# A STUDY IN THE BIOSYNTHESIS OF STEPHANIA ALKALOIDS

A thesis submitted by

ADRIAAN WIECHERS

in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

of

The University of London

Imperial College,
LONDON, S.W.7.

June, 1966.

## ABSTRACT

A new bisbenzylisoquinoline alkaloid, stebisimine, has been isolated from Stephania japonica Miers. Stebisimine has been shown by physical methods and chemical degradations to be N-nor-1,2-dehydroepistephanine.

The idea that bisbenzylisoquinoline alkaloids might be formed in Nature by the oxidative phenol coupling of coclaurine derivatives has so far lacked experimental support. However, tracer studies have now shown that half of the epistephanine molecule is derived exclusively from (-)-N-methylcoclaurine. This confirms the absolute configuration of the alkaloid. Parallel feeding experiments show that racemization of the (+)-enantiomer is unimportant in this plant. Proposals have been advanced to account for the biosyntheses of bisbenzylisoquinoline alkaloids in general.

The biosyntheses of protostephanine and hasubanonine are discussed and the results from several tracer experiments are interpreted.

ACKNOWLEDGEMENTS

I am deeply grateful to Professor D.H.R. Barton, F.R.S., for the privilege and the stimulation of research under his guidance and for the appointment as Visiting Lecturer in the Department of Chemistry.

I am greatly indebted to Dr. Gordon W. Kirby for his sustained help and in particular for the numerous fruitful and stimulating discussions we have had.

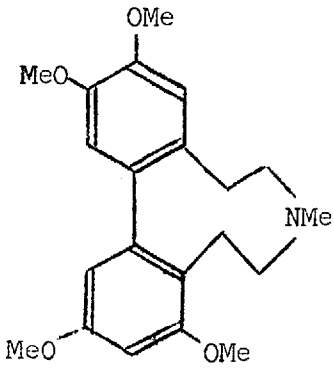
I wish to thank many members of the Imperial College staff for their assistance, particularly Dr. E.S. Waight, Mr. F. Boshoff, Mr. and Mrs. R. Young, Mr. D. Aldrich and his assistants, Mrs. I. Boston, and the staff of the organic chemical stores.

I acknowledge with gratitude the leave granted to me by the University of Pretoria.

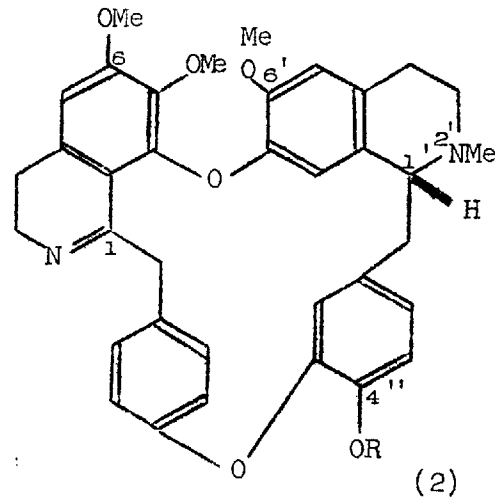
# TABLE OF CONTENTS

# INTRODUCTION

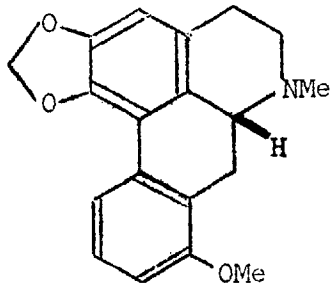
Stephania japonica Miers (family Menispermaceae) is a valuable plant in that it contains many alkaloids. From the Formosan variety were isolated[1] protostephanine[2] (1), epistephanine[2] (2; R = Me), stephanine[3] (3) and metaphanine[3] (4) as the non-phenolic tertiary bases, together with the phenolic base hypoepistephanine[4] (2; R = H) and bases designated as A, B, and C respectively. Hasubanonine[5] (5; R = Me) and homostephanoline[3] (5; R = H), contained in the Japanese Stephania japonica, were not isolated or even detected in the Formosan plant. In addition, the alkaloids prometaphanine[6] (6), insularine[7] (7), steponine[8] (8), and stepholine[9] (9; identical with obamegine) are known constituents of S.japonica.



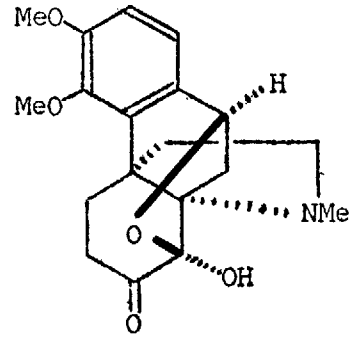

(1)



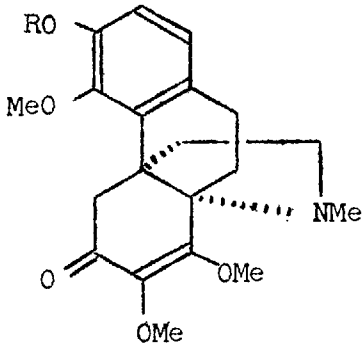

(2)


O
O
NMe
H
OMe

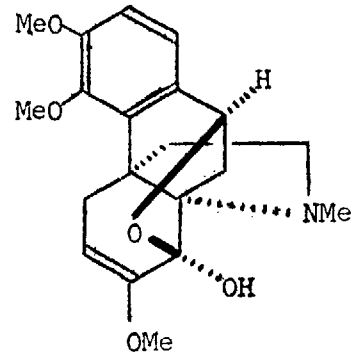
(3)


MeO
MeO
H
NMe
O
OH
O

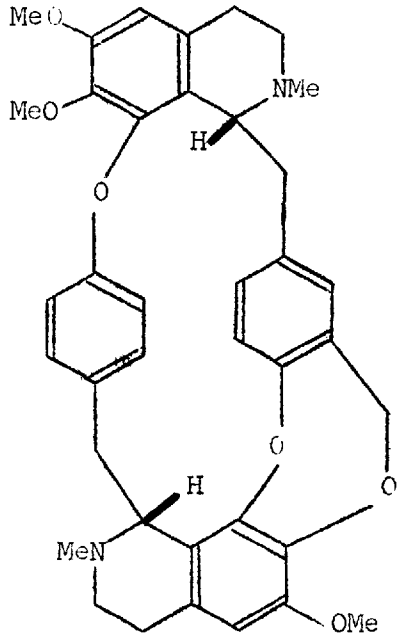
(4)


RO
MeO
NMe
O
OMe
OMe

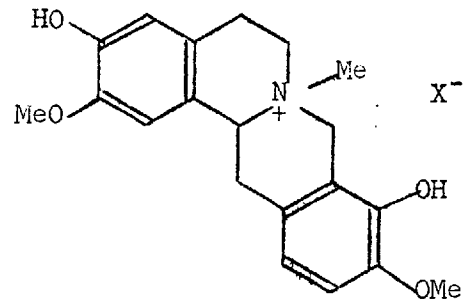
(5)


MeO
MeO
H
NMe
O
OH
OMe

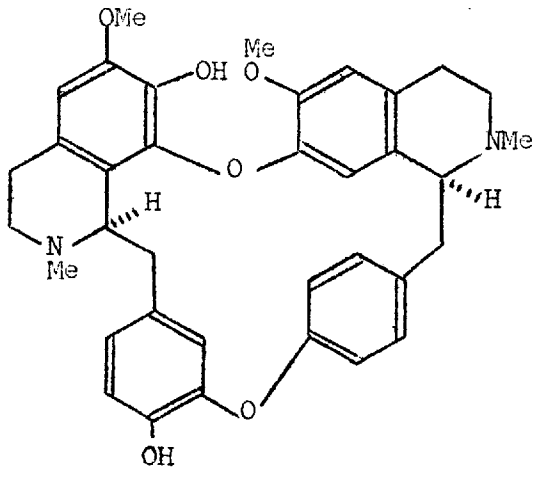
(6)


MeO
MeO
NMe
H
O
O
H
O
MeN
OMe

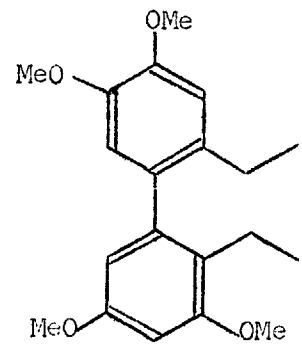
(7)


HO
MeO
Me
N
+
X⁻
OH
OMe

(8)


OMe
OH
Me
O
NMe
O
H
H
N
Me
O
OH

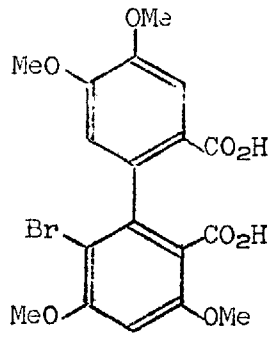
(9)


OMe
MeO
MeO
OMe

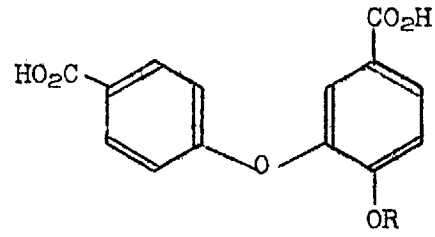
(10)

I. The constitution of some Stephania alkaloids

Protostephanine (1) was first isolated[2] by Kondo and Sanada in 1927. A first-stage Hofmann degradation of protostephanine afforded[10] two isomeric α- and β-methines. The dihydro derivatives obtained from the two separate methines were subjected individually to a second-stage Hofmann degradation, followed by catalytic hydrogenation. Both pathways gave the same tetramethoxybiphenyl derivative (10). N.m.r. assignments[11] completely justified the proposed formulae. The n.m.r. spectrum of protostephanine (1) showed a singlet at τ 3.26 (1H), a singlet at τ 3.33 (1H), an AB quartet at τ 3.55 (1H), 3.68 (1H) and four methoxyl resonances at τ 6.12 (3H), 6.20 (6H) and 6.23 (3H). The methylimino resonance occurred at τ 7.70. The same low field pattern of two singlets and an AB quartet persisted in the n.m.r. spectrum of the biphenyl derivative (10), with two magnetically different ethyl groups at higher field. Attempted synthesis[11] of protostephanine (1) starting from the intermediate (11) gave in low yield a product which appeared, at least chromatographically, to be protostephanine (1).



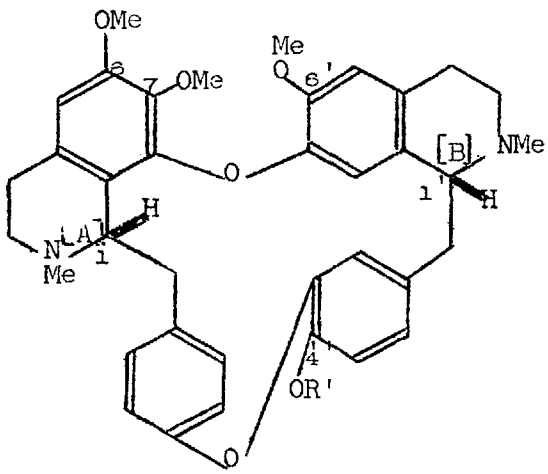

(11)



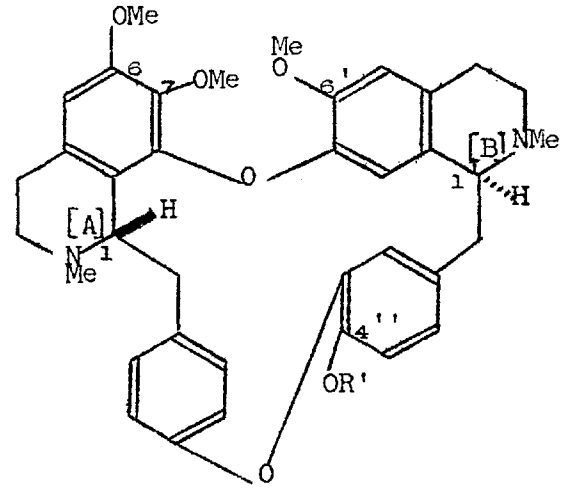

(12)

Epistephanine (2; R = Me) and hypoepistephanine (2; R = H)

(+)-Epistephanine (2; R = Me), a bisbenzylisoquinoline alkaloid, was first isolated[2] from S.japonica Miers by Kondo and Sanada. Potassium permanganate oxidation[12] of epistephanine (2; R = Me) gave 2-methoxydiphenylether-5,4'-dicarboxylic acid (12; R = Me). Dissolving zinc in sulphuric acid reduction[13] of epistephanine (2; R = Me) gave an alleged mixture of diastereomeric dihydroepistephanine-A and -B. This result was questioned[14] and subsequently dismissed. The reduction (vide supra) gave stereospecifically dihydroepistephanine-A. N-Methyldihydroepistephanine-A was identified[15] as O-methyloxyacanthine (13; R' = Me). [Note added in proof: Furukawa recently[152] confirmed Kondo and Tanaka's original[13] observation.]

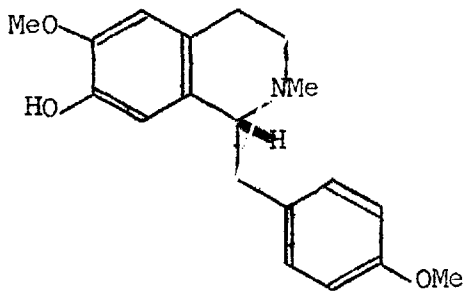


(13)(SR;+-)

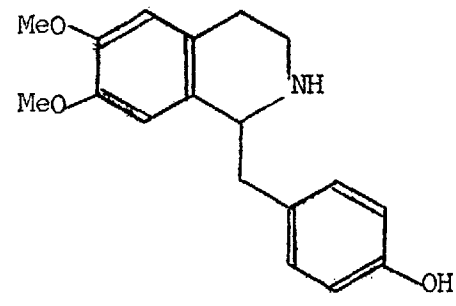


(14)(SS;++)

Furthermore, either sodium borohydride[16] or dissolving zinc in sulphuric acid reduction[14] of epistephanine dimethiodide gave stereospecifically N-methyldihydroepistephanine-B methiodide. The corresponding non-crystalline dimethiodide (RR;--) was the antipode[17,18] of O-methylrepandine dimethiodide (14; R' = Me). Also, sodium in liquid ammonia reductive fission[19] of epistephanine gave (-)-4'-ON-dimethylcoclaurine (15) and (±)-7-O-methylcoclaurine (16).



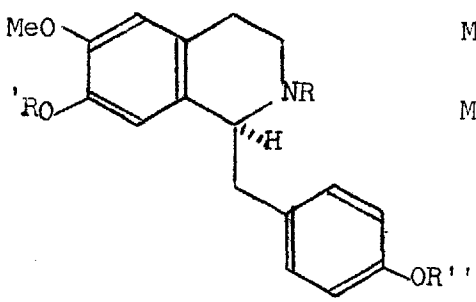
(15)



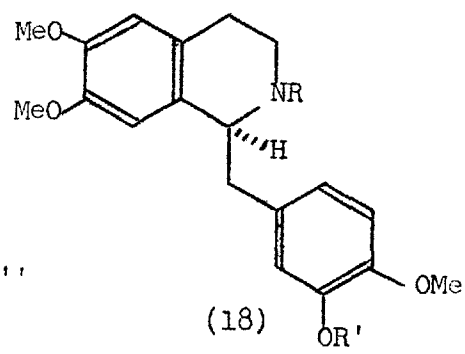
(16)

(+)-Hypoepistephanine (2; R=H) gave[20] epistephanine (2; R=Me) on treatment with ethereal diazomethane, whereas O-ethylhypoepistephanine (2; R=Et) afforded the di-acid (12; R=Et) on oxidation with potassium permanganate. Also, hypoepistephanine (2; R=H) on reduction with dissolving zinc in sulphuric acid[18] gave dihydrohypoepistephanine-A, the N-methyl derivative of which was identical with oxyacanthine (13; R'=H). Sodium borohydride reduction[18] of hypoepistephanine (2; R=H), followed by N-methylation, gave the enantiomer of repandine (14; R'=H).

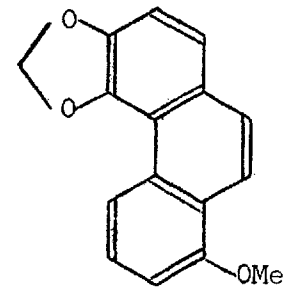
The absolute configurations[21] of the two asymmetric centres [A] and [B] in both compounds (13; R'=H or Me) and (14; R'=H or Me) follow from reductive cleavage experiments, since the configurational interrelationships of the corresponding mono-bases are known. For example, (+)-NOO-trimethylcoclaurine (17; R = R' = R'' = Me) has the same absolute configuration[21,22] as (+)-laudanosine (18; R = R' = Me) and (+)-N-methylcoclaurine[23] (17; R' = R'' = H, R = Me) is derived from (-)-coclaurine (17; R = R' = R'' = H).




(17)




(18)



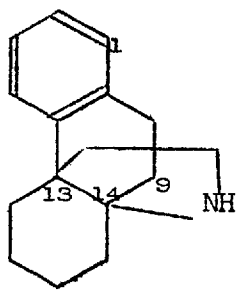

(19)

Stephanine (3)

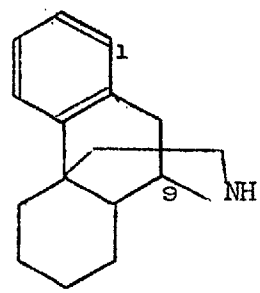
(-)-Stephanine (3) was first isolated[3] in 1924. When oxidized with permanganate[25] it generated 3-methoxyphthalic acid. Hofmann degradation of stephanine (3)[25] followed by oxidation and decarboxylation gave 1-methoxy-5,6-methylenedioxyphenanthrene (19), the structure of which was proved synthetically.[26] The absolute configuration[27] of stephanine (3) is derived from the sign of its specific rotation at 589 mμ.

Hasubanonine (5; R=Me) and homostephanoline (5; R=H)

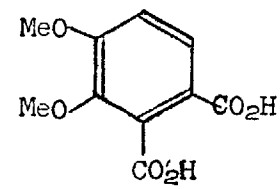
(-)-Hasubanonine (5; R=Me) and its phenolic counterpart, (-)-homostephanoline (5; R=H) are two very interesting alkaloids, both chemically and biosynthetically. They are the first representatives of a new class of alkaloids containing the novel hasubanan skeleton (20) [c.f. morphinan (21)]. Hasubanonine (5; R=Me) was




(20)

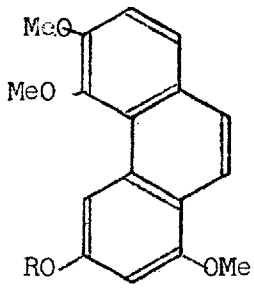



(21)

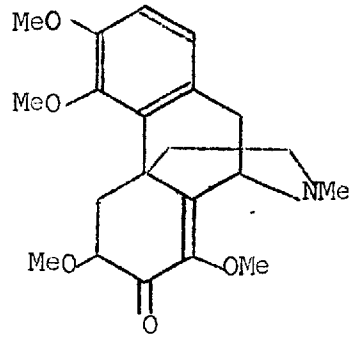



(22)

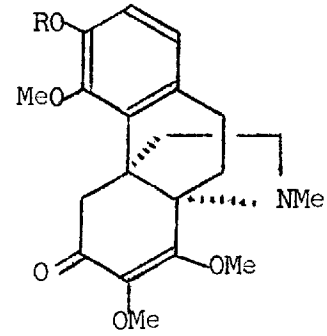
first[5] isolated in 1951. It contains four methoxyl groups, one N-methyl group and a conjugated carbonyl group. Hasubanonine gave hemipinic acid (22) on permanganate oxidation. Hofmann degradation of its methiodide[5,28] afforded a methine base, which on heating with acetic anhydride generated NN-dimethylaminoethanol and O-acetylhasubanol (23; R=Ac). The structure of O-ethylhasubanol



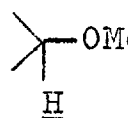

(23)




(24)



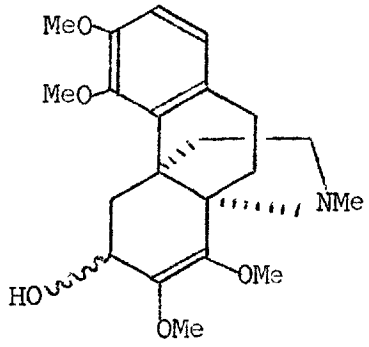

(5)

(23; R=Et) was confirmed[29] by direct comparison with an authentic synthetic specimen. Recent n.m.r. evidence[30] confirmed the presence of the original assigned functional groups (vide supra). In addition to a methylene group [$\tau$ 6.62 (1H, doublet, J = 16 c/s); $\tau$ 7.27 (1H, doublet, J = 16 c/s)] $\alpha$ to a carbonyl group and two aromatic hydrogens at $\tau$ 3.28 (2H, singlet), there was no signal attributable to a proton geminal to a methoxyl group ( 

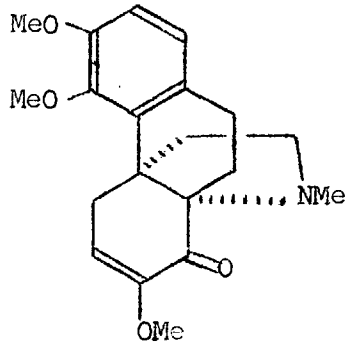
). The structure[31] (24) proposed by Bentley

for hasubanonine was thus ruled out.

Hasubanonine (5; R=Me) was reduced with sodium borohydride[30] and the resulting epimeric allylic alcohols separated by alumina column chromatography to give dihydrohasubanonine-A (25a) and -B (25b). Spectral (i.r.

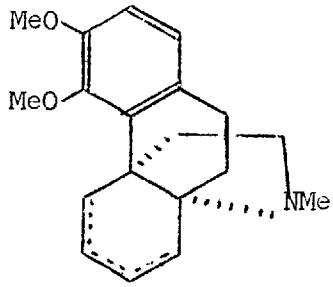



(25a)
(25b)

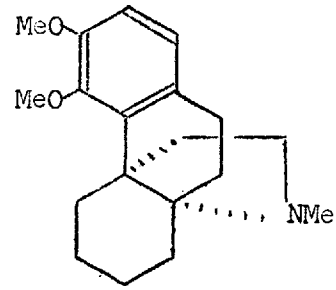



(26)

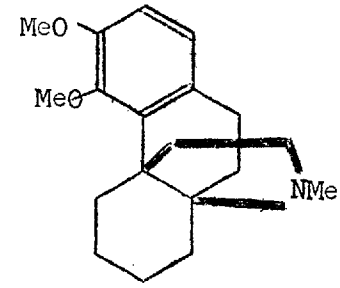
and n.m.r.) data and their respective chromatographic behaviour, indicated that the hydroxyl group of dihydrohasubanonine-A (25a) is quasi-axial and that of -B (25b) is quasi-equatorial. Both -A (25a) and -B (25b) were reoxidized to hasubanonine (5; R=Me). Dihydrohasubanonine-A (25a) and -B (25b), when treated separately with dilute hydrobromic acid under very mild conditions, underwent demethanolisation and gave the conjugated carbonyl compound (26). Clemmensen reduction of this compound (26) afforded an olefinic (27) and a saturated compound (28).




(27)

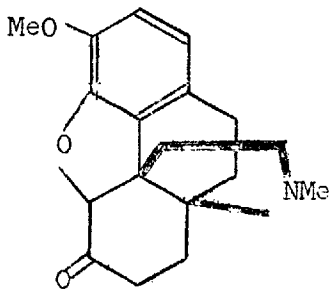



(28)

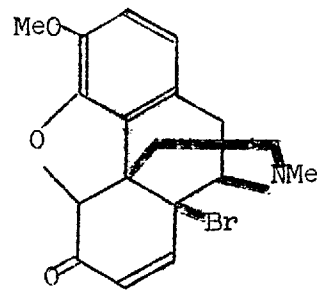



(29)

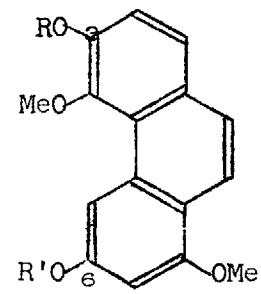
The former compound (27) on catalytic hydrogenation gave compound (28). This compound (28) was shown to be enantiomeric with a product[32] (29) obtained by successive Huang-Minlon reduction and O-methylation of dihydroindoline codeinone (30), which previously[32] was obtained by reduction of 14-bromocodeinone (31) with sodium borohydride.




(30)




(31)




(32)

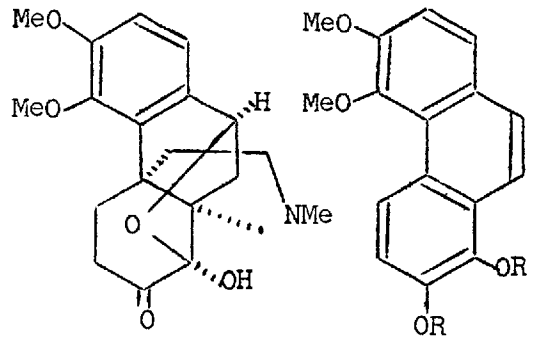
Homostephanoline ( 5; R=H) was first isolated[3] in 1924. Methylation of homostephanoline[33] (5; R=H) with diazomethane gave hasubanonine (5; R=Me). Furthermore, O-ethylhomostephanoline[34] (5; R=Et) was subjected to a Hofmann degradation and the resulting methine acetolysed to give 6-O-acetyl-3-O-ethylhomostephanol (32; R=Et; R'=Ac) which was hydrolysed to O-ethylhomostephanol (32; R=Et, R'=H) and thence converted to the methyl ether (32; R=Et, R'=Me), the structure of which was proved by synthesis. Alternatively, the orientation of the phenolic hydroxyl group in homostephanoline (5; R=H) could be established from a base-catalysed deuterium-exchange experiment[35]. The hydrogen atom ortho to the phenolic hydroxyl group would exchange.

Metaphanine (4) and prometaphanine (6)

The alkaloid (-)-metaphanine (4) was first isolated[3] in 1924 and was given the correct empirical formula $C_{19}H_{23}NO_5$[36] in 1960. Subsequently the alkaloid "neostephanine"[37] from Stephania abyssinica was identified[38] as metaphanine. It is anticipated that both metaphanine (4) and prometaphanine (6) are biogenetically and chemically closely related to hasubanonine (5; R=Me).

Metaphanine (4) resisted[39] acetylation and was

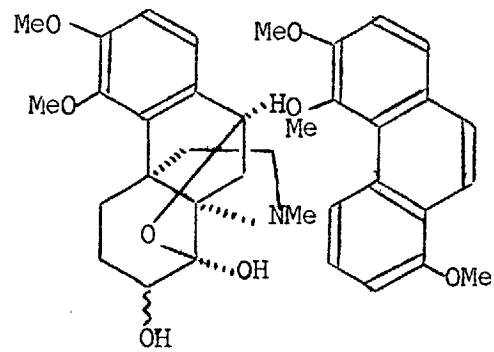
recovered unchanged upon treatment with mineral acid. However, acetolysis[40] of metaphanine (4) with acetic anhydride gave a neutral compound (33; R=Ac), which, upon hydrolysis and subsequent ethylation, afforded 3,4-dimethoxy-7,8-diethoxyphenanthrene (33; R=Et). Also, dihydro-metaphanine (34) upon treatment[39] with acetic anhydride, followed by hydrolysis and subsequent methylation, gave 3,4,7,8-tetramethoxyphenanthrene (33; R=Me) together with compound (35).




(4)

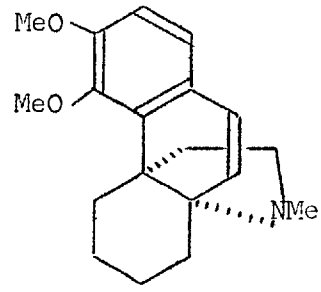



(33)

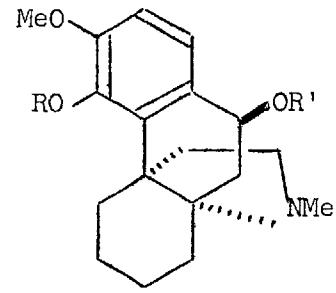



(34) (35)

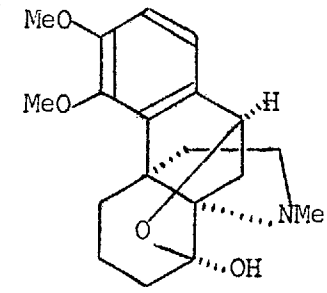
Huang-Minlon reduction[39] of metaphanine (4) gave four deoxo derivatives, deoxometaphanine-A (36), -B (37, R=Me, R'=H), -C (37; R=R'=H), and -D (38). Their relationships and interconversions are set out below. Of special importance is the enantiomeric relationship




(36)




(37)



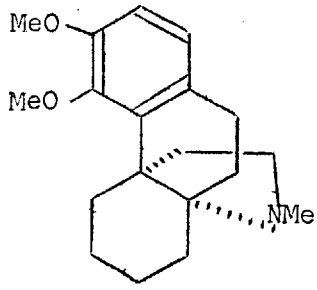

(38)



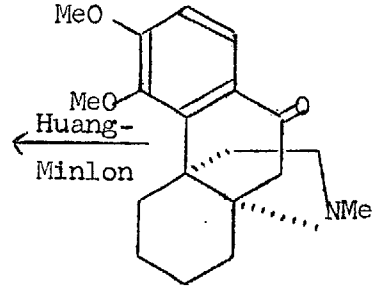




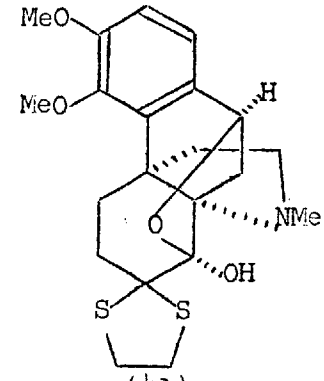



(39)

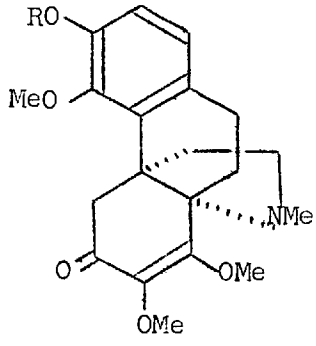



(40)

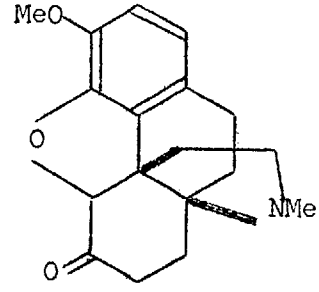



(41)

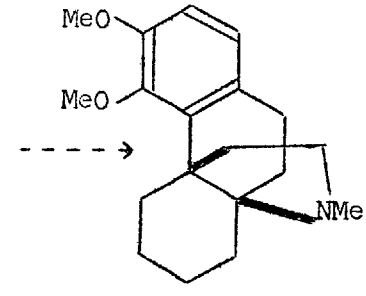





(5)



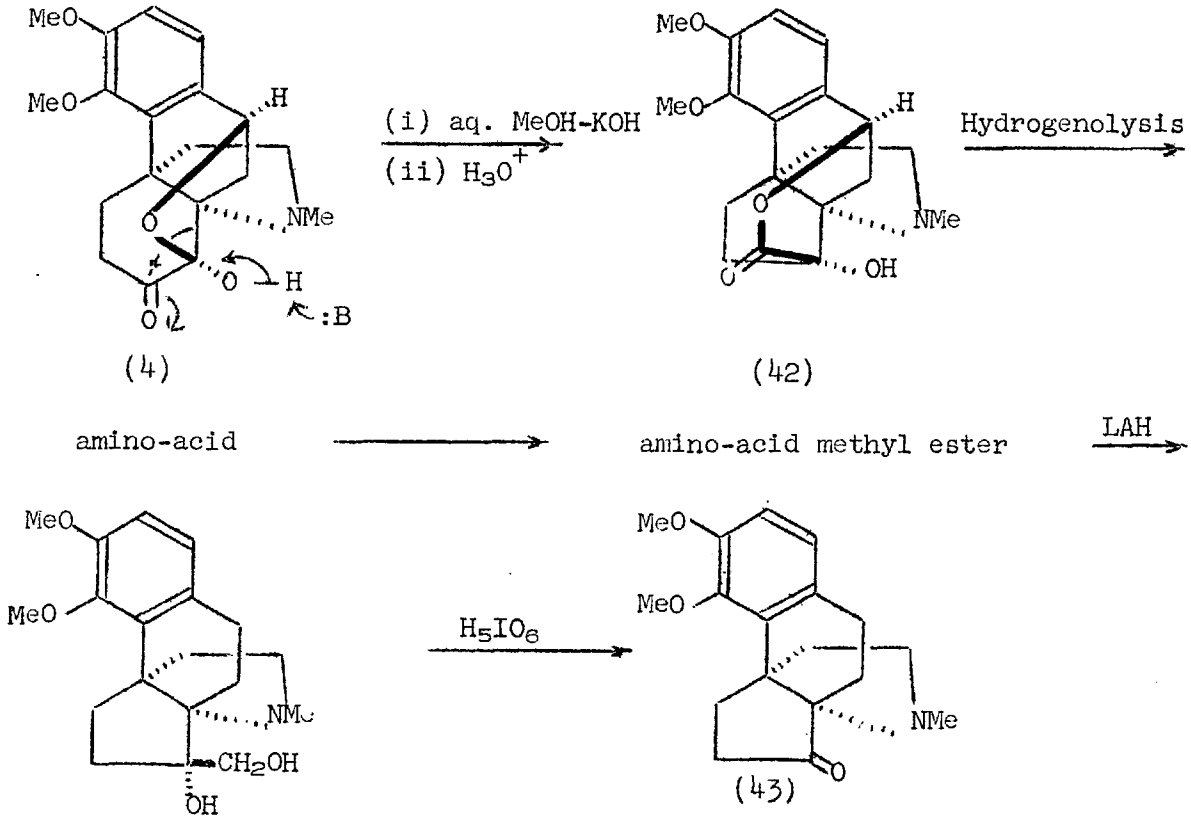

(30)

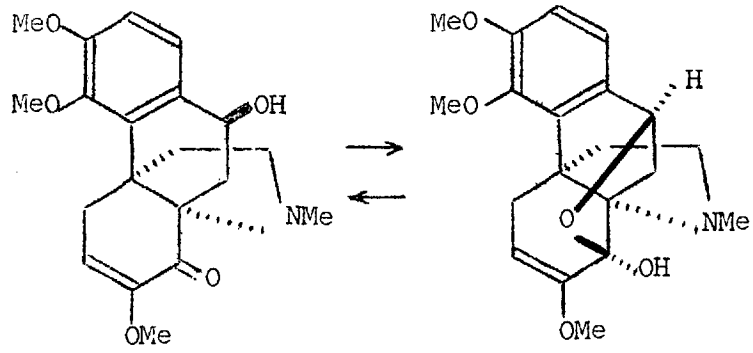



(29)

between dihydrodeoxometaphanine-A (39) and the compound (29), derived from dihydroindolinecodeinone (30). Compound (39) previously[30] was derived from hasubanonine (5; R=Me).

When metaphanine was allowed to react with aqueous methanolic potassium hydroxide, a benzilic acid-type rearrangement took place to give a hydroxy δ-lactone (42) in which the δ-lactone ring and the ethanamine bridge are <u>trans</u> oriented. That indeed a ring contraction had occurred during treatment with alkali was proved by degrading the δ-lactone (42) to a cyclopentanone (43), as is shown in the accompanying reaction scheme.





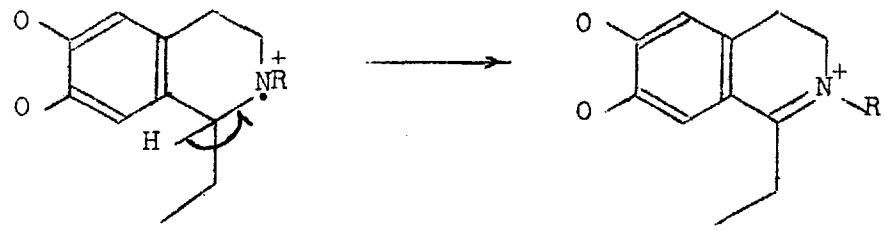

(6) (44)

Prometaphanine (6), an amorphous, but chromatographically pure, solid was recently isolated[41] from Stephania japonica and was characterised as its methiodide salt. The n.m.r. spectra[41] of prometaphanine (6) in various solvent combinations suggested that it exists as an equilibrium mixture of the ketone (6) and the hemiketal (44). Prometaphanine (6), when treated with dilute mineral acid under very mild conditions, gave metaphanine (4) in quantitative yield. A similar degradation sequence of acetolysis, hydrolysis and alkylation of the corresponding phenanthrol applies to prometaphanine (6).

II. The constitution of stebisimine

From Indian Stephania japonica we isolated, in addition to the known bisbenzylisoquinoline alkaloid epistephanine[12,13,19,56] (2; R = Me), a new alkaloid, called "stebisimine" for reasons that will soon become apparent. Subsequently stebisimine was isolated as a minor constituent of Stephania japonica (of Japanese origin) grown at Imperial College.

Stebisimine, m.p. 233-235°, analysed for $C_{36}H_{34}N_2O_6$. Examination and comparison of the mass spectra obtained from stebisimine and its derivatives and those obtained from authentic specimens of bisbenzylisoquinoline alkaloids[42] proved instructive. The most characteristic feature of the mass spectrum of stebisimine was the very intense molecular ion peak at m/e 590, which was also the base peak in the spectrum. Mass measurement confirmed the previously derived (vide supra) molecular formula. The $(M^+ - 1)$ peak, characteristic of the loss of a hydrogen atom from C-1[43] [c.f. epistephanine (2; R=Me)], relatively was of only very low intensity. Apart from



simple methyl and methoxyl radical losses, originating from the molecular ion as was shown by the appearance of the appropriate metastable ions, no further significant fragmentations occurred. In all of the recorded mass spectra we observed a group of peaks around ($M^+$ + 14) of very low intensity, which could arise from thermally induced intermolecular methyl transfer.[44,45] Recently, a similar observation was reported in the literature[46] but ascribed to the presence of some methylated impurities. A specimen of isochondodendrine[42] (46) appeared to be contaminated with an O-methyl homologue. In addition to the expected base peak at m/e 298,[46] it showed a strong peak at m/e 312.[47] The m/e values and their assignments of some bisbenzylisoquinoline alkaloids are summarized below.

## Mass spectra of bisbenzylisoquinoline alkaloids

m/e values

Fangchinoline (45; R=H). 608 ($M^+$, base peak), 607, 593 ($M^+$ - Me), 577 ($M^+$ - OMe), 501 ($M^+$ - 107), 487 ($M^+$ - 121), 486, 471 (486 - Me), 418, 417, 416, 382, 381, 367 (382 - Me), 304 ($M^{++}$), 192, and 191 (isotope peak 191.5).

Isochondodendrine (46). 594 ($M^+$), 593, 487 ($M^+$ - 107), 298 (base peak).

Contaminant: 608 ($M^+$), 607, 501 ($M^+$ - 107), 312.

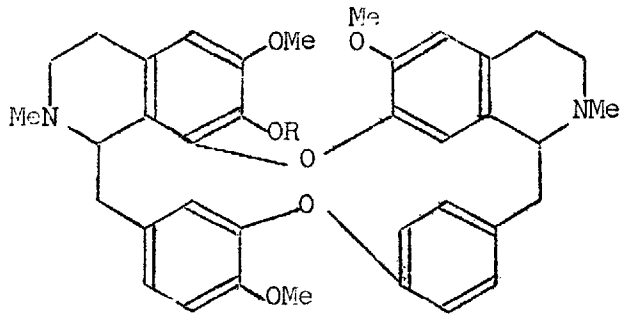
Epistephanine (2; R=Me). 606 ($M^+$ and base peak), 605 (95% of base peak), 591 ($M^+$ - Me), 575 ($M^+$ - OMe), 561, 559, 545, 485 ($M^+$ - 121), 483, 381, 379, 303 ($M^{++}$, isotope peak 303.5), 190, 174, and 145.

Stebisimine (48). 590 ($M^+$ and base peak), 575 ($M^+$ - Me), 559 ($M^+$ - OMe), 370, 295 ($M^{++}$, isotope peak at 295.5), 221, 206, 192, 175.

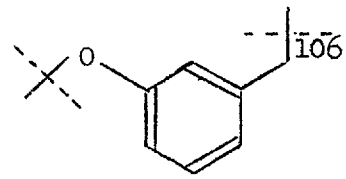
NN-Dimethyltetrahydrostebisimine (mixture of diastereoisomeric racemates).

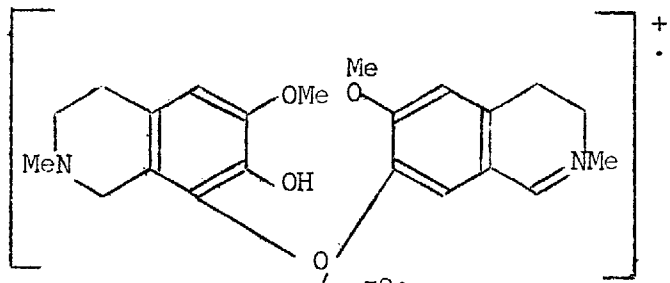
622 ($M^+$), 621, 607 ($M^+$ - $CH_3$), 591 ($M^+$ - OMe), 516, 515 ($M^+$ - 107), 431 ($M^+$ - 191), 396, 395 (base peak), 381 (396 - Me), 379, 364, 358, 349, 311 ($M^{++}$, isotope peak 311.5), 198 (isotope peak 198.5), 175, and 174.

Fangchinoline (45 ; R=H )

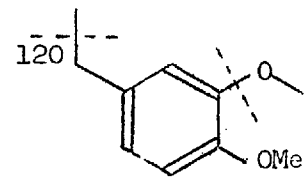


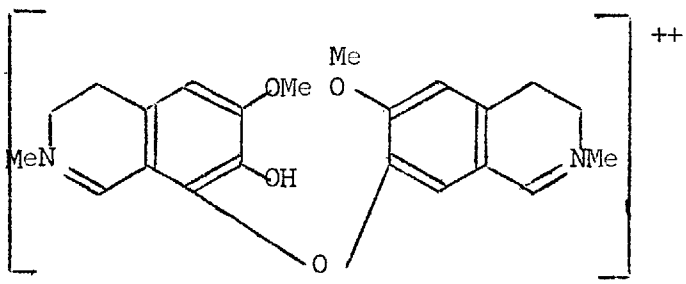

(45)



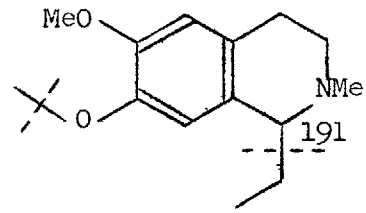




m/e 382

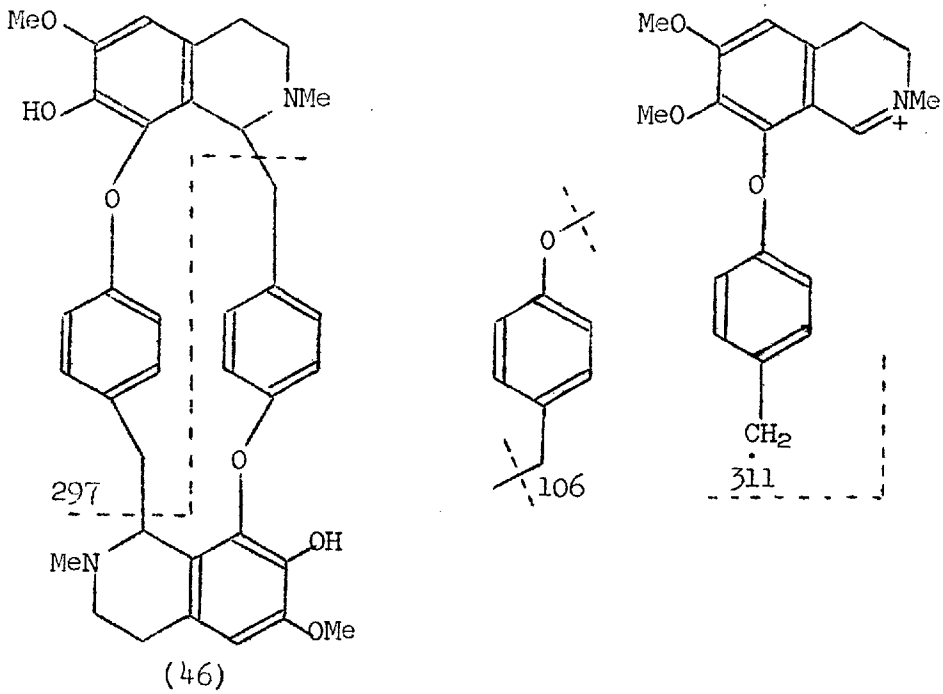



m/e 191

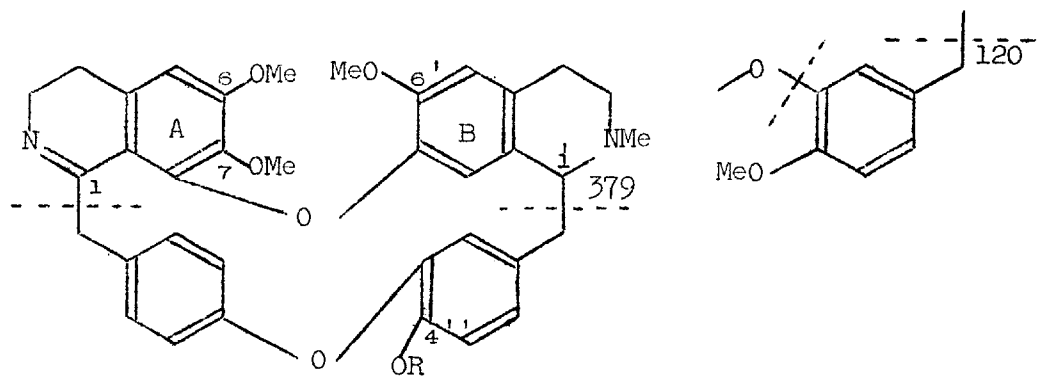


Isochondodendrine (46)



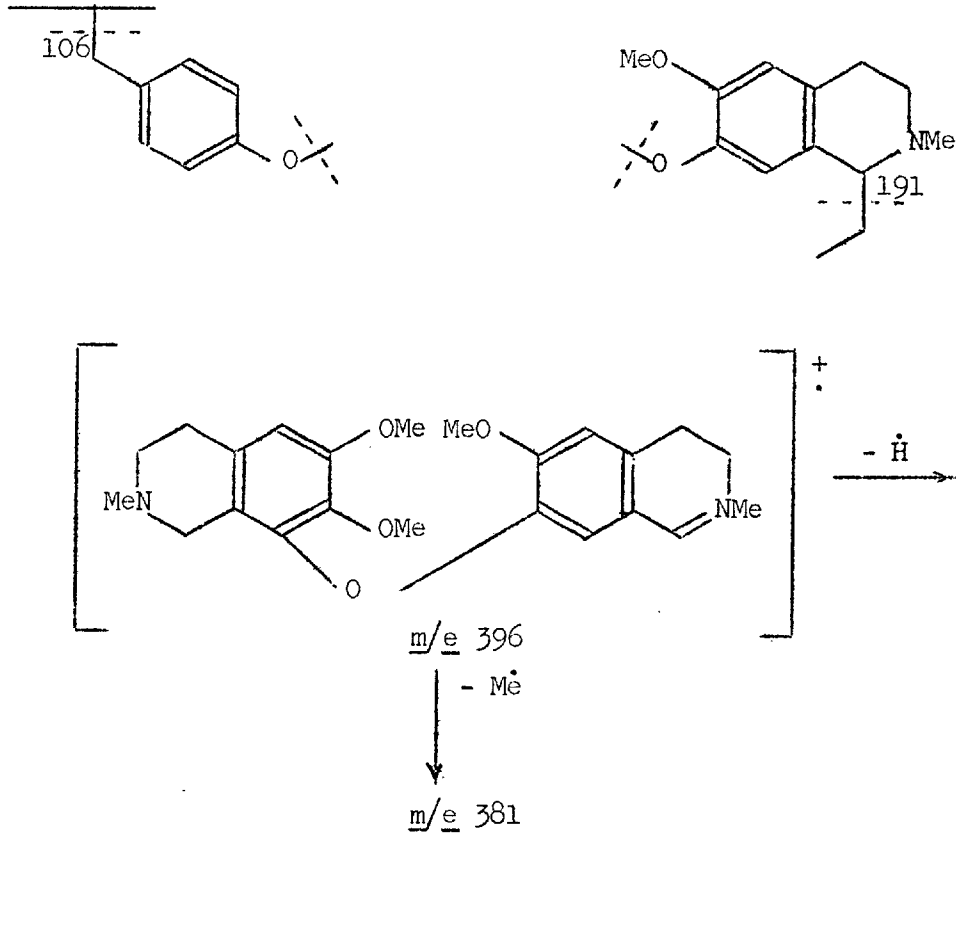

(46)

Epistephanine ( 2 ; R = Me )



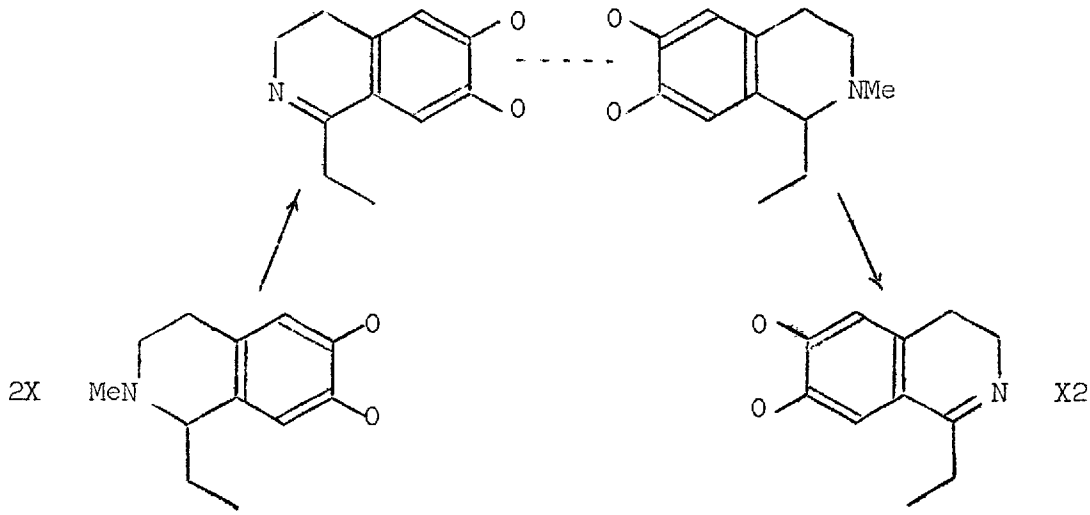

(2)

NN-Dimethyltetrahydrostebisimine ( mixture of diastereoisomeric racemates )



The infrared spectrum of stebisimine (48) in chloroform solution showed an absorption band at 1610 $cm.^{-1}$, which shifted to 1620 $cm.^{-1}$ in a Nujol mull. These absorptions partly arose from C = N stretch as was evident from the two absorption bands at 1643 and 1603 $cm.^{-1}$ in the Nujol spectrum of stebisimine dihydrochloride.

The ultraviolet absorption spectrum of stebisimine (48) resembled the spectra obtained from known[42] bis-benzylisoquinoline alkaloids, both as far as shape and absorption band positions were concerned. Inspection of the intensities of the long wavelength absorption band at ± 280 mμ revealed a continuous hyperchromic shift in passing through the series oxyacanthine (13; R'=H), epistephanine (2; R=Me), and stebisimine (48). The following chromophoric changes therefore seemed reasonable, e.g.



Ultraviolet spectra of bisbenzyltetrahydroisoquinoline alkaloids containing two diphenyl ether linkages[48]

| Compound | $\lambda_{max.}$ (mμ) | $\varepsilon_{max.}$ |
|---|---|---|
| Oxyacanthine[49] (13; R'=H) | 282 | 8,400 |
| | 238 sh. | 28,300 |
| | 206 | 87,500 |
| O-Methylrepandine[49] (14; R'=Me) | 282 | 6,500 |
| | 233 sh. | 45,000 |
| | 206 | 128,000 |
| (+)-Tetrandrine[42] (45; R=Me) | 281 | 9,600 |
| Epistephanine (2; R=Me) | 282 | 14,600 |
| | 233 | 34,100 |
| Stebisimine (48) | 308 infl. | 12,500 |
| | 279 | 24,200 |
| | 238 | 51,900 |
| NN-Dimethyltetrahydrostebisimine mixture | 281 | 7,280 |

Earlier n.m.r. work on bisbenzyltetrahydroisoquinoline alkaloids[50] showed that the line positions of the methoxyl and methylimino groups were characteristic both of the

Methoxyl and methylimino resonances ($\tau$ values) in the n.m.r. spectra of bisbenzylisoquinoline alkaloids

| Compound | Conf. | OMe | | | | NMe | |
|---|---|---|---|---|---|---|---|
| | | 4'' | 6 | 6' | 7 | 2' | 2 |
| O-Methyloxyacanthine[50] (13; R'=Me) | (+-) | 6.10 | 6.21 | 6.40 | 6.80 | 7.35 | 7.45 |
| O-Methylrepandine[50] (14; R'=Me) | (++) | 6.05 | 6.25 | 6.60 | 6.95 | 7.45 | 7.45 |
| Epistephanine (2; R=Me) | (o-) | 6.12 | 6.12 | 6.14 | 6.64 | 7.47 | |
| Stebisimine (48) | (oo) | 6.04 | 6.10 | 6.12 | 6.75 | | |
| NN-Dimethyltetrahydrostebisimine mixture | | 6.05 | 6.24 | 6.57 | 6.98 | 7.43 | 7.43 |
| | and | 6.11 | 6.21 | 6.38 | 6.80 | 7.33 | 7.43 |

mode of linkage of the benzylisoquinoline units and of the relative stereochemistry at the two asymmetric centres. The n.m.r. spectra both of epistephanine (2; R=Me) and stebisimine (48) showed, in addition to three ordinary methoxyl groups, a "shielded" methoxylgroup at a higher field value. A molecular model of epistephanine (2; R=Me) showed the C-7 methoxyl group to pass closely over the top of the adjacent aromatic ring B. Significantly the n.m.r. spectrum of stebisimine (48) showed no N-methyl signal.

The zero specific rotation of stebisimine (48) at the D-line and the flatness of the o.r.d. curve[51] of stebisimine dihydrochloride over the wavelength region 250-400 mμ proved its optical inactivity.

These properties suggested that stebisimine might be N-nor-1',2'-dehydroepistephanine (2; R=Me) and this was confirmed chemically.

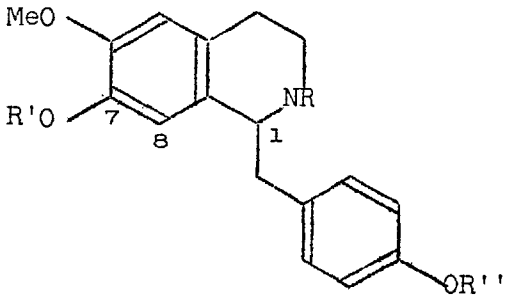
When stebisimine dimethiodide was reduced with sodium borohydride a mixture of diastereoisomeric racemates was obtained. The infrared spectrum in chloroform solution showed an absorption band at 1610 $cm.^{-1}$ of reduced intensity compared with stebisimine. The ultraviolet spectrum of the mixture resembled that of an ordinary NN-dimethyl bisbenzyltetrahydroisoquinoline

alkaloid, with an expected decrease of the molar absorptivity value for the ± 280 mμ absorption band. The n.m.r. spectrum of this racemic mixture showed methoxyl signals at τ 6.05, 6.24, 6.57, and 6.98, whilst the two methylimino groups resonated at τ 7.43. These line positions resembled those reported for O-methylrepandine[50] (14; R'=Me). Furthermore, signals at τ 6.11, 6.21, 6.38, 6.80, 7.33, and 7.43, the last two somewhat broadened and obscured by the methylene proton resonances, resembled those reported for O-methyloxyacanthine (13; R'=Me).[50] Equally instructive was the mass spectrum of the racemic mixture. The molecular ion peak appeared at m/e 622 and thus confirmed the expected mass shift, △ m = 32. The overall fragmentation pattern summarized previously, with the base peak at m/e 395, was the same as those reported[47] in the mass spectra of either O-methylrepandine (14; R'=Me) or its diastereoisomer O-methyloxyacanthine (13; R'=Me).

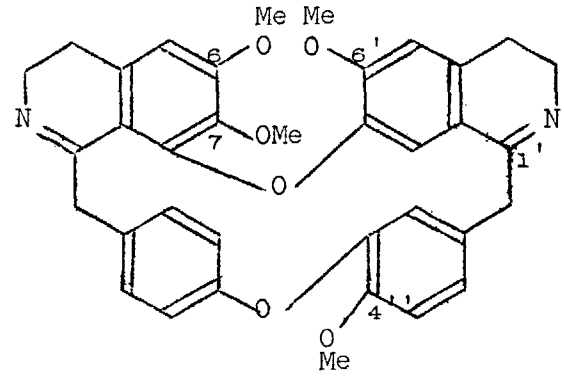
Catalytic reduction of stebisimine (48) [two equivalents absorbed] gave, after N-methylation of the reaction mixture and separation of the two components, a major component with an expected molecular ion peak at m/e 622 and the same fragmentation pattern noted previously[47] for either O-methylrepandine (14; R'=Me) or

O-methyloxyacanthine (13; R' = Me).

Finally, reduction of stebisimine (48) with sodium in liquid ammonia[52] gave, after N-methylation of the reaction mixture, (±)-armepavine (47; R=R'=Me, R''=H), the




(47)




(48)

hydrochloride of which was identical (i.r. and mixed m.p.) with synthetic racemic armepavine hydrochloride[53], and (±)-NO-dimethylcoclaurine (47; R=R''=Me, R'=H). The n.m.r. spectrum[54] of the latter compound (47; R=R''=Me, R'=H) showed an $A_2B_2$ quartet at τ 2.98, 3.48 (J = 8.5 c/s), two aromatic hydrogens at τ 3.48, 3.66, two methoxyl groups at τ 6.17, 6.23 and an N-methyl group at τ 7.52. The n.m.r. spectrum[54] of the corresponding O-methyl ether

(47; R=R'=R''=Me) showed both a shielded C-8 hydrogen at $\tau$ 4.01 and a shielded C-7 methoxyl group at $\tau$ 6.45. The syrupy base was characterized and identified (no mixed m.p. depression) as (±)-NOO-trimethylcoclaurine methiodide.[55]

## The Biosynthesis of some Stephania alkaloids

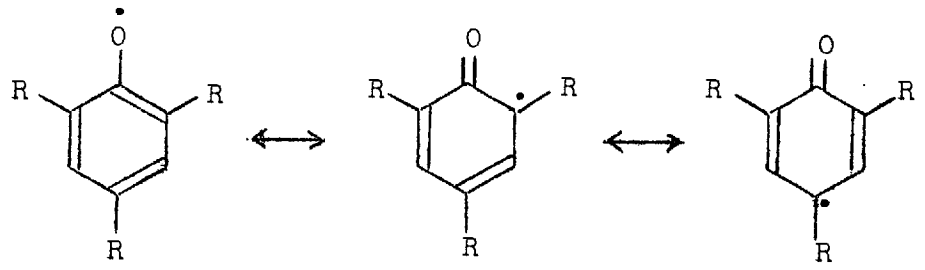
The subject of the biosynthesis of alkaloids has been reviewed in several places.[57,58,59,60,61,62,63,64] However, here no general review of the biosynthesis of alkaloids will be made. In the subsequent discussion of the biosynthesis of some Stephania alkaloids frequent reference will be made to experimentally proven biosynthetic theories.

Many[65,66] biosynthetic hypotheses and structural correlations of certain natural products have been based on the unifying mechanistic principle of the coupling of phenolate radicals.

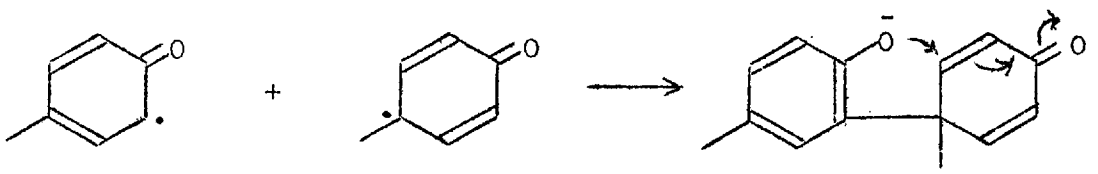
Phenolate radicals may be generated by the oxidation of phenols or phenolate anions with one electron-transfer oxidizing reagents such as ferric chloride, potassium ferricyanide, etc.[66,67] The subsequent free radical either dimerises, internally couples or reacts with another compound. However, the production of a stable phenolate radical (49; R = $Bu^t$) becomes possible where bulky 2,4,6-trisubstitution prevents further reaction by coupling.[68] If two phenolate radicals were to couple, either intra- or intermolecularly, one can envisage

either carbon-carbon or carbon-oxygen bonds being formed due to the spreading of the odd electron over oxygen as well as over the two ortho carbon and the para carbon atoms as in (49).

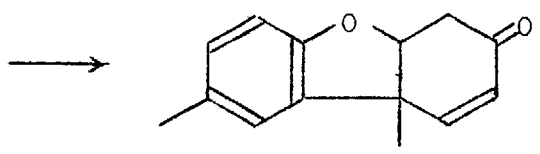
Barton showed[69] that "Pummerer's ketone"[70] is represented by structure (52) and he represented its



(49)



(50) (51)



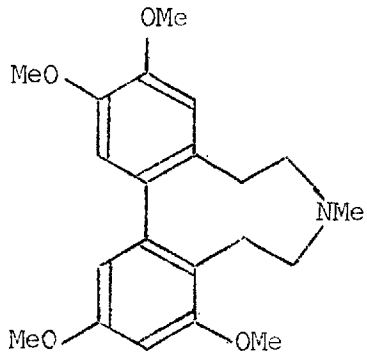
(52)

formation as a carbon-carbon coupling of two p-cresolate radicals (50) to give the intermediate dienone (51), which by β-addition of the phenolate anion affords the nicely crystalline enone (52). This scheme for the synthesis of "Pummerer's ketone" is important because it indicates a mode of biogenesis for a good number of alkaloids.[66]

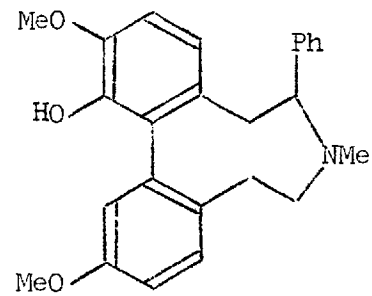
Recent progress in free-radical oxidation reactions, both for biogenetic schemes and for biogenetic-type laboratory syntheses, has been reviewed.[66,71,72,73]

Protostephanine (1)

Protostephanine (1) is the only naturally occurring compound containing the dibenz-[d,f]-azonine ring system.




(1)



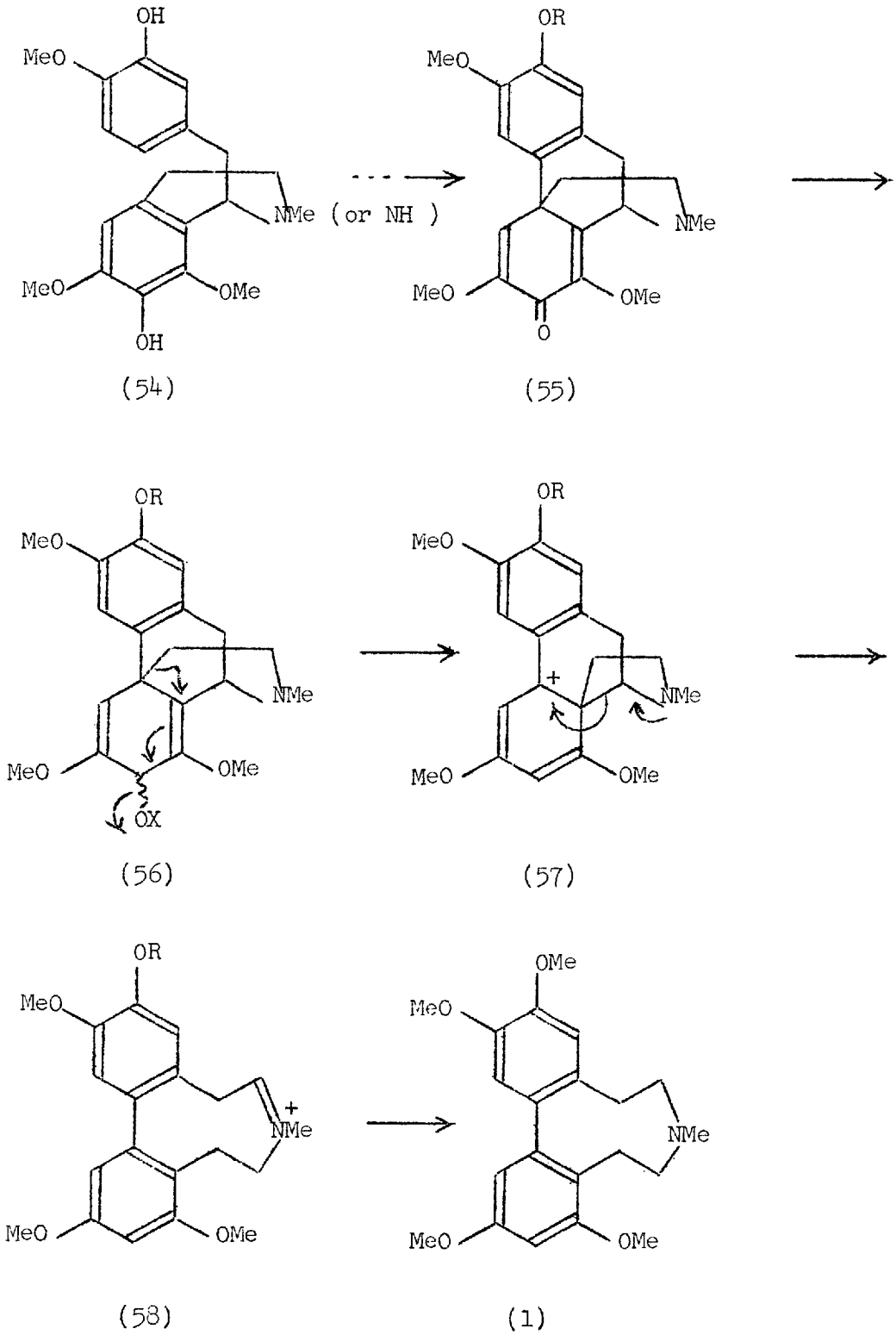

(53)

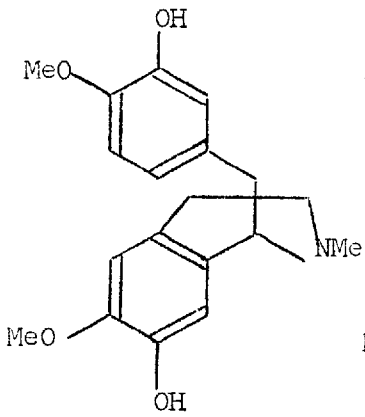
However, it is closely similar in structure to phenyldihydrothebaine[74](53).

On the basis of the previously mentioned concept of a carbon-carbon dimerization of phenol radicals, Barton proposed[75] a biogenetic scheme for protostephanine, which is summarized below. Oxidative coupling of the diphenolic base (54; either NMe or NH), which we synthesized for the first time, could yield the dienone (55; R = H or Me). These dienones (55; R = H and R = Me) have recently been synthesized by Professor A. R. Battersby and Mr. P. Hackett.[76] Reductive elimination[77] of the carbonyl oxygen and aromatization, indicated by the sequence (55) ⟶ (56; X = phosphate) ⟶ (57), could yield an immonium ion (58; R = H or Me) which by direct or indirect reduction would give protostephanine (1). Alternatively the versatile and biologically important[66,77,78,79] diphenolic base reticuline (59) could possibly be hydroxylated at the dienone level (60a) [which is closely related to the dienone amurine[85](60b)] or some later stage, to undergo a similar series of transformations noted previously to give protostephanine (1).

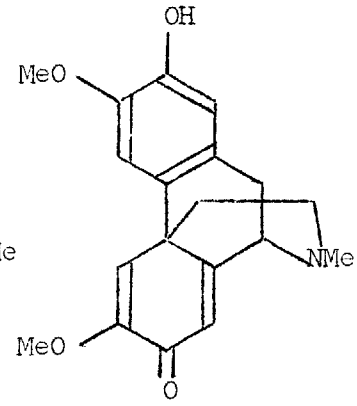
An alternative biogenetic scheme for the formation of protostephanine (1) was proposed[80] by Boit.

## Barton's biogenetic scheme[75]

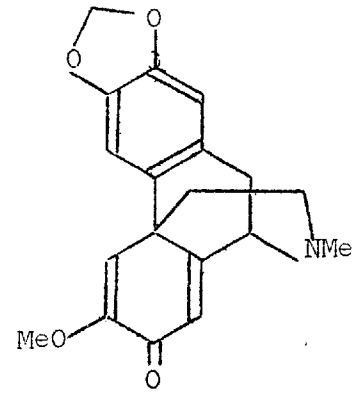




(59)

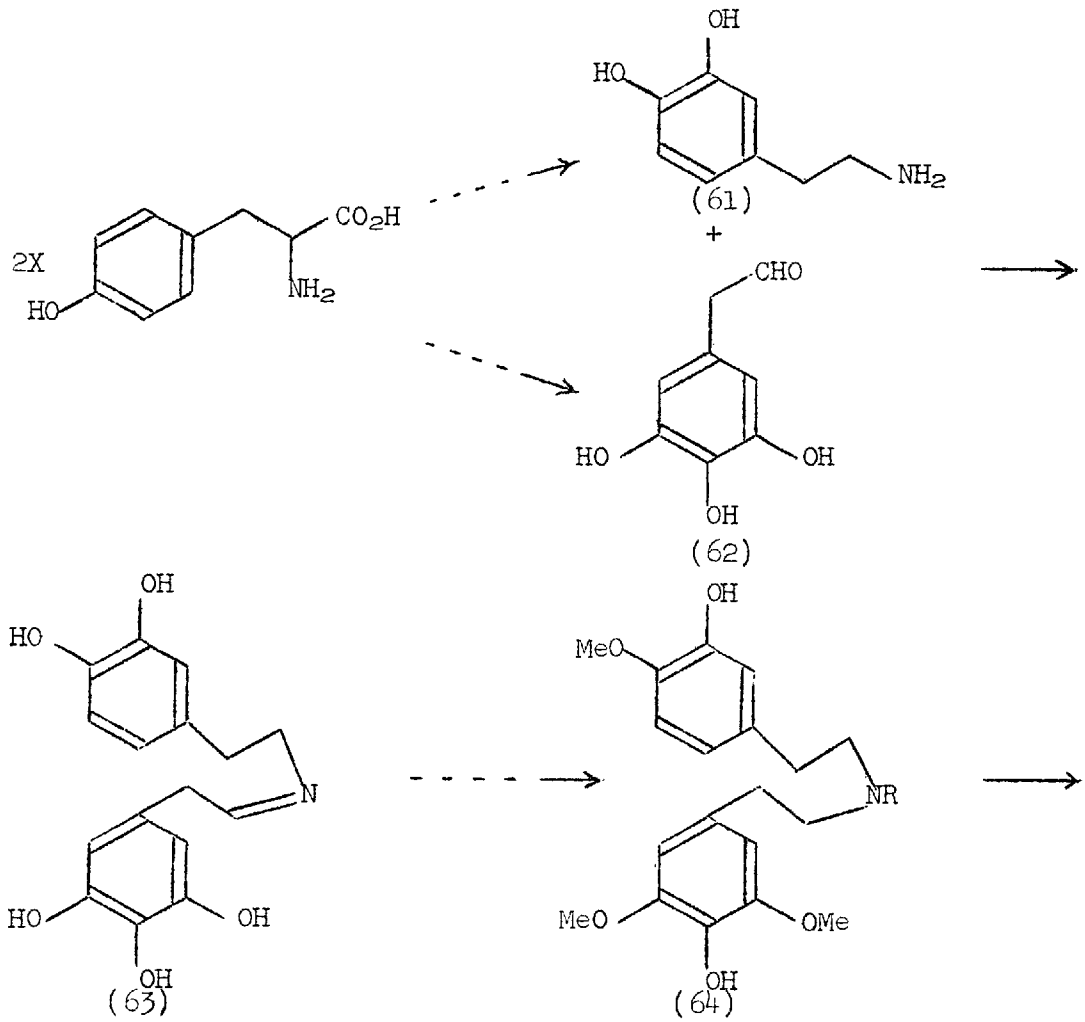


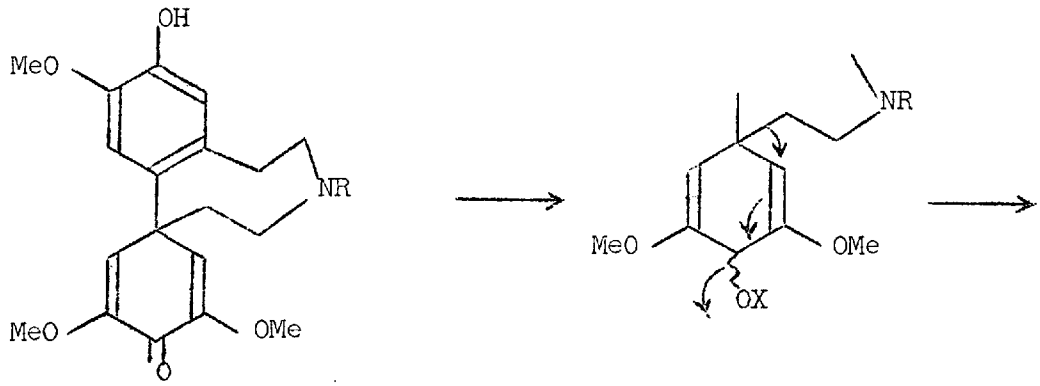
(60a)



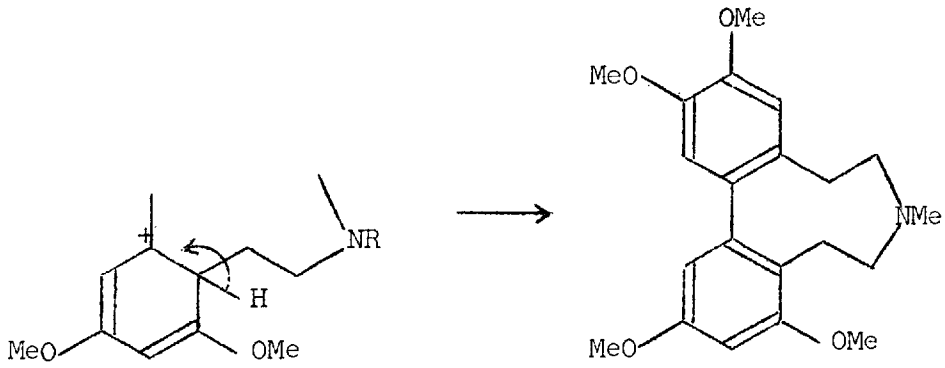
(60b)

Boit's biogenetic scheme[80]






(65) (66)

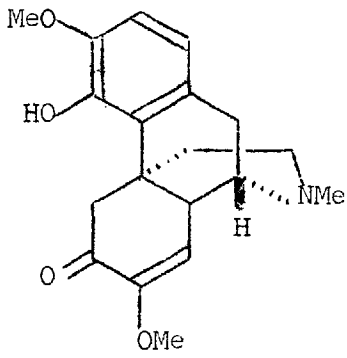



(67) (1)

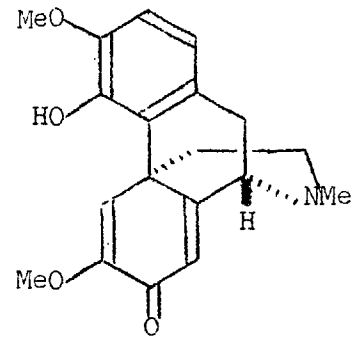
Condensation of dopamine (61) with 3,4,5-trihydroxyphenylacetaldehyde (62) or their biological equivalents, formally derivable from tyrosine,[81,82,83] would yield the Schiff's base (63), which on reduction and methylation (to ensure that the subsequent phenolic coupling occurs in the desired sense) would give the bis-[β-phenethyl]-amine (64; R = H or Me). Para-para oxidative coupling of the diphenolic base (64; R = H or Me) could yield the dienone (65; R = H or Me) which similarly by reduction to the dienol (66), allylic elimination with bond migration and aromatization could give protostephanine (1). The reaction sequence is summarized in the sense (64) ⟶ (65) ⟶ (66) ⟶ (67) ⟶ (1).

Our results obtained from feeding experiments are summarized below. In a preliminary experiment with (±)-[2-$^{14}$C]tyrosine an incorporation of 0.04% into protostephanine in Stephania japonica Miers was obtained. In the following season (1965) when (±)-[2-$^{14}$C]tyrosine was fed, no protostephanine was produced at the time of feeding. Also, neither of the large phenolic precursors (54; NMe; 2-methyl-$^{14}$C labelled), (54; NH; 2',6'-$^{3}H_2$ labelled) nor (±)-reticuline (59; 2',6',8-$^{3}H_3$ labelled) were incorporated. Although it is difficult to interpret negative results, they may, however, be a reflection

of poor transfer of these precursors to the actual site of protostephanine synthesis in the plant. An apparently analogous poor transfer at the diphenolic stage is provided by the formation[84] of sinomenine (68) from reticuline (59) via the dienone sinoacutine (69). Although sinoacutine (69) was incorporated well into sinomenine (68), the incorporation of reticuline (59) was much less efficient. Experiments are in hand to test the validity of Boit's proposed biogenetic scheme for the synthesis of protostephanine in the plant. Also, possible feeding experiments




(68)




(69)

of the diphenolic bases (54) and reticuline (59) to a different Stephania plant species under different environmental conditions might prove instructive. In conclusion, from our results it appears that we are probably not

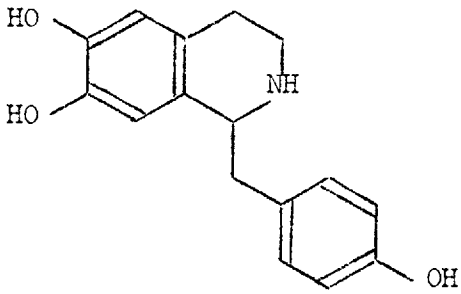
observing the normal biosynthetic route to protostephanine.

PROTOSTEPHANINE

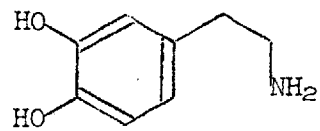
| Precursor | nc | year | weight (mg.) | incorp. (%) |
|---|---|---|---|---|
| (±)-[2-$^{14}$C]tyrosine | 0.02 | 1964 | 9.7 | 0.04 |
| (±)-[2-methyl-$^{14}$C]-1-(3'-hydroxy-4'-methoxybenzyl)-2-methyl-6,8-dimethoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline(54) | 0.0157 | 1965 | 28 | 0.0 |
| (±)-[2',6'-$^{3}H_2$]-1-(3'-hydroxy-4'-methoxybenzyl)-6,8-dimethoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline(54) | 0.183 | 1965 | 31 | 0.0002 |
| (±)-[2',6',8-$^{3}H_3$]-reticuline(59) | 0.147 | 1965 | 23 | 0.0 |

Epistephanine (2; R=Me)

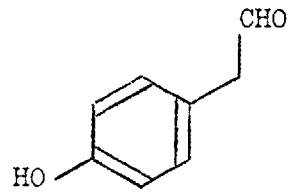
The chemistry of the bisbenzylisoquinoline alkaloids has been reviewed in several places.[52,86,87] Biogenetically[52,66,88] they appear to be derived in Nature from norcoclaurine (70) and its simple N- and O-methyl derivatives, by oxidative phenol coupling, to give bases containing respectively one, two and three diphenyl ether linkages.




(70)




(71)

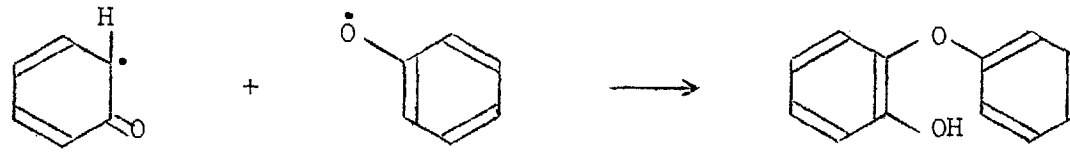



(72)

Selected examples from approximately seventy naturally occurring bisbenzyl- and bisalkylisoquinoline alkaloids are summarized below, both to illustrate some obvious deviations from the normal norcoclaurine oxygenation

pattern and the rich diversity of structural types which formally could be derived from the coupling of phenolate radicals in the sense,

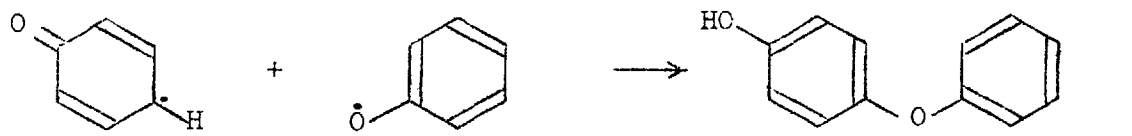
(i) <u>ortho</u> C - O coupling to give an



(73)

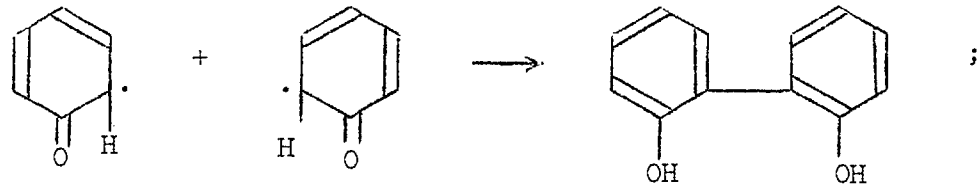
<u>o</u>-hydroxydiphenyl ether (73);

(ii) <u>para</u> C - O coupling to give a <u>p</u>-hydroxy-diphenyl ether (74)



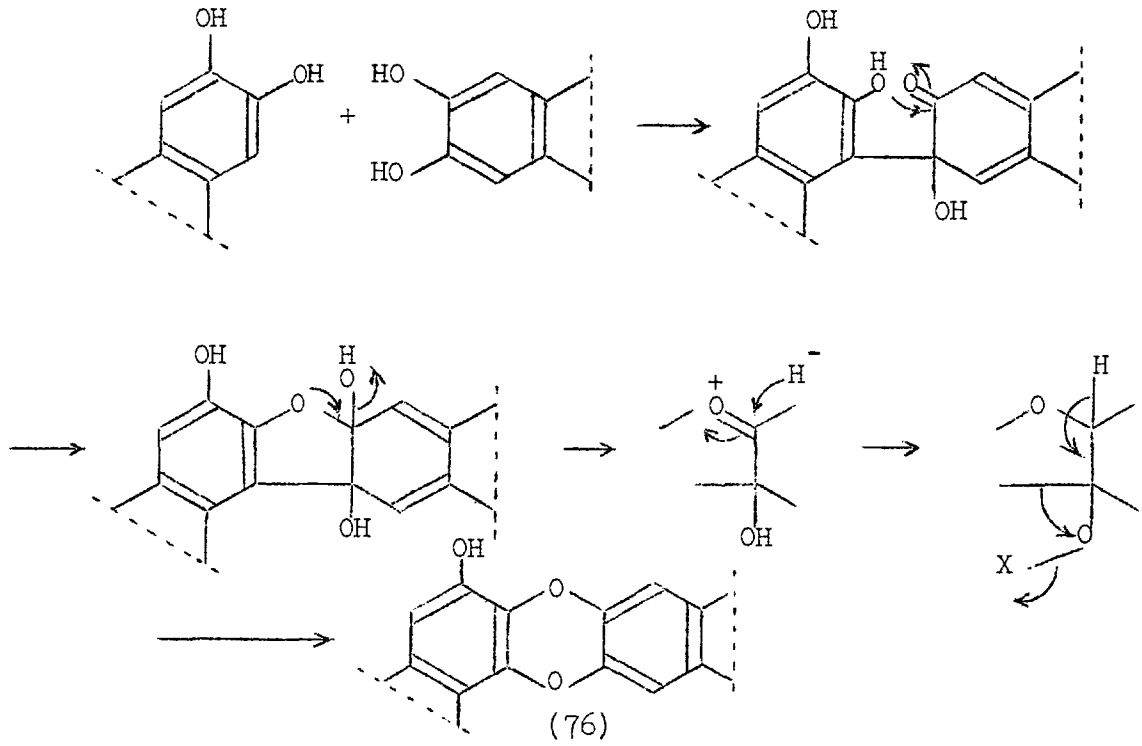
(74)

(iii) ortho-ortho C - C coupling to give an o,o'-dihydroxybiphenyl (75)



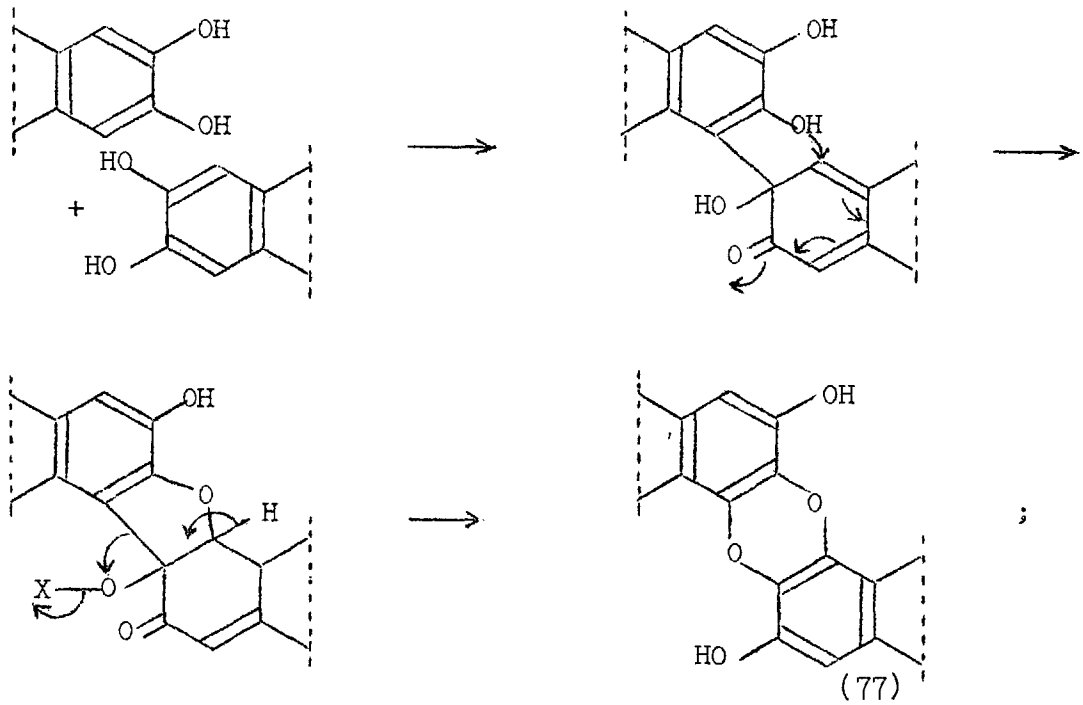
(75)

(iv) ortho-ortho C - C coupling and a subsequent migration induced by the departure of a strongly electron-attracting residue X to give either dibenzo-p-dioxin (76) or (77)[66]



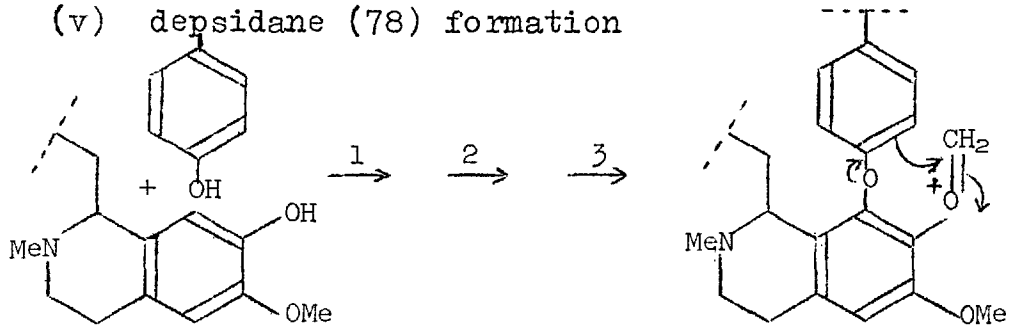
(76)

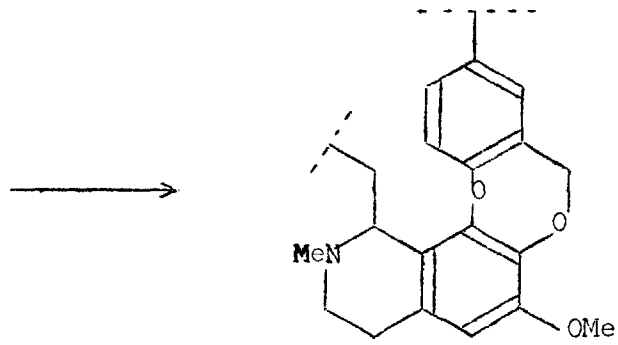
and



(77)

(v) depsidane (78) formation



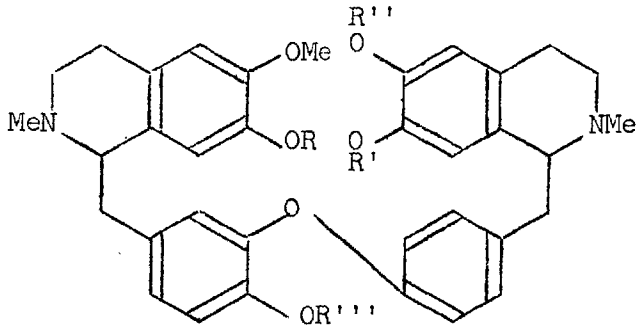


(78)

1. oxidative coupling
2. methylation
3. oxidative cyclisation of the O-methyl group

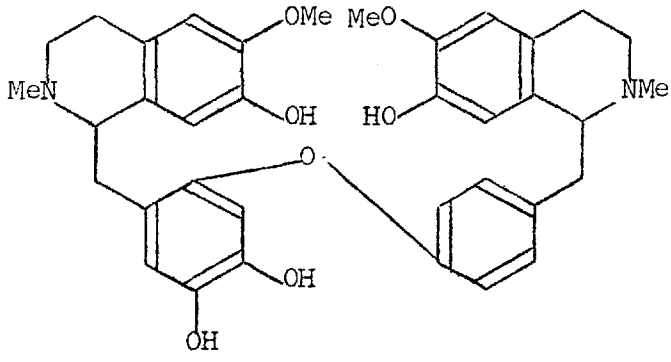
The absolute configurations at the relevant asymmetric centres of numerous bisbenzylisoquinoline alkaloids have been summarized elsewhere[21,49] and are therefore partly omitted in the subsequent discussion.

## Bases containing one diphenyl ether linkage

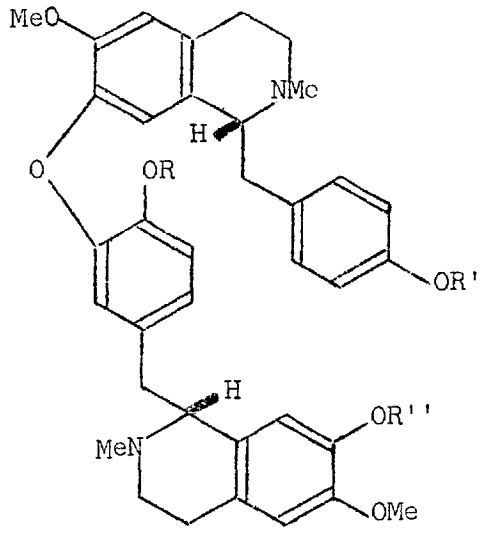



(79)

dauricine[21] (79; R=R'=R''=Me, R'''=H)
magnoline[21] (79; R=R'=R'''=H, R''=Me)
daurinoline[89] (79; R=R'=Me, R''=R'''=H)




magnolamine[21] (80)

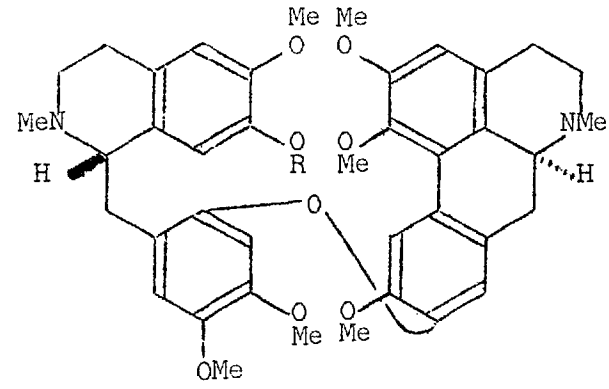


(81)

liensinine[90](81;R=R'=H,R''=Me)

isoliensinine[90](81;R=R''=H,R'=Me)

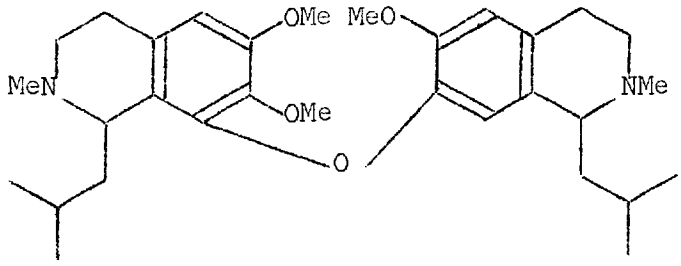
neferine[135](81;R=H,R'=R''=Me)



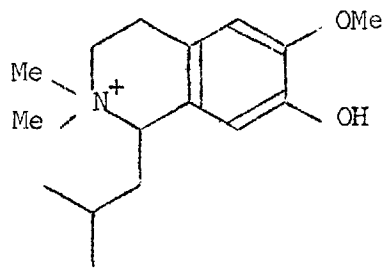
(82)

thalicarpine[91,136](82;R=Me)

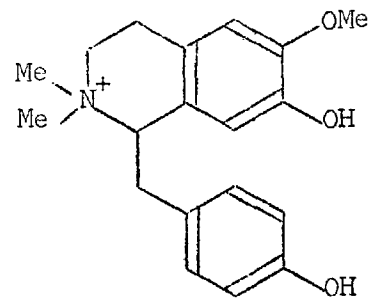
thalmelatine[136,137](82;R=H)



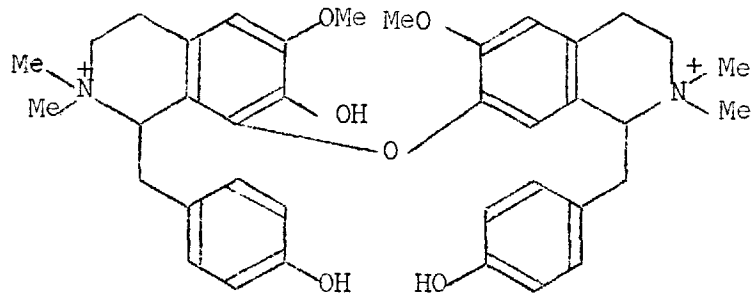
isopilocereine[92,93](83)



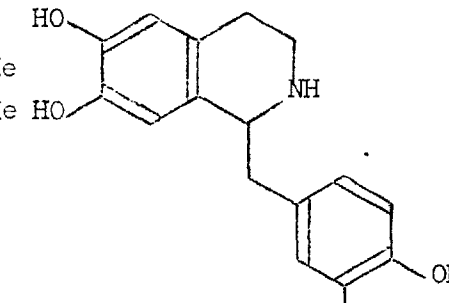
(84)



(85)



(86)



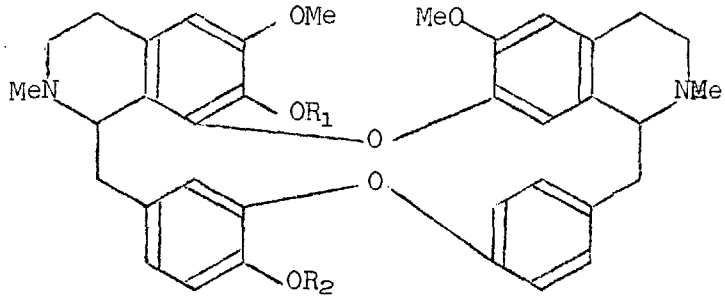
(87)

Apparently bases of the magnoline-type (79; R = R' = R''' = H, R'' = Me) are formed firstly in the plant and could act as precursors for those bases belonging to the berbamine-oxyacanthine series [(88) and (89)]. Similarly, curine (94) and related alkaloids [vide infra] could possibly be derived in Nature from liensinine-type (81) bases. The variety of oxidative phenol coupling in these dimeric bases containing one diphenyl ether linkage, combined with the configurational possibilities at the two asymmetric carbon atoms, would account for all of the oxyacanthine-berbamine [(88) and (89)] and curine-type [(94) and (95)] bases. Interestingly, the quaternary base magnocurarine (85), when oxidized[72] with one mole equivalent of potassium ferricyanide, gave the crystalline dimer (86). Oxidation therefore occurs exclusively at the phenolic hydroxyl group of the isoquinoline residue. Similarly, lophocerine methiodide (84) when oxidized[72] with one mole equivalent of alkaline potassium ferricyanide,

gave in excellent yield the dimeric compound isopilocereine dimethiodide (83). Although it was suggested[72] that oxidative condensation of quaternary bases are possibly involved in the biosynthesis of alkaloids, we would rather think they represent end products on the metabolic pathway - as yet neither the magnocurarine dimer (86) nor its close relatives have been found in Nature.

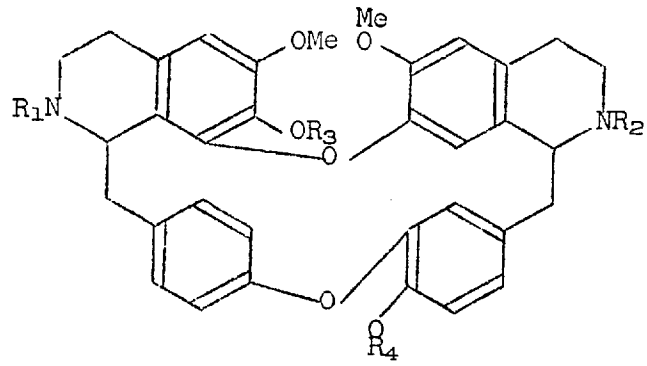
The left hand benzylisoquinoline units both in magnolamine (80) and the novel compound thalicarpine (82; R = Me) are possibly derived from norlaudanosoline (87) and some methylated derivatives thereof.

Bases containing two diphenyl ether linkages




(88)

tetrandrine[21](88;$R_1$=$R_2$=Me)
berbamine[21](88;$R_1$=Me,$R_2$=H)
obamgine[21](88;$R_1$=H,$R_2$=H)
fangchinoline[21](88;$R_1$=H,$R_2$=Me)

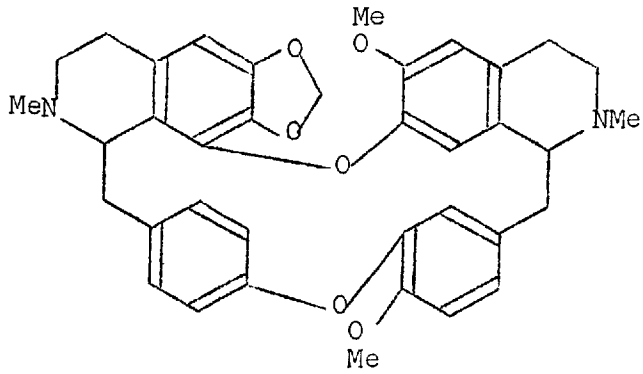


(89)

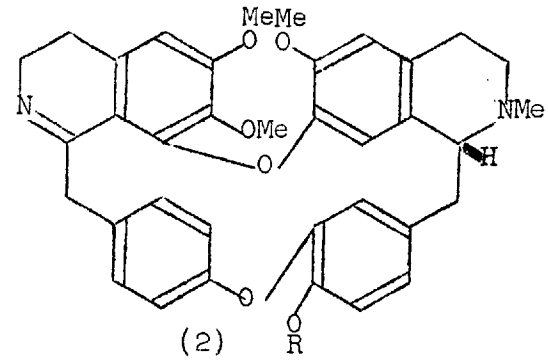
obaberine[21](89; $R_1=R_2=R_3=R_4=Me$)

oxyacanthine[21](89; $R_1=R_2=R_3=Me, R_4=H$)

trilobamine[21](89; $R_1=Me, R_2=R_3=R_4=H$)



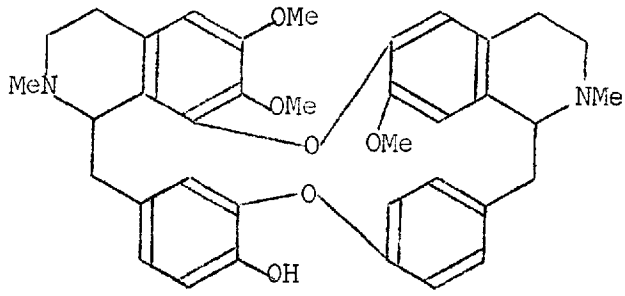
cepharanthine[21](90)



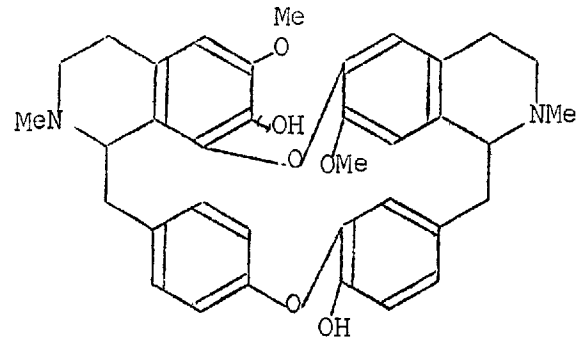
(2)

epistephanine[19](2; R=Me)

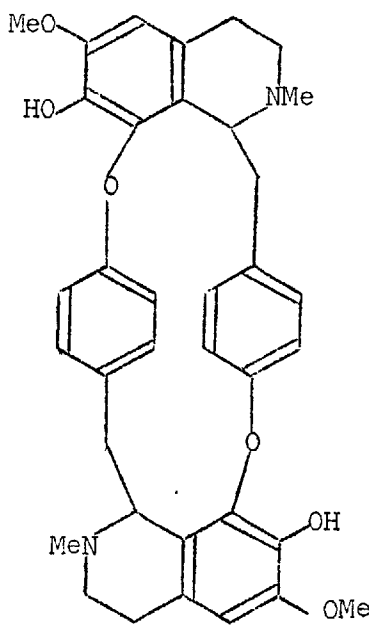
hypoepistephanine(2; R=H)



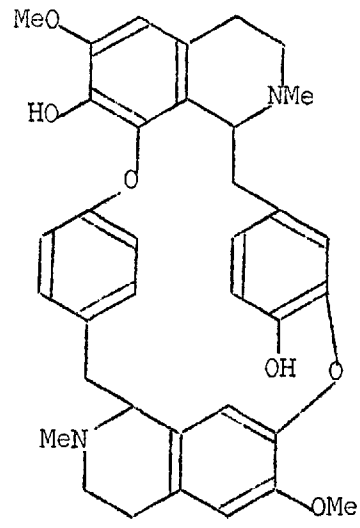
thalicberine[21](91)



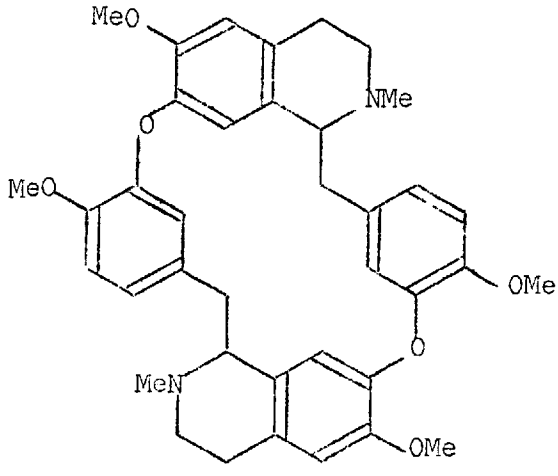
thalicrine[21](92)



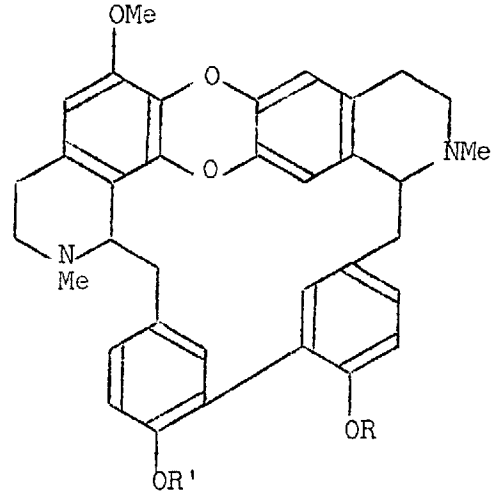

isochondodendrine[21](93)



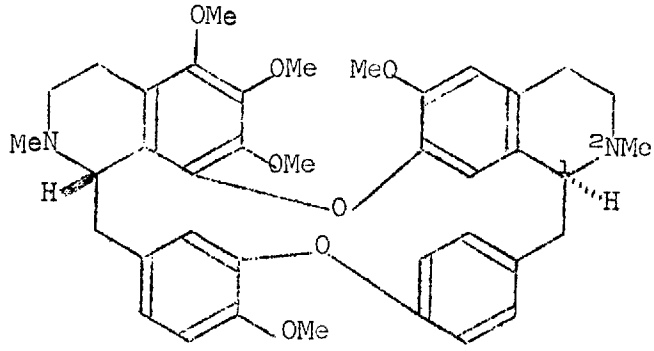

curine[21](94)




OOdimethylhayatine[94](95)

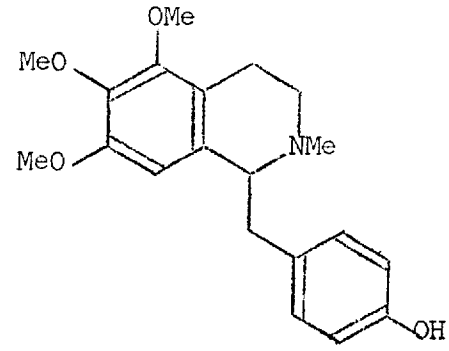



tiliacorine[95](96)
(R=Me,R'=H or R=H,R'=Me)

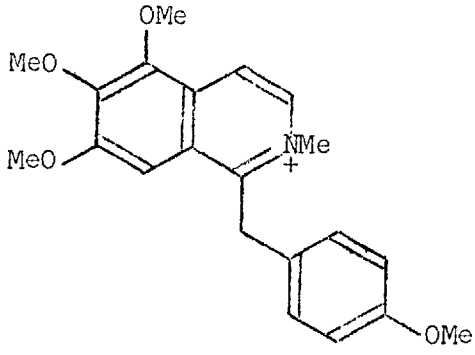



(+)-hernandezine[96](97)

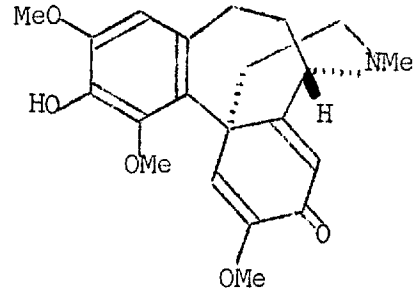
thalsimine[96](N-nor-1,2-dehydrohernandezine)



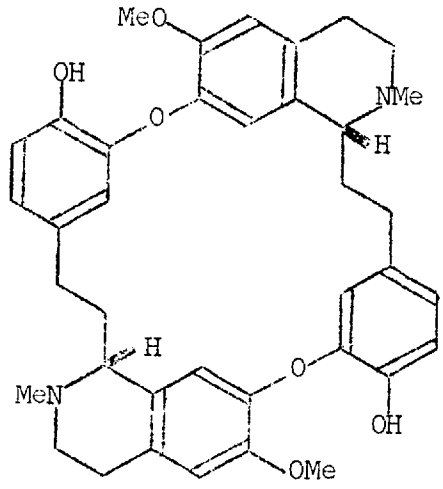

thalifendlerine[97](98)



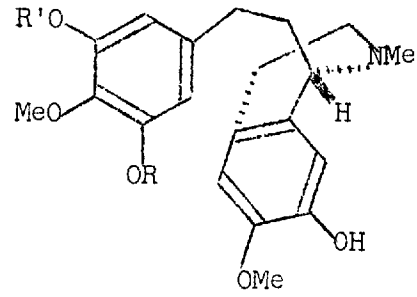

takatonine[98](99)




androcymbine[99](100)



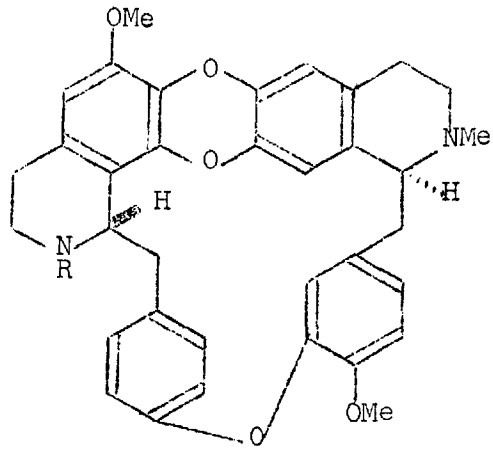

melanthioidine[100](101)




(102)

As was mentioned previously (vide supra) all the constitutions (88) to (96) probably are biosynthesised by phenol oxidation from norcoclaurine (70) or its suitably methylated derivatives. The constitution[95] (96), assigned to the alkaloid tiliacorine, biogenetically and chemically, is rather suspect. It is biogenetically significant that (+)-hernandezine[96] (97) occurs in Thalictrum fendleri along with the novel C-5 oxygenated 1-benzyltetrahydroisoquinoline alkaloid thalifendlerine[97] (98), the C-7 phenolic counterpart which probably provides half the hernandezine molecule. This same oxygenation pattern occurs in the quaternary base takatonine[98] (99). Melanthioidine[100] (101) is the first representative of a new class of alkaloids, the bisphenethylisoquinoline alkaloids. Recently[99] the dienone alkaloid androcymbine (100) was proved to have the constitution and absolute stereochemistry (100). It was suggested[99] that androcymbine probably is biosynthesised by phenol oxidation from the phenethylisoquinoline base (102; R = Me, R' = H or R = H, R' = Me).

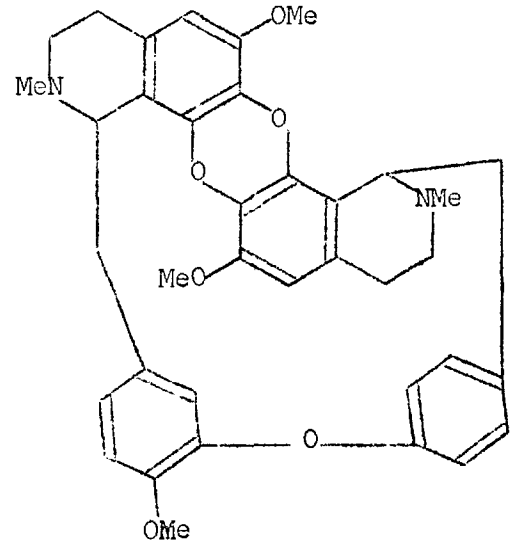
## Bases containing three diphenyl ether linkages




(103)

trilobine[101](103;R=H)

isotrilobine[102](103;R=Me)



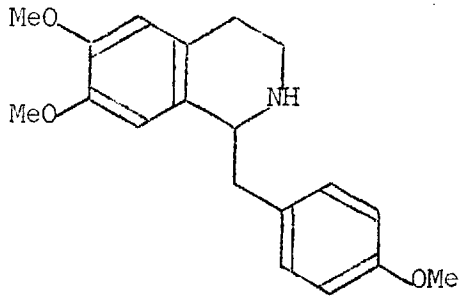

(104)

OON-trimethylmicranthine[103]

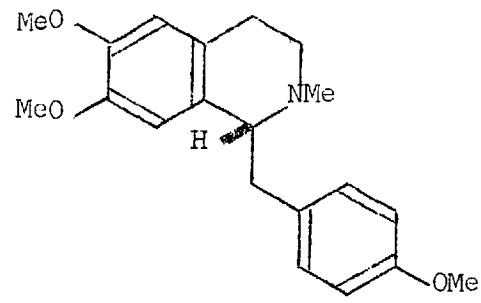
As has already been pointed out[66] one can still use oxidative phenol coupling followed by subsequent secondary changes to account for the biosynthesis of alkaloids such as trilobine (103; R = H) and micranthine (104). A possible mechanism, analogous to the proposed[78] mechanism for the formation of the so-called "berberine bridge" by the oxidative cyclisation of an N-methyl group, to account for the third ethereal linkage in insularine (7) was given previously (cf. p. 48) under reaction scheme (v).

We now wish to report and discuss appropriate tracer studies on epistephanine[19] (2; R = Me), a bisbenzylisoquinoline alkaloid belonging to the oxyacanthine series (89).

(±)-[2-$^{14}$C]Tyrosine was incorporated (0.17%) into epistephanine in Stephania japonica Miers. Cleavage[19] of the alkaloid with sodium in liquid ammonia[52] gave, after methylation with diazomethane of the product mixture, (±)-OO-dimethylcoclaurine[104] (105) and (-)-NOO-trimethylcoclaurine[19] (106). The latter compound (106) has the




(105)

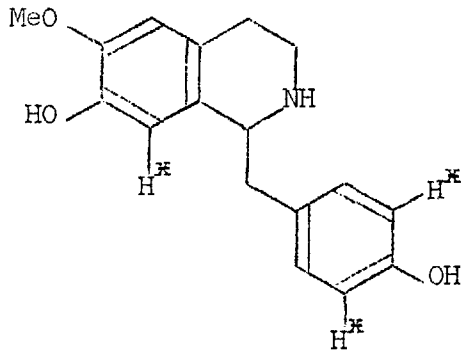



(106)

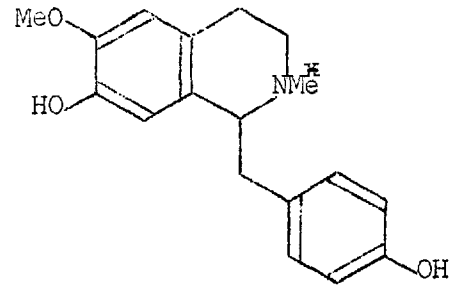
indicated absolute stereochemistry.[21,22,23,24] These compounds (105) and (106) contained, respectively, 50 and 51% of the total activity. Biogenetically,[105,106,107] norcoclaurine (70) is probably formed from dopamine (71)

and the p-hydroxyphenacetaldehyde (72) or their biological equivalents. Although the two tetrahydrobenzylisoquinoline halves of the molecule contained equal amounts of radioactivity (the actual positions of the labelled carbon atoms were not determined), a later result will show the two halves actually involved in the oxidative coupling steps to be different. Recently[50] Dr. I.R.C. Bick has kindly informed us of his unpublished work on the biosynthesis of berbamine (88; $R_1$ = Me, $R_2$ = H) in Atherosperma moschatum. The feeding of (±)-[2-$^{14}$C]-tyrosine gave radioactive berbamine (0.1% incorporation) containing approximately equal amounts of radioactivity in the two halves of the molecule.

In the following season (1965) incorporation of (±)-[2-$^{14}$C]tyrosine (0.084%), (±)-[8,3',5'-$^{3}H_3$]coclaurine[24] (107) (0.008%), and (±)-[N-methyl-$^{14}$C]N-methylcoclaurine[108]




(107)



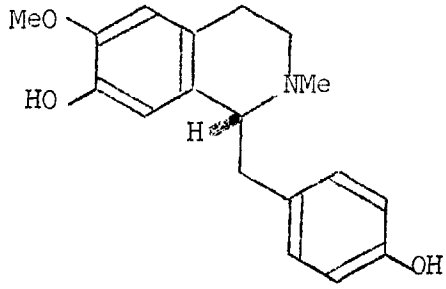

(108)

## Epistephanine

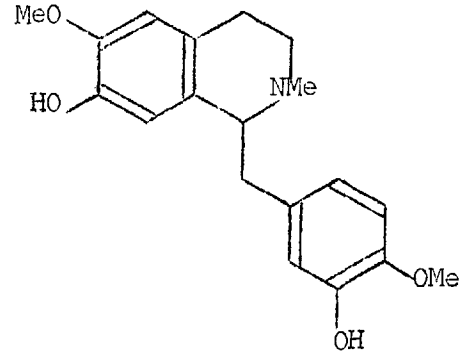
| Precursor | mc | year | incorp. (%) |
|---|---|---|---|
| (±)-[2-$^{14}$C]tyrosine | 0.02 | 1964 | 0.17 |
| (±)-[2-$^{14}$C]tyrosine | 0.02 | 1965 | 0.084 |
| (±)-[8,3',5'-$^{3}H_3$]-coclaurine (107) | 0.0123 | 1965 | 0.008 |
| (±)-[N-methyl-$^{14}$C]-N-methylcoclaurine (108) | 0.0188 | 1965 | 0.05 |
| (+)-[8,3',5'-$^{3}H_3$]-N-methylcoclaurine | 0.0872 | 1965 | 0.003 |
| (-)-[8,3',5'-$^{3}H_3$]N-methylcoclaurine (109) | 0.066 | 1965 | 0.06 |

(108) (0.05%) was observed. These and later incorporations have been corrected for loss of tritium from those positions involved in the oxidative coupling. Herzig-Meyer demethylation[109] of epistephanine, derived from the N-methyl labelled precursor, located 98% of the activity in the N-methyl group.

Both enantiomers[110] of [8,3',5'-$^{3}H_3$]N-methylcoclaurine were fed, in parallel, to Stephania japonica. The (-)-enantiomer[24] (109) (which recently was isolated[111]

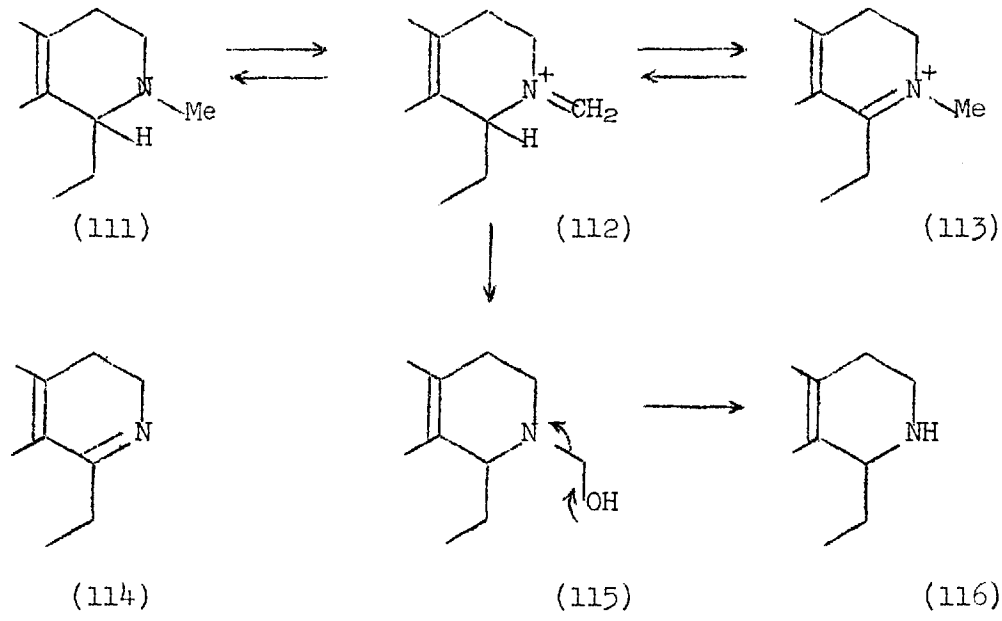



(109)




(110) = (59)

from Phylica rogersii ) was incorporated (0.06%) into epistephanine (2; R = Me) much more efficiently than its antipode (0.003%). This confirms[21,23,24] the R-absolute configuration (2; R = Me) of the alkaloid and shows that racemisation[112,113] of the precursor, presumably by oxidation-reduction [(111) $\rightleftharpoons$ (112)] via the dehydro derivative (113), is unimportant in this plant. Treatment of generally tritiated (±)-reticuline (110) with sodium in liquid ammonia under those conditions which will effect reductive cleavage of epistephanine, caused no loss of radioactivity in the [$^{3}$H]reticuline. Therefore, degradation of epistephanine




(111) (112) (113)

(114) (115) (116)

derived from (-)-N-methylcoclaurine gave (-)-NOO-trimethylcoclaurine (106) containing 95% of the total activity. The other fragment, (±)-OO-dimethylcoclaurine (105), was inactive. Clearly (-)-N-methylcoclaurine provides only half the epistephanine molecule and is not demethylated in the plant to give coclaurine, in the sense (111) ⟶ (112) ⟶ (115) ⟶ (116), or any other metabolically active derivative, for example the imine (114). Furthermore, stebisimine (48) which was isolated in this feeding experiment was practically inactive. The imine half in epistephanine probably is derived from either (+)- or

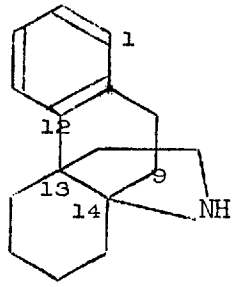
(-)-coclaurine, either which could be directly involved in the oxidative coupling step with (-)-N-methylcoclaurine (109), followed by dehydrogenation, or perhaps more likely the imine (as 114) is formed first and then undergoes coupling to give epistephanine. Further work is necessary to determine the precise origin of the imine half in epistephanine.

Hasubanonine (5; R = Me)

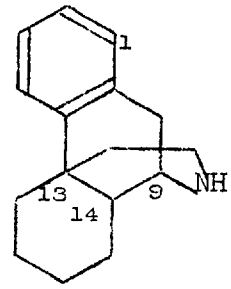
The chemistry both of hasubanonine (5; R = Me) and metaphanine (4) has been discussed previously. Closely related chemically are the phenolic base homostephanoline (5; R = H) and the enol ether prometaphanine [(6) $\rightleftarrows$ (44)].

Barton's original proposals,[75] concerning the possible biosyntheses of hasubanonine and metaphanine, were based on the constitutions[5,28,29,114] (117) and (118)[36] respectively for hasubanonine and metaphanine, both of which, since then, have been shown to be differently oxygenated hasubanan (20) derivatives. Inspection of the hasubanan skeleton (20) suggests either a $C_9$ - N bond migration in morphinan (21) or an initial coupling of phenolate radicals, using an appropriately oxygenated benzyltetrahydroisoquinoline precursor, in such a way as to provide a reasonable driving force for carbon-nitrogen bond migration. Tracer studies designed to test these ideas were carried out on hasubanonine (5; R = Me).

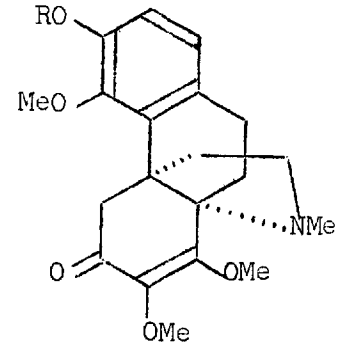
(±)-[2-$^{14}$C]Tyrosine was incorporated (0.64%) into hasubanonine in Stephania japonica Miers. In the following season (1965) a greater incorporation (1.7%) was obtained. Subsequently possible degradation procedures were explored to determine the positions of the labelled



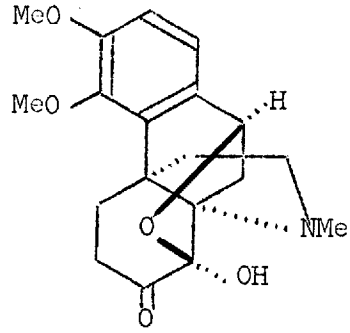

(20)



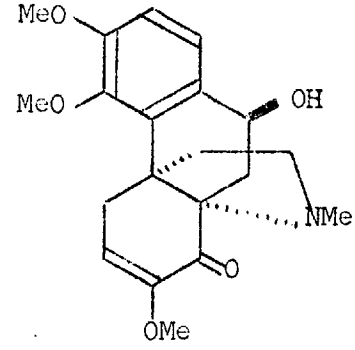

(21)



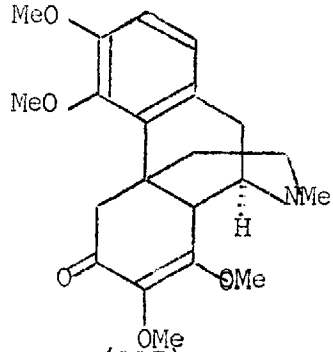

(5)



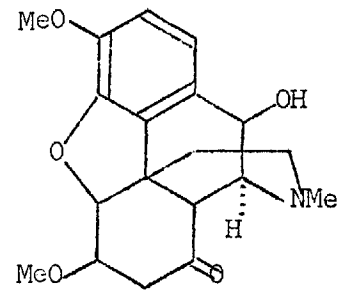

(4)




(6) ⇆ (44)

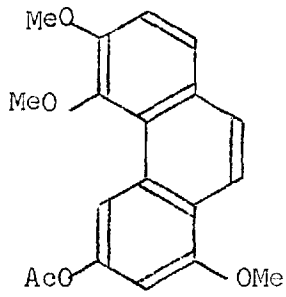


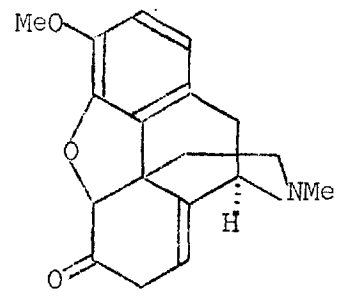

(117)

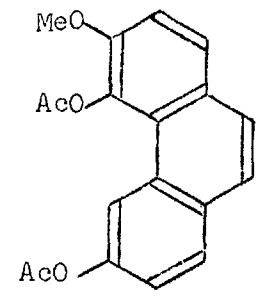



(118)

carbon atoms. Acetolysis of hasubanonine directly gave a complex reaction mixture, from which no O-acetylhasubanol[5,29] (119) could be isolated. On the contrary, acetolysis of neopinone (120) gave the nicely crystalline 3,5-diacetoxy-

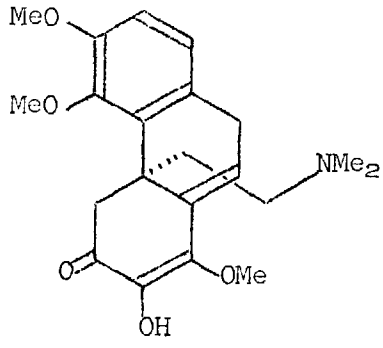


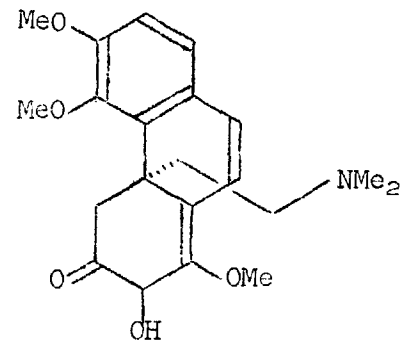




(119) = (23;R=Ac) (120) (121)

6-methoxyphenanthrene[115] (121). However, acetolysis of hasubanonine methine[5,28,114], tentatively formulated either as (122) or (123), gave crystalline O-acetylhasubanol[5,29] (119). Radioactive hasubanonine (1.7% incorporation) in this way gave radioactive O-acetylhasubanol (119) which contained 41% of the total activity. Although the position of the label, probably position 9, has to

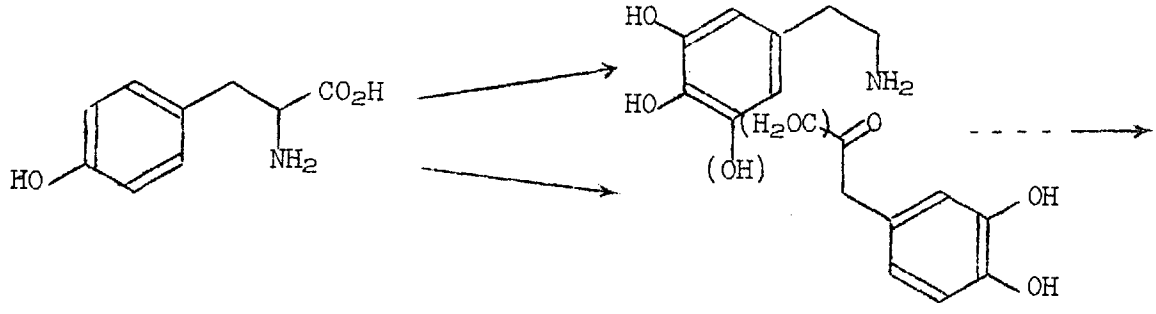


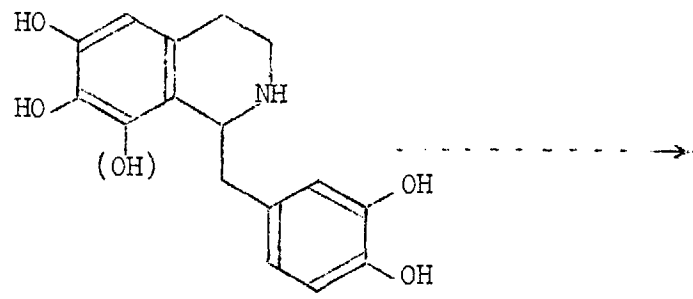

(122)




(123)

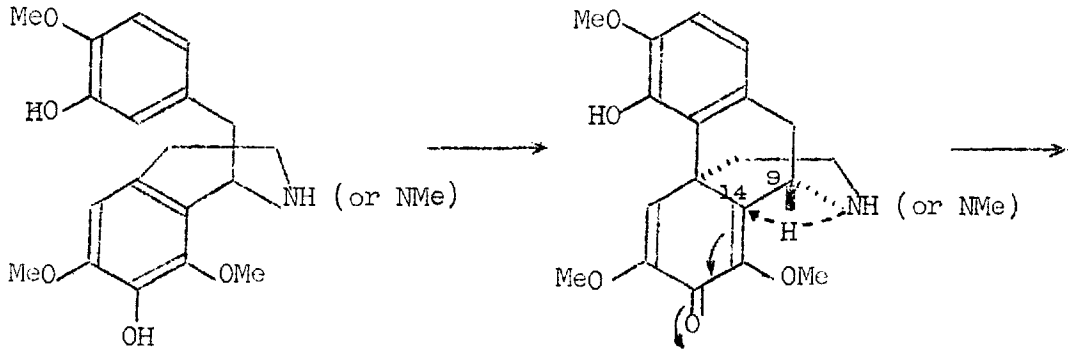
be determined by degradation and the ethanamine bridge residue, probably[5] NN-dimethylaminoethyl acetate, has to be isolated, it is anticipated that, like[116,117] morphine, two aromatic $C_6 - C_2$ units are used, through the involvement of a 1-benzyltetrahydroisoquinoline precursor, to build the hasubanan group of bases, e.g.:



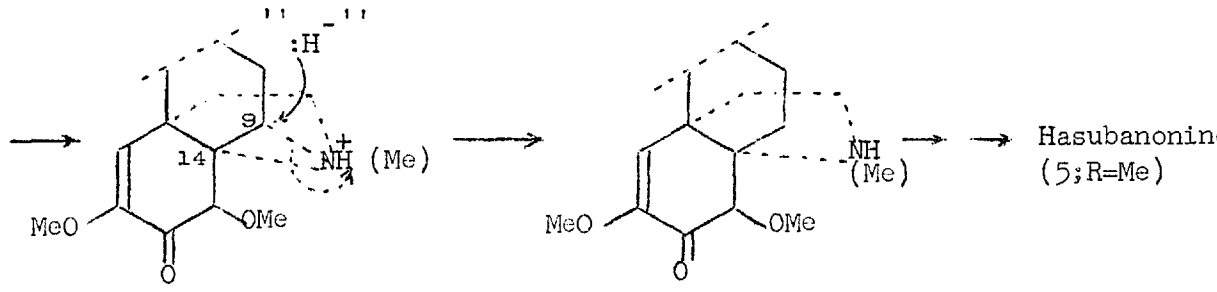



Hasubanonine(5;R=Me)

Further research was aimed at determining some of the 1-benzyltetrahydroisoquinoline intermediates[80] which possibly precede, at a later stage on the metabolic pathway, the formation of hasubanonine (5; R = Me). Oxidative phenol coupling either of the diphenolic base (54; NH) or (54; NMe), which previously was discussed in connection with the biosynthesis of protostephanine, could give the isomeric dienone (124). β-Addition of



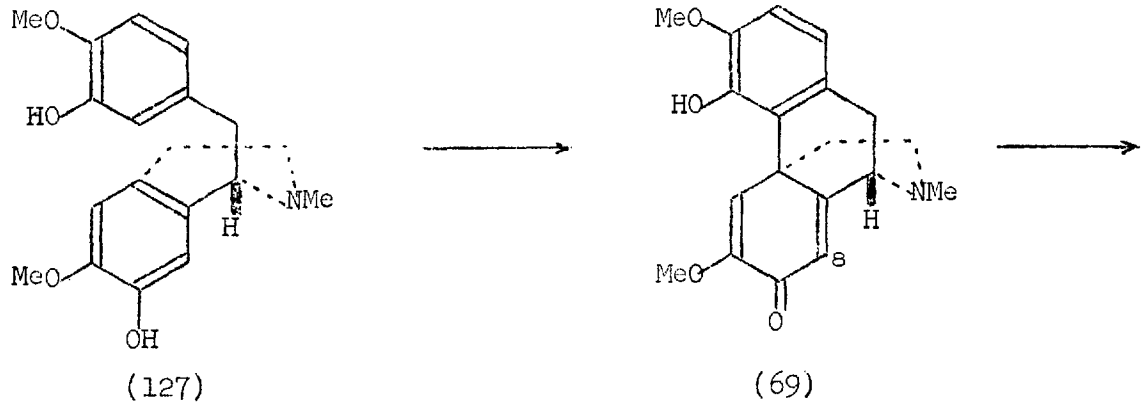

(54) (124)



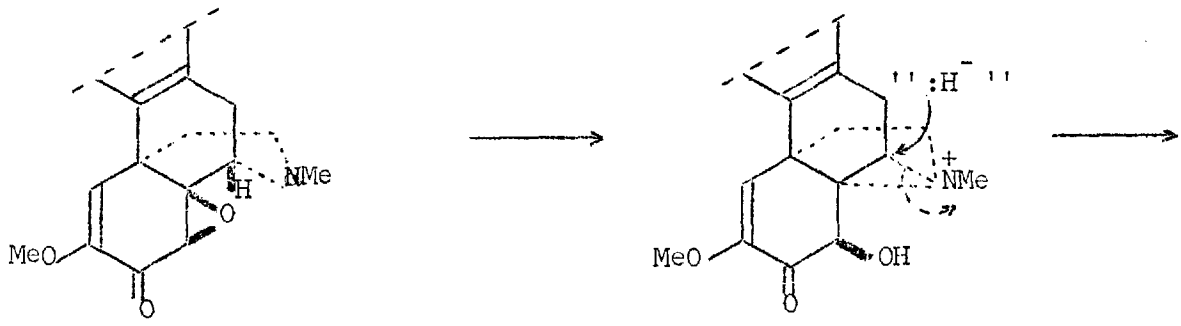

(125) (126)

the imino nitrogen [in the sense (124) ⟶ (125)], however unlikely, could give an aziridinium ion (125) which, by direct or indirect reduction ["biological $H^-$ equivalent" attack at C-9], hydrolysis of the enol ether (126), and subsequent methylation of the diosphenol, could give hasubanonine (5; R = Me). Rather expectedly, neither the diphenolic base (54; NH) nor (54; NMe) were incorporated into hasubanonine.

Alternatively, oxidative phenol coupling of (+)-reticuline[118] (127), of opposite absolute configuration to the morphine alkaloids, would give (-)-sinoacutine[84] (69). Selective epoxidation of (-)-sinoacutine (69), followed by ring opening and subsequent reduction of the aziridinium ion (129), in the sense (69) ⟶ (128) ⟶ (129), eventually could give hasubanonine (5; R = Me).

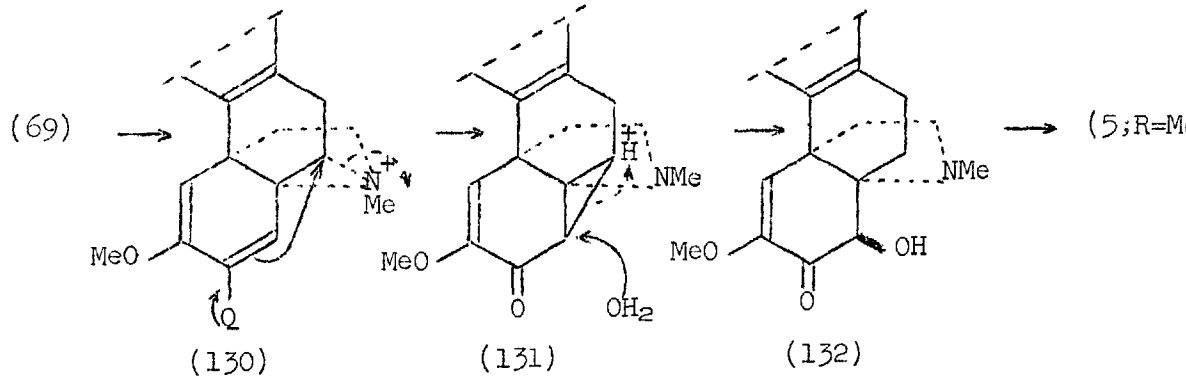



(127) (69)




(128) (129)

- - - - - - - - → Hasubanonine(5;R=Me)

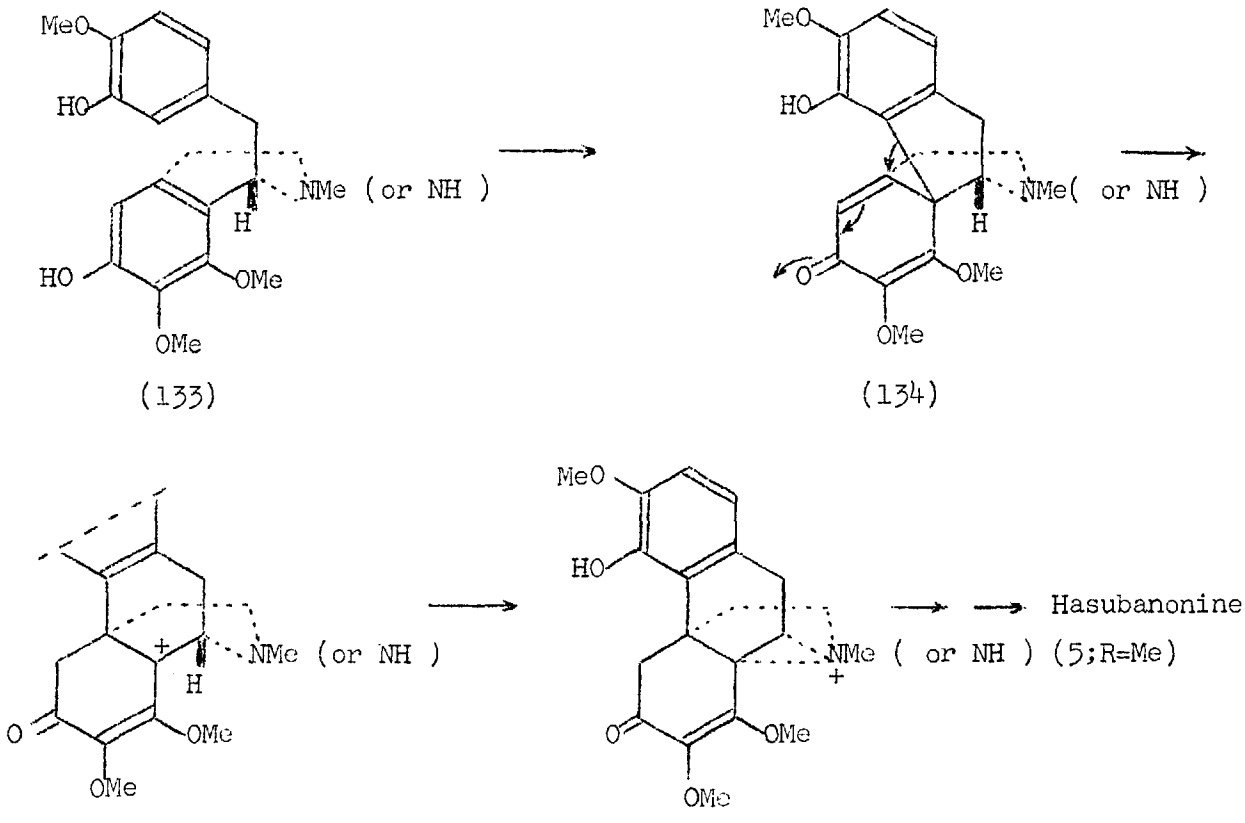



(130) (131) (132)

An unlikely alternative mechanism for the introduction of the additional hydroxyl group at C-8 in sinoacutine (69), with subsequent carbon-nitrogen bond migration, is shown in the sequence (69) ⟶ (130) ⟶ (131) ⟶ (132). Although neither (±)-[2',6',8-$^3H_3$]reticuline (59) nor (-)-[1-$^3H$]sinoacutine (69) were incorporated into hasubanonine (5; R = Me), there were two additional, chromatographically identical and structurally very similar, components [see experimental section] present in the hasubanonine fraction either of which could be derived from both (±)-reticuline and sinoacutine. Only after rigorous purification and dilution was the hasubanonine, isolated from the work-up of these two individual feeding experiments, obtained radio-inactive. The radiochemical impurities in the hasubanonine fraction were only slowly and incompletely oxidized by alkaline potassium ferricyanide. Also, a mixture of two very minor components, with molecular ion peaks at m/e 371 and 373 respectively, was consistently isolated from inactive plant material. In both the abovementioned feeding experiments this mixture was found to be highly radioactive. It is important to identify these minor constituents in Stephania japonica.

Alternatively, oxidative phenol coupling of the

diphenolic base (133), isomeric with (54), could give the dienone (134). Rearrangement of this dienone, in

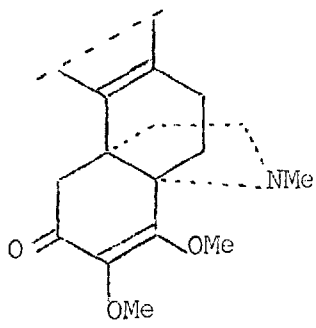



the sense (134) ⟶ (135), would give the carbonium ion (135) and subsequently the aziridinium ion (136) which, on reduction and methylation, could give hasubanonine (5; R = Me). The synthesis of the possible precursor (133) is currently in progress.

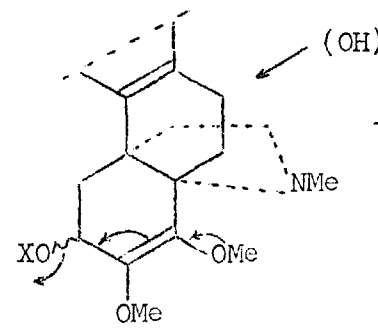
## Hasubanonine (5; R = Me)

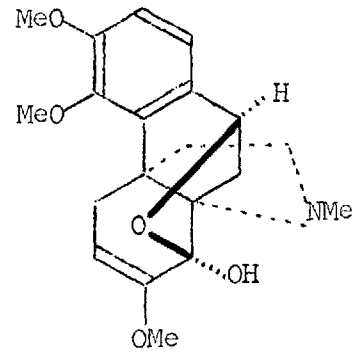
| Precursor | year | mc | incorp. (%) |
|---|---|---|---|
| (±)-[2-$^{14}$C]tyrosine | 1964 | 0.02 | 0.64 |
| | 1965 | 0.02 | 1.70 |
| (±)-[2',6'-$^{3}H_2$]-1-(3'-hydroxy-4'-methoxybenzyl)-6,8-dimethoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline (54; NH) | 1965 | 0.183 | 0.0 |
| (±)-[N-methyl-$^{14}$C]-1-(3'-hydroxy-4'-methoxybenzyl)-2-methyl-6,8-dimethoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline (54; NMe) | 1965 | 0.0157 | 0.0 |
| (±)-[2',6',8-$^{3}H_3$]reticuline (59) | 1965 | 0.147 | 0.0 |
| (-)-[1-$^{3}$H]sinoacutine (69) | 1965 | 0.045 | 0.0 |

It is anticipated that hasubanonine (5; R=Me) is formed first in the plant. Reduction, demethylation with allylic elimination, and hydroxylation at C-10 would give prometaphanine [(6) $\rightleftharpoons$ (44)], which is closely related to metaphanine (4).




(5)




(6) ⇄ (44)

Metaphanine(4)

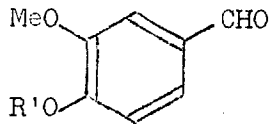
## EXPERIMENTAL

All melting points were obtained using a micro Kofler hotstage, and all are reported uncorrected. Micro-analyses, unless otherwise stated, were performed at Imperial College initially under the direction of Miss J. Cuckney, and thereafter of Mr. K. I. Jones. Organic extracts containing neutral or acidic substances were dried over sodium sulphate. Those containing basic substances were dried over potassium carbonate. Nuclear magnetic resonance spectra were run on Varian A-60 and Varian HA-100 spectrometers in deuterochloroform. The multiplicities of lines in n.m.r. spectra are designated by the following abbreviations: s. (singlet), d. (doublet), and m. (multiplet). Mass spectra were obtained on an A.E.I. M S.9 mass spectrometer with direct probe insertion. Ultraviolet spectra refer to absolute ethanol solutions, unless otherwise stated. Chromatography, unless specified to the contrary, refers to neutral alumina of Brockmann activity III. Distilled and sodium dried benzene was employed throughout chromatographical purifications and separations. Light petroleum ether refers to the b.p. 40-60$^{o}$ fraction.

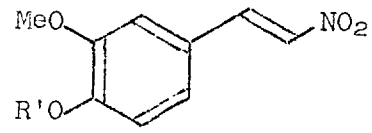
## Synthesis of precursors and degradation products

Reductive cleavage both of epistephanine (2; R = Me) and stebisimine (48) with sodium in liquid ammonia gave simple coclaurine derivatives. Owing to the limited quantities of alkaloids available it was necessary to synthesize these bisected benzylisoquinolines. This proved valuable in so far as thin layer chromatographical comparisons and spectroscopical correlations were concerned.

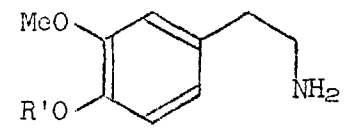
In the labelled penta-oxygenated benzylisoquinoline derivatives (54; NH) and (54; NMe), both carbon-14 or tritium were introduced at the end of the synthesis.



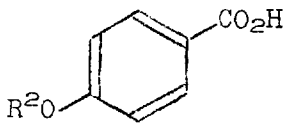

(137a)R'=Me
(137b)R'=$CH_2Ph$



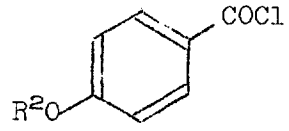

(138a)R'=Me
(138b)R'=$CH_2Ph$



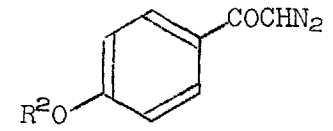

(139a)R'=Me
(139b)R'=$CH_2Ph$




(140a)$R^2$=$CH_2Ph$
(140b)$R^2$=Me



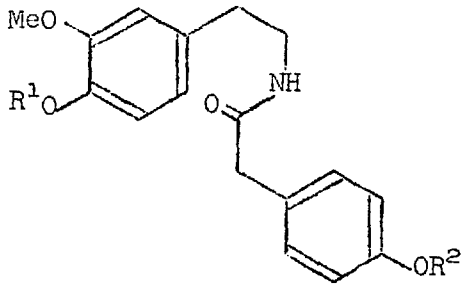

(141a)$R^2$=$CH_2Ph$
(141b)$R^2$=Me




(142a)$R^2$=$CH_2Ph$
(142b)$R^2$=Me

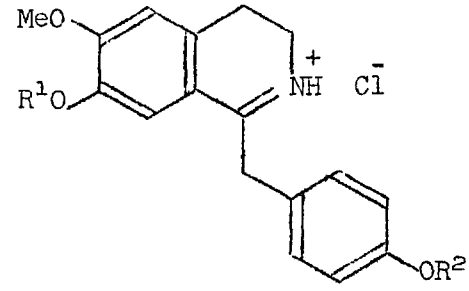
The benzylisoquinolines were synthesized by well-established Bischler-Napieralski methods.[119] The phenethylamines (139a) and (139b) were derived from suitably OO-dialkyl protocatechuic aldehyde derivatives (137a) and (137b). The diazoketones (142a) and (142b) were obtained from the corresponding p-methoxy or p-benzyloxy substituted benzoic acids (140a) and (140b). Photochemical rearrangement[120] of these diazoketones in the presence of the required phenethylamines gave the corresponding amides (143). In the syntheses of these simple coclaurine derivatives, the cyclodehydration of the amides (143) with phosphorus oxychloride in refluxing toluene proceeded smoothly and in excellent yields. A method applicable to all known instances requires a large excess of freshly distilled phosphorus oxychloride, whilst dry nitrogen gas is bubbled through the refluxing reaction mixture. In one particular case (vide infra) a reaction time of five minutes sufficed, which was evident from the disappearance of the amide N-H stretching vibration in the infrared spectrum.

In all cases the imines were generated from the corresponding 3,4-dihydroisoquinoline hydrochlorides (144a) and (144b) and subsequently reduced with sodium boro-hydride in methanol containing a trace of potassium



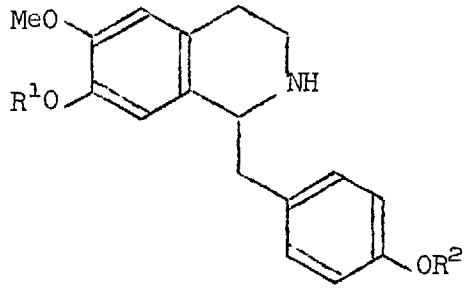
(143a)$R^1$=Me,$R^2$=$CH_2Ph$

(143b)$R^1$=$CH_2Ph$,$R^2$=Me



(144a)$R^1$=Me,$R^2$=$CH_2Ph$

(144b)$R^1$=$CH_2Ph$,$R^2$=Me

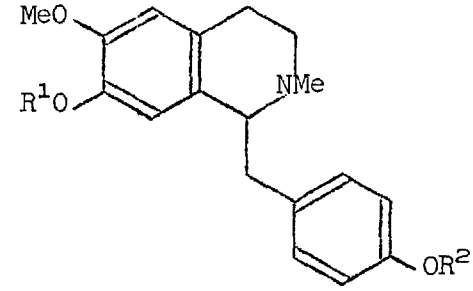


(145a)$R^1$=Me,$R^2$=$CH_2Ph$

(145b)$R^1$=$CH_2Ph$,$R^2$=Me

(145c)$R^1$=Me,$R^2$=H

(145d)$R^1$=H,$R^2$=Me



(146a)$R^1$=Me,$R^2$=$CH_2Ph$

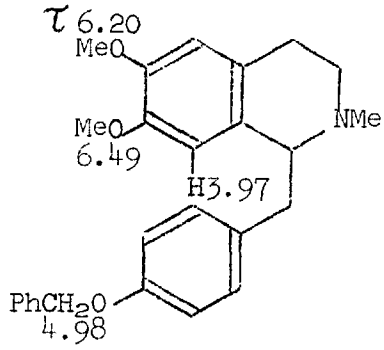
(146b)$R^1$=$CH_2Ph$,$R^2$=Me

(146c)$R^1$=Me,$R^2$=H

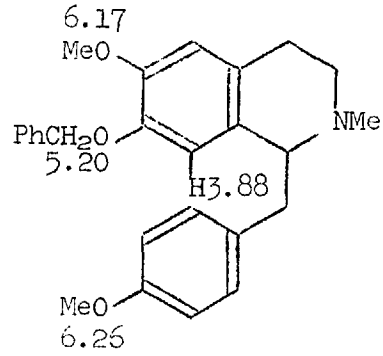
(146d)$R^1$=H,$R^2$=Me

hydroxide. The N-methyl group was introduced at this stage by reaction of the secondary amine with formaldehyde and formic acid.[121,122] This reaction proceeded rapidly and in practically quantitative yield.

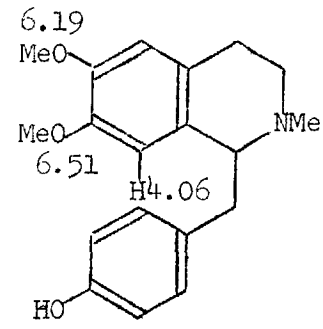
The final step required to complete the syntheses of these coclaurine derivatives was the removal of the protecting benzyl groups. It was found that, by using a moderate excess of palladium on charcoal, hydrogenolysis proceeded reliably and efficiently.



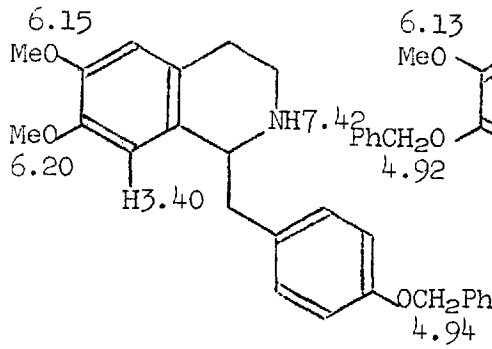

(146a)



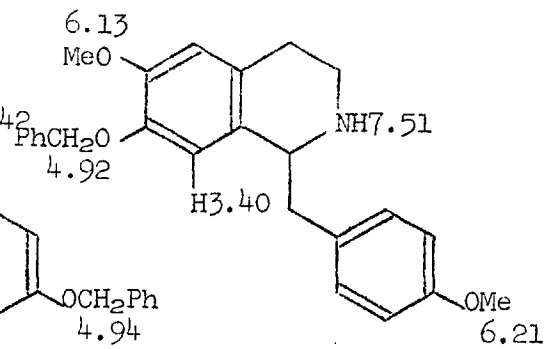

(146b)




(146c)




(145a)



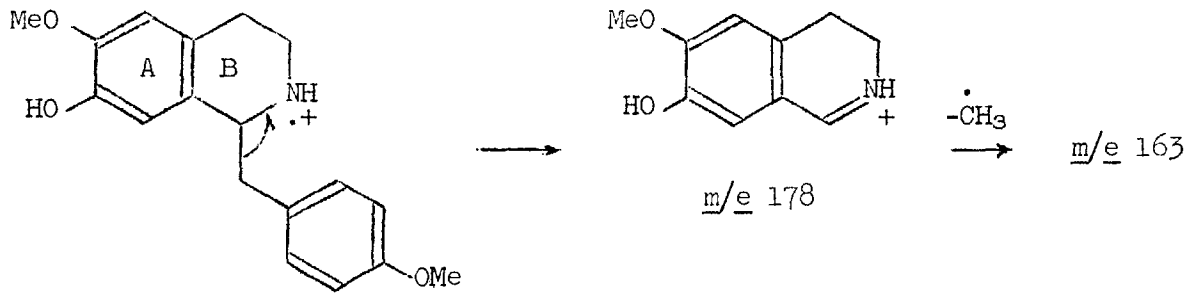

(145b)

Examination of the n.m.r. spectra of a series of benzylisoquinolines proved instructive. In the series of tertiary amines the C-7 methoxyl or benzyloxyl and the C-8 proton resonances are shifted upfield as a result of the shielding induced by the C-1 benzylic aromatic ring.[50,54,78,123,12] Observation of this shielding effect is a valuable means for the identification of an alkoxyl group at C-7 in N-methyltetrahydroisoquinoline bases. In the secondary amines (145a) and (145b) the resonances of the imino hydrogens are shifted upfield. Therefore the molecules exist in such a conformation in which the benzyl ring lies close to the nitrogen atom.

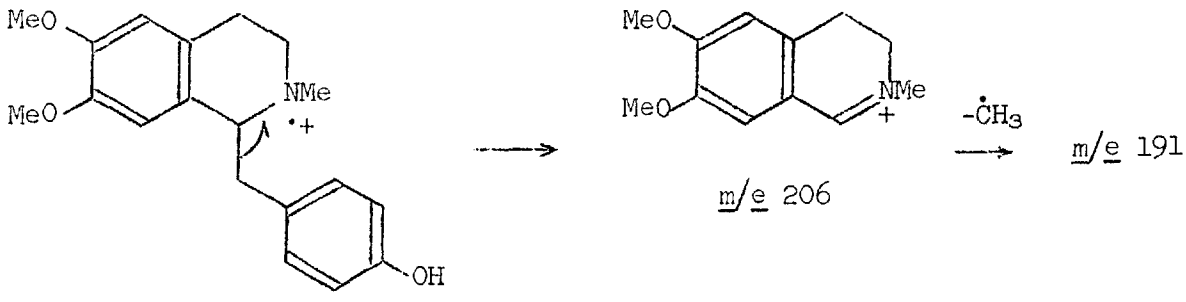
The difference between the position and the appearance of the hydroxyl absorption frequency in the infrared, clearly distinguishes a 7-hydroxy-1-benzyl from a 4'-hydroxybenzyl substituted 1,2,3,4-tetrahydroisoquinoline.

Finally, the benzyltetrahydroisoquinolines have very characteristic mass spectra.[124] In the mass spectrum of compound (145d) the molecular ion (m/e 299) is only of very low abundance. The origin of the $(M^+ - 2)$ and $(M^+ - 3)$ peaks is not clear.[125] The base peak occurs at m/e 178, due to the loss of the C-1 benzyl substituent. In the mass spectrum of armepavine (146c) and (47; R = R' = Me, R'' = H) the base peak shifts to m/e 206.

Mass spectrometry thus serves ideally to distinguish between differently substituted ring A or C-2 tetrahydroisoquinolines. Although the crystalline 3,4-dihydroisoquinoline derivative (148) was claimed[53] to be a stable

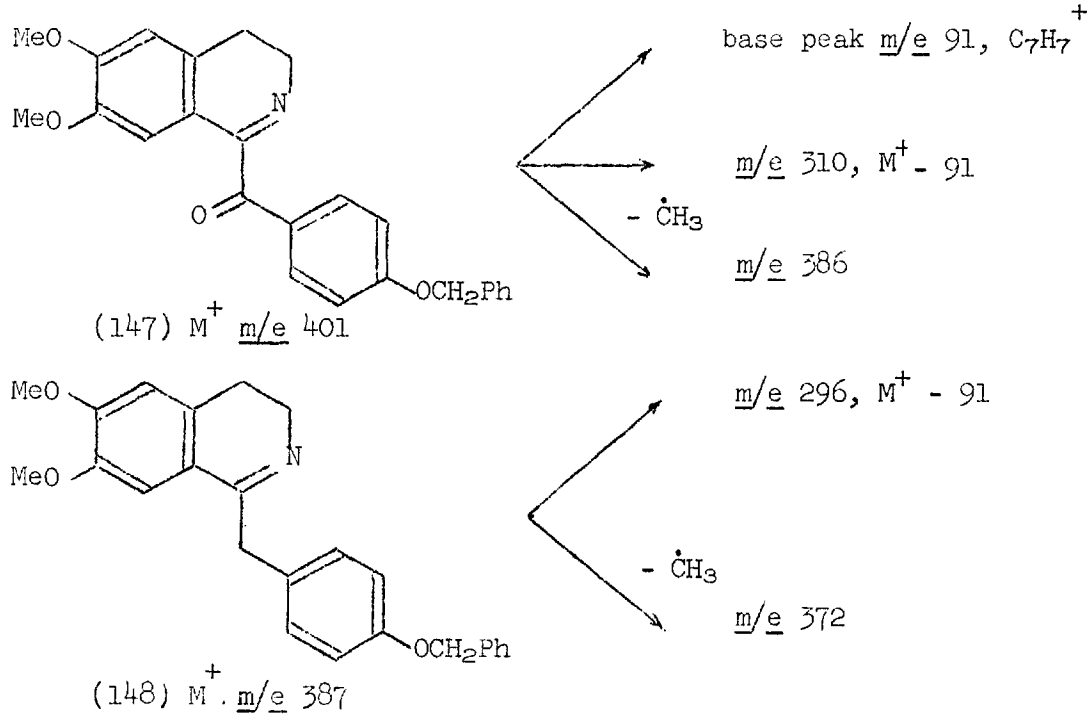



(145d) $M^+$, m/e 299



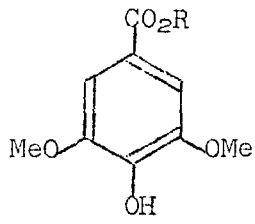

(146c) $M^+$, m/e 313

compound, its mass spectrum shows it to be admixed with the ketone (147).

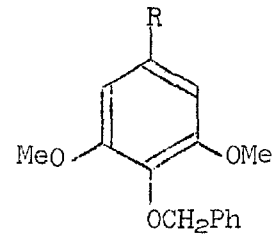



The penta-oxygenated 1-benzyltetrahydroisoquinoline precursors[75] (154c) and (154d) were synthesized by standard methods. The benzyl chloride/potassium hydroxide benzylation[126] of syringic acid (149a) gave variable results. A better yield was obtained when methyl syringate (149b) reacted with sodium hydride in dimethylformamide and benzyl chloride. The O-benzyl methyl ester (150a) was smoothly reduced by lithium aluminium hydride and the resulting benzylic alcohol (150b) oxidized with active manganese dioxide to give nicely crystalline O-benzyl

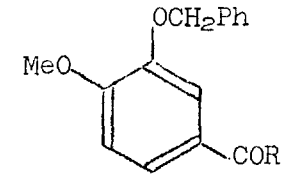
syringaldehyde (150c). This was converted into the nitrostyrene (150d), followed by lithium aluminium hydride reduction to give the required amine (150e).



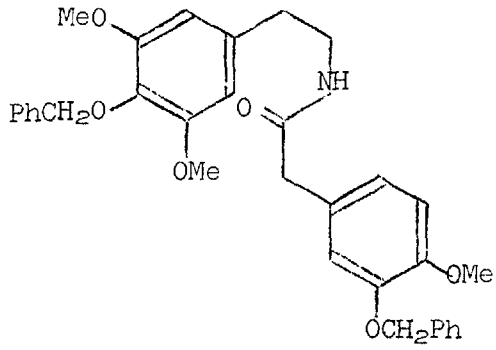

(149a) R=H
(149b) R=Me



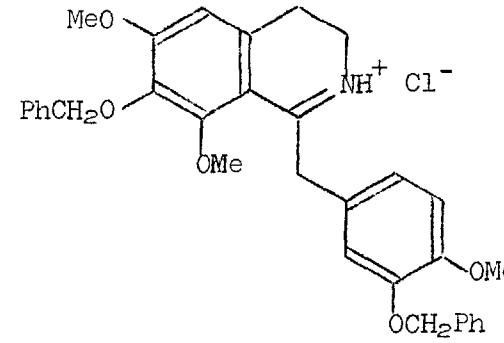

(150a) R=$CO_2Me$
(150b) R=$CH_2OH$
(150c) R=CHO
(150d) R=$CH{:}CH{\cdot}NO_2$
(150e) R=$CH_2CH_2NH_2$



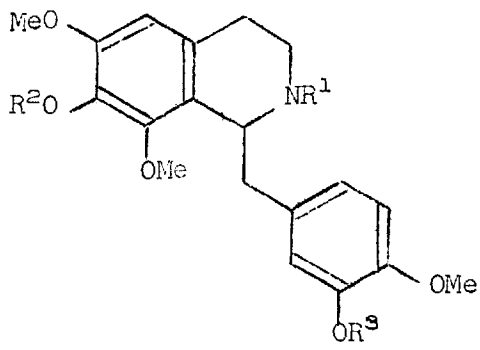

(151a) R=H
(151b) R=OH
(151c) R=Cl
(151d) R=$CHN_2$




(152)



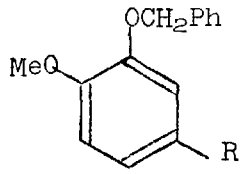

(153)




(154a) $R^1$=H, $R^2$=$R^3$=$CH_2Ph$
(154b) $R^1$=Me, $R^2$=$R^3$=$CH_2Ph$
(154c) $R^1$=$R^2$=$R^3$=H
(154d) $R^1$=Me, $R^2$=$R^3$=H

Initially, serious difficulties were encountered in the cyclodehydration of the amide (152). However, application of the improved ring-closure procedure discussed above, gave the non-crystalline 3,4-dihydroisoquinoline hydrochloride (153) which subsequently was converted into the nicely crystalline secondary amine (154a). The [N-methyl-$^{14}$C]N-methyl group was introduced at this late stage with [$^{14}$C]paraformaldehyde and formic acid. In both cases removal of the benzyl groups was accomplished with concentrated hydrochloric acid. The diphenolic secondary amine (154c) deteriorated on keeping in air or in solution and was tritiated[35] directly. A similar experiment with deuterium oxide showed (n.m.r. control) exchange at the positions ortho and para to the 3'-phenolic hydroxyl group.

Finally, possible synthetic pathways leading to the precursors (160a) and (160b) have been explored. Photochemical rearrangement of the diazoketone (156d) in the

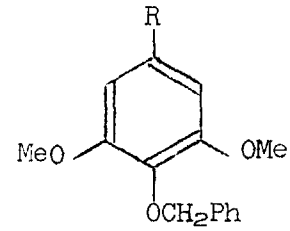


(155a) R=CHO

(155b) R=CH:CH·$NO_2$

(155c) R=$CH_2CH_2NH_2$

(155d) R=$CH_2CH_2NHCH_2Ph$

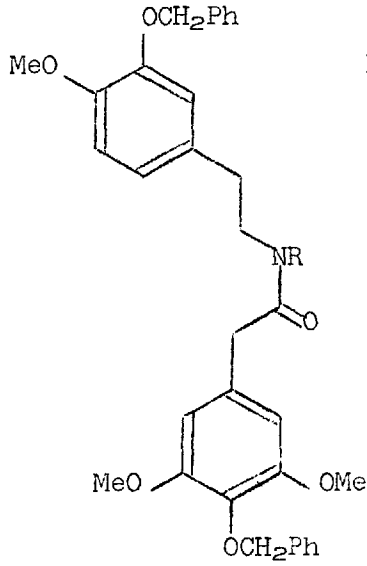


(156a) R=$CO_2Me$

(156b) R=$CO_2H$

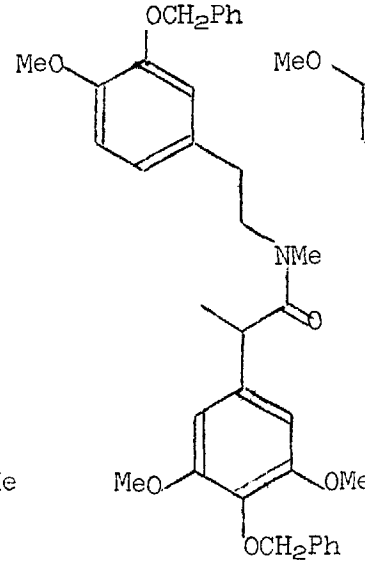
(156c) R=COCl

(156d) R=CO·$CHN_2$

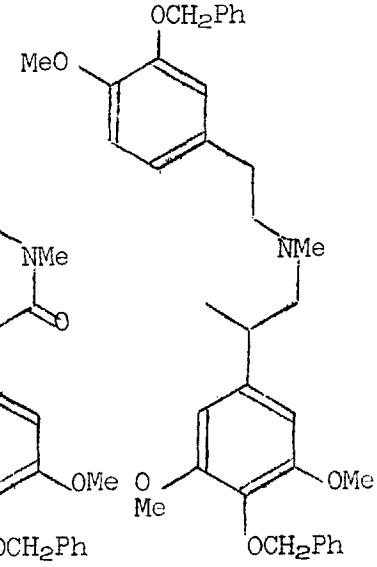


(157a) R=H

(157b) R=$CH_2Ph$



(158)



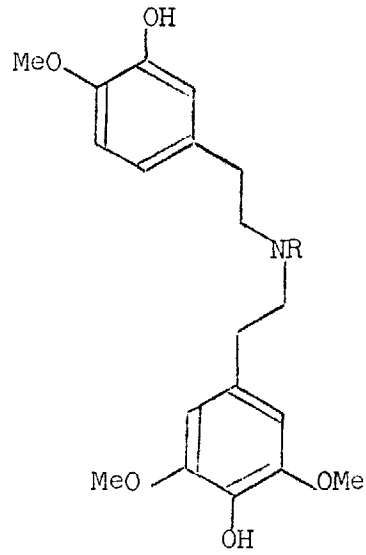
(159)

presence of the phenethylamine (155c) gave the amide (157a). When the amide (157a) reacted[127] with one equivalent of sodium hydride in dimethylformamide and excess methyl iodide at room temperature for 24 hrs., most of the starting material was recovered unchanged. Similarly, when the reaction was conducted at 80° for 5 hrs., a mixture containing some unreacted secondary amide and two additional components was obtained. However, when a large excess sodium hydride was used and the reaction carried out at 80° for one hr., one reaction product was obtained. This proved to be a tertiary amide (i.r. and n.m.r.) which subsequently was reduced with lithium aluminium hydride. The n.m.r. spectrum of the tertiary amine showed, in addition to three methoxyl, two benzyloxyl and one methylimino resonances, a doublet (J = 6.3 c/s) at $\tau$ 8.76, the integral rise of which corresponded to three protons. Although no proper degradation has been done, it seems reasonable to formulate the tertiary amide, formed under these particular reaction conditions, as structure (158) and the corresponding tertiary amine as structure (159).

Lithium aluminium hydride reduction of the secondary amide (157a) gave mixtures of varying complexity. It is

known[128] in some cases of the LAH reduction of amides that cleavage of the carbon-nitrogen bond to yield the alcohol, can become an important side reaction.

Although diborane reduces[128] primary, secondary, and, especially, tertiary amides under relatively mild conditions, to give the corresponding amines in practically quantitative yields, an alternative synthesis[129] of the secondary amine (160a) has been explored. Photochemical rearrangement of the diazoketone (156d) in the presence of the N-benzyl phenethylamine (155d) gave the tertiary amide (157b). LAH reduction, hydrogenolysis, and N-methylation, would give the phenolic amines (160a) and (160b) respectively.




(160a) R=H
(160b) R=Me

1-(3'-Hydroxy-4'-methoxybenzyl)-6,8-dimethoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline(154c), and 1-(3'-hydroxy-4'-methoxybenzyl)-2-methyl-6,8-dimethoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline(154d).

O-Benzylisovanillin (151a)

To a solution of isovanillin (10 g.) in ethanol (120 ml.), containing potassium hydroxide (4.8 g., 30% excess) was added freshly distilled benzyl chloride (10 g., 20% excess). The resulting mixture was refluxed for 5 hrs. The solution was then cooled and filtered. The filtrate was evaporated and the residue poured with stirring into cold water (100 ml.) to give O-benzylisovanillin (14.9 g., 93%), which crystallized from ether-light petroleum ether, m.p. 61-62° (lit.,[130] m.p. 63.5°).

O-Benzylisovanillic acid (151b)

A solution of potassium permanganate (6.7 g.) in water (150 ml.) was added dropwise to a stirred solution of O-benzylisovanillin (151a) (10.2 g.) in acetone (150 ml.). The stirring was continued overnight after complete addition. Sulphur dioxide was bubbled through the reaction mixture and the acetone was removed under

reduced pressure. The solid was collected and redissolved in ether (150 ml.). The ethereal solution was then extracted with 10% aqueous sodium carbonate solution (4 x 25 ml.) and the combined aqueous alkaline extract acidified (2N HCl; 30 ml.). The precipitated solid was recrystallized from alcohol to give the acid (6.6 g., 60%), m.p. 177-9° (lit.,[131] m.p. 180°).

3-Benzyloxy-4-methoxy-ω-diazoacetophenone (151d)

A suspension of O-benzylisovanillic acid (151b) (6.4 g.) in dry benzene (40 ml.) containing oxalyl chloride (5 ml.) was left at room temperature for 24 hrs. The solvent and excess reagent was then evaporated under reduced pressure. More benzene was added and evaporated. This process was repeated several times. The resulting acid chloride (6.8 g.), which solidified, was dissolved in the minimum volume of dry benzene and added dropwise with efficient stirring to a cold ethereal solution of diazomethane (from 20 g. nitrosomethylurea). The stirring was continued overnight whilst room temperature was slowly reached. The solution yielded 3-benzyloxy-4-methoxy-ω-diazoacetophenone (6.14 g., 88%), m.p. 109-111° (lit.,[131] m.p. 114-115°), $\nu_{max.}$ (in $CHCl_3$) 2140 $cm.^{-1}$

Methyl syringate (149b)

Syringic acid (149a) (20 g.) was suspended in anhydrous methanol (100 ml.). Dry hydrochloric acid gas was passed in until a clear solution was obtained. After refluxing for two hours, with continuous hydrochloric acid gas saturation, the mixture was left overnight and then evaporated to near dryness. The methanolic residue was poured into water (300 ml.) containing sodium hydrogen carbonate (4 g.), and the solid filtered off. Recrystallisation from aqueous methanol afforded methyl syringate (19.2 g., 89%), m.p. 106-107° (lit.,[132] m.p. 107-108°).

Methyl O-benzylsyringate (150a)

A solution of methyl syringate (149b) (12 g.) in anhydrous dimethylformamide (40 ml.) was added dropwise to a stirred suspension of 50% sodium hydride (3.5 g., 30% excess) in dimethyl formamide (30 ml.). The stirring was continued for an additional hour at room temperature, followed by the dropwise addition of distilled benzyl chloride (9.4 g., 30% excess). This mixture was stirred for five hours at 80°. The solvent was removed under reduced pressure and the residue treated with ice. Crystallization from methanol gave the O-benzylated ester (10.3 g., 60%), m.p. 70-71° (lit.,[126,133] m.p. 71°).

4-Benzyloxy-3,5-dimethoxybenzyl alcohol (150b)

A solution of methyl O-benzylsyringate (150a) (10.3 g.) in anhydrous tetrahydrofuran (40 ml.) was added dropwise to a stirred and refluxing suspension of lithium aluminium hydride (2 g.) in dry tetrahydrofuran (40 ml.). The stirring and refluxing were continued for eight hours whence the reaction mixture was cooled and the excess lithium aluminium hydride decomposed with water. The suspension was diluted with tetrahydrofuran and filtered through Celite. The filter cake was washed with boiling tetrahydrofuran and the combined filtrate was evaporated. The oily residue was treated with water and extracted with ether (4 x 50 ml.). The ethereal extract gave the viscous alcohol (9.25 g., 99%), $\nu_{max.}$ ($CHCl_3$) 3350-3550 (broad), 3610 $cm.^{-1}$

O-Benzylsyringaldehyde (150c)

A mixture of 4-benzyloxy-3,5-dimethoxybenzyl alcohol (150b) (32.3 g.) and active manganese dioxide (320 g.) in anhydrous benzene (400 ml.) was shaken at room temperature for one hour (i.r. control). The brown suspension was diluted with benzene and filtered. The filtrate was evaporated and the residue crystallized from alcohol to give the aldehyde (20.7 g., 64%),

m.p. 62-63°(lit.,[134] m.p.63°), $\nu_{max.}$ ($CHCl_3$) 1693, 2740 cm.$^{-1}$

4-Benzyloxy-3,5-dimethoxy-ω-nitrostyrene (150d)

A mixture of O-benzylsyringaldehyde (150c) (3.6 g.), freshly distilled nitromethane (0.9 g.), methylamine hydrochloride (0.15 g.), and anhydrous sodium carbonate (0.12 g.) was heated at 40° (bath temperature) for fifteen hours. The yellowish reaction mixture was diluted with water and filtered. The product was washed thoroughly with water and ether to give the nitrostyrene (3 g., 72%), m.p. 130-132° (lit.,[134] m.p. 133°).

4-Benzyloxy-3,5-dimethoxyphenethylamine (150e)

A solution of 4-benzyloxy-3,5-dimethoxy-ω-nitrostyrene (150d) (3 g.) in dry tetrahydrofuran (40 ml.) was added dropwise to a well-stirred, refluxing suspension of lithium aluminium hydride (3 g.) in dry tetrahydrofuran (40 ml.). After seven hours at reflux temperature, the mixture was treated as usual to give the oily amine (2.6 g., 98%) which was purified as the oxalate (EtOH), m.p. 195-197°.

N-(4-Benzyloxy-3,5-dimethoxyphenethyl)-3-benzyloxy-4-methoxyphenylacetamide (152)

A solution of 4-benzyloxy-3,5-dimethoxyphenethylamine (150e) (0.48 g.) and 3-benzyloxy-4-methoxy-ω-diazoacetophenone (151d) (0.5 g.) in anhydrous benzene (60 ml.) was irradiated under nitrogen using a high pressure mercury lamp. After two hours the reaction was terminated (i.r. control). The benzene solution successively was washed with 5% sodium hydroxide solution (3 x 15 ml.), 2N-hydrochloric acid solution (2 x 15 ml.), and water (15 ml.). The dried benzene solution was evaporated and the product (0.69 g., 75%) was crystallized from benzene/n-hexane, m.p. 124-126°, $\nu_{max.}$ 3440, 1663 cm.$^{-1}$, $\tau$ 3.17 (3H, d., J = 2.5 c/s), 3.64 (2H, s.), 4.47 (1H, broad, CO.NH), 4.83 (2H, s., $OCH_2Ph$), 4.97 (2H, s., $OCH_2Ph$), 6.10 (3H, s., $OCH_3$), and 6.19 (6H, s., 2 x $OCH_3$). (Found: C, 72.85; H, 6.18; N, 2.23. $C_{33}H_{35}NO_6$ requires C, 73.17; H, 6.51; N, 2.59%).

1-(3'-Benzyloxy-4'-methoxybenzyl)-7-benzyloxy-6,8-dimethoxy-3,4-dihydroisoquinoline hydrochloride (153)

To a solution of the amide (152) (0.2 g.) in dry, refluxing toluene (5 ml.) was added freshly-distilled phosphorus oxychloride (2 ml.) whilst dry nitrogen gas

was bubbled through the reaction mixture. After fifteen minutes (i.r. control) the solvent and excess reagent were evaporated under reduced pressure. The brownish residue was redissolved in toluene and evaporated under reduced pressure. This process was repeated three times, after which time the residue was triturated with dry petroleum ether to give an amorphous yellowish solid, which could not be induced to crystallize. It was, however, successfully converted into the requisite tetrahydroisoquinoline derivative (154a), vide infra.

1-(3'-Benzyloxy-4'-methoxybenzyl)-7-benzyloxy-6,8-dimethoxy-1,2,3,4-tetrahydroisoquinoline (154a)

The amorphous iminohydrochloride (153) (0.23 g.) was dissolved in chloroform (25 ml.) and the chloroform solution, flushed with nitrogen, was washed with saturated aqueous sodium hydrogen carbonate solution (2 x 10 ml.). The oily residue from the evaporated chloroform solution was dissolved in methanol (8 ml.) and 1N-methanolic potassium hydroxide solution (0.2 ml.). The solution was cooled in an ice-bath. Sodium borohydride (0.15 g.) was added in small portions and the stirring, under nitrogen, was continued for an additional two hours, after complete addition. The solvent was evaporated under

reduced pressure. The residue was treated with 4N-sodium hydroxide solution (10 ml.) and exhausted with chloroform (4 x 20 ml.). The combined chloroform extract was washed with water (1 x 20 ml.), dried, and evaporated. The resulting oily amine (0.22 g.) was chromatographed and eluted with benzene/chloroform (9:1; v/v). The purified amine (0.17 g., 88%) crystallized from ether/light petroleum ether, m.p. 94°, $\tau$ 3.18 (3H, slightly broad s.), 3.62 (1H, s.), 4.85 (2H, s., $OCH_2Ph$), 5.00 (2H, s., $OCH_2Ph$), 6.09 (3H, s., $OCH_3$), 6.14 (3H, s., $OCH_3$), and 6.22 (3H, s., $OCH_3$). (Found: C, 75.50; H, 7.20; N, 2.41. $C_{33}H_{35}NO_5$ requires C, 75.41; H, 6.71; N, 2.66%).

1-(3'-Hydroxy-4'-methoxybenzyl)-6,8-dimethoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline (154c)

A solution of the OO-dibenzyl secondary amine (154a) (0.25 g.) and concentrated hydrochloric acid (5 ml.) was heated, under nitrogen, on the steam-bath for 3½ hours (t.l.c. control). The acidic solution was cooled and basified with 4N-sodium hydroxide solution (pH 10). The alkaline solution was washed with ether (2 x 15 ml.) and then acidified with concentrated hydrochloric acid (pH 2). This acidic solution was washed

with ether (2 x 15 ml.), basified with solid sodium carbonate and extracted with chloroform (3 x 15 ml.) to give a foam (0.16 g., 95%), $\nu_{max.}$ 3550 cm.$^{-1}$, $\tau$ 3.25 (3H, slightly broad s.), 3.66 (1H, s.), 5.24 (3H, broad s., complete deuterium exchange), 6.15 (3H, s., $OCH_3$), and 6.25 (6H, s., 2 x $OCH_3$). The phenolic base is rather unstable - a chloroform solution rapidly deteriorates.

(±)-[2',6'-$^2H_2$]1-(3'-Hydroxy-4'-methoxybenzyl)-6,8-dimethoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline (2',6'-$^2H_2$154c)

The phenol (154c) (73.4 mg.) in deuterium oxide (99.7%; 0.3 ml.) containing potassium t-butoxide (50 mg., 2 mole equivalent) was heated[35] in a sealed, nitrogen-filled Carius tube at 100° for six days. The tube, when cooled off, was opened and 2N-hydrochloric acid (5 ml.) was added. The acidic solution was washed with ether (2 x 15 ml.), basified with solid sodium carbonate (pH 8-9) and was extracted with chloroform (4 x 25 ml.). The combined chloroform extract was washed with water (20 ml.), dried and evaporated. The residual foam (42 mg.) was chromatographed and the product eluted with chloroform/ethanol (49:1; v/v) to give a product (23 mg.), $\tau$ 3.28 (1H, s.) and 3.64 (1H, s.).

(±)-[2',6'-$^3H_2$]1-(3'-Hydroxy-4'-methoxybenzyl)-6,8-dimethoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline (2',6'-$^3H_2$ 154c)

When the phenol (154c) (89.7 mg.) in tritiated water (0.3 ml., 0.06 c) containing potassium t-butoxide (54 mg., ca. 2 mole equivalent) was heated[35] in a sealed, nitrogen-filled Carius tube at 100° for six days and treated as usual (vide supra), a brownish foam (54 mg., spec. activity 4.07 x $10^5$ dps/mg. [spec. molar activity 3.8 mc/m mole; theory requires 3.6 mc/m mole]) was obtained.

1-(3'-Benzyloxy-4'-methoxybenzyl)-2-methyl-7-benzyloxy-6,8-dimethoxy-1,2,3,4-tetrahydroisoquinoline (154b)

A suspension of the OO-dibenzyl ether secondary amine (154a) (108 mg.) in water (2 ml.) was treated with formic acid (0.3 ml.) and the pH of the solution was adjusted to 5 with 4N-sodium hydroxide solution. Formalin solution (37-41% w/v; 0.2 ml.) was added and the mixture was heated, under nitrogen, on the steam-bath for twenty minutes (t.l.c. control) with occasional shaking. The solution was evaporated under reduced pressure and the residue treated with 4N-sodium hydroxide solution (5 ml.). Ether extraction afforded an oil

(107 mg., 96%) which was purified by chromatography. The product was eluted with benzene to give a colourless syrup (87 mg.), $\tau$ 3.23 (3H, s.), 3.65 (1H, s.), 4.90 (2H, s., $OCH_2Ph$), 5.02 (2H, s., $OCH_2Ph$), 6.15 (3H, s., $OCH_3$), 6.17 (3H, s., $OCH_3$), 6.23 (3H, s., $OCH_3$), and 7.70 (3H, s., $NCH_3$).

1-(3'-Hydroxy-4'-methoxybenzyl)-2-methyl-6,8-dimethoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline (154d)

A solution of the requisite OO-dibenzyl ether (154b) (87 mg.) and concentrated hydrochloric acid (3 ml.) was heated, under nitrogen, on the steam-bath for twenty minutes (t.l.c. control). The acidic solution was cooled and basified with 4N-sodium hydroxide solution (6 ml.). The alkaline solution was washed with ether (2 x 10 ml.), acidified with concentrated hydrochloric acid (0.4 ml.), washed with ether (2 x 10 ml.) and basified with solid sodium carbonate. Chloroform extraction (3 x 15 ml.) gave the syrupy tertiary amine (150 mg., 94%), which crystallized from alcohol, m.p. 142°, $\nu_{max.}$ 3530 $cm.^{-1}$, $\tau$ 3.11 (1H, s.), 3.28 (2H, slightly broad s.), 3.64 (1H, s.), 4.66 (2H, s., complete deuterium exchange), 6.07 (3H, s., $OCH_3$), 6.18 (3H, s., $OCH_3$), 6.20 (3H, s., $OCH_3$), and 7.68 (3H, s., $NCH_3$).

(Found: C, 67.16; H, 6.94; N, 4.36. $C_{20}H_{25}NO_5$ requires C, 66.84; H, 7.01; N, 3.90%.)

(±)-[2-methyl-$^{14}$C]1-(3'-Hydroxy-4'-methoxybenzyl)-2-methyl-6,8-dimethoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline (2-$^{14}$C 154d)

A suspension of the OO-dibenzyl ether secondary amine (154a) (58 mg.) in water (1 ml.) was treated with formic acid (0.15 ml.) and the pH of the solution was adjusted to 5 with 4N-sodium hydroxide solution. [$^{14}$C]-Paraformaldehyde (0.71 mg., 0.1 mc.) was added and the mixture was heated, under nitrogen, on the steam-bath for twenty minutes with occasional shaking. Inactive formalin solution (37-41% w/v; 0.2 ml.) was added to the reaction mixture and the heating and shaking processes were continued for an additional thirty minutes. The mixture was cooled off and worked up as previously to give the oily tertiary amine (59 mg.).

A solution of the requisite [2-$^{14}$C]OO-dibenzyl ether (vide supra) (59 mg.) and concentrated hydrochloric acid (3 ml.) was heated, under nitrogen, on the steam-bath for two hours (t.l.c. control). The reaction mixture was treated as usual to give the oily amine (37.5 mg., 0.045 mc) which was chromatographed and crystallized from alcohol, m.p.140-142°.

## (±)-N-Norarmepavine (145c) and (±)-armepavine (146c)

3,4-Dimethoxy-ω-nitrostyrene (138a) (method of Dr. D. S. Bhakuni).

To a solution of veratric aldehyde (137a) (20 g.) in freshly distilled nitromethane (30 ml.) was added methylamine hydrochloride (4 g.) and anhydrous potassium acetate (4 g.). The mixture was shaken at room temperature for three hours. Water was added and the insoluble product was filtered off. The bright yellow product was washed thoroughly with water and ether to give the nitrostyrene (23.8 g., 76%), m.p. 141° (lit.[53] m.p.145°).

β-[3,4-Dimethoxyphenyl]ethylamine (139a)

A solution of 3,4-dimethoxy-ω-nitrostyrene (138a) (10 g.) in anhydrous tetrahydrofuran (160 ml.) was added dropwise to a stirred, refluxing suspension of lithium aluminium hydride (6 g.) in dry tetrahydrofuran (100 ml.). After five hours the mixture was cooled and the excess reagent was decomposed with water. The suspension was diluted with tetrahydrofuran and filtered. The filter cake was washed thoroughly with several portions of hot tetrahydrofuran. The filtrates were combined, evaporated under pressure, and the residual oil was extracted with

ether to give the oily amine. An ethanolic solution of the amine was treated with oxalic acid (6.2 g.) in ethanol. The first-formed precipitate was filtered off and recrystallized from alcohol to give the amine oxalate (6.6 g.; 51%), m.p.180-182° (lit.,[53] m.p. 178°).

4-Benzyloxy-ω-diazoacetophenone (142a)

A suspension of 4-benzyloxybenzoic acid (7.7 g.) in anhydrous benzene (50 ml.) containing oxalyl chloride (5 ml.) was left overnight at room temperature. The solvent and excess reagent were then evaporated under reduced pressure. More benzene was added and the evaporation process repeated. The solid acid chloride (8 g.) was dissolved in dry benzene (10 ml.) and added dropwise to a cold and well-stirred ethereal diazomethane solution (from 40 g. nitrosomethylurea). The reaction mixture was left stirring overnight. The solvent was evaporated in the cold. The residue was dissolved in a small volume of hot benzene and diluted with petroleum ether to give the diazoketone (5.9 g.; 69%), m.p.115-117° (lit.,[138] m.p.117°).

N-(3,4-Dimethoxyphenethyl)-4-benzyloxy-phenylacetamide (143a)

A solution of β-[3,4-dimethoxyphenyl]ethylamine

(139a) (3.3 g.) and 4-benzyloxy-ω-diazoacetophenone (142a) (5.1 g., 10% excess) in anhydrous benzene (160 ml.) was irradiated under nitrogen, using a high-pressure mercury lamp. After five hours the reaction was terminated (i.r. control). The benzene solution was washed successively with 1N-sodium hydroxide solution (2 x 20 ml.), 2N-hydrochloric acid solution (2 x 20 ml.), and water (20 ml.). The benzene solution was dried and evaporated. The solid residue (5 g.; 68%) was recrystallized from benzene/petroleum ether to give the amide (3.7 g.), m.p.117-118° (lit.,[53] m.p.116°), $\nu_{max.}$ 3494, 1662 cm.$^{-1}$, $\tau$ ("$A_2B_2$" quartet) 2.91 (2H, d., J = 8.5 c/s), 3.09 (2H, d., J = 8.5 c/s); 3.38 (3H, slightly broad s.), 4.29 (1H, broad s., .CONH), 4.97 (2H, s., $OCH_2Ph$), and 6.20 (6H, s., 2 x $OCH_3$).

1-(4'-Benzyloxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline hydrochloride (144a)

To a solution of the requisite amide (143a) (3.2 g.) in dry, refluxing toluene (40 ml.) was added freshly-distilled phosphorus oxychloride (10 ml.), whilst dry nitrogen gas was bubbled through the reaction mixture. After twenty minutes (i.r. control) the reaction mixture was cooled. The imine hydrochloride crystallized

spontaneously. The reaction mixture was diluted with ether and the product was filtered off. Recrystallization from ethanol/ether gave the hydrochloride as plates (2.95 g., 87%), m.p.210° (lit.,[53] m.p. 210.5-211°), $\nu_{max.}$ (Nujol) 1650 cm.$^{-1}$ (Found: C, 70.62; H, 6.36; Cl, 8.37; N, 3.33. Calc. for $C_{25}H_{26}ClNO_3$: C, 70.81; H, 6.18; Cl, 8.36; N, 3.30%).

1-(4'-Benzyloxybenzyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (145a)

To a methanolic solution (20 ml.) of the 3,4-dihydroisoquinoline (144a) (recovered from 1.0 g. hydrochloride), cooled in ice, sodium borohydride (0.8 g.) was added in small portions. The reaction mixture was left stirring overnight. The solvent was evaporated under reduced pressure and the residue treated with 4N-sodium hydroxide solution (20 ml.). The product was extracted with chloroform (3 x 20 ml.) and the combined chloroform extract washed with water (15 ml.) to give an oily amine (1.1 g., "100%"), $\tau$ ($A_2B_2$ quartet) 2.82 (2H, d., J = 9 c/s), 3.03 (2H, d., J = 9 c/s); 3.40 (2H, s.), 4.94 (2H, s., $OC\underline{H}_2Ph$), 6.15 (3H, s., $OC\underline{H}_3$), 6.20 (3H, s., $OC\underline{H}_3$), and 7.42 (1H, s., N<u>H</u>, complete deuterium exchange). The amine hydrochloride, recrystallized from ethanol/ether,

had m.p. 198-200°. (Found: C, 70.87; H, 6.51; Cl, 8.58; N, 3.28. $C_{25}H_{28}ClNO_3$ requires C, 70.49; H, 6.62; Cl, 8.32; N, 3.29%.)

1-(4'-Hydroxybenzyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline[(±)-N-norarmepavine] (145c)

The O-benzyl ether hydrochloride (145a) (132 mg.) was added to a suspension of pre-reduced 10% palladium on carbon (36 mg.) in ethanol (10 ml.), containing one drop of concentrated hydrochloric acid. Upon shaking, there was a slow uptake of hydrogen. It continued until one equivalent had been absorbed (seven hours). The catalyst was filtered off and washed with hot ethanol. The filtrates were combined and the ethanol was evaporated under reduced pressure to give a white foam. The oily amine (100 mg.), which was recovered from its hydrochloride, had $\nu_{max.}$ 3600, 3325 (bonded OH) cm.$^{-1}$, $\tau$ ($A_2B_2$ quartet) 3.01 (2H, d., J = 8.5 c/s), 3.36 (2H, d., J = 8.5 c/s); 3.37 (1H, s.), 3.40 (1H, s.), 4.28 (2H, broad s., complete deuterium exchange), 6.16 (3H, s., $OCH_3$) and 6.21 (3H, s., $OCH_3$). The oxalate which crystallized from alcohol had m.p. 222° (lit.,[139] m.p.205-7°; lit.,[140] 210°; lit.,[19] 222-7°).

1-(4'-Benzyloxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (146a)

The O-benzyl ether secondary amine (145a) (342 mg.) was dissolved in formic acid (1.5 ml.). The pH of the solution was adjusted to 5 with 4N-sodium hydroxide solution. Formalin solution (37-41% w/v; 1.5 ml.) was added and the mixture was heated, under nitrogen, on the steam-bath for ninety minutes (t.l.c. control). The solution was evaporated under reduced pressure and the residue treated with 4N-sodium hydroxide solution. Chloroform extraction gave an oil which was purified by chromatography. The product was eluted with benzene/chloroform (4:1; v/v) to give the syrupy amine (353 mg.; 99%), $\tau$ ($A_2B_2$ quartet) 2.97 (2H, d., J = 9 c/s), 3.11 (2H, d., J = 9 c/s); 3.45 (1H, s.), 3.97 (1H, s.), 4.98 (2H, s., $OCH_2Ph$), 6.20 (3H, s., $OCH_3$), 6.49 (3H, s., $OCH_3$), and 7.47 (3H, s., $NCH_3$). The hydrochloride which crystallized from ethanol/ether, had m.p. 195-7°. (Found: C, 71.31; H, 6.97; Cl, 8.63; N, 3.40. $C_{26}H_{30}ClNO_3$ requires C, 70.98; H, 6.87; Cl, 8.06; N, 3.18%).

1-(4'-Hydroxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline[(±)-armepavine] (146c)

The O-benzyl ether hydrochloride (146a) (101 mg.) was added to a suspension of pre-reduced 10% palladium on carbon (25 mg.) in ethanol (10 ml.), containing one drop of concentrated hydrochloric acid. Upon shaking, there was a slow uptake of hydrogen. It continued until one equivalent had been absorbed (eight hours). The catalyst was filtered off and was washed with hot ethanol. The filtrates were combined and the solvent evaporated under reduced pressure to give a white foam. The hydrochloride was dissolved in 5% aqueous sodium carbonate solution and the amine extracted with chloroform (3 x 20 ml.) to give an oily product (80 mg.) which crystallized from acetone/ether, m.p.164-166° (lit.,[53,141] m.p.166°), $\nu_{max.}$ 3604, 3283 (bonded OH) cm.$^{-1}$, base peak m/e 206, $\tau$[54] 2.07 (1H, broad s., complete deuterium exchange); ($A_2B_2$ quartet) 3.10 (2H, d., J = 8.6 c/s), 3.33 (2H, d., J = 8.6 c/s); 3.43 (1H, s.), 4.06 (1H, s.), 6.19 (3H, s., $OCH_3$), 6.51 (3H, s., $OCH_3$), 7.45 (3H, s., $NCH_3$). The hydrochloride crystallized from ethanol/ether, m.p. 209-211° (dec.).

1-(4'-Methoxybenzyl)-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline[(±)-4'-O-methylcoclaurine] (145d) and 1-(4'-methoxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline[(±)-4'-ON-dimethylcoclaurine] (146d)

4-Methoxy-ω-diazoacetophenone (142b)

Anisoyl chloride, derived from anisic acid (140b) (7.52 g.), was dissolved in benzene (15 ml.) and added dropwise to an ethereal solution of diazomethane (from 41 g. nitrosomethylurea). After stirring overnight, the solution was evaporated under reduced pressure. The residue was dissolved in a small volume of hot benzene and this solution was diluted with petroleum ether to give the diazoketone (4.7 g.; 60%), $\nu_{max.}$ 2130 $cm.^{-1}$, m.p. 87° (lit.,[142] m.p. 90-91°).

N-(4-Benzyloxy-3-methoxyphenethyl)-4-methoxyphenacetamide (143b)

A solution of β-[4-benzyloxy-3-methoxyphenyl]ethylamine (139b) (from 3.02 g. amine hydrochloride, kindly supplied to me by Mr. G.M. Chapman) and 4-methoxy-ω-diazoacetophenone (142b) (1.94 g., 10% excess) in anhydrous benzene (120 ml.) was irradiated under nitrogen,

using a high-pressure mercury lamp. After three hours the reaction was terminated (i.r. control). The benzene solution was treated as usual to give the amide (3.04 g., 82%) which, recrystallized from benzene/petroleum ether, had m.p. 114-116° (lit.,[143] m.p.114-115°; lit.,[144] m.p. 116-117°), $\nu_{max.}$ 3489, 1660 cm.$^{-1}$, $\tau$ 2.8-3.6 (7 aromatic H's), 4.55 (1H, broad s., .CONH), 4.89 (2H, s., $OCH_2Ph$), 6.18 (3H, s., $OCH_3$), and 6.22 (3H, s., $OCH_3$).

1-(4'-Methoxybenzyl)-7-benzyloxy-6-methoxy-3,4-dihydroisoquinoline hydrochloride (144b)

To a solution of the amide (143b) (2.07 g.) in dry, refluxing toluene (40 ml.) was added freshly distilled phosphorus oxychloride (10 ml.) whilst dry nitrogen gas was bubbled through the reaction mixture. After thirty minutes (i.r. control) the excess reagent and toluene were evaporated under reduced pressure. The residue was redissolved in toluene and the toluene evaporated. Recrystallization from ethanol/ether gave the hydrochloride (1.78 g., 82%), m.p.215-217° (lit.,[145] m.p. 215-216.5°). (Found: C, 70.89; H, 6.13; Cl, 8.49; N, 3.39. Calc. for $C_{25}H_{26}ClNO_3$: C, 70.81; H, 6.18; Cl, 8.36; N, 3.30%). The corresponding oily imine showed $\tau$ (limiting $A_2B_2$ quartet) 2.97 (4H); 3.16 (1H, s.), 3.30

(1H, s.), 4.97 (2H, s., $OC\underline{H}_2Ph$), 6.11 (3H, s., $OC\underline{H}_3$), and 6.24 (3H, s., $OC\underline{H}_3$); $\lambda_{max.}$ 231, 276, and 311 mμ.

1-(4'-Methoxybenzyl)-7-benzyloxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline (145b).

To an ice-cooled methanolic solution (30 ml.) of the 3,4-dihydroisoquinoline (144b) (recovered from 1.02 g. hydrochloride) was added sodium borohydride (0.8 g.) in small portions. The reaction mixture was left stirring overnight. The solvent was evaporated under reduced pressure and the residue treated with 4N-sodium hydroxide solution. The product was extracted with chloroform (3 x 30 ml.) and the combined chloroform extract washed with water (30 ml.) to give an oily amine (0.91 g., 97%), $\tau$ ($A_2B_2$ quartet) 2.90 (2H, d., J = 8.5 c/s), 3.15 (2H, d., J = 8.5 c/s); 3.34 (1H, s.), 3.40 (1H, s.), 4.92 (2H, s., $OC\underline{H}_2Ph$), 6.13 (3H, s., $OC\underline{H}_3$), 6.21 (3H, s., $OC\underline{H}_3$), and 7.51 (1H, s., N$\underline{H}$, complete deuterium exchange). The amine hydrochloride crystallized as needles from abs. alcohol, m.p.177-178° (lit.,[144] m.p. 179.5°) and as clusters from acetone, m.p.196-198°. (Found: Cl, 8.35. Calc. for $C_{25}H_{28}ClNO_3$: Cl, 8.32%).

1-(4'-Methoxybenzyl)-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline (145d)

The O-benzyl ether hydrochloride (145b) (105 mg.) was added to a suspension of pre-reduced 10% palladium on carbon (21 mg.) in ethanol (10 ml.), containing one drop of concentrated hydrochloric acid. Upon shaking there was an immediate uptake of hydrogen. It continued until one equivalent had been absorbed (ninety minutes). The shaking was continued for one hour during which practically no additional hydrogen was taken up. The catalyst was filtered off and washed with hot ethanol. The filtrates were combined and the solvent was evaporated under reduced pressure to give a white foam which, on treatment with 5% aqueous sodium carbonate solution and chloroform extraction (3 x 20 ml.), gave the amine (74 mg.) which crystallized from acetone, m.p.173-174°, $\nu_{max.}$ 3566 cm.$^{-1}$, base peak at m/e 178, $\tau$ ($A_2B_2$ quartet) 2.89 (2H, d., J = 8.7 c/s), 3.17 (2H, d., J = 8.7 c/s); 3.30 (1H, s.), 3.48 (1H, s.), 5.28 (2H, broad singlet, NH, OH), 6.24 (3H, s., $OCH_3$), and 6.28 (3H, s., $OCH_3$).

1-(4'-Methoxybenzyl)-7-benzyloxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (146b)

The O-benzyl ether secondary amine (145b) (429 mg.) was dissolved in formic acid (1.5 ml.). The pH of the

solution was adjusted to 5 with 4N-sodium hydroxide solution. Formalin solution (37-41% w/v; 1.5 ml.) was added and the mixture was heated, under nitrogen, on the steam-bath for one hour (t.l.c. control). The solution was evaporated under reduced pressure and the residue was treated with 4N-sodium hydroxide solution. Chloroform extraction gave the oily amine (405 mg. ; 91%) which was chromatographed on alumina and eluted with benzene/chloroform (4:1; v/v), $\tau$ ($A_2B_2$ quartet) 3.03 (2H, d., J = 8.5 c/s), 3.21 (2H, d., J = 8.5 c/s); 3.43 (1H, s.), 3.88 (1H, s.), 5.20 (2H, s., $OCH_2Ph$), 6.17 (3H, s., $OCH_3$), 6.26 (3H, s., $OCH_3$), and 7.49 (3H, s., $NCH_3$). The amine hydrochloride crystallized from ethanol/ether, m.p. 183-185°. (Found: Cl, 8.39; N, 3.19. $C_{26}H_{30}ClNO_3$ requires Cl, 8.06; N, 3.18%.)

1-(4'-Methoxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (146d)

The O-benzyl ether hydrochloride (146b) (109 mg.) was added to a suspension of pre-reduced 10% palladium on carbon (20 mg.) in ethanol (10 ml.) containing one drop of concentrated hydrochloric acid. Upon shaking there was an immediate uptake of hydrogen. It continued until one equivalent had been absorbed (two hours).

The shaking was continued for an additional hour. The catalyst was filtered off and washed with several portions of hot ethanol. The filtrates were combined and the solvent was evaporated under reduced pressure to give a white foam which, after treatment with 5% aqueous sodium carbonate solution and chloroform extraction (3 x 20 ml.), gave the oily amine[145,146] (81 mg.), $\nu_{max.}$ 3570 cm.$^{-1}$, $\tau$[54] ($A_2B_2$ quartet) 2.98 (2H, d., J = 8.7 c/s), 3.22 (2H, d., J = 8.7 c/s); 3.49 (1H, s.), 3.67 (1H, s.), 3.92 (1H, broad s., O<u>H</u>), 6.21 (3H, s., OC<u>H</u>$_3$), 6.27 (3H, s., OC<u>H</u>$_3$), and 7.56 (3H, s., NC<u>H</u>$_3$).

A methanolic solution of the oily amine (80 mg.) was treated with excess ethereal diazomethane and the mixture was kept at room temperature for two days (t.l.c. control). The solvent and excess reagent were removed under reduced pressure and the oily residue was chromatographed. The product (45 mg.), eluted with benzene/chloroform (25:1), was dissolved in methanol (2 ml.) and excess methyl iodide was added. The mixture was refluxed on the steam-bath for two hours. Evaporation of the methanol and excess methyl iodide left a reddish residue, which crystallized from acetone to give (±)-<u>NOO</u>-trimethylcoclaurine methiodide, m.p.120-122° (sinters at 115°) [lit.,[55] m.p.120-125° (sinters at 115°)].

N-(4-Methoxy-3-hydroxyphenethyl)β-(3,5-dimethoxy-4-hydroxyphenyl)ethylamine (160a)

O-Benzylsyringic acid (156b)

Methyl O-benzylsyringate (156a) (8.6 g.) and sodium hydroxide (2 g.) in water (20 ml.) was heated on the steam-bath for two hours. The solution was diluted with water (10 ml.) and washed with ether (2 x 15 ml.). The aqueous phase was acidified with 2N-hydrochloric acid (30 ml.) and the precipitate recrystallized from aqueous alcohol to give the acid (7.1 g., 86%), m.p.155-157° (lit.,[133] m.p.157°).

4-Benzyloxy-3,5-dimethoxy-ω-diazoacetophenone (156d)

A suspension of O-benzylsyringic acid (156b) (3.1 g.) in anhydrous benzene (10 ml.) containing oxalyl chloride (5 ml.) was left at room temperature for two days. The solvent and excess reagent were evaporated under reduced pressure. The acid chloride (156c) (3.1 g.), freshly crystallized from light petroleum ether, m.p. 42-44° (lit.,[133] m.p.45°), was dissolved in ether (15 ml.) and added dropwise to an ethereal solution of diazomethane (from 10.3 g. of nitrosomethylurea) maintained at 0°. The reaction mixture was left stirring overnight.

The ether was evaporated under reduced pressure and the residue crystallized from benzene/light petroleum ether to give the diazoketone (2.62 g., 83%), m.p.126° (lit.,[133] m.p. 122-123°).

3-Benzyloxy-4-methoxy-ω-nitrostyrene (155b)

A mixture of O-benzylisovanillin (155a) (20 g.), anhydrous sodium acetate (10 g.), and methylamine hydrochloride (10 g.) in freshly distilled nitromethane (20 ml.) was shaken at room temperature for three hours. The reaction mixture was diluted with water and the insoluble product was filtered off. The yellow solid was washed thoroughly with water and ether to give the nitrostyrene (20 g., 85%), m.p.129-130° (lit.,[147] m.p. 128°).

β-(3-Benzyloxy-4-methoxyphenyl)ethylamine (155c)

A solution of 3-benzyloxy-4-methoxy-ω-nitrostyrene (155b) (10 g.) in anhydrous tetrahydrofuran (120 ml.) was added dropwise to a refluxing suspension of lithium aluminium hydride (10 g.) in tetrahydrofuran (120 ml.). After two hours the mixture was worked up as usual to give the oily amine, which was treated with saturated ethereal hydrogen chloride. The product (8.4 g., 81%) crystallized from ethanol/ethyl acetate, m.p.166-168° (lit.,[147] m.p.166°).

N-(3-Benzyloxy-4-methoxyphenethyl)-4-benzyloxy-3,5-dimethoxyphenacetamide (157a)

A solution of β-(3-benzyloxy-4-methoxyphenyl)ethylamine (155c) (1.76 g.) and 4-benzyloxy-3,5-dimethoxy-ω-diazoacetophenone (156d) (2.13 g.) in anhydrous benzene (85 ml.) was irradiated under nitrogen, using a high pressure mercury lamp. After two and a half hours the reaction was terminated (i.r. control). The benzene solution was washed successively with 1N-hydrochloric acid solution (2 x 15 ml.), 1N-sodium hydroxide solution (2 x 15 ml.), and water (2 x 15 ml.). The benzene solution was dried and evaporated to give a syrupy product (4 g.) which was chromatographed twice on alumina (150 g.). The product was eluted with benzene/chloroform (1:1) and crystallized from alcohol to give the amide (3.1 g.), m.p.87-89°, $\nu_{max.}$ 3450, 1660 $cm.^{-1}$, τ 4.50 (.CONH), 4.92 ($OCH_2Ph$), 5.01 ($OCH_2Ph$), 6.19 ($OCH_3$), and 6.26 (2 x $OCH_3$).

Lithium aluminium hydride reduction of the secondary amide (157a)

A solution of the amide (157a) (0.5 g.) in anhydrous tetrahydrofuran (10 ml.) was added dropwise to a refluxing suspension of lithium aluminium hydride (0.3 g.) in the same solvent (10 ml.). After five hours the reaction

mixture was treated as usual to give an oily product (0.3 g.). T.l.c. showed at least five compounds to be present.

N-Methylation of the secondary amide (157a) and lithium aluminium hydride reduction of the tertiary amide (158)

"50%" Sodium hydride (1 g.) was added to anhydrous dimethylformamide (10 ml.) and the reaction flask was flushed with dry, deoxygenated nitrogen. A solution of the amide (157a) (1.64 g.) in dry dimethylformamide (12 ml.) was added dropwise, followed by a solution of methyl iodide (1 g.) in dimethylformamide (1 ml.). The mixture was stirred at room temperature for two hours (persistent $\nu_{N-H}$) and at 80° for an additional hour (i.r. and t.l.c. control). The reaction mixture was cooled in ice and water (10 ml.) was added. Benzene extraction (3 x 20 ml.) gave a product which was chromatographed on alumina and eluted with benzene/ethyl acetate (9:1). The chromatography was repeated to give the amide (1.25 g.), $\nu_{max.}$ 1640 $cm.^{-1}$.

A solution of the amide (158) (1.2 g.) in anhydrous tetrahydrofuran (15 ml.) was added dropwise to a refluxing suspension of lithium aluminium hydride (0.33 g.) in the same solvent (15 ml.). After two hours the excess LAH

was decomposed as usual. Ether extraction gave an oily amine (1 g.) which was chromatographed on alumina (40 g.). The oily tertiary amine (159) (0.71 g.), eluted with benzene, had $\tau$ 3.25 (3 aromatic H's), 3.61 (s., two aromatic H's), 4.90 (2H, $OC\underline{H}_2Ph$), 5.02 (2H, $OC\underline{H}_2Ph$), 6.17 (3H, $OC\underline{H}_3$), 6.20 (6H, 2 x $OC\underline{H}_3$), and 8.76 (3H, d., $J$ = 6.3 c/s, $CHC\underline{H}_3$). The amine acid oxalate crystallized from alcohol, m.p.103-105°. (Found: C, 68.96; H, 6.72; N, 2.01. $C_{37}H_{43}NO_9$ requires C, 68.82; H, 6.71; N, 2.17%).

## Isolation and degradation of stebisimine (48)

### Isolation

*Stephania japonica* leaves and stems (of Indian origin) were disrupted in 2% methanolic tartaric acid solution (4.5 l.). The resulting pulp was left soaking at room temperature for three days, with occasional stirring, after which time it was filtered through Celite. The filter cake was resoaked for an additional three days and then filtered off. The combined methanolic filtrates and washings were concentrated to a small bulk (340 ml.) on a rotatory evaporator and the tarry residue diluted with water (600 ml.) and 2N-hydrochloric acid solution (100 ml.). The acidic aqueous solution was filtered through Celite and the filtrate repeatedly extracted with ether (17 x 200 ml.). The aqueous phase was basified with sodium carbonate and exhausted with chloroform (25 x 250 ml.). The volume of the combined chloroform extracts was reduced on a rotatory evaporator and the remaining chloroform solution (400 ml.) washed with 3% sodium hydroxide solution (7 x 30 ml.), water (30 ml.), and dried, to give the total non-phenolic base fraction (8 g.). T.l.c. on silica (methanol as developing solvent)

revealed a major, lower - $R_F$, and a minor, higher - $R_F$, component.

The total non-phenolic base fraction (8 g.) was chromatographed on alumina (150 g.) and eluted successively with benzene (fractions 1 to 37; 50 ml. fractions were collected), benzene/dichloromethane (1:1, v/v; fractions 38 to 41), and dichloromethane (fractions 42 to 52). Finally the column was washed out with methanol. Fractions 22 to 37 (t.l.c. control) were combined to give episteph-anine (2 g.) which, after several recrystallizations from methanol, had m.p.188-206°, 200-204° (dried *in vacuo* at 100° for 48 hrs); $\nu_{max.}$ 1603 $cm.^{-1}$; $\lambda_{max.}$ 232.5 and 282 mμ ( ε 34, 100 and 14, 600); τ 6.12 (6H, 2 x $OCH_3$), 6.14 (3H, $OCH_3$), 6.64 (3H, $OCH_3$), and 7.47 (3H, $NCH_3$); *m/e* 606 (molecular ion and base peak), 605 (95% of base peak), 591, 575, 561, 559, 545, 485, 483, 381, 379, 303 ($M^{++}$, isotope peak at 303.5), 190, 174, 145, with an additional, relatively low abundant and temperature dependent, peak at *m/e* ($M^+$ + 14); $[\alpha]_D^{25}$ = + 226° (*c* 1.04 in $CHCl_3$) (Found: N, 4.56. $C_{37}H_{38}N_2O_6$ requires N, 4.62%). *Hydrochloride*, m.p. > 300° (gradual dec.), $\lambda_{max.}$ 284 mμ ( ε 13,110). *Picrate*, m.p. 174-176° (EtOH). *Methiodide*, m.p. 235° (dec.). Direct comparisons (t.l.c. and mixed t.l.c.; m.p. and mixed m.p.188-206°; identical i.r.'s in both chloroform and Nujol) with an

authentic specimen[56] of epistephanine were made.

Combined fractions 38 to 47 gave, after rigorous purification, an additional batch (0.5 g.) of epistephanine.

The combined mother-liquors from the second batch of epistephanine (vide supra) and combined fractions 48 and onwards gave, after repeated dissolution in a small volume of ethanol and then dilution with methanol, a chromatographically pure, crystalline compound (0.43 g.), stebisimine (48), m.p.233-235° (dried in vacuo at 100° for 12 hrs); $\nu_{max.}$ 1610 cm.$^{-1}$, $\nu_{max.}$ (Nujol) 1620 cm.$^{-1}$; $\lambda_{max.}$ 238, 279 mμ ( $\varepsilon$ 51,900 and 24,200), $\lambda_{infl.}$ 308 mμ ( $\varepsilon$ 12,500); $\tau$ 2.8 - 3.8 (8H), 4.09 (an aromatic H, confirmed by spin decoupling), 6.04 ($OCH_3$), 6.10 ($OCH_3$), 6.12 ($OCH_3$), 6.75 ($OCH_3$), and no N-methyl resonance; m/e 590 (molecular ion and base peak), 575 ($M^+ - CH_3$, metastable peak at m/e 560) 559 ($M^+$ - OMe, metastable peak at m/e 530), 370, 295 ($M^{++}$, isotope peak at m/e 295.5) 221, 206, 192, 175, with a very low abundant peak at m/e ($M^+ + 14$); mass measurement for $C_{36}H_{34}N_2O_6$ requires 590.241671. Found: 590.241734; $[\alpha]_D^{25} = 0°$ (c 1.26 in $CHCl_3$) (Found: C, 73.33; H, 6.13; N, 4.76, 4.64. $C_{36}H_{34}N_2O_6$ requires C, 73.20; H, 5.80; N, 4.74%).

Hydrochloride, m.p. > 290° (dec.); $\nu_{max.}$ (Nujol) 1643 $cm.^{-1}$. Picrate, m.p. 254-256°. Methiodide, m.p. > 290° (gradual dec.), $\lambda_{max.}$ 340 mμ ( $\varepsilon$ 18,450).

Sodium in liquid ammonia reduction of Stebisimine (48)

Liquid ammonia (30 ml.) was distilled from sodium metal directly into the reaction flask. Stebisimine (48) (54 mg.) in anhydrous toluene (10 ml.) was added dropwise, with vigorous stirring, followed by small pieces of freshly cut sodium metal (ca. 20 mg., theory requires 16.7 mg.). The stirring was continued for an additional three hours after complete addition. Ammonium chloride was added to the reaction mixture (discharge of the blue colour) and the ammonia evaporated. The residue was taken up in 1N-sodium hydroxide solution (10 ml.) and the alkaline solution was washed with ether (2 x 10 ml.). The aqueous phase was acidified with concentrated hydrochloric acid, then basified with sodium carbonate and exhausted with chloroform (5 x 15 ml.) to give a yellowish oily product (50 mg.). T.l.c. (alumina, EtOAc) showed two components, the $R_F$-values of which matched those of the authentic mono-bases.

The oily product (50 mg.) was dissolved in 98% formic acid (0.5 ml.). 4N-Sodium hydroxide solution

was added dropwise (pH 5), followed by 38% formalin solution (0.5 ml.). The reaction mixture, flushed with nitrogen, was heated on the steam-bath for six hours (t.l.c. control) and evaporated under reduced pressure. The residue was treated with saturated aqueous sodium hydrogen carbonate solution and extracted with chloroform (5 x 15 ml.) to give an oil (46 mg.). T.l.c. (alumina EtOAc) revealed, in addition to the two major and expected N-methyl derivatives, two very minor components of intermediate $R_F$-values. The reaction mixture (46 mg.) was chromatographed on alumina (8 g.) and eluted with chloroform to give, firstly, syrupy 4'-NO-dimethylcoclaurine (17 mg.) (47; R'=H, R=R''=He), $\nu_{max.}$ 3550 cm.$^{-1}$, $\tau$ 2.98 (d., J = 8.5 c/s), 3.48 (d., J = 8.5 c/s) [$A_2B_2$ quartet], 3.48 (s.,H), 3.66 (s.,H), 4.73 (br., OH), 6.17 (s., $OCH_3$), 6.23 ($OCH_3$), and 7.52 ($NCH_3$). This syrupy product in methanol (2 ml.) was treated with an excess ethereal diazomethane solution and left at room temperature for 24 hours to give, after chromatography on alumina, syrupy (±)-NOO-trimethylcoclaurine (14 mg.), $\tau$ 3.00 (d., 2H, J= 8.5 c/s), 3.22 (d., J = 8.5 c/s) [$A_2B_2$ quartet], 3.43 (s., H), 4.00 (s., H), 6.16 ($OCH_3$), 6.23 ($OCH_3$), 6.45 ($OCH_3$), and 7.42 ($NCH_3$), which was characterized as its methiodide, m.p. and mixed m.p. 121-123° (sinters at 115°)

(MeOH) [lit.,[55] m.p.120-125° (sinters at 115°)]. Secondly, was eluted (see chromatographical separation above) a product (18 mg.), $\nu_{max.}$ 3600, 3330 $cm.^{-1}$, m/e 206 (base peak), which was further purified by chromatography and then converted into its hydrochloride (recrystallized from ethanol/ether), identical [m.p. and mixed m.p.208-210°; i.r. (Nujol)] with authentic (±)-armepavine hydrochloride.

Sodium borohydride reduction of stebisimine dimethiodide

Sodium borohydride (60 mg.) was added portionwise to a solution of stebisimine dimethiodide (62 mg.) in methanol (8 ml.) and the solution stirred for an additional seven hours after complete addition (u.v. control). The methanol was evaporated under reduced pressure and the residue treated with water (10 ml.) and 4N-sodium hydroxide solution (5 ml.). The alkaline solution was extracted with chloroform (3 x 15 ml.) and the combined chloroform extract washed with water (10 ml.), dried, and evaporated to give an amorphous powder (41 mg.) [t.l.c. (silica gel, MeOH) showed two components to be present], $\nu_{max.}$ 1610 $cm.^{-1}$ (of reduced intensity compared with stebisimine); $\lambda_{max.}$ 281 mμ ( ε 7,280); τ 6.05 (OMe), 6.24 (OMe), 6.57 (OMe), 6.98 (OMe), and 7.43 (2 x NMe) [methoxyl and

methylimino resonances of the major, slower running, component ]; τ 6.11 (OMe), 6.21 (OMe), 6.38 (OMe), 6.80 (OMe), 7.33 (NMe), and 7.43 (NMe) [methoxyl and methylimino resonances of the minor, slightly faster running, component]; m/e 622 (molecular ion $M^+$), 621, 607 ($M^+ - CH_3$), 591 ($M^+ - OCH_3$), 516, 515, 431 ($M^+ - 191$), 396, 395 (base peak), 381 (396 - $CH_3$), 379, 364, 358, 349, 311 ($M^{++}$, isotope peak 311.5), 198 (isotope peak 198.5), 175, and 174.

Catalytic hydrogenation of stebisimine (48)

Stebisimine (48) (15.8 mg.) was added to a suspension of pre-reduced Adams catalyst (8 mg.) in ethanol (5 ml.). Upon shaking, the uptake of hydrogen continued until two equivalents had been absorbed (3 hours). The catalyst was filtered off and the ethanol evaporated under reduced pressure to give a white foam (15.6 mg.). T.l.c. ($SiO_2$, MeOH) showed a major, slower running, and a minor, faster running, components. This foam was dissolved in 98% formic acid (0.2 ml.) and water (1 ml.) 4N-Sodium hydroxide solution was added dropwise (pH 5), followed by 38% formalin solution (0.2 ml.). The reaction mixture, flushed with nitrogen, was heated on the steam-bath for one hour and evaporated under reduced pressure. The

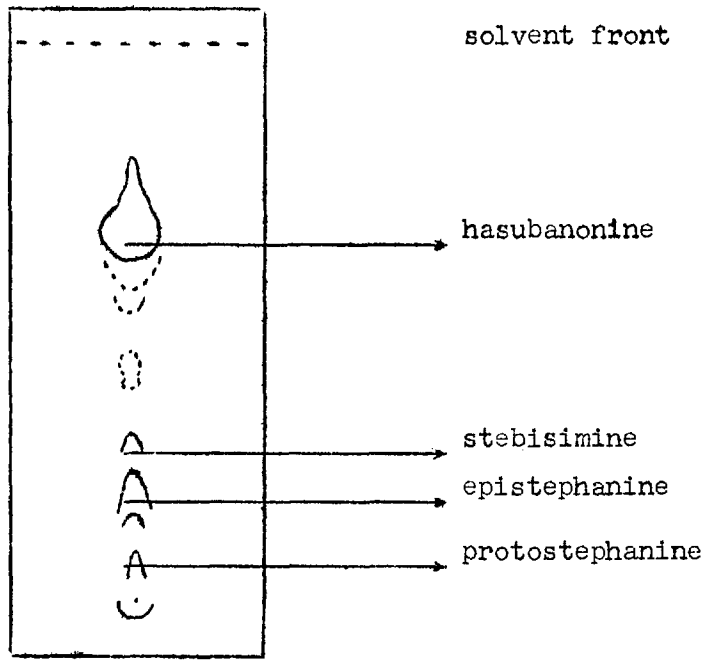
residue was treated with dilute alkali and extracted with dichloromethane (4 x 10 ml.) to give a foam (16 mg.), which was washed through an alumina column. The major, slower running, component, separated from the minor component on an ordinary $SiO_2$ thin layer plate, showed m/e 622 ($M^+$), 621, 607, 591, 516, 515, 431, 396, 395 (base peak), 381, 364, 349, 311 ($M^{++}$), 198, and 175.

## General isolation procedure of the alkaloids and degradative procedures

### General isolation procedure

Typically, one whole, fresh *Stephania japonica* plant (wet weight 87 g.), grown at Imperial College, was disrupted in 2% methanolic tartaric acid solution (1 l.). The resulting pulp was left soaking at room temperature for three days, with occasional stirring, after which time it was filtered through Celite. The filter cake was washed with methanol, resoaked for an additional three days and then filtered off. The combined methanolic filtrates and washings were concentrated to a small bulk volume (*ca.* 100 ml.) on a rotatory evaporator and the dark green residue diluted with water (100 ml.) and 2*N*-hydrochloric acid solution (40 ml.). The acidic aqueous solution was filtered through Celite and the filtrate (positive Mayer test for alkaloids) repeatedly extracted with ether (5 x 50 ml.). The aqueous phase was basified with sodium carbonate (pH 8-9) and exhausted, successively, with dichloromethane (4 x 50 ml.) and chloroform (4 x 50 ml.). The extracts were combined, concentrated to *ca.* 100 ml. on a rotatory evaporator, dried ($K_2CO_3$),

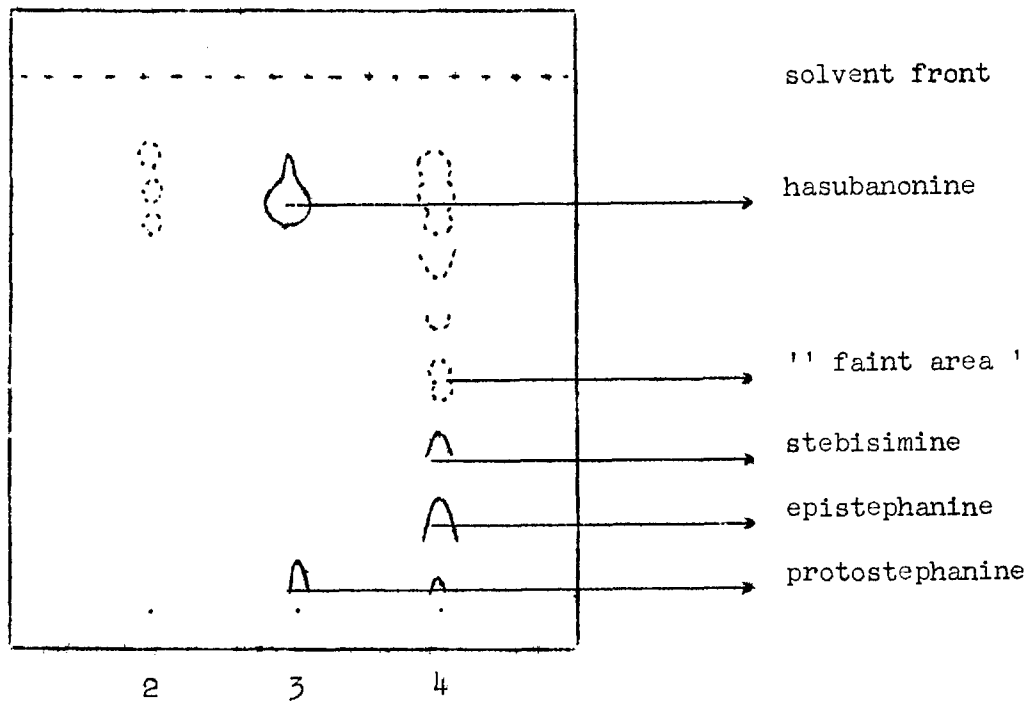
filtered, and evaporated under reduced pressure to give the total bases as a brownish syrup (0.28 g.). T.l.c. on silica $GF_{254}$ (methanol as developing solvent) showed the following distribution pattern and relative proportions of the components present in the total base fraction, e.g.




A solution of the total bases in dichloromethane (50 ml.) was washed with 3% aqueous sodium hydroxide solution (5 x 10 ml.) and water (10 ml.). The dichloromethane layer was dried ($K_2CO_3$) and evaporated under

reduced pressure to give the total non-phenolic bases (0.18 g.). The combined aqueous sodium hydroxide solution gave, after work-up, the total phenolic bases (35 mg.).

The total non-phenolic bases (0.18 g.) were chromatographed on alumina (grade III, 25 g., the column was packed in anhydrous benzene) and eluted successively with benzene (fraction 1, 250 ml.), benzene-dichloromethane (1:1 v/v, fraction 2, 250 ml.), dichloromethane (fraction 3, 250 ml.), dichloromethane-ethyl acetate (1:1 v/v, fraction 4, 250 ml.), and ethyl acetate (fraction 5, 250 ml.). T.l.c. on silica $GF_{254}$ (MeOH as developing solvent) of the syrupy products obtained from the work-up of fractions 1 to 5 individually, showed the following component distribution:



The syrupy product (63 mg.) obtained from fraction 3 was applied to a silica $GF_{254}$ thick layer plate (65 g. adsorbent). The two components were separated preparatively (MeOH as the developing solvent) and isolated individually by eluting the separate blocks with methanol, containing a few drops of diethylamine. The product (51 mg.), obtained from the uppermost block, was chromatographed on alumina III (8 g.) and eluted with benzene-chloroform (2:1 v/v) to give syrupy hasubanonine (5; R = Me) (31 mg.). This syrup in absolute ethanol (2 ml.) was treated dropwise with saturated ethanolic picric acid solution to give hasubanonine picrate which, after four recrystallizations from absolute ethanol, had m.p. and mixed m.p. 209-211° (dec.) [lit.,[5] m.p.210° (dec.)]. A chloroform solution of hasubanonine picrate was applied to an alumina grade III (8 g.) column and eluted with ethanol to give syrupy hasubanonine (14 mg.), $\lambda_{max.}^{EtOH}$ 266 mμ ( ε 10,700); τ 3.28 (s., 2H), 5.94 (s., 3H, $OCH_3$), 6.09 (s., 3H, $OCH_3$), 6.22 (s., 3H, $OCH_3$), 6.37 (s., 3H, $OCH_3$), 7.46 (s., 3H, $NCH_3$), and an A B quartet at 6.67 (1H, d., J = 16 c/s), 7.27 (1H, d., J = 16 c/s); m/e 373 ($M^+$), 358, 342, 315 (base peak), 314, 284, 258, 245, 244, 243, 230, and 213 [identical with the mass spectrum of authentic[56] hasubanonine; however, the

published[148] mass spectrum of hasubanonine shows the base peak at m/e 245. In addition, "hasubanonine methanolate", recovered from the hasubanonine picrate mother-liquors, has $M^+$ at m/e 405 and the base peak at m/e 245. It is conceivable that two different, temperature dependent, fragmentation pathways are operative]. Furthermore, hasubanonine hydrobromide had m.p. 206° (MeOH - $Et_2O$) [lit.,[5] m.p.207-208° (dec.)], $\nu_{max.}$ (Nujol) 2400-2500 ($\overset{+}{N}H$), 1680 (α,β-unsat. C = O), and 1630 (C = C) $cm.^{-1}$; hasubanonine methiodide had m.p. 177-179° ($Me_2CO$-$nC_6H_{14}$) [lit.,[5] m.p.177-178°], $\lambda_{max.}^{MeOH}$ 267 mμ.

Similarly the syrupy product (58 mg.) originally obtained from fraction 4 (see above) was applied to a second silica $GF_{254}$ thick layer plate. The individual components (see below) were isolated by eluting the appropriate blocks with methanol. The combined product, eluted from the two lowermost blocks, gave, after chromatography an alumina grade III (8 g.), protostephanine (1) (6 mg.) which was directly compared with an authentic[56] specimen (identical t.l.c. and mixed t.l.c.; identical i.r.'s in chloroform); protostephanine picrate had m.p. and mixed m.p. 207-209° (lit.,[2] m.p.209°). The free base (from a separate isolation) had $\tau$[11] 3.21 (s., 1H), 3.28 (s., 1H), 3.49 (1H, d., J = 2.5 c/s), 3.61 (d., 1H,

J = 2.5 c/s), 6.06 (s., 3H, $OCH_3$), 6.14 (s., 6H, 2 x $OCH_3$), 6.18 (s., 3H, $OCH_3$), and 7.68 (s., 3H, $NCH_3$).

Isolation of the product from the epistephanine block gave the syrupy base (24 mg.), which, after chromatography on alumina grade III, crystallized from methanol. Direct comparisons (identical t.l.c. and mixed t.l.c.; m.p. and mixed m.p. 188-206°; identical i.r.'s in both chloroform and Nujol) with an authentic[56] specimen of epistephanine (2; R = Me) were made. Also, stebisimine (48), freshly chromatographed on alumina, was identical (i.r.'s in chloroform) with the diimine which previously was isolated from the Indian Stephania japonica.

Finally, a product mixture (ca. 5 mg.) of two slightly resolved components was eluted from the "faint area" (see above). This mixture had molecular ion peaks $M_1^+$ at m/e 373 and $M_2^+$ at m/e 371 respectively. The ultraviolet spectrum of the mixture ($\lambda_{max.}$ 266 mμ) resembles that of hasubanonine but differs from those of salutaridine, isosinomenine,[122] and the dienone[76] (55; R = Me; $M^+$ at m/e 371).

## Degradative procedures

### Sodium in liquid ammonia reduction of epistephanine (2; R = Me)

Liquid ammonia (ca. 30 ml.) was distilled from sodium

metal directly into the reaction flask. Epistephanine (26 mg.) in anhydrous toluene (10 ml.) was added dropwise, with vigorous stirring, followed by small pieces of freshly cut sodium metal (ca. 10 mg.). The stirring was continued for an additional three hours after complete addition. The liquid ammonia was allowed to evaporate overnight. The residue was taken up in water (10 ml.) and the alkaline solution washed with ether (2 x 5 ml.). The aqueous phase was acidified (pH 2), then basified with sodium carbonate and extracted with chloroform (4 x 10 ml.) to give a yellowish oily product (24 mg.). T.l.c. (alumina, EtOAc) showed two components, the $R_F$-values of which matched those of the synthetic mono-bases.

The mixture in methanol (0.5 ml.) was treated with excess ethereal diazomethane and left at room temperature for 48 hours (t.l.c. control). The solvent and excess reagent were evaporated under reduced pressure. The residue (25 mg.) was chromatographed on alumina III (3 g.) and eluted with benzene-chloroform (7:3 v/v) to give firstly, syrupy (-)-NOO-trimethylcoclaurine[19] (106) (9 mg.), base peak[149] at m/e 206; methiodide, m.p. 132-134° (sinters at 125°) [lit.,[19] m.p.134-136° (sinters at 123°)]; and secondly (±)-OO-dimethylcoclaurine (105)(6 mg.), base peak[149] at m/e 192; oxalate, m.p.205-207° (lit.,[104] m.p. 206-207°).

O-Acetylhasubanol (119)

Hasubanonine methiodide (35.6 mg.) in methanol (5 ml.) and 5% methanolic potassium hydroxide solution (3 ml.) was heated, under nitrogen, on the steam-bath for one hour (u.v. control). The reaction mixture was cooled and treated with solid carbon dioxide. The insoluble inorganic salts were filtered off, and the filtrate evaporated, in the cold, under reduced pressure to give chromatographically pure hasubanonine methine[5,28,150] (23 mg.). This labile methine, anhydrous sodium acetate (11 mg.), and acetic anhydride (0.5 ml.) were heated at 170-180° (furnace temperature) in a sealed and nitrogen-filled Carius tube for two hours. The reaction mixture was poured into water (10 ml.) and the heterogeneous mixture heated on the steam-bath for ten minutes. The acetic acid was neutralized with solid sodium hydrogen carbonate and the solution extracted with ethyl acetate (3 x 15 ml.). The combined ethyl acetate extract was washed with water (15 ml.), dried ($Na_2SO_4$) and evaporated under reduced pressure to give impure O-acetylhasubanol (18.3 mg.), which was chromatographed on alumina (5 g.) and eluted rapidly with benzene-chloroform (9:1 v/v) to give O-acetylhasubanol[29] (119) which, crystallized from methanol, had m.p. 123° (lit.,[5] m.p.123-124°), $\nu_{max.}$

1750 cm.$^{-1}$ (phenolic OAc), and $M^+$ peak at m/e 326 ($C_{19}H_{18}O_5$) with peaks at m/e 285 and 284 (base peak).

## Tracer Experiments

### Counting techniques

Both [$^{14}C$] and [$^{3}H$]-containing specimens were assayed by liquid-scintillation techniques. The radio-active sample was dissolved in anhydrous dimethylformamide (0.1 ml.). Nuclear Enterprises Ltd. scintillator type NE 213 (1.2 ml.) was then added and homogeneity of the mixture assured. All related samples were counted at the same time and in duplicate. The counting procedure was calibrated using either standard [1,2 -$^{3}H_2$]- or [1-$^{14}C$]n-hexadecane, with efficiencies of ca. 20% and 75% respectively being customary. Any substantial quenching was corrected by internal calibration.

### Method of feeding

All the feeding experiments were by the "wick method". The precursor was dissolved in water (3 ml.) with the aid of a trace of 1N-hydrochloric acid solution (pH of the aqueous precursor solution 5-7). An untreated cotton thread was run through the stem of the plant and then drawn back and forth to sever the tissues. The

ends of the thread were placed in the precursor solution, contained in a small poly-topped vial. Usually the precursor solution was divided between three or four vials. After all the solution had passed into the plant, water was added to the vial and allowed to pass *via* the wick into the plant. This washing-in procedure was repeated several times.

*The alkaloids from (±)-[2-$^{14}$C]tyrosine*

(±)-[2-$^{14}$C]Tyrosine (0.02 mc) was administered (28th October, 1964) to a *Stephania japonica* plant. After seven days the plant (167 g. wet weight) was worked up to give the total non-phenolic bases (0.3 g., 3% incorporation of the activity fed). From these hasubanonine (35.4 mg.), epistephanine [10.8 mg., which was diluted with inactive epistephanine (41 mg.) and chromatographed to give purified epistephanine (45.5 mg.)], and protostephanine [9.7 mg., which, after dilution with inactive protostephanine (27 mg.) and chromatography, gave purified, crystalline protostephanine (24.2 mg.)] were isolated.

| | Specific activity (μc/mmole) |
|---|---|
| Hasubanonine (35.4 mg., after recrystallization of hasubanonine picrate twice from ethanol and chrom.of the base recovered from its picrate) | $2.96 \times 10^6$ |
| Hasubanonine methiodide | $3.20 \times 10^6$ |
| Recrystallized | $3.15 \times 10^6$ |
| Recrystallized | $3.05 \times 10^6$ |
| Incorporation: | 0.64% |

| | Specific activity (dis./100 sec/mg.) |
|---|---|
| Epistephanine (45.5 mg.) | 2680 |
| Recrystallized | 2860 |
| Mother-liquor | 2820 |
| Converted into dihydrochloride and recrystallized | 2270 |
| Epistephanine recovered from the dihydrochloride, chromatographed and crystallized | 2800 |
| Incorporation: | 0.17% |

The epistephanine (11.93 mg.) was recounted in 1966 (2650 dis./100 sec/mg.), then diluted with inactive epistephanine (11.89 mg.) and recrystallized [19 mg.; 1312 dis./100 sec/mg. ($7.95 \times 10^5$ dis./100 sec/mmole). Calculated: 1326 dis./100 sec/mg. ($8.035 \times 10^5$ dis./100 sec/mmole)]. The epistephanine was then cleaved[19] with sodium in liquid ammonia to give, after O-methylation of the product mixture (17 mg.) with diazomethane, and separation of the two components,

(±)-OO-dimethylcoclaurine (4 mg.), 1220 dis./100 sec/mg. ($3.82 \times 10^5$ dis./100 sec/mmole, 47.5% of the total activity); converted into the oxalate and recrystallized, 1120 dis./100 sec/mg. ($4.015 \times 10^5$ dis./100 sec/mmole, 50% of the total activity),

and

(-)-NOO-trimethylcoclaurine (8 mg.), 1183 dis./100 sec/mg. ($3.873 \times 10^5$ dis./100 sec/mmole, 48.2% of the total activity); converted into the methiodide and recrystallized, 867 dis./100 sec/mg. ($4.071 \times 10^5$ dis./100 sec/mmole, 51% of the total activity).

| | Specific activity (dis./100 sec/mg.) |
|---|---|
| Protostephanine (24.2 mg.) | 1190 |
| Recrystallized | 1250 |
| Mother-liquor | 1230 |
| Incorporation: | 0.044% |

The alkaloids from the duplicate (±)-[2-$^{14}$C]tyrosine feeding experiment

(±)-[2-$^{14}$C]Tyrosine (0.02 mc) was administered (8th July, 1965) to a Stephania japonica plant. After eleven days the plant (107 g., wet weight) was worked up to give the total non-phenolic bases (0.3 g., 6910 dis./100 sec/mg., 2.8% incorporation) and subsequently the individual alkaloids.

| | Specific activity (dis/100 sec/mg.) |
|---|---|
| Hasubanonine fraction (0.1 g.) | 21600 |
| Converted into hasubanonine picrate, recrystallized and the base recovered from its picrate (38 mg.) | 26600 |

| | Specific activity (dis./100 sec/mg.) |
|---|---|
| Converted again into hasubanonine picrate, recrystallized and the base recovered from its picrate | 33300 ($1.24 \times 10^7$ dis./100 sec/mmole) |
| Converted into hasubanonine hydrobromide and recrystallized twice from MeOH - $Et_2O$ | 27290 ($1.24 \times 10^7$ dis./100 sec/mmole) |

Incorporation: 1.7%

The hasubanonine hydrobromide (12.1 mg.; $2.729 \times 10^4$ dis./100 sec/mg.) was diluted with inactive hasubanonine hydrobromide (83.3 mg.) and recrystallized from MeOH - $Et_2O$ [92.5 mg., $3.38 \times 10^3$ dis./100 sec/mg. ($1.535 \times 10^6$ dis./100 sec/mmole); calculated: $3.46 \times 10^3$ dis/100 sec/mg.]. The free base (78 mg.), recovered from the hydrobromide, was converted into its methiodide (67 mg.) and recrystallized from $Me_2CO$-$\underline{n}C_6H_{14}$ [2.71 x

$10^3$ dis./100 sec/mg. ($1.40 \times 10^6$ dis./100 sec/mmole)]. Hofmann degradation of hasubanonine methiodide (41.3 mg.) gave the syrupy methine (29.1 mg.), which was acetolysed to give impure O-acetylhasubanol (26.6 mg.). Chromatography and recrystallization from methanol gave O-acetylhasubanol (119), m.p.122°, $1.93 \times 10^3$ dis./100 sec/mg.; recrystallized, $1.91 \times 10^3$ dis./100 sec/mg. ($0.623 \times 10^6$ dis./100 sec/mmole, 41% of the total activity).

Epistephanine, initially eluted from the thick layer plate, was diluted with inactive epistephanine (24 mg.) and chromatographed to give the syrupy base (44.3 mg., $2.15 \times 10^3$ dis./100 sec/mg.) which crystallized and was recrystallized from methanol: $1.374 \times 10^3$ dis./100 sec/mg.; recrystallized twice from methanol: $1.40 \times 10^3$ dis./100 sec/mg.

Incorporation: 0.084%.

Hasubanonine and protostephanine from (±)-[2',6'-$^3H_2$]1-(3'-hydroxy-4'-methoxybenzyl)-6,8-dimethoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline (54)

The proposed precursor (0.183 mc) was fed (26th September 1965) to a S.japonica plant. After nine days the whole plant (217 g., wet weight) was worked up to give the total non-phenolic bases ($0.33$ g., $1.312 \times 10^4$

dis./100 sec/mg., 0.63% incorporation) and subsequently the two individual alkaloids.

| | Specific activity (dis./100 sec/mg.) |
|---|---|
| Hasubanonine fraction (103 mg.) | 1200 |
| Converted into hasubanonine picrate, recrystallized twice from ethanol, and the base recovered from its picrate | 0.0 |

Incorporation: none

The protostephanine fraction (1020 dis./100 sec/mg.) was diluted with inactive protostephanine (25 mg.) and separated on an alumina column from admixed epi-stephanine. The protostephanine (312 mg.) was converted into its picrate and then recrystallized twice from methanol.

| | |
|---|---|
| Protostephanine recovered from picrate | 46 |
| Protostephanine recovered from the picrate mother-liquors | 45 |

Incorporation: 0.0002% (negligible)

Hasubanonine and protostephanine from (±)-[N-methyl-$^{14}$C]1-(3'-hydroxy-4'-methoxybenzyl)-2-methyl-6,8-dimethoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline (54; NMe)

The proposed precursor (0.0157 mc) was administered (4th September 1965) to a S.japonica plant. After nine days the whole plant (200 g., wet weight) was worked up to give the total non-phenolic bases (0.37 g., $9.23 \times 10^2$ dis./100 sec/mg., 0.6% incorporation) which was chromatographed on alumina as usual. The original dichloromethane fraction (fraction 3) was diluted with inactive protostephanine (26 mg.) and then separated preparatively on a silica gel thick layer plate from hasubanonine.

| | Specific activity (dis./100 sec/mg.) |
|---|---|
| Protostephanine chromatographed (28 mg.) | 0.0 |
| Converted into protostephanine picrate, recrystallized from methanol, and the base recovered | 5.0 |

Incorporation: none

| | |
|---|---|
| Hasubanonine chromatographed (89 mg.) (0.01% incorp.) | 63 |

| | Specific activity (dis./100 sec/mg.) |
|---|---|
| Converted into hasubanonine picrate, recrystallized from ethanol, and base recovered (46 mg.) | 38 |
| Converted into hydrobromide and recrystallized twice from MeOH-$Et_2O$ (44 mg., m.p.206-208°) | 25 |

Incorporation: 0.002% (negligible)

Hasubanonine and protostephanine from (±)-[2',6',8-$^3H_3$]-reticuline[151] (59)

The precursor (0.147 mc) was fed (10th June 1965) to a S.japonica plant. After seven days the whole plant (69 g., wet weight) was worked up to give the total non-phenolic bases (0.16 g.) and the total phenolic bases (36 mg., 2.38 x $10^6$ dis./100 sec/mg.). The total non-phenolic bases were chromatographed on alumina. The combined product, obtained from the dichloromethane and dichloromethane-ethyl acetate fractions, was applied to a silica gel thick layer plate and the components were separated preparatively.

| | Specific activity (dis./100 sec/mg.) |
|---|---|
| Hasubanonine chromatographed (38 mg.) | $5.13 \times 10^4$ |
| Converted into hasubanonine hydrobromide and recrystallized | $3.21 \times 10^4$ |
| Recrystallized | $1.84 \times 10^4$ |
| Diluted with inactive hasubanonine hydrobromide (9.5 mg.) and recrystallized | $7.35 \times 10^3$ |
| The hydrobromide successively was recrystallized five times from MeOH-$Et_2O$: 3980, 2730, 1850, 1310, and 160 dis./100 sec/mg. | |

Incorporation: <0.001% (negligible)

| | |
|---|---|
| Protostephanine, diluted with inactive protostephanine (16.5 mg.) and chromatographed (22.6 mg.) | 1830 |
| Converted into protostephanine picrate, recrystallized from MeOH and the base recovered (15 mg.) | 0.0 |

Incorporation: none

| | |
|---|---|
| The product (3.2 mg.) recovered from the "faint area" (see general isolation procedure) | $1.22 \times 10^5$ |

Hasubanonine from (-)-[1-$^{3}$H]sinoacutine[151] (69)

The precursor (0.046 mc) was administered (13th May 1965) to a S.japonica plant. After seven days the whole plant (88 g., wet weight) was worked up to give the total bases (0.28 g., $4.743 \times 10^{4}$ dis./100 sec/mg., 7.91% incorp.), which were separated into the total non-phenolic bases (0.18 g., $7.175 \times 10^{4}$ dis./100 sec/mg.) and the total phenolic bases (35.2 mg., $8.34 \times 10^{5}$ dis./100 sec/mg.). The total non-phenolic bases were chromatographed on alumina. The products, obtained from the dichloromethane (63.4 mg., $8.60 \times 10^{4}$ dis./100 sec/mg.) and the dichloromethane-ethyl acetate (58 mg., $2.9 \times 10^{5}$ dis./100 sec/mg.) fractions, were applied to two silica gel thick layer plates and the components separated preparatively.

| | Specific activity (dis./100 sec/mg.) |
|---|---|
| Hasubanonine chromatographed (31.2 mg., 1.52% incorp.) | $8.242 \times 10^{4}$ |
| Converted into hasubanonine picrate, recrystallized from methanol and the base recovered (12.7 mg.) | $2.46 \times 10^{4}$ |
| Base recovered from the picrate mother-liquors (14.3 mg.) | $1.334 \times 10^{5}$ |

| | Specific activity (dis./100 sec/mg.) |
|---|---|
| Product oxidized with alkaline ferricyanide and chromatographed (10.7 mg.) | $1.86 \times 10^4$ |
| Re-oxidized with alkaline ferricyanide (7.9 mg.) | $8.26 \times 10^3$ |
| Reconverted into hasubanonine picrate (20 mg.), diluted with inactive hasubanonine picrate (24 mg.), recrystallized from absolute ethanol (37.6 mg.) and the base recovered (23.7 mg.) | 8070 |
| Reconverted again into hasubanonine picrate, recrystallized from abs. ethanol (31 mg.picrate), and the base recovered (23 mg.) | 2050 |
| Converted into hasubanonine hydrobromide and recrystallized from MeOH-$Et_2O$ (19.4 mg.) | 520 |
| Recrystallized | 371 |
| Recrystallized | 210 |

Incorporation: < 0.004%

| | |
|---|---|
| The product (5.4 mg.) eluted from the "faint area" on the thick layer plate of fraction 4 | $2.88 \times 10^6$ |

Epistephanine (2; R = Me) from (±)-[3',5',8-$^3H_3$]-coclaurine[110] (107)

The precursor ($4.38 \times 10^5$ dps./mg., 10.4 mg.; 0.0123 mc) was administered (20th June 1965) to a S.japonica plant. After eight days the whole plant (36 g., wet weight) was worked up to give the total non-phenolic bases (88 mg., $4.82 \times 10^4$ dis./100 sec/mg.) and the total phenolic bases (14 mg., $3.3 \times 10^6$ dis./100 sec/mg.). The total non-phenolic bases were diluted with inactive epistephanine (22.8 mg.). The components of the crude alkaloid mixture were separated preparatively on a silica gel thick layer plate (the usual preliminary chromatography of the total non-phenolic bases was discarded in this and in subsequent feeding experiments).

| | Specific activity (dis./100 sec/mg.) |
|---|---|
| Epistephanine chromatographed (27.8 mg.) | 5296 |
| Crystallized and recrystallized from methanol | 1111 |

| | Specific activity (dis./100 sec/mg.) |
|---|---|
| Recrystallized | 833 |
| Recrystallized | 920 |
| Mother-liquors | 895 |
| Converted into dihydrochloride and recrystallized | 895 |

Incorporation: 0.0084%

Epistephanine (2; R=Me) from (±)-[N-methyl-$^{14}$C]N-methylcoclaurine[108] (108)

The precursor (9.7 mg., 7.17 x $10^4$ dps/mg., 0.0188 mc) was fed (7th July 1965) to a S.japonica plant. After nine days the whole plant (67 g., wet weight) was worked up to give the total non-phenolic bases (178 mg.) and the total phenolic bases (26 mg., 3.72 x $10^5$ dis./100 sec/mg.). The total non-phenolic bases were diluted with inactive epistephanine (27 mg.). The components of the crude alkaloid mixture were separated preparatively on a silica gel thick layer plate.

| | Specific activity (dis./100 sec/mg.) |
|---|---|
| Epistephanine chromatographed (40 mg.) | 2500 |
| Crystallized and recrystallized from methanol | 639 |

| | Specific activity (dis./100 sec/mg.) |
|---|---|
| Recrystallized twice from MeOH | 676 |

Incorporation: 0.05%

| | |
|---|---|
| The epistephanine (6.46 mg.) was diluted with inactive epistephanine (17.42 mg.) and converted into epistephanine dihydrochloride | 160.4 ($1.09 \times 10^5$ dis./100 sec/mmole |
| Calculated | 163 ($1.11 \times 10^5$ dis./100 sec/mmole |
| Triethylmethylammonium iodide | 441 ($1.07 \times 10^5$ dis./100 sec/mmole |

NMe: 97% of the total activity

Epistephanine (2; R=Me) from (+)-[8,3',5'-$^3H_3$]N-methylcoclaurine[110] and (-)-[8,3',5'-$^3H_3$]N-methylcoclaurine[110] (109)

Two matched S.japonica plants were chosen. To one was fed (29th August 1965) (+)-[8,3',5'-$^3H_3$]N-methylcoclaurine (7.25 mg., $4.45 \times 10^5$ dps./mg.; 0.0872 mc). To the other plant was administered (29th August 1965)

(-)-[8,3',5'-$^{3}H_{3}$]N-methylcoclaurine (6.06 mg., 4.05 x $10^{5}$ dps./mg.; 0.066 mc). Both plants were sacrificed after nine days and worked up separately.

The whole plant (130 g., wet weight) from the (+)-enantiomer feeding experiment gave, after the usual work-up procedure, the total non-phenolic bases (0.3 g.) which were diluted with inactive epistephanine (33.5 mg.). The epistephanine was separated preparatively from the other components on a silica gel thick layer plate.

| | Specific activity (dis./100 sec/mg.) |
|---|---|
| Epistephanine chromatographed (32 mg.) | 6510 |
| Crystallized and recrystallized from methanol | 307 |
| Recrystallized twice from MeOH | 220 |
| Mother-liquors | 215 |

Incorporation: 0.003%

The whole plant (150 g., wet weight) from the (-)-enantiomer feeding experiment gave, as usual, the total non-phenolic bases (0.32 g.) which were diluted with inactive epistephanine (32 mg.). The epistephanine was separated preparatively from the other components on a silica gel thick layer plate.

| | Specific activity (dis./100 sec/mg.) |
|---|---|
| Epistephanine chromatographed (35.5 mg.) | 16620 |
| Crystallized and recrystallized from MeOH | 2120 |
| Recrystallized twice from MeOH | 2800 |
| | ($1.7 \times 10^6$ dis./100 sec/mmole) |
| Evaporated mother-liquors | 2780 |
| Converted into epistephanine dihydrochloride and recrystallized from EtOH-$Et_2O$ | 2480 |
| | ($1.69 \times 10^6$ dis./100 sec/mmole) |

Incorporation: 0.06%

The epistephanine dihydrochloride (6.5 mg.) was diluted with inactive epistephanine dihydrochloride (10.9 mg.) and recrystallized from ethanol-ether (1110 dis./100 sec/mg.). The epistephanine (9.4 mg., 1310 dis./100 sec/mg. or $7.94 \times 10^5$ dis./100 sec/mmole; calculated: 1240 dis./100 sec/mg. or $7.52 \times 10^5$ dis/100 sec/mmole), recovered from the dihydrochloride and chromatographed, was cleaved[19] with sodium (ca. 3 mg.)

in liquid ammonia (ca. 30 ml.) to give, after O-methylation of the product mixture (5.7 mg.) and quantitative separation of the two components:

(±)-OO-dimethylcoclaurine[104] (105)(1.89 mg.), 0.0 dps/mg.; converted into oxalate, 0.4 dps./mg.

and

(-)-NOO-trimethylcoclaurine[19] (106)(1.3 mg.), 2192 dis./100 sec/mg. or $7.17 \times 10^5$ dis./100 sec/mmole (95% of the original total activity, e.g. $7.52 \times 10^5$ dis./100 sec/mmole).

The residue from the evaporated methanolic mother-liquors (21.2 dps./mg., see above) was diluted with inactive stebisimine (27.5 mg.) and chromatographed. The syrupy product mixture of stebisimine and epistephanine was diluted with more inactive stebisimine (15.3 mg.) and crystallized from methanol.

| | Specific activity (dis./100 sec/mg.) |
|---|---|
| Stebisimine | 106 |
| Recrystallized | 68 |
| Recrystallized | 74 |

Incorporation: 0.0013% (negligible)

## V. REFERENCES


1. M. Tomita and T. Ibuka, J.Pharm.Soc.Japan, 1963, 83, 996.
2. H. Kondo and T. Sanada, J.Pharm.Soc.Japan, 1927, 47, 177.
3. H. Kondo and T. Sanada, J.Pharm.Soc.Japan, 1924, 44, 1034.
4. H. Kondo and T. Sanada, J.Pharm.Soc.Japan, 1928, 48, 1141.
5. H.Kondo, M. Satomi, and T. Odera, Ann.Rep.ITSUU Lab., 1951, 2, 35.
6. M. Tomita, T. Ibuka, and Y Inubushi, Tetrahedron Letters, 1964, 3617.
7. M. Satomi, Ann.Rep.ITSUU Lab., 1955, 6, 31.
8. M. Tomita, Y. Watanabe, and M. Fuse, J.Pharm.Soc. Japan, 1957, 77, 274.
9. M. Tomita and T. Ibuka, J.Pharm.Soc.Japan, 1965, 85, 557.
10. K. Takeda, Bull.Agric.Chem.Soc.Japan, 1956, 20, 165.
11. K. Takeda, Ann.Rep.ITSUU Lab., 1963, 13, 45.
12. H. Kondo and K. Tanaka, J.Pharm.Soc.Japan, 1943, 63, 267.

13. H. Kondo and K. Tanaka, J.Pharm.Soc. Japan, 1943, 63, 273.

14. M. Tomita and Y. Watanabe, Pharm.Bull.(Japan), 1956, 4, 124.

15. E. Fujita, J.Pharm.Soc. Japan, 1952, 72, 213 and 217.

16. W.M. Whaley and C.N. Robinson, J.Amer.Chem.Soc., 1953, 75, 2008.

17. E. Fujita and T. Saijoh, J.Pharm.Soc.Japan, 1952, 72, 1232.

18. Y. Watanabe, J.Pharm.Soc.Japan, 1960, 80, 166.

19. M. Tomita and E. Fujita, Pharm.Bull.(Japan), 1954, 2, 378.

20. H. Kondo and T. Nozoe, J.Pharm.Soc.Japan, 1943, 63, 333.

21. M. Tomita and J. Kunitomo, J.Pharm.Soc.Japan, 1962, 82, 734 and 741; and references cited therein.

22. H. Corrodi and E. Hardegger, Helv.Chim.Acta, 1956, 39, 889.

23. H. Yamaguchi, J.Pharm.Soc.Japan, 1958, 78, 678.

24. L.J. Haynes, K.L. Stuart, D.H.R. Barton, D.S. Bhakuni, and G.W. Kirby, Chem.Comm., 1965, 141.

25. H. Shirai, J.Pharm.Soc.Japan, 1944, 64, 44.

26. H. Shirai and N. Oda, J.Pharm.Soc.Japan, 1956, 76, 1287.

27. M. Shamma, Experientia, 1962, 18, 64.

28. H. Kondo and M. Satomi, Ann.Rep.ITSUU Lab., 1957, 8, 41.

29. Y. Watanabe and H. Matsumura, J.Pharm.Soc.Japan, 1963, 83, 991.

30. M. Tomita, T. Ibuka, Y. Inubushi, Y. Watanabe, and M. Matsui, Tetrahedron Letters, 1964, 2937; Idem., Pharm.Bull.(Japan), 1965, 13, 538.

31. K.W. Bentley, Experientia, 1956, 12, 251.

32. S. Okuda, K. Tsuda, and S. Yamaguchi, J.Org.Chem., 1962, 27, 4121; Idem., Pharm.Bull.(Japan), 1965, 13, 1092.

33. M. Tomita and Y. Watanabe, J.Pharm.Soc.Japan, 1956, 76, 856.

34. Y. Watanabe, M. Matsui, and K. Ido, J.Pharm.Soc. Japan, 1965, 85, 584.

35. G.W. Kirby and L. Ogunkoya, J.Chem.Soc., 1965, 6914.

36. K. Takeda, Ann.Rept.ITSUU Lab., 1960, 11, 61.

37. H.L. de Waal and E. Weideman, Tydskr.Natuurwetenskappe, 1962, 2, 12.

38. Personal communication, Professor H.L. de Waal.

39. M. Tomita, T. Ibuka, Y. Inubushi, and K. Takeda, Tetrahedron Letters, 1964, 3605; Idem., Pharm. Bull.(Japan), 1965, 13, 695 and 704.

40. T. Ibuka, J.Pharm.Soc.Japan, 1965, 85, 579.

41. M. Tomita, T. Ibuka, and Y. Inubushi, Tetrahedron Letters, 1964, 3617.

42. We thank Professor S. Morris Kupchan for several authentic specimens of bisbenzylisoquinoline alkaloids.

43. H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry. Vol.I: Alkaloids" , Holden-Day Inc., London, 1964, p.173.

44. D.W. Thomas and K. Biemann, J.Amer.Chem.Soc., 1965, 87, 5447.

45. Personal communication, Dr. R.T. Aplin.

46. D.C. DeJongh, S.R. Shrader, and M.P. Cava, J.Amer. Chem.Soc., 1966, 88, 1052.

47. M. Tomita, T. Kikuchi, K. Fujitani, A. Kato, H. Furukawa, Y. Aoyagi, M. Kitano, and T. Ibuka, Tetrahedron Letters, 1966, 857; J. Baldas, Q.N. Porter, I.R.C. Bick, and M.J. Vernengo, Tetrahedron Letters, 1966, 2059.

48. A.W. Sangster and K.L. Stuart, Chem.Revs., 1965, 65, 69.

49. A.R. Battersby, I.R.C. Bick, W. Klyne, J.P. Jennings, P.M. Scopes, and M.J. Vernengo, J.Chem.Soc., 1965, 2239.

50. I.R.C. Bick, J. Harley-Mason, N. Sheppard, and M.J. Vernengo, J.Chem.Soc., 1961, 1896; Dr. I.R.C. Bick, University of Tasmania, lecture given at Imperial College on 16th February, 1966.

51. We thank Professor W. Klyne and Dr. P.M. Scopes, Westfield College, for the o.r.d. measurement.

52. M. Tomita, "Progress in the Chem. of Organic Natural Products" , Springer-Verlag, Vienna, 1952, p.175.

53. M. Tomita and H. Yamaguchi, Pharm.Bull.(Japan), 1953, 1, 10.

54. M. Tomita, T. Shingu, K. Fujitani, and H. Furukawa, Pharm.Bull.(Japan), 1965, 13, 921.

55. M. Tomita and Y. Sasaki, Pharm.Bull.(Japan), 1954, 2, 375.

56. We thank Professor M. Tomita for numerous authentic specimens.

57. A.R. Battersby, Quart.Revs., 1961, 15, 259.

58. A.R. Battersby, Tilden Lecture, Proc.Chem.Soc., 1963, 189.

59. D.H.R. Barton, Hugo Muller Lecture, Proc.Chem.Soc., 1963, 293.

60. K. Mothes and H.R. Schütte, Angew.Chem., Int.Ed., 1963, 2, 341 and 441.

61. E. Leete, "Biogenesis of Natural Compounds" , ed. P. Bernfeld, Pergamon, London, 1963, p.739.

62. E. Leete, "Technique of Organic Chemistry" , Vol.XI, ed. A. Weissberger, Interscience Publishers, New York, 1963, p.437.

63. S.F. Dyke, The Royal Inst.of Chem., Lecture Series 1965, No.1, p.22.

64. L. Kühn and S. Pfeifer, Die Pharmazie (Berlin), 1965, 20, 659.

65. H. Erdtman and C.A. Wachtmeister, Festschr.Arthur Stoll, Birkhäuser A.G., Basel, 1957, p.144.

66. D.H.R. Barton and T. Cohen, Festschr.Arthur Stoll, Birkhäuser A.G., Basel, 1957, p.117.

67. W.A. Waters, "The Chemistry of Free Radicals" , Oxford University Press, 1946.

68. C.D. Cook and N.D..Gilmour, J.Org.Chem., 1960, 25, 1429.

69. D.H.R. Barton, A.M. Deflorin, and O.E. Edwards, J.Chem.Soc., 1956, 530.

70. R. Pummerer, D. Melamed, and H. Puttfarcken, Ber., 1925, 55, 3116.

71. J.R. Lewis, Chem.and Ind.(London), 1962, 159; 1964, 1672.

72. B. Franck, G. Blaschke, and G. Schlingloff, Angew. Chem., Int.Ed., 1964, 3, 192.

73. A.I. Scott, Quart.Revs., 1965, 19, 1.

74. K.W. Bentley and R. Robinson, J.Chem.Soc., 1952, 947.

75. D.H.R. Barton, Pure and Appl.Chem., 1964, 9, 35.

76. We thank Professor A.R. Battersby for a specimen of the dienone (55; R = Me).

77. D.H.R. Barton, G.W. Kirby, W. Steglich, G.M. Thomas, A.R. Battersby, T.A. Dobson, and H. Ramuz, J.Chem.Soc., 1965, 2423.

78. D.H.R. Barton, R.H. Hesse, and G.W. Kirby, J.Chem. Soc., 1965, 6379.

79. A.R. Battersby, R.J. Francis, E.A. Ruveda, and J. Staunton, Chem.Comm., 1965, 89.

80. H-G. Boit, "Ergebnisse der Alkaloid-Chemie bis 1960", Akademie-Verlag, Berlin, 1961, p.402.

81. G. Rosenfeld, L.C. Leeper, and S. Udenfriend, Arch. Biochem.Biophys., 1958, 74, 252.

82. J. Massicot and L. Marion, Canad.J.Chem., 1957, 35, 1.

83. E. Leete, Chem.and Ind.(London), 1959, 604.

84. D.H.R. Barton, (Mrs.) A.J. Kirby, and G.W. Kirby, Chem.Comm., 1965, 52.

85. H. Flentje, W. Döpke, and P.W. Jeffs, Naturwiss., 1965, 52, 259.

86. M. Kulka, "The Alkaloids" , Vol.IV, ed. R.H.F. Manske and H.L. Holmes, Academic Press, New York, 1954, p.199.

87. H-G. Boit, "Ergebnisse der Alkaloid-Chemie bis 1960" , Akademie-Verlag, Berlin, 1961, p.230.

88. F. Faltis and H. Frauendorfer, Ber., 1930, 63, 806.

89. M. Tomita and Y. Okamoto, J.Pharm.Soc.Japan, 1965, 85, 456.

90. M. Tomita, H. Furukawa, T-H. Yang, and T-J. Lin, Tetrahedron Letters, 1964, 2637; Idem., Chem. Pharm.Bull.(Japan), 1965, 13, 39.

91. S.M. Kupchan and N. Yokoyama, J.Amer.Chem.Soc., 1964, 86, 2177.

92. C. Djerassi, T. Nakano, and J.M. Bobbitt, Tetrahedron, 1958, 2, 58.

93. M. Tomita, T. Kikuchi, and K. Bessho, Tetrahedron Letters, 1963, 127.

94. K.P. Agarwal, S. Rakhit, S. Bhattarcharji, and M.M. Dhar, J.Sci.Ind.Research, 1960, 19B, 479.

95. B. Anjaneyulu, K.W. Gopinath, T.R. Govindachari, and B.R. Pai, J.Sci.Ind.Research, 1962, 21B, 602.

96. M. Shamma, B.S. Dudock, M.P. Cava, K.V. Rao, D.R. Dalton, D.C. DeJongh, and S.R. Shrader, Chem. Comm., 1966, 7.

97. M. Shamma, M.A. Greenberg, and B.S. Dudock, Tetrahedron Letters, 1965, 3595.

98. S. Kubota, T. Masui, E. Fujita, and S. Morris Kupchan, Tetrahedron Letters, 1965, 3599; Idem., J.Org. Chem., 1966, 31, 516.

99. A.R. Battersby, R.B. Herbert, L. Pijewska, and F. Santavy, Chem.Comm., 1965, 228.

100. A.R. Battersby, R.B. Herbert, and F. Santavy, Chem. Comm., 1965, 415.

101. M. Tomita and H. Furukawa, J.Pharm.Soc.Japan, 1963, 83, 760.

102. Y. Inubushi, K. Nomura, and M. Miyawaki, J.Pharm.Soc. Japan, 1963, 83, 282.

103. I.R.C. Bick and A.R. Todd, J.Chem.Soc., 1950, 1606.

104. M. Tomita and F. Kusuda, J.Pharm.Soc.Japan, 1952, 72, 793.

105. R. Robinson, "The Structural Relations of Natural Products", Clarendon Press, Oxford, 1955.

106. R.H.F. Manske, J.Chem.Soc., 1954, 2987.

107. E. Winterstein and G. Trier, "Die Alkaloide", Borntträger, Berlin, 1910.

108. We thank Mr. G. Chapman for the N-methyl labelled specimen.

109. J. Grant, "Quantitative Organic Microanalysis, F. Pregl", J. and A. Churchill Ltd., London, 1951, p.194.

110. We thank Dr. D.S. Bhakuni for the resolved labelled specimens and for labelled coclaurine.

111. R.R. Arndt, J.Chem.Soc., 1963, 2547.

112. A.R. Battersby, D.M. Foulkes, and R. Binks, J.Chem. Soc., 1965, 3323.

113. A.R. Battersby and M. Hirst, Tetrahedron Letters, 1965, 669.

114. M. Satomi, Ann.Rep.ITSUU Lab., 1952, 3, 37.

115. S.P. Findlay and L.F. Small, J.Amer.Chem.Soc., 1951, 73, 4001.

116. E. Leete and S.J.B. Murrill, Tetrahedron Letters, 1964, 147.

117. A. R. Battersby and R.J. Francis, J.Chem.Soc., 1964, 4078.

118. A.R. Battersby, R. Binks, D.M. Foulkes, R.J. Francis, D.J. McCaldin, and H. Ramuz, Proc.Chem.Soc.(London), 1963, 203.

119. W.M. Whaley and T.R. Govindachari, "Organic Reactions", ed. R. Adams, John Wiley and Sons, New York, Vol.6, 1951, p.83.

120. L. Horner, E. Spietschka, and A. Gross, Ann., 1951, 573, 17.

121. A.R. Surrey, A. Mooradian, R.A. Cutler, C.M. Suter, and J.S. Buck, J.Amer.Chem.Soc., 1949, 71, 2421.

122. Personal communication, Mrs. A. J. Kirby.

123. D.R. Dalton, M.P. Cava, and K.T. Buck, Tetrahedron Letters, 1965, 2687.

124. M. Ohashi, J.M. Wilson, H. Budzikiewicz, M. Shamma, W.A. Slusarchyk, and C. Djerassi, J.Amer.Chem.Soc., 1963, 85, 2807.

125. L.J. Haynes, E.J. Herbert, and J.R. Plimmer, J.Chem.Soc.(C), 1966, 615.

126. W. Bradley and R. Robinson, J.Chem.Soc., 1928, 1541.

127. W.S. Fones, J.Org.Chem., 1949, 14, 1099.

128. H.C. Brown and P. Heim, J.Amer.Chem.Soc., 1964, 86, 3566.

129. Personal communication, Dr. D.A. Widdowson.

130. K.W.Merz and J. Fink, Arch.Pharm., 1956, 289, 347.

131. K.W. Gopinath, T.R. Govindachari, and N. Viswanathan, Chem.Ber., 1959, 92, 1657.

132. M.T. Bogert and J. Ehrlich, J.Amer.Chem.Soc., 1919, 41, 798.

133. W. Bradley and R. Robinson, J.Chem.Soc., 1928, 1541.

134. K. Kratzl, T. Horejschi, and G. Billek, Monatsch, 1954, 85, 1154.

135. H. Furukawa, J. Pharm.Soc.Japan, 1966, 86, 75.

136. M. Tomita, H. Furukawa, S.T.Lu, and S. Morris Kupchan, Tetrahedron Letters, 1965, 4309.

137. N.M. Mollov and H.B. Dutschewska, Tetrahedron Letters, 1966, 853.

138. J.A.D. Jeffreys, J.Chem.Soc., 1956, 4453.

139. H. Yamaguchi and K. Nakano, J.Pharm.Soc.Japan, 1959, 79, 1106.

140. M. Tomita and M. Kozuka, J.Pharm.Soc.Japan, 1964, 84, 362.

141. L. Marion, L. Lemay, and V. Portelance, J.Org.Chem., 1950, 15, 216.

142. A. Burger and S. Avakian, J.Org.Chem., 1940, 5, 606.

143. L.V. Volkova, O.N. Tolkachev, and N.A. Preobrazhenskii, Doklady Akad.Nauk.S.S.S.R., 1955, 102, 521.

144. M. Tomita, K. Nakaguchi, and S. Takagi, J.Pharm. Soc.Japan, 1950, 70, 152.

145. M. Tomita, Y. Watanabe, and H. Furukawa, J.Pharm. Soc.Japan, 1961, 81, 1644.

146. T. Kikuchi, J.Pharm.Soc.Japan, 1958, 78, 576.

147. R. Robinson and S. Sugasawa, J.Chem.Soc., 1931, 3163.

148. M. Tomita, A. Kato, and T. Ibuka, Tetrahedron Letters, 1965, 1019.

149. M. Tomita, H. Furukawa, T. Kikuchi, A. Kato, and T. Ibuka, Chem.Pharm.Bull.(Japan), 1966, 14, 232.

150. M. Satomi, Ann.Rep.ITSUU Lab., 1952, 3, 37.

151. We thank Mrs. A.J. Kirby for the labelled specimen.

152. H. Furukawa, J.Pharm.Soc.Japan, 1966, 86, 253.