through the solution. It was recrystallized from ethanol to give needles (0.35 g., 21%), m.p. 282 - 284 ° (decomp. lit.[128] 285°).

When the diazonium compound in quartz apparatus at 0° was irradiated with a high-pressure mercury lamp for one hour no (±)-anonaine could be detected on TLC after reduction of the N-nitroso group.

1-(1-Nitrobenzyl)-1,2,3,4 - tetrahydro - 6,7 - methylenedioxyisoquinoline (187)

To the dihydroisoquinoline (185, 1.0 g.) dissolved in methanol (200 ml.) sodium borohydride (0.37 g.) was added during half an hour and the solution was stirred at room temperature for a further hour.

The methanol was removed, sodium hydroxide (4N, 40 ml.) added and the base was extracted into ether (3 x 25 ml.). The ether solution was reduced in volume and ethanolic hydrogen chloride was added, giving a crystalline hydrochloride, which was recrystallized from ethanol to give plates (0.96 g., 85%), m.p. 234 - 235°. M.p of free base (from ether) 98 - 99°.

(Found C, 58·93; H, 5·11; N, 7·81; Cl, 10·19; $C_{17}H_{17}N_2O_4Cl$ requires C, 58·60; H, 4·88; N, 8·04; Cl, 10·20).

n.m.r. τ 1·90 - 2·70 (4H, m), 3·30 (1H, s), 3·50 (1H, s) 4·15 (2H, s), 5·7 - 7·5 (8H, m).

1-(1-Nitrophenyl)-2-acetyl - 1,2,3,4 - tetrahydro - 6,7 - methylenedioxy isoquinoline (188).

The tetrahydroisoquinoline (1 g.) was left overnight at room temperature in acetic anhydride (5 ml.) and pyridine (5 ml.).

After removal of the solvents *in vacuo* the *N*-acetyl compound crystallized from ethanol (0.84 g., 83%), m.p. 156-157°.

(Found C,64·52; H,5·35; N,8.30.

$C_{19}H_{18}N_2O_5$ requires C,64·39; H,5·12; N,8·00).

n.m.r. τ 2·00 - 3·20 (4·0 H, m), 3·30 (0·5H,s), 3·58 (1.5H, broad s), 4·21 (1H,s), 4·23 (1H,s), 4·5 - 7·7 (6·6H, m) 8.05 (1·4H, s), 8·60 (1·5 H,s).

1-(1-Nitrobenzyl)-2-methyl - 1,2,3,4 - tetrahydro - 6,7 - methylenedioxy isoquinoline (192).

The tetrahydroisoquinoline hydrochloride (0.6g.) was dissolved in formic acid (97%; 10 ml.) and formalin (40 %, 10 ml.) and heated on a steam bath for 45 mins.

After the excess reagents had been removed *in vacuo*, *N* sodium hydroxide was added to make the solution basic, and the liberated product was extracted into ether (3x25 ml.). The ether solution was extracted with water, and, after being reduced in volume, ethanolic hydrogen chloride was added. The precipitated hydrochloride was recrystallized from methanol giving pale yellow plates (0.57 g., 92%), m.p. 204 - 205°.

(Found C,59.55; H,5.07; N,7.77; Cl,10.11,
$C_{18}H_{18}NO_4Cl$ requires C,59.60; H,5.27; N,7.72; Cl, 9.77)

n.m.r. (in carbon tetrachloride), τ, 2.3-3.5 (4H,m), 3.43 (1H,s), 3.61 (1H,s), 4.17 (2H,s), 5.03-7.68 (7H,m), 7.73 (3H,s).

<u>1-(1-Aminobenzyl)-2-methyl - 1,2,3,4-tetrahydro-6,7-methylene-dioxyisoquinoline (193)</u>

Zinc dust (0.86 g.) was added over half an hour to a warm solution of the nitro compound (192,2.0 g.) in hydrochloric acid (1 part conc. HCl: 2 parts water, 86.5 ml.) and the mixture was stirred for a further half hour.

After filtering off the excess zinc, the free base was liberated by the addition of ammonia (0.880) and it was extracted into ether (3x40 ml.). After concentration the dihydrochloride was precipitated by adding methanolic hydrogen chloride, and recrystallized from methanol to give needles (1.7 g., 83%). m.p. 282-283° (lit.[169] 283-284°).

n.m.r. 2.86-3.76 (6H, peaks at 3.51 and 3.57) 4.16 (2H,s), 5.36 - 7.51 (9H,m), 7.55 (3H,s).

<u>(±)-Roemerine (82)</u>

The diamine (193) as its dihydrochloride (1 g.) was dissolved in sulphuric acid (2N, 50 ml.) and cooled to 0°. Sodium nitrite (0.25 g.) was added during half an hour, after which the solution was allowed to stand at 0° for five hours.

After heating at 100° until nitrogen ceased to be evolved (approximately three minutes), ammonia (0.880) was added precipitating the bases, which were extracted into ether (3 x 20 ml.). After drying (KOH pellets) and removal of the solvent, the bases were chromatographed over alumina, roemerine being eluted with carbon tetrachloride-benzene (1:1) - TLC control.

Roemerine (24.6 g., 29%) was crystallized as its hydrochloride from ethanolic hydrochloric acid, m.p. 262-267° (decomp., lit.[197] 262-3° for (-)-roemerine hydrochloride).

If cuprous iodide (1 g.) were added before decomposition of the diazonium salt, and the reaction worked up as before, the yield of roemerine was 35%.

SYNTHESES OF PRECURSORS

Synthesis of N-Methylcoclaurine.

Protocatechuic aldehyde. (Method of Lange[185])

Pyridine (78 ml.) was added slowly with stirring to a solution of vanillin (20 g.) and anhydrous aluminium trichloride (19 g.) in methylene dichloride (200 ml.). The solution, protected from moisture, was refluxed under nitrogen for 18 hours.

Dilute acid was added until the solution was acidic (pH 4) and the aqueous layer was separated. After extraction with ether (3 x 100 ml.) and evaporation of the ether, protocatechuic aldehyde (11.1 g., 61%) crystallized, m.p. 154 - 155° (lit.[185] 153-154°).

3-Hydroxy-4-benzyloxybenzaldehyde (194)

Potassium hydroxide (8.5 g.) was dissolved in ethanol (60 ml.). Protocatechuic aldehyde (20 g.) and benzyl chloride (19.2g.) were added and the solution was refluxed for 1½ hrs. under nitrogen.

The potassium chloride was filtered off and the solvent was removed under reduced pressure from the filtrate, leaving a residue which was dissolved in water and extracted with ether (3 x 40 ml.). The combined ether extracts were extracted into sodium hydroxide (N, 40 ml.), which was acidified and the liberated phenols were taken up into ether (3 x 40 ml.). After removing the ether the

residue was dissolved in sodium hydroxide and sodium hydroxide pellets were added, precipitating the sodium salt of the para-benzyl ether. This salt was filtered off, dissolved in water (50 ml.) which was acidified, and extracted with ether (3 x 30 ml.). The ether solution was treated with charcoal and evaporated. p-O-Benzyl protocatechuic aldehyde was recrystallized from ethanol to give rods (6.9 g., 21%), m.p. 119 - 120° (lit.[136] 122°).

3-Methoxy [^{14}C]-4-benzyloxybenzaldehyde (195)

3-Hydroxy-4-benzyloxybenzaldehyde (200 mg.) was dissolved in dimethylformamide (dried, 3 ml.) under nitrogen and sodium hydride (57 mg. 53.3% in mineral oil) was added. To it in vacuo methyl iodide (0.93 mg., 0.1 mc.) was distilled from a break-seal ampoule. Methyl iodide (44 mg.) in dimethylformamide (1 ml.) was distilled in vacuo into the ampoule and from there into the reaction vessel. The reaction vessel was sealed and the solution stirred for two days at room temperature.

Methyl iodide (1 ml.) was added and the solution stirred for one hour at room temperature. Water (5 ml.) and sodium hydroxide were added and the product was extracted into ether (3 x 5 ml.). The ether solution was washed with water (5 ml.),

dried (Na_2SO_4) and the solvent removed. The residue crystallised from diisopropyl ether as plates (156 mg., 75%), m.p. 65° (lit.[187] 64-65°). Radiochemical yield 61%.

3-Methoxy-4-benzyloxybenzaldehyde (205) (Method of Burger [188])

Potassium hydroxide (4.8 g.) was dissolved in ethanol (96 ml.) on warming. Vanillin (12 g.) and benzyl chloride (13 ml.) were added and the mixture was refluxed under nitrogen for 6 hrs.

The potassium chloride was filtered off and the solvent was removed from the filtrate. Water (20 ml.) was added to the resulting cake, and this was extracted with ether (3 x 30 ml.). The ether solution was extracted with N sodium hydroxide solution (15 ml.) and water (15 ml.), dried (Na_2SO_4) and evaporated. The residue was crystallised from diisopropyl ether to give plates (13.6 g., 71%) m.p. 65° (lit. [187] 64-65°)

3-Methoxy-4-benzyloxy-ω-nitrostyrene (196) (Method of Bhakuni [167]).

To O-benzyl vanillin (20 g.), in redistilled nitromethane (100 ml.), sodium acetate (anhydrous, 2.5 g.) and methylamine hydrochloride (2.5 g.) were added and the mixture was shaken for 24 hrs. at room temperature.

The nitrostyrene was dissolved in chloroform and filtered. After removing the chloroform the nitrostyrene was recrystallised from ethanol, containing a few drops of acetic acid, to give needles (21.4 g., 92%), m.p. 123° (lit.[189] 122-123°).

3-Methoxy-4-benzyloxy-β-phenethylamine (197)

A solution of the nitrostyrene (10 g.) in tetrahydrofuran (100 ml.) was slowly added to a suspension of lithium aluminium hydride (40 g.) in refluxing tetrahydrofuran (200 ml.). After the addition was complete the mixture was refluxed for a further hour.

The excess lithium aluminium hydride was destroyed by the addition of ethyl acetate followed by water. Sodium hydroxide was added to precipitate the inorganic salts and the tetrahydrofuran solution was decanted off. The residue was extracted with ether (2 x 100 ml.) and the combined tetrahydrofuran and ether solutions were removed *in vacuo*. The residue was dissolved in ethanol and ethanolic hydrochloric acid was added, from which the phenethylamine crystallized as its hydrochloride (6.96 g., 66%), m.p. 176-177° (lit. [189] 173-175°)

In the small-scale radioactive reaction the excess lithium aluminium hydride was destroyed with wet ether. The inorganic salts were dissolved in an aqueous solution of Rochelle's salt (potassium sodium tartrate) and the phenethylamine in ether was separated from this. The aqueous solution was further extracted with ether and the procedure as above used.

4-Hydroxyphenylacetic acid (199)

The cyanohydrin of p-hydroxybenzaldehyde (198) was prepared by the method of Londenburg, Folkers and Major [190]. p-Hydroxybenzaldehyde (50 g.) was dissolved in 10% aqueous sodium bisulphite (430 ml.), cooled to 0° and ether (220 ml.) was added. 10% aqueous sodium cyanide (100 ml.) was slowly added and the mixture was stirred for one hour.

After separation of the layers and extraction of the aqueous layer with ether (2 x 50 ml.), the combined ether solutions were washed with 10% aqueous sodium bisulphite (100 ml.). Removal of the ether left an oil of the cyanohydrin.

The cyanohydrin was converted to the phenylacetic acid by the method of Barton and Kirby[191]. To the oil of the cyanohydrin, hydrogen iodide (d. 1.94, 110 ml.) was added and refluxed for 45 mins. After cooling, the solution was poured into 10% aqueous sodium bisulphite (700 ml.) and the insoluble by-product was filtered off. The bisulphite solution was extracted with ether (3 x 100 ml.), and the other solution was washed water, dried ($MgSO_4$), and treated with charcoal. After removal of the ether a few drops of water were added and the mixture was left at 0° overnight. Needles (10.5 g., 17%) were filtered off, m.p. 150-151° (lit.[191] 149-152°).

4-Benzyloxyphenylacetic acid (200) (Method of Barton and Kirby[191])

Potassium hydroxide (4 g.) was dissolved in ethanol (50 ml.) and p-hydroxyphenylacetic acid (4 g.) and benzyl chloride (4 g.) were added. The solution was refluxed under nitrogen for five hours.

Potassium chloride was filtered off and the ethanol removed from the filtrate. Water (50 ml.) was added to the residue and it was extracted with ether (2 x 30 ml.). The aqueous solution was acidified to give plates of p-O-benzylphenylacetic acid (41 g., 64%), m.p. 120 - 121° (lit.[170c] 121°).

N-(3-Methoxy-4-benzyloxyphenethyl)-p- benzyloxyphenylacetamide (201)

p-Benzyloxyphenylacetyl chloride was prepared by refluxing the acid (200, 160 mgs.) in oxalyl chloride (2.5 ml.) and benzene (dry, 2 ml.) for two hours. The excess oxalyl chloride and benzene were removed *in vacuo*; dry benzene was added and removed.

The acid chloride was dissolved in benzene and added dropwise over ½ hr. to a stirred mixture of the phenethylamine hydrochloride (102 mg.), 2 N aqueous sodium hydroxide (1 ml.) and benzene (1 ml.). It was left stirring for a further hour.

The two layers were separated and the aqueous layer was extracted with a further portion of benzene (2 ml.). The combined benzene solutions were extracted with water (1 ml.) and dried (Na_2SO_4). Evaporation of the solvent gave the crystalline amide (135 mg., 81%), m.p. 118° (lit.[170b] 118°).

6-Methoxy-7-benzyloxy-1-(p-benzyloxybenzyl)-3,4-dihydroisoquinoline (202).

The amide (135 mg.) was refluxed under nitrogen in freshly distilled phosphorus oxychloride (1 ml.) and toluene (2 ml.) for 20 mins.

The solvents were removed under reduced pressure, toluene was added and again evaporated. The product was triturated with ether and crystallized from ethanol-ether to give needles (102 mg., 73%), m.p. 163-165° (lit. [170a] 164°).

6-Methoxy-7-benzyloxy-1-(p-benzyloxybenzyl)-1,2,3,4 - tetrahydroisoquinoline (203)

Sodium borohydride (38 mg.) was added during 30 mins. to an ice-cold solution of the dihydroisoquinoline hydrochloride (80 mg.) in methanol (2 ml.) to which 2 drops of 4N aqueous sodium hydroxide had been added. The solution was stirred for a further 45 mins.

The methanol was removed and water (5 ml.) was added. This was extracted with ether (3x3ml.) and the combined ether extracts were shaken with water, and dried (K_2CO_3). After removal of the ether the hydrochloride (62.6 mg., 78%) was crystallized from ethanolic hydrochloric acid and ether, m.p. 190-193 (decomp., lit. [170a] 192°).

(±)-Coclaurine (221)

OO-Dibenzylcoclaurine (62.6 mg.) in ethanol (3.5 ml.), to which 2 drops of concentrated hydrochloric acid had been added, was hydrogenolysed in the presence of 10% palladium-charcoal.

The catalyst was filtered off and the solvent removed. (±)-Coclaurine hydrochloride (34 mg., 80%) crystallized from methanol-ether, m.p. 256-258° (lit.[170b] 255-256°).

(±)-*N*-methylcoclaurine (224)

Coclaurine hydrochloride (33.9 mg.) was dissolved in formic acid (0.4 ml.), formalin (40%, 0.4 ml.) and sodium hydroxide (0.3 ml.). The solution was heated at 100° under nitrogen for 15 mins.

The solvent was removed under reduced pressure and the acid was neutralised with saturated aqueous sodium bicarbonate. This solution was extracted with chloroform (3 x 3 ml.), and the chloroform solution was extracted with water (2 ml.) and dried (Na_2SO_4). After removing the chloroform the hydrochloride crystallized from methanolic hydrochloric acid - ether to give needles (31.5 mg., 93%), m.p. 250-253° (decomp., lit.[192] 252-254°).

(±)-N-Methyl [^{14}C] coclaurine (211)

(±)-Coclaurine hydrochloride (40 mg.) was dissolved in formic acid (0.5 ml.) and 4N sodium hydroxide (0.3 ml.), and radioactive paraformaldehyde (0.71 mg., 0.1 mc) was added. The solution was heated at 100° under nitrogen for 10 mins. Inactive paraformaldehyde (30 mg.) was added and the solution heated for 15 mins. Finally formalin (40%, 0.3 ml.) was added and the reaction completed in 7 mins.

(±)-N- methylcoclaurine hydrochloride was obtained as before - radiochemical yield 73%.

3-Methoxy-4-benzyloxybenzyl Alcohol. (206) (Method of Battersby et al.[55])

To a solution of O-benzylvanillin (10 g.) in methanol (50 ml.) was added sodium borohydride (1.8 g.) over half an hour, and the solution was stirred at room temperature for a further 1½ hrs.

After removal of the solvent water (50 ml.) was added and the benzyl alcohol was extracted into ether (3 x 30 ml.). The ether solution was washed with water (20 ml.), dried (Na_2SO_4), and the ether was partially removed to give the benzyl alcohol (8.42 g., 85%), m.p. 71-72° (lit.[55] 72-73°).

3-Methoxy-4-benzyloxybenzyl Chloride (207) (Method of Tiwari[171]).

The benzyl alcohol (206, 8 g.) in dry benzene (60 ml.) was added slowly to a solution of repurified thionyl chloride (40 ml.) and pyridine (0.8 ml.) in refluxing benzene (80 ml.).

After refluxing for one hour, the solution was cooled and iced

water was added. The benzene layer was washed successively with N $NaHCO_3$ and water, dried (Na_2SO_4) and the solvent was removed. After being treated with charcoal the benzyl chloride (5.5 g., 47%) crystallized from ether, m.p. 74° (lit.[55] 71.5 - 72.5,[193] 72-74°).

3-Methoxy-4-benzyloxyphen [1-^{14}C] ethylamine (209)

3-Methoxy-4-benzyloxybenzyl chloride (100 mg.) was dissolved in freshly distilled dimethylsulphoxide (2 ml.) and radioactive potassium cyanide (1.77 mg., 1 mc) was added. The solution was heated on a steam bath for 15 mins. Potassium cyanide (17.2 mg.) was added and the solution was heated for 3 hrs.

The solution was cooled, water (10 ml.) was added and the product was extracted into ether (4 x 4 ml.), carrier nitrile (18 mg.) being added to the first ether portion. The combined ether solutions were washed with water (5 ml.), dried (Na_2SO_4), and the ether was removed.

The residue was dissolved in anhydrous ether (5 ml.) and added slowly to a suspension of lithium aluminium hydride (400 mg.) in refluxing ether (5 ml.).

After one hour the excess lithium aluminium hydride was destroyed with wet ether and water. The ether layer was decanted off and the residue washed with ether (2 x 5 ml.). The combined ether solutions were washed with water (5 ml.), dried (K_2CO_3), and the ether removed.

The amine hydrochloride (18 mg.) crystallized from ethanolic hydrogen chloride-ether, m.p. 176-177° (lit.[189] 173-175°)

During the larger-scale non-radioactive synthesis the intermediate benzyl cyanide was crystallized from ether as plates, m.p. 67-69° (lit.[193] 67-68°).

Synthesis of N-methyl [^{14}C] norcoclaurine

3,4 - Dibenzyloxybenzaldehyde

Protocatechuic aldehyde (16.0 g.) was dissolved in dry acetone (160 ml.); anhydrous potassium carbonate (32 g.) and benzyl chloride (40 ml.) were added, and the mixture was refluxed with stirring under nitrogen for 24 hours. The inorganic salts were filtered off and the filtrate was steam-distilled until the distillate was clear in order to remove excess benzyl chloride. The deposited oil was extracted into ether (3 x 50 ml.); the ether solution was extracted with sodium hydroxide solution and dried (Na_2SO_4). After removing the ether dibenzyloxybenzaldehyde crystallized from methanol as plates (29.7 g., 80%), m.p. 90-91° (lit.[195] 91°).

3,4-Dibenzyloxy-ω-nitrostyrene (Method of Bhakuni[167])

3,4-Dibenzyloxybenzaldehyde (10 g.) was dissolved in nitromethane (30 ml.) and methylamine hydrochloride (1.2 g.) and anhydrous sodium acetate (1.2 g.) were added. The mixture was shaken in a stoppered vessel at room temperature for 16 hrs.

The crystalline nitrostyrene, which was filtered off and washed with water and ether, was recrystallized from ethanol-glacial acetic acid (19:1) to give needles ((.7 g., 86%), m.p. 122° (lit.[196] 118-119°).

3,4-Dibenzyloxy-β-phenethylamine.

The nitrostyrene (12 g.) in dry tetrahydrofuran was added to a suspension of lithium aluminium hydride (10 g.) in refluxing tetrahydrofuran (200 ml.) and the solution was refluxed for a further 45 minutes.

The excess lithium aluminium hydride was destroyed by adding wet ether and water, and the layers were separated by the addition of sodium hydroxide. The ether layer was decanted off and the remaining aqueous layer was extracted by stirring it with ether (2 x 50 ml). After removal of the ether and tetrahydrofuran in vacuo the resulting oil was dissolved in ether and dried (K_2CO_3).

After removal of the ether, ethanolic hydrogen chloride was added and the hydrochloride was recrystallized from ethanol to give needles (7.7 g., 63%), m.p. 132-133° (lit.[196] 133°).

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

The acid chloride, prepared as before from p-benzyloxyphenylacetic acid (0.85 g.), was added dropwise to a vigorously stirred mixture of the amine hydrochloride (1.2 g.), sodium hydroxide (2 N, 36 ml.), and benzene (3.0 ml.). The mixture was stirred for a further 45 minutes.

The benzene layer was separated from the aqueous layer and the aqueous layer was extracted with benzene (2 x 10 ml.). The combined benzene solutions were dried (Na_2SO_4), and the benzene was removed, leaving crystalline amide (1.3 g., 71% based on amine), m.p. 124-125° (lit.[167] 125°).

1-(p-Benzyloxybenzyl)-3,4-dihydro-6,7-dibenzyloxyisoquinoline.

The amide (13.0 g.) was dissolved in toluene (20 ml.) and phosphorus oxychloride (10 ml.) and the solution was refluxed under nitrogen for 20 minutes.

The toluene and phosphorus oxychloride were removed under reduced pressure. The oil was triturated with ether, and the hydrochloride (10 g., 81%) was precipitated from ethanol with ether, m.p. 168° - 169° (lit.[167] 167 - 168°).

1-(p-Benzyloxybenzyl)-1,2,3,4 - tetrahydro-6,7-dibenzyloxyisoquinoline (212).

The dihydroisoquinoline hydrochloride (1 g.) was dissolved in methanol (30 ml.) and sodium hydroxide (2 ml.), and sodium borohydride (0.8 g.) was added during 45 minutes. The solution was left stirring for a further hour.

After removing the methanol, water was added, and the tetrahydroisoquinoline was extracted into ether. The ether was extracted with water and dried (Na_2CO_3). The free base (0.68 g., 73%) crystallized from ether, m.p. 89° (lit.[167] 89°).

1-(p-Benzyloxybenzyl)-1,2,3,4 - tetrahydro-[2-methyl - ^{14}C] 6,7-dibenzyloxyisoquinoline (214).

The tetrahydroisoquinoline (66 mg.) was dissolved in formic acid (1.3 ml.), and sodium hydroxide was added until the solution was permanently cloudy. Radioactive paraformaldehyde (0.71 mg., 0.1 m was added and the solution was heated at 100° for 10 minutes. Further paraformaldehyde (2.75 mg.) was added and the mixture heated for 15 minutes. Finally formalin (40%, 1 ml.) was added and the reaction completed in 10 minutes.

After removing the solvent under reduced pressure, the solution was made alkaline with aqueous sodium bicarbonate and extracted with ether (3 x 5 ml.). From the ether solution, after extraction with water (5 ml.), drying (K_2CO_3), and reduction in volume the N-methyl compound crystallized (54.3 mg., 80%), m.p. 96°.

[N-methyl - ^{14}C] norcoclaurine (215)

The tri-O-benzyl compound (0.25 g.), dissolved in ethanol (12 ml.), methanol (3 ml.) and conc. hydrochloric acid (0.24 ml.) was hydrogenolysed in the presence of 10% palladium on carbon for three hours.

After filtering off the catalyst, and removal of the solvent, N-methylnorcoclaurine (0.12 g., 82%) was precipitated as its hydrochloride from methanol with ether.

Synthesis of (±)-Armepavine.

1-(p-Benzyloxybenzyl)-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline (217).

To a solution of the dihydroisoquinoline hydrochloride (216, 1.2 g.; kindly supplied by Dr. A. Wiechers) in methanol (15 ml.) and 4 N aqueous sodium hydroxide (1 ml.) at 0°C, sodium borohydride was added over one hour. The solution was left stirring for a further 1½ hours.

The solvent was removed, water (20 ml.) was added and the tetrahydroisoquinoline was extracted into ether (3 x 15 ml.). After extraction of the ether solution with water, and removal of the solvent the amine (0.92 g., 77%) was crystallized as its hydrochloride from ethanol-ether, m.p. 198-201° (lit.[79] 198-200°).

[N-Methyl-^{14}C]-O-benzylarmepavine (218)

The tetrahydroisoquinoline (34 mg.) was dissolved in formic acid (1.0 ml.) and sodium hydroxide (0.3 ml.). Radioactive paraformaldehyde (0.77 mg., 0.1 mc) was added and the solution under nitrogen was heated at 100°. After 15 minutes paraformaldehyde (2.0 mg.) was added, and after a further 10 minutes formalin (40%, 0.5 ml.) was introduced to complete the reaction.

After removal of the solvent under reduced pressure, N aqueous sodium hydroxide (4 ml.) was added and the amine was extracted into ether (3 x 4 ml.). After being washed with water (5 ml.) and dried

(K_2CO_3), the amine (28 mg., 80%) was crystallized as its hydrochloride from ethanol-ether, m.p. 221 - 224° (lit.[79] 195-197°). Radiochemical yield 35%.

(±)-[N-Methyl-^{14}C] armepavine (219)

O-benzylarmepavine hydrochloride (28 mg.), dissolved in ethanol (2 ml.) and conc. hydrochloric acid (2 drops), was hydrogenolysed in the presence of 10% palladium-carbon.

After filtration and evaporation of the solvent, the resulting foam was dissolved in 5% aqueous sodium bicarbonate (5 ml.) which was extracted with chloroform (3 x 3 ml.). The chloroform solution was washed with water (2 ml.), and after removal of the solvent (±)-armepavine hydrochloride (17 mg., 77%) crystallized from ethanol-ether, m.p. 210 - 212° (lit.[79] 209-211°).

Feeding and Work-up Procedure with Anona Reticulata Plants

The Anona reticulata plants were wick-fed (in three separate places on the stem) with the hydrochlorides of the precursors in water and left for ten days. The plant was washed and then blended with ethanol (4 l.) and allowed to stand for three days.

After removing the ethanol 0.1 N hydrochloric acid was added. The acid solution was filtered, extracted with ether (25 ml.) and basified with sodium hydroxide. The basic solution was extracted with ether (3 x 25 ml.) and removal of the solvent gave the crude non-phenolic alkaloids.

The phenolic alkaloids were obtained by adding carbon dioxide to the basic solution until it was neutral and extracting the alkaloids into chloroform (3 x 25 ml.).

The crude non-phenolic alkaloids were chromatographed over alumina, the anonaine being eluted with benzene-chloroform (1:1), Anonaine hydrochloride was prepared by precipitation from ethanolic hydrogen chloride with ether. In one case (±)-anonaine hydrochloride was added.

The anonaine hydrochloride was converted to its free base and treated with formic acid and formalin (0.3 ml.) for 15 minutes. After removing the solvent under reduced pressure and adding 2N sodium hydroxide (until basic) roemerine was extracted into ether (2 x 5 ml.). The ether solution was washed with water, dried

(Na_2CO_3) and evaporated. The resulting gum was dissolved in methanol and methyl iodide (0.1 ml.) was added.

The resulting methiodide was treated as described under the feedings to Papaver dubium plants.

Feeding of (±)-[8,3',5' - ^{3}H$_3$] coclaurine

Fed 9.20 mg. (±)-coclaurine hydrochloride, 0.109 mc.

Wet weight of plant 119 g.

Diluted with 9.3 mg. (±)-anonaine hydrochloride

Compound	Amount in mg.	Activity in d.p.s/mmole	Incorporation %
Non-phenolic alkaloids	53		0.59
Phenolic alkaloids			0.27
Anonaine hydrochloride	17.8	6.11×10^5	
Roemerine methiodide	12.0	2.60×10^4	
Methine base hydrochloride	5.0	2.22×10^4 1.98×10^4 2.01×10^4	0.32
Methine base methiodide	1.3	1.97×10^4	0.29

Incorporation allowing for loss of tritium 0.44%.

Feeding of (±)-[5,8,3',5' - $^3H_4$] norcoclaurine

Fed 6.43 mg. (±) -norcoclaurine hydrochloride, 0.07 mc.

Wet weight of plant 37.1 g.

Compound	Amount in mg.	Activity in d.p.s/mmole x 10^5	Incorporation %
Non-phenolic alkaloids	31		0.67
Phenolic alkaloids			0.73
Anonaine hydrochloride	16.5	9.29	
Roemerine methiodide	14.2	9.73	
Methine hydrochloride	8.7	(6.58 6.38 6.33)	
Methine methiodide	2.4	6.00	0.37

Incorporation allowing for loss of tritium 0.49%.

Feeding and Work-up Procedure with Papaver Dubium Feedings.

The precursors, except with tyrosine, as their hydrochlorides in water were injected into the seed-pods of Papaver dubium plants after the petals had dropped. Ten days later the plants were harvested.

The plants were washed and blended with ethanol (2 l.) and left in ethanol for three days. They were then worked up as in the large-scale extraction. The phenols were obtained by treating the basic solution with carbon dioxide and extraction into chloroform.

When the alkaloid of the plant was diluted with (±)-roemerine the roemerine was converted to its methine base, which was crystallized to constant activity. The activity of this was checked by making its methiodide.

Roemerine Methine Base (220)

Roemerine methiodide was prepared by dissolving roemerine (free base) in the minimum volume of methanol, adding a few drops of methyl iodide and allowing it to stand at room temperature for one day. The methiodide, m.p. 222-225° (lit.[197] 215-216°), was filtered off.

The roemerine methiodide (about 15 mg.) was dissolved in methanolic potassium hydroxide (20% 4 ml.) and refluxed for three and a half hours. After removal of the methanol, water (5 ml.) was added and the methine base was extracted into ether (3 x 3 ml.). The ether solution was extracted with water (2 ml.), dried, and the ether was removed. The resulting methine base (generally about 80% yield) was crystallized from ethanolic hydrogen chloride, m.p. 220-225°, free base m.p. 74° (lit.[197] 73-74°) The methine base hydrochloride was recrystallized from ethanol to constant activity.

The methine base methiodide was prepared from the methine free base as with preparation of roemerine methiodide. The methine base methiodide, m.p. 283-4° (lit.[197] 274 - 275°) was filtered off.

In the case of the triply-labelled feeding, the activity of the C-3 position was determined by converting roemerine to the vinyl phenanthrene (227). This indicated the activity of the C-3 and methylenedioxy position combined. The activity at C-3 was then found by oxidation to the phenanthrene carboxylic acid (228).

<u>1,2-Methylenedioxy-4-vinylphenanthrene (227).</u>

The methine base methiodide (15 mg.) was refluxed in methanolic potassium hydroxide (20%, 2 ml.) for 3 hours.

The solvent was removed, water (5 ml.) was added, and the vinylphenanthrene extracted into chloroform (3x3 ml.). After washing the chloroform solution with water (3 ml.) the solvent was removed and the product was crystallized from methanol (5.75 mg. 67%), m.p. 87° (lit.[197] 86-87°).

<u>1,2-Methylenedioxyphenanthrene-4-carboxylic acid (228).</u>

To the vinylphenanthrene (8 mg.) in acetone (2 ml.) potassium permanganate (21 mg.) was added.

After 20 minutes <u>N</u>/10 sodium hydroxide (5 ml.) was added and the solution was extracted with ether (3 ml.). Concentrated hydrochloric acid was added and the precipitate was extracted into benzene (3x4 ml.). After washing with water (4 ml.) and drying (Na_2SO_4) the acid (5.8mg., 68%) was crystallized from benzene as needles, m.p. 245-247° (lit.[128] 240°,[197] 263-264°).

Formaldehyde dimedone derivative from roemerine.

100 ml. of water was distilled from roemerine (15 mg.) in sulphuric acid (35%, 40 ml.) into an aqueous dimedone solution (0.6%, 25 ml.), the concentration of the acid being kept constant.

After being left at 0° overnight, the dimedone derivative was filtered off and recrystallized from ethanol.

Triethylmethyl ammonium iodide from roemerine.

The *N*-methyl group of roemerine was determined by the standard Herzig-Meyer method.

Feeding of $(\pm)$- $[2 - ^{14}C]$ Tyrosine.

Fed $(\pm)$ - tyrosine, 0.0085 me.

Wet weight of plants 42 g.

Weight of ethanol extract 2.69 g.

Diluted with $(\pm)$-roemerine hydrochloride 24 mg.

Compound	Amount in mg.	Activity d.p.s./mmole x 10^3	Incorporati %
Total bases			3.45
Roemerine hydrochloride	27.0		
Roemerine methiodide	12.7	1.53	
Methine base hydrochloride	10.3	1.77	
Methine base methiodide	2.1	1.77	0.17

Incorporation 0.17%.

Feeding of (±)-[8,3',5' - $^3H_3$] Coclaurine.

Fed (±) - coclaurine hydrochloride, 0.12 mc.

Wet weight of plants 72.5 g.

Weight of ethanol extract 3.56 g.

Dilute with (±)- roemerine hydrochloride 25.7 mg.

Compound	Amount in mg.	Activity d.p.s./mmole x 10^4	Incorporation %
Non-phenolic bases	25.8		1.4
Phenolic bases			1.0
Roemerine hydrochloride	29.1	2.13	
Roemerine methiodide	34.0	1.34	
Methine base hydrochloride	17.2	1.72	
Methine base methiodide		1.60	0.041

Incorporation allowing for loss of tritium 0.062%

Feeding of (±)-$[5,8,8-{}^{3}H_{3}]$ isococlaurine

Fed (±) - isococlaurine 0.12 mg.

Wet weight of plants 44.4 g.

Weight of ethanol extract 2.2 g.

Diluted with (±)- roemerine hydrochloride 22.16 mg.

Compound	Amount in mg.	Activity d.p.s./mmole	Incorporation %
Non-phenolic bases	20.8		0.05
Phenolic bases			2.0
Roemerine hydrochloride	21.0	140	0.0002
		0	0.00

Incorporation 0.00%

Feeding of (±) -[5,8,3',5' - $^3H_4$] norcoclaurine.

Fed (±) - norcoclaurine 0.16 mc.

Wet weight of plants 61.5 g.

Weight of éthanol extract 3.60 g.

Diluted with (±) - roemerine hydrochloride 22.7 mg.

Compound	Amount mg.	Activity d.p.s./mmole x 10^5	Incorporation %
Non-phenolic bases	27.8		1.12
Phenolic bases			0.67
Roemerine hydrochloride	27.9	1.4	
Roemerine methiodide	27.4	2.1	
Methine base hydrochloride	10.6	1.6	
Methine base methiodide	4.1	1.6	0.23

Incorporation allowing for loss of tritium 0.34%.

Feeding of (±) -[8,3', 5' - $^3H_3$]- N-Methylcoclaurine

Fed (±) - N-methylcoclaurine 0.12 mc.

Wet weight of plants 42.6 g.

Weight of ethanol extract 3.25 g.

Diluted with (±) - roemerine hydrochloride 23.71 mg.

Compound	Amount mg.	Activity d.p.s./mmole x 10^5	Incorporation %
Non-phenolic bases	36		3.25
Phenolic bases			1.1
Roemerine hydrochloride	21.5	2.9	
Roemerine methiodide	20.7	2.1	
Methine base hydrochloride	12.4	1.9	
Methine base methiodide	2.6	1.9	0.32

Incorporation allowing for loss of tritium 0.46%.

Feeding of (±) -[8,3',5' - $^3H_3$] - Coclaurine (1965)

Fed (±) - Coclaurine 0.083 mc.

Wet weight of plants 29.0 g.

Weight of ethanol extract 2.87 g.

Diluted with (+)-roemerine hydrochloride 17.6 mg.

Compound	Amount mg.	Activity d.p.s./mmole x 10^4	Incorporation %
Non-phenolic bases			0.34
Phenolic bases			0.02
Roemerine hydrochloride	21.7	6.58	
Roemerine methiodide	14.1	5.33	
Methine base hydrochloride	7.0	5.47	
Methine base methiodide	2.9	5.48	0.10

Incorporation allowing for loss of tritium 0.15%.

Feeding of (±)-[N-methyl - O-methyl - ^{14}C] coclaurine

Total activity fed 0.018 mc.

Labelling pattern: O - Methyl 19.0%

N - Methyl 81.0%

Wet weight of plants 31.2 g.

Weight of ethanol extract 2.9 g.

Diluted with (+)-roemerine hydrochloride (83 mg.)

Compound	Amount mg.	Activity d.p.s/mmole x 10^2	Incorporation %
Non-phenolic bases	280		9.7
Phenolic bases			0.22
Roemerine hydrochloride	67.2	20.8	0.19
Roemerine methiodide		19.5	0.18
Triethylmethylammonium iodide		17.7	
Formaldehyde dimedone		2.24	

Labelling pattern in roemerine:

N-methyl 87%

Methylenedioxy 11%

Incorporation 0.19%.

Feeding of (−)-[8,3',5'-$^3H_3$] N-methylcoclaurine

Fed (−)-N-methylcoclaurine 0.07 mc.

Wet weight of plants 37.1 g.

Weight of ethanol extract 3.1 g.

Diluted with (±)-roemerine hydrochloride (12.0 mg.)

Compound	Amount in mg.	Activity d.p.s./mmole x 10^3	Incorporation %
Non-phenolic bases			0.0092
Phenolic bases			0.0088
Roemerine hydrochloride	8.6 mg.	3.6	0.0034
Roemerine methiodide	5.3	2.7	0.0026
Methine hydrochloride	3.4	0.25	0.0002

Incorporation 0.0003 %

Feeding of (+)- [8, 3', 5' - $^{3}H_3$] N-methylcoclaurine (225)

Fed (+)-N-methylcoclaurine 0.072 mc.

Wet weight of plants 14.4 g.

Weight of ethanol extract 3.0 g.

Diluted with (±) - roemerine hydrochloride (18.3 mg.)

Compound	Amount in mg.	Activity d.p.s/mmole x 10^4	Incorporation %
Non-phenolic bases			0.56
Phenolic bases			0.037
Roemerine hydrochloride	15.6	4.4	
Roemerine methiodide	15.7	3.6	
Methine hydrochloride		3.4	0.074
Methine methiodide		3.4	0.074

Incorporation allowing for loss of tritium 0.11%

Feeding of (±)-[N-methyl-^{14}C] norcoclaurine (226)

Fed (±)-N-methylnorcoclaurine 0.0074 mc.

Wet weight of plants 30.9 g.

Weight of ethanol extract 0.5 g.

Diluted with (+)-roemerine hydrochloride (42 mg.).

Compound	Amount mg.	Activity d.p.s/mmole x 10^2	Incorporation %
Non-phenolic bases			5.2
Phenolic bases			0.03
Roemerine hydrochloride	30.5	9.7	0.10
Roemerine methiodide		10.8	
Triethylmethylammonium iodide		10.4	

Incorporation 0.10%.

Feeding of (±)-[N-methyl-^{14}C] armepavine (219)

Fed (±)-armepavine 0.014 mc.

Wet weight of plants 11.5 g.

Weight of ethanol extract 0.47 g.

Diluted with (+)-roemerine hydrochloride (10.0 mg.)

Compound	Amount mg.	Activity d.p.s/mmole x 10	Incorporation %
Non-phenolic bases			1.95
Phenolic bases			7.53
Roemerine hydrochloride	5.0	3.8	< 0.001

Feeding of $[^3H]$- mecambrine

Fed mecambrine 0.011 mc.

Wet weight of plants 8.9 g.

Weight of ethanol extract 0.3 g.

Diluted with (±)-roemerine hydrochloride (17.2 mg.)

Compound	Amount mg.	Activity d.p.s/mmole x 10^4	Incorporation %
Non-phenolic bases			7.1
Phenolic bases			0.24
Roemerine hydrochloride	16.0	5.73	
Roemerine methiodide	14.9	4.64	
Methine hydrochloride	8.2	4.691	
Methine methiodide		4.65	2.34

Incorporation 2.34%

Feeding of (±)-[N-methyl - O-methyl-3-^{14}C] coclaurine.

Fed (±)-N-methylcoclaurine 0.034 mc.

Labelling pattern: N-methyl 61.6%

C-3 25.4%

O-methyl 13.0%

N-methyl : C-3 = 2.42:1

Wet weight of plants 23.0 g.

Weight of ethanol extract 1.2 g.

Diluted with (+)-roemerine hydrochloride (100 g.)

Compound	Amount mg.	Activity d.p.s/mmole x 10^3	Incorporation %
Non-phenols			0.46
Phenols			1.4
Roemerine hydrochloride	60	5.9	0.19
Roemerine methiodide		5.6	
Triethylmethylammonium iodide		4.2	
Formaldehyde dimedone		0.064	
Vinylphenanthrene		1.8	
Phenanthrene carboxylic acid		0.071	

Labelling pattern of roemerine:

N-methyl 72%

C-3 29%

Methylenedioxy 1.2%

N-methyl: C-3 = 2.44:1

Incorporation 0.19%

Feeding and work-up procedure with *Meconopsis Cambrica*.

The precursors were fed as with the feedings to *Papaver dubium*, and the plants were worked up as with the large-scale extraction of mecambrine.

Mecambrine was crystallized to constant activity as its free base from ether. The *N*-methyl group and methylenedioxy group were determined as with roemerine. The position of the tritium in the mecambrine, derived from (±)-$[8,3',5'-^3H_3]$-coclaurine, was determined by converting mecambrine to mecambroline and exchanging with aqueous base. A trial experiment, exchanging mecambroline with base in deuterium oxide, showed that two protons exchanged.

The activity at the C-3 position of mecambrine, derived from triply-labelled *N*-methylcoclaurine, was determined by conversion to roemerine and the same series of reactions as with the aporphine.

Mecambroline was isolated from the phenols by precipitation from an aqueous solution with concentrated hydrochloric acid and recrystallization from water. Its activity was checked by crystallization of its free base.

Mecambroline (78) from mecambrine(75).

Mecambrine was dissolved in hydrochloric acid (1 part conc. HCl: 4 parts water) and heated at 100° for 20 minutes. Mecambroline hydrochloride was filtered off and recrystallized from water.

Exchange of mecambroline with deuterium oxide.

Mecambroline (free base, 10mg.) was dissolvea in dimethylformamide (1ml.), and potassium tert.-butoxide (2.5mg.) in deuterium oxide (0.5ml.) was added under nitrogen. The solution was heated at 100° for 2 days.

Isolation of mecambrine and inspection of its mass spectrum showed two protons had exchanged.

(+)-Roemerine (147) from mecambrine (75).

To mecambrine(64.5mg.) in ether (21ml.) lithium aluminium hydride (50mg.) was added during 20 minutes. The solution was stirred for a further half hour at room temperature.

After destroying the excess reducing agent with wet ether and water, the ether layer was decanted off. The aqueous layer was extracted with ether, and, after drying (K_2CO_3) and removal of the solvent, (+)-roemerine (57.5mg., 83%) crystallised as its hydrochloride from ethanolic hydrochloric acid.

Feeding of [N-methyl-O-methyl^{14}C]coclaurine.

Fed (±)-N-methylcoclaurine 0.017mc.

Wet weight of plants 20.12g.

Diluted with (-)-mecambrine (52.6mg.).

Compound	Amount	Activity d.p.s./mmole	Incorporation %
Non-phenolic bases			0.18
Phenolic bases			0.58
Mecambrine	47.8mg.	1.0×10^3	0.028

Incorporation 0.028%.

Feeding of [^3H]mecambrine.

Fed (-)-mecambrine 0.0023mc.

Wet weight of plants 64g.

Weight of ethanol extract 2.3g.

Diluted with (+)-mecambroline hydrochloride (28.4mg.).

Compound	Amount mg.	Activity d.p.s./mmole 10^3	Incorporation %
Mecambroline hydrochloride	10.0	6.35	
Mecambroline free base		7.55	2.76

Incorporation 2.76%.

Feeding of [$8,3',5'-^3H_3$]coclaurine.

Fed (±)-coclaurine hydrochloride 0.094mc.

Wet weight of plants 11.5g.

Diluted with (-)-mecambrine (26.0mg.)

Compound	Amount mg.	Activity d.p.s./mmole 10^3	Incorporation %
Non-phenols			0.15
Phenols			0.017
Mecambrine	25.4	4.85	0.045
Mecambroline hydrochloride		4.15	
Mecambroline hydrochloride after exchange		0.00	

Incorporation 0.066%.

Feeding of [N-methyl-O-methyl-3-^{14}C]coclaurine.

Fed (±)-N-methylcoclaurine 0.023mc.

Labelling pattern: N-Methyl 61.6%

C-3 25.4%

O-Methyl 13.0%

N-Methyl:C-3 = 2.42:1.

Wet weight of plants 116.7g.

Weight of ethanol extract 7.7g.

Diluted with (-)-mesembrine (50.0mg.)

Compound	Amount mg.	Activity d.p.s./mmole 10^3	Incorporation %
Non-phenolic bases			0.098
Phenolic bases			0.85
Mesembrine	104.3	1.6	0.090
Roemerine hydrochloride		1.7	
Triethylmethyl-ammonium iodide		1.2	
Formaldehyde dimedone derivative		0.04	
Vinylphenanthrene		0.56	
Phenanthrene-carboxylic acid		0.03	

Labelling pattern: N-Methyl 72.1%

C-3 32.0%

Methylenedioxy 1.8%

N-Methyl: C-3 = 2.22:1

Incorporation 0.090%.

Feeding and work-up procedure with Croton linearis.

The feeding of the precursor and isolation of crotonosine were carried out in the West Indies by Professor Haynes and Dr. Stuart.

Crude crotonosine was purified by conversion to its hydrochloride. Reconversion to the free base and crystallization from chloroform gave crotonosine, the activity of which was not reduced by recrystallization from isopropanol.

The activity was checked by making diacetylcrotonosine, and it was on this compound that the Herzig-Meyer O-methyl determination was performed.

Diacetylcrotonosine.

Crotonosine (32mg.), in pyridine (2.5ml.) and acetic anhydride (1.5ml.), was left at room temperature for 12 hours. After evaporating off the solvents, diacetylcrotonosine was crystallized from ethyl acetate.

Feeding of $(\pm)$-[8,3',5'-$^{3}H_{3}$-O-methyl-^{14}C]coclaurine.

Fed $(\pm)$-coclaurine hydrochloride.

^{3}H : ^{14}C ratio in precursor 13.0 : 1.

Compound	^{14}C Activity d.p.s./mmole	^{3}H Activity d.p.s./mmole
Crotonosine		1.78×10^{3}
Diacetylcrotonosine		1.68×10^{3}
Triethylmethyl-ammonium iodide	78	

^{3}H : ^{14}C ratio in crotonosine 21.5 : 1.

Incorporation 0.034%.

(±)-Glaziovine.

(±)-N-Methylcoclaurine hydrochloride (138 mg.) was converted to its free base and dissolved in chloroform (500 ml.). Potassium ferricyanide (270 mg.) and sodium bicarbonate (2.5 g.) in water (50 ml.) were added, and the mixture was stirred vigorously for one hour.

The chloroform was separated and the aqueous layer was extracted with chloroform (2x25 ml.). After drying (K_2CO_3) the chloroform was removed, and the residue was chromatographed over alumina. (±)-Glaziovine was eluted with chloroform and crystallized from ether to give needles (1.3 mg., 1.1%), m.p.177-179° (lit.[95] for (-)-glaziovine 235-237°), identical on TLC with an authentic specimen.

Mass spec. M^+(m/e 297) 100%, M-1 (m/e 296) 35%, M-29 (m/e 268) 95%, M-43 (m/e 254) 52%.

Mass of molecular ion 297.1382416

$C_{18}H_{19}NO_3$ requires 297.136485.

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