ALKALOIDS OF MONIMIACEOUS PLANTS

by

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Submitted in fulfilment of the requirements
for the Degree of
Doctor of Philosophy.

UNIVERSITY OF TASMANIA,

HOBART.

October, 1965.

Except as stated herein, this thesis contains no material which has been accepted for the award of any other degree or diploma in any university, and, to the best of my knowledge, this thesis contains no copy or paraphrase of material previously published or written, except when due reference is made in the text of the thesis.

G. K. Douglas.

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SUMMARY.

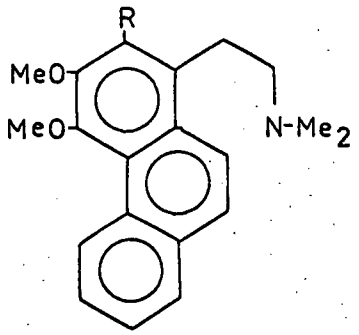
In connection with their studies on the alkaloids of Atherosperma moschatum Labill., Bick, Clezy and Crow[10] reported the isolation of three new alkaloids, atherosperminine, spermatheridine and atherospermidine, which they characterized but on which they carried out no structural investigations.

Atherosperminine has now been shown to be identical with the known alkaloid, 1-N,N-dimethylaminoethyl-3,4-dimethoxyphenanthrene[57] (i) which had been previously isolated from Cryptocarya angulata C.T.White. Spermatheridine was identified with liriodenine[64] (ii). The chemical and spectral properties of atherospermidine indicated structures (iii) or (iv) for this compound; synthetic experiments proved the former was correct.

Of the further bases isolated from A.moschatum, chemical, spectral and synthetic evidence allowed the assignment of structures (v) and (vi) to methoxyatherosperminine and atheroline, respectively. The tentative structures (vii), (viii) and (ix) were proposed for atherospermoline, spermatherine and moschatoline, respectively, on the basis of chemical and spectrographic properties.

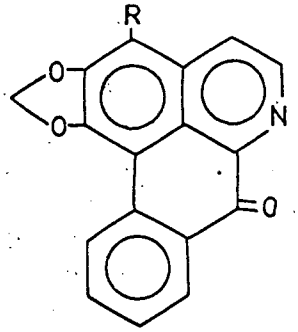
It was suggested that the yellow colour of the heartwood of A.moschatum, which restricts its use in the paper-pulp industry, was due to the presence of the yellow alkaloids spermatheridine (ii) and atherospermidine (iv).

The major alkaloid of Dryadodaphne novoguineensis (Perk.)



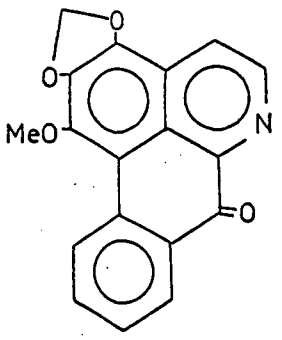
i. R = H

v. R = OMe



ii. R = H

iii. R = OMe



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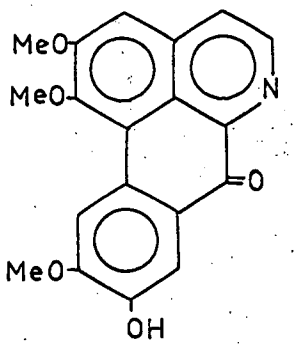

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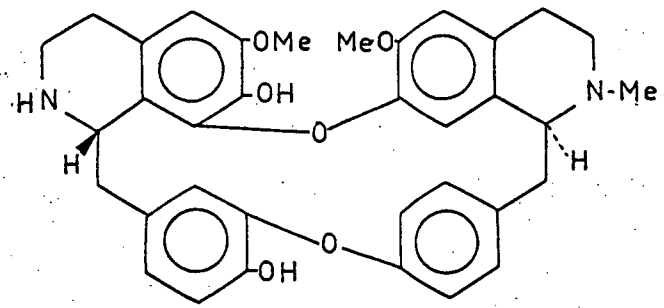

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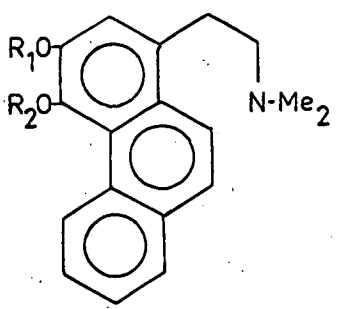
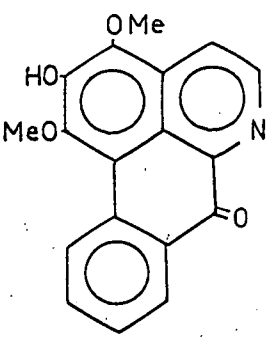


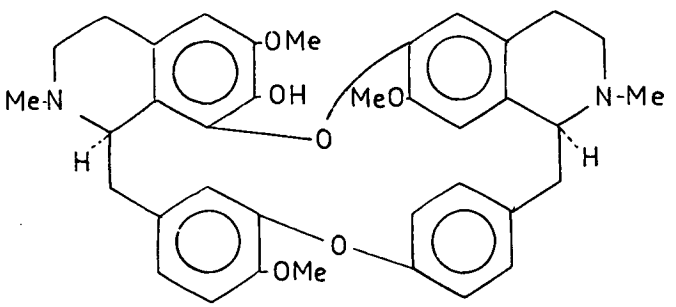
viii. R = H; R = Me or vice versa



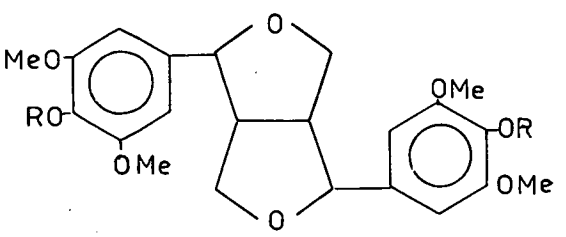
ix

Smith, dryadine, was reisolated and the Hofmann degradation of this compound was repeated. Analytical, n.m.r. and mass spectral data, together with chemical evidence which had previously been obtained[25], allowed the tentative structure (x) to be proposed for this compound.

Three minor alkaloids were isolated from this plant; one was shown to be identical with spermatheridine (ii) and one with atheroline (vi). The third, alkaloid D, was isolated in minute amount and its spectral properties indicated a novel structure.



x



xi. R = glucose

A non-alkaloidal constituent of *Atherosperma moschatum* proved identical with liriodendrine (xi), a lignan glycoside previously isolated[93] from *Liriodendron tulipifera*, L.

Some taxonomic problems associated with certain New South Wales *Daphnandra* species were investigated.

ACKNOWLEDGEMENTS.

I wish to record my heart-felt thanks to Dr.I.R.C.Bick for his stimulating discussions, encouragement and calm endurance throughout the course of these investigations.

I am particularly grateful to Dr.D.H.S.Horn (Division of Organic Chemistry, C.S.I.R.O.) who determined the majority of the n.m.r. spectra. Mr.F.P.Johnson (University of Sydney) determined the n.m.r. spectrum of O-acetylatheroline, Dr. Eugene A.Pier (Varian Associates) that of atherospermoline and Professor L.M. Jackman that of Alkaloid D; to these people I record my appreciation.

I am indebted to Dr.W.I.Taylor (CIBA) for the gift of O-methylatheroline and liriodenine, to Dr.E.E.Dickey (Institute of Paper Chemistry, Wisconsin) for the gift of liriodendrine, and to Dr.R.G.Cooke (University of Melbourne) for the gift of atherosperminine picrate and hydriodide.

I am also indebted to Professor W.Klyne who determined the O.R.D. curves for atherospermoline and dryadine.

I also wish to thank Mr.D.T.Kitchener (Australian Newsprint Mills, Ltd.) for arranging the supply of the bark and leaves of Atherosperma moschatum and Mr.J.L.Somerville (Australian Newsprint Mills, Ltd.) for arranging the supply of ground sassafras heartwood. I am grateful to Dr.C.C.J.Culvenor and colleagues of the Division of Organic Chemistry, C.S.I.R.O., who arranged for the collection and extraction of the bark of Dryadodaphne novoguineensis and the

extraction of the bark and leaves of *Atherosperma moschatum*.

I acknowledge the award of a Commonwealth Post-Graduate Scholarship and financial support from a University Post-Graduate Scholarship and the University Grants Committee.

MEMORANDUM.

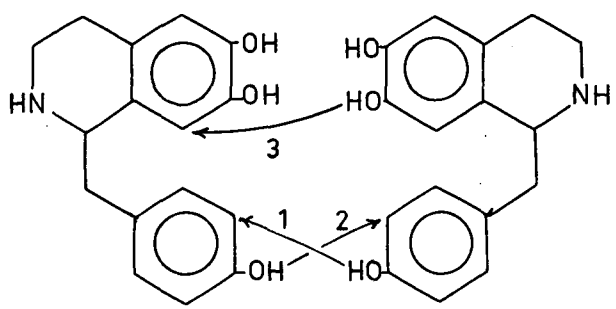
Throughout this work analytical samples were dried at 90°/0.03 mm Hg over phosphorus pentoxide for 12 hours unless stated otherwise. Microanalyses were carried out by the Australian Microanalytical Service. Infra-red Spectra were determined in nujol mulls or in chloroform solution on a Perkin-Elmer Model 221 i.r. Spectrophotometer. Ultra-violet and visible spectra were determined on a Perkin-Elmer Model 4000 A Spectrometer. N.m.r. spectra were determined on a Varian A 60 Spectrometer (unless otherwise stated) using tetramethylsilane as internal reference. The chemical shifts were expressed in parts per million on the δ-scale.

All evaporations were carried out under reduced pressure unless stated otherwise.

1. INTRODUCTION.

Alkaloid-bearing plants of the Australian Monimiaceae have generally yielded alkaloids of the bisbenzylisoquinoline type. For this reason a brief outline of the chemistry of this class of compound will be presented; a more detailed account may be found in a complete and thorough review by Grundon[1].

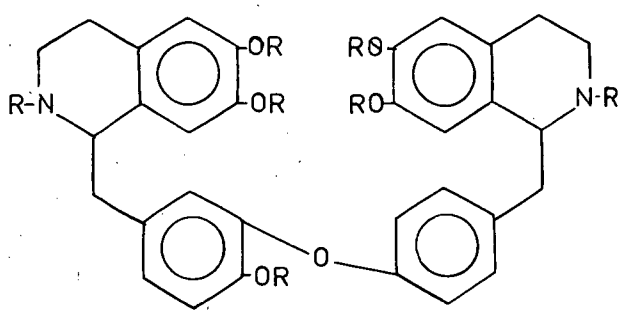
The bisbenzylisoquinoline molecule consists of two benzylisoquinoline moieties linked by one, two or three oxygen atoms; these ether bridges are believed to arise by intermolecular dehydrogenation of two molecules of 1-(4-hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline[13] in which the new C-O bond is formed by phenolic coupling at nuclear positions ortho to hydroxyl groups. The route which the dehydrogenation follows leads to the classification of bisbenzylisoquinoline alkaloids into several different classes.



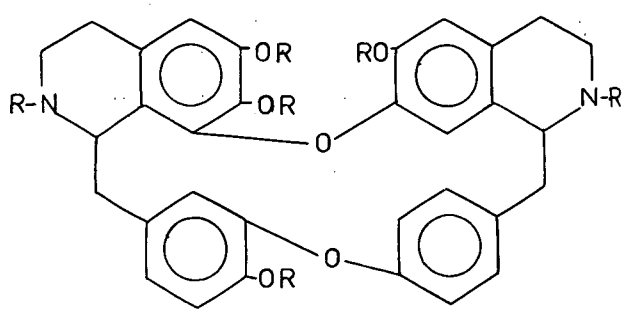
Scheme 1.I

Dehydrogenation via pathway 1 (Scheme 1.I) leads to the structure (1.I) containing one diphenylether linkage (dauricine class); the

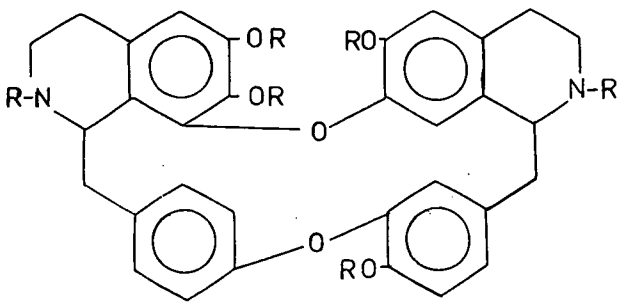
formation of two ether bridges by pathways 1 and 3 or by pathways 2 and 3 leads to structure (1.II) (berbamine class) and structure (1.III) (oxyacanthine class) respectively. In all three molecules the two isoquinoline portions and/or the two benzyl portions are linked ("head-to-head" linkage).



1.I

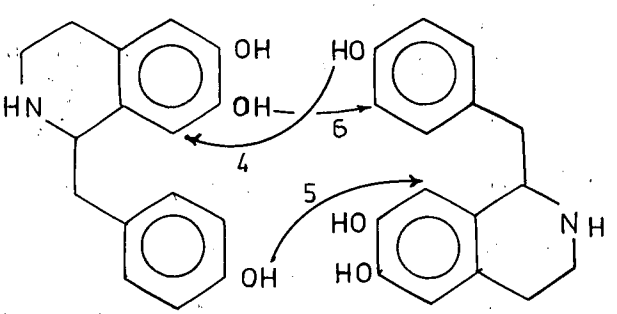


1.II



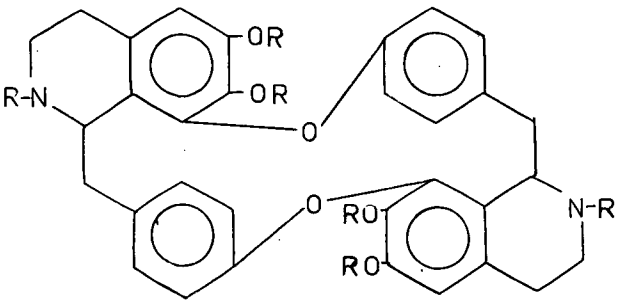
1.III

An alternative method of linkage is illustrated in Scheme 1.2. Here an isoquinoline and benzyl portion are linked by ether bridges ("head-to-tail" linkage).

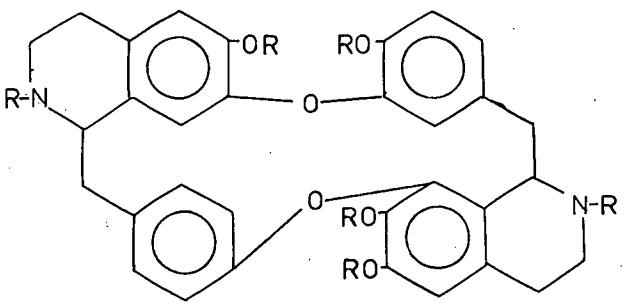


Scheme 1.2

Coupling via pathways 4 and 5 leads to the structurally symmetrical molecule 1.IV (isochondrodendrine class) whilst that by pathways 5 and 6 gives the unsymmetrical structure 1.V (curine class).

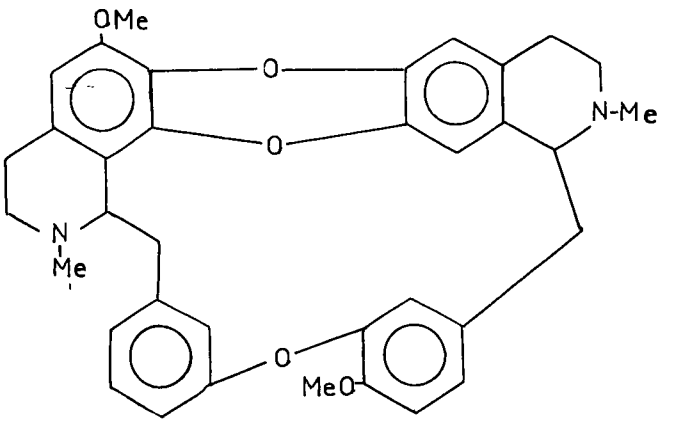


1.IV

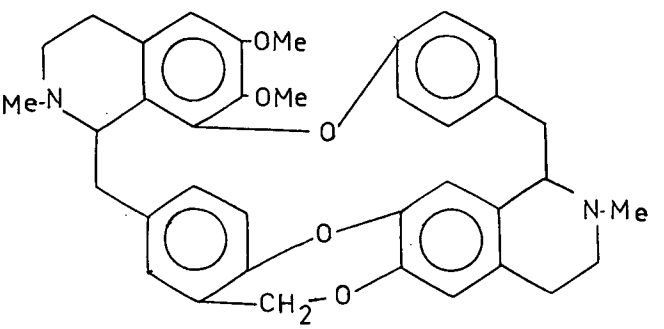


1.V

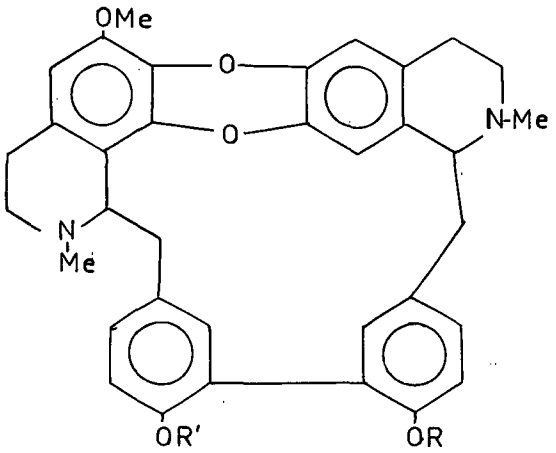
A small number of bisbenzylisoquinoline bases have been shown to contain three diaryl ether bridges, two of which are contained in a dibenzo-1,4-dioxin system, e.g., N-methyltrilobine[2](1.VI). Insularine[3] (1.VII) contains three ether bridges, two of which are in a depsidan nucleus whilst tiliacorine[4] (1.VIII) contains a dibenzo-1,4-dioxin system and the benzylic portions are linked directly rather than through the usual ether bridge.



1.VI

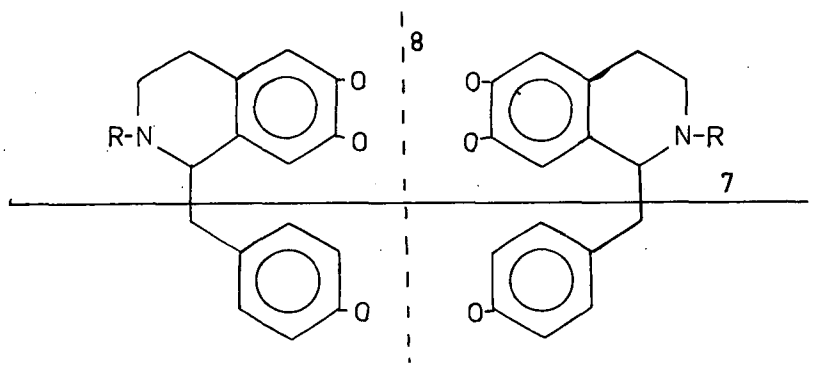


1.VII



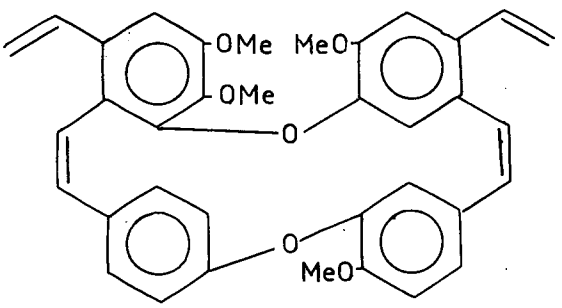
1, VIII (R = H, R' = Me or vice versa)

The structural investigation of a bisbenzylisoquinoline by chemical means involves splitting the molecule horizontally (pathway 7 in Scheme 1.3) with the preservation of the ether bridges and vertically (pathway 8 in Scheme 1.3) in which the ether bridges are broken.

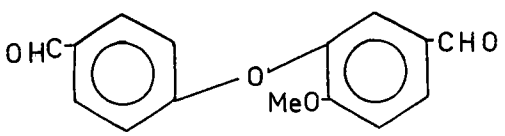


Scheme 1.3

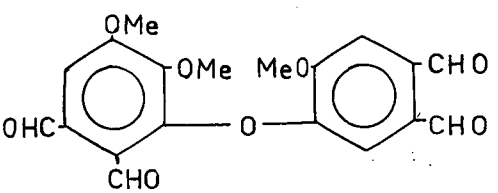
The horizontal cleavage is achieved by exhaustive methylation which leads to distilbene derivatives (e.g. 1.IX) which upon ozonolysis yield 5,4'-diformyl-2-methoxy-diphenylether (1.X) and 4,5,5',6'-tetraformyl-2,2',3'-trimethoxydiphenylether (1.XI) in the case of compounds linked "head-to-head" by two diphenyl ether linkages. In the case of compounds linked "head-to-tail" (e.g. curine class) a similar sequence of reactions leads to 5,6,4'-triformyl-2,3-dimethoxydiphenylether (1.XII) and 4,5,5'-triformyl-2,2'-dimethoxy-diphenylether (1.XIII).



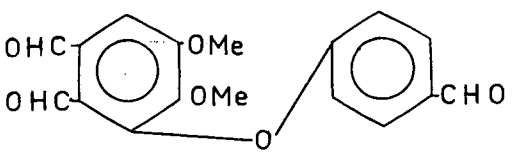
1.IX



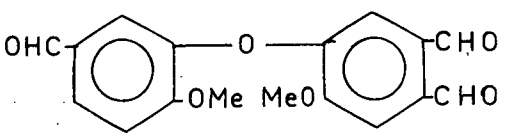
1.X



1.XI

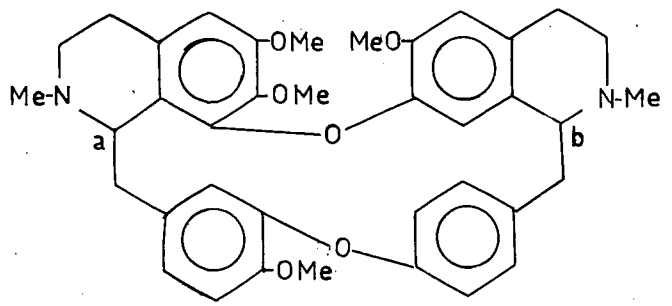


1.XII



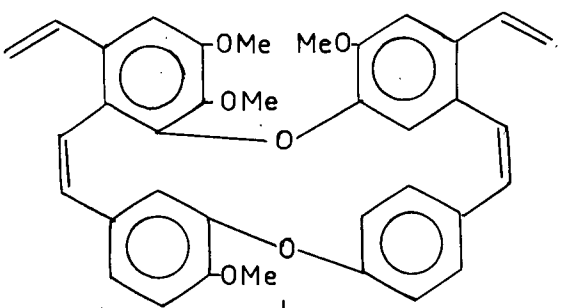
1.XIII

It is readily seen (Scheme 1,4) that this series of reactions alone would not distinguish between alkaloids of the berbamine class (1.XIV) and those of the oxyacanthine class (1.XV). Furthermore the loss of the asymmetric centres a and b means that no conclusions can **be drawn as to the stereochemistry of the original molecule.**



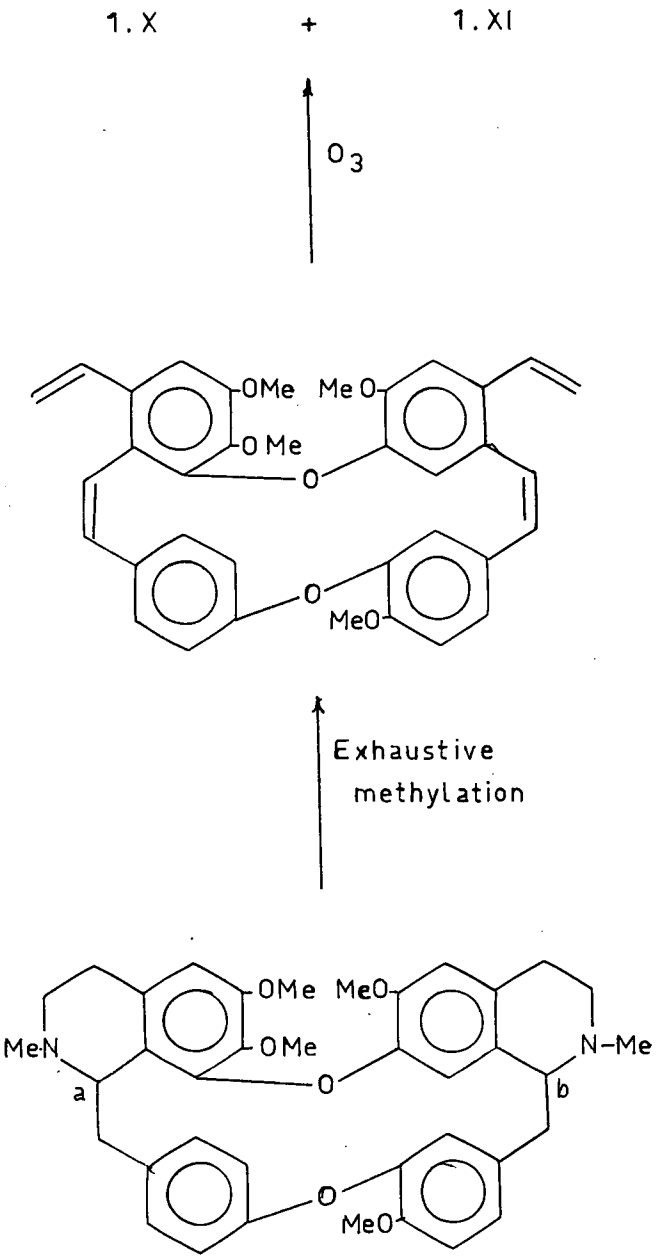
1.XIV

Exhaustive methylation



O_3

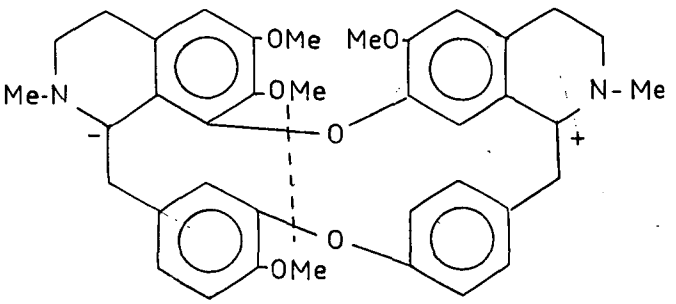
1.X 1.XI



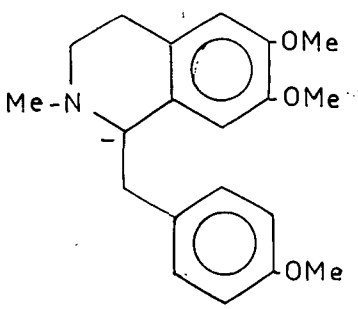
1.XV

Scheme 1.4

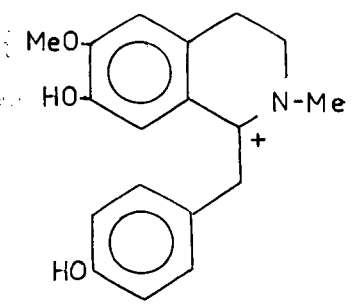
Both these problems were resolved by Tomita[5] who applied the reductive fission of diphenylether linkages with sodium in liquid ammonia to molecules of the bisbenzylisoquinoline type. From O-methylberbamine[6] (1.XIV) he isolated a non-phenolic and a phenolic product; the former was shown to be (-)-1-(4-methoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (1.XVI). The phenolic product (1.XVII) was dextrorotatory and contained two phenolic groups; it was converted to the known compound (1.XVIII).



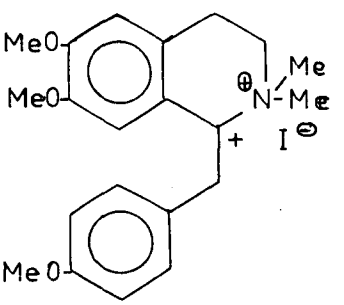
1.XIV



1.XVI

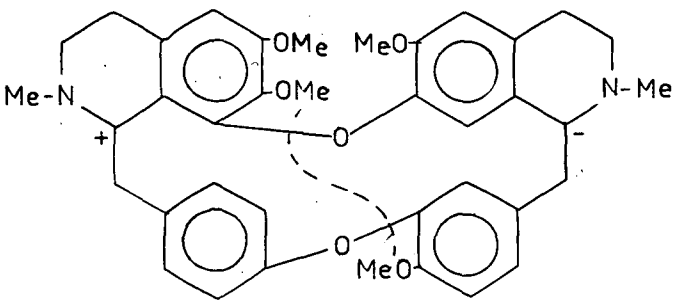


1.XVII

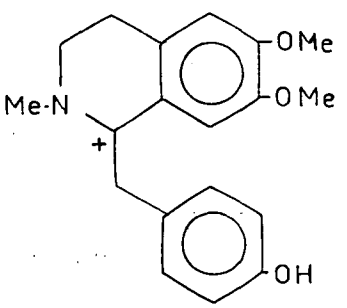


1.XVIII

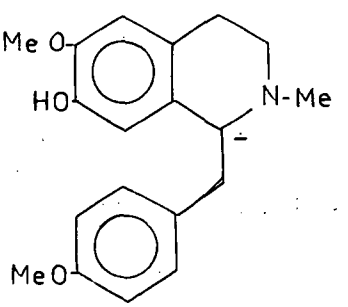
On the other hand O-methyloxyacanthine[7] (1.XV) yielded two phenolic products, one dextrorotatory and the other leavorotatory; the former (1.XIX) was identified as the enantiomer of the known alkaloid (-)-armepavine whilst the latter (1.XX) was converted to the trimethoxy compound (1.XVI). O-Methyloxyacanthine was therefore assigned the structure 1.XV in which the asymmetric centres a and b were paired (+,-), whilst O-methylberbamine had structure 1.XIV in which the asymmetric centres a and b were paired (-,+).



1.XV

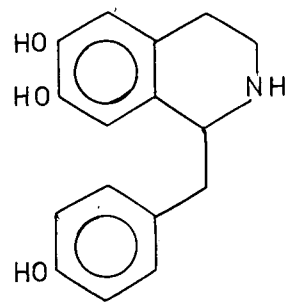


1.XIX



1.XX

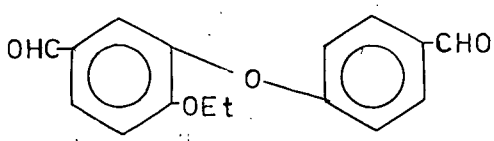
After this reaction was applied to several bisbenzylisoquinolines there emerged the important conclusion that reductive fission of bisbenzylisoquinolines occurs at specific C-O bonds to produce derivatives of 1-(4-hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (1.XXI), i.e. this reaction is the reversal of the suggested biosynthetic pathway[13]. Furthermore, the cleavage furnished units which retained the configuration about the asymmetric centres (a and b) and since the absolute configuration of key (mono)benzyltetrahydroisoquinolines had been established[8], the absolute configuration about the asymmetric centres a and b in the bisbenzylisoquinoline molecule automatically followed[9].



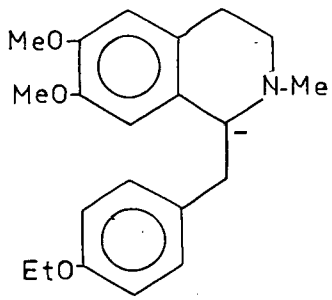
1.XXI

The positions of phenolic hydroxy groups in the molecule are established by labelling these groupings by O-ethylation followed by a repetition of the above degradations and identification of the products. Thus O-ethylberbamine[10] furnished 5,4'-diformyl-2-ethoxydiphenylether (1.XXII) after Hofmann degradation and (-)-1-(4-ethoxybenzyl)-6-7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (1.XXIII)

upon cleavage with sodium in liquid ammonia; the phenol group of berbamine is, therefore, at position 4".



1.XXII



1.XXIII

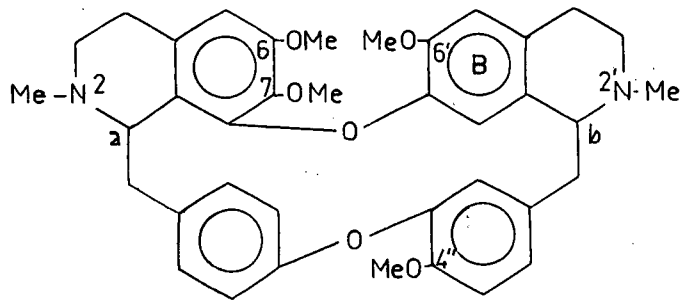
Physical methods of structure determination have emerged as valuable tools in the elucidation of the structure of bisbenzylisoquinolines. Useful correlations between the resonance positions of methoxyl and methylimino groups and the chemical and stereochemical structure of bisbenzylisoquinoline molecules have been obtained from a study of the n.m.r. spectra of several bases[11].

Normally methoxyl resonances occur around δ 3.8 ppm but in the spectrum of a bisbenzylisoquinoline they may be found between δ 4.00 ppm and δ 3.00 ppm. Table 1.I shows the resonance positions of methoxyl and N-methyl protons for selected compounds of the berbamine and oxyacanthine classes.

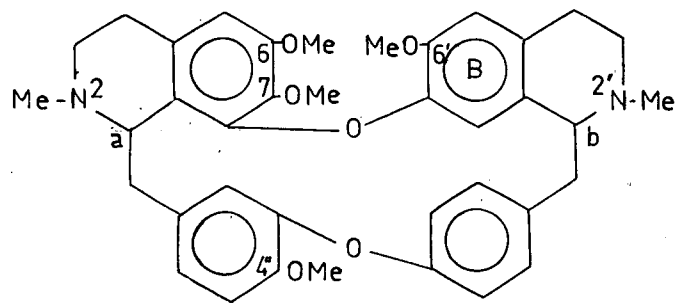
TABLE 1.I.

N.M.R. Spectra of Bisbenzylisoquinolines[11] (PPM, δ).

Name	Formula	OMe				NMe	
		4"	6	6'	7	2'	2
O-Methylrepandine	1.XXIV(+,+)	3.95	3.75	3.40	3.05	2.55	2.55
O-Methyloxyacanthine	1.XXIV(+,-)	3.95	3.79	3.60	3.20	2.65	2.55
Isotetrandrine	1.XXV(-,+)	3.90	3.78	3.63	3.18	2.60	2.28
Tetrandrine	1.XXV(+,+)	3.95	3.73	3.35	3.18	2.59	2.30



1.XXIV



1.XXV

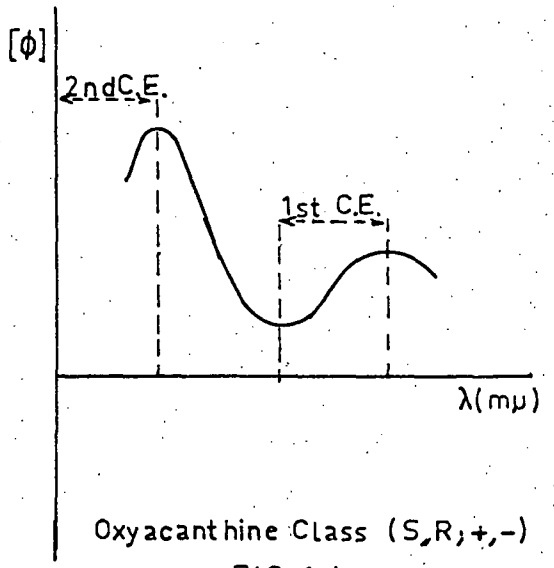
The assignments were made when an examination of molecular models showed that methoxyl groups on position 4" were least likely to be affected by ring-currents generated in adjacent aromatic rings whilst that on position 7 was able, and in some cases forced, to pass closely over the adjacent aromatic ring B and was therefore assigned the high-field resonance. By the same token, resonances around δ 3.50 ppm were assigned to the methoxyl group on position 6' rather than that on position 6 because the former is held in an environment more closely analogous to that on position 7. For bases of the oxyacanthine class the N-methyl resonances both occurred around δ 2.55 ppm whilst bases of the berbamine class gave well-separated peaks around δ 2.55 ppm and δ 2.30 ppm; the latter was assigned to the methylimino group on position 2. A further useful regularity was that when the asymmetric centres were paired (+,+) or (-,-) the 6'-methoxyl resonance occurred around δ 3.40 ppm whilst the pairing (+,-) or (-,+) gave a value near δ 3.60 ppm.

The detailed interpretation of the spectra of bases of the curine/isochondodendrine classes was more difficult because molecular models of these classes of alkaloids showed considerably more flexibility than for the berbamine-oxyacanthine classes. In all cases molecular conformations could be found which accounted for the regularities in the spectral data but it was not immediately obvious why these conformations were more probable than others which would be expected to lead to different results. All resonances of N-methyl groups were found between δ 2.50 ppm and δ 2.13 ppm.

In a recent paper[12], empirical correlations were drawn between the O.R.D. curves and the configuration of bisbenzylisoquinolines of known structure and configuration, thus enabling predictions to be made as to the configuration of new alkaloids.

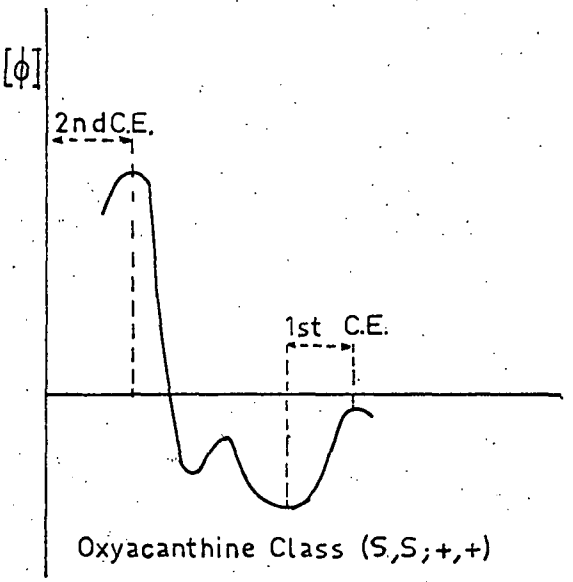
All the bases gave rise to broadly similar O.R.D. curves, except for alkaloids of the oxyacanthine class in which the asymmetric centres a and b were paired (S,S;+,+), and for alkaloids of the berbamine class with the pairing (S,R;+,-) or (R,S;-,+). Alkaloids of the oxyacanthine class with the pairing (S,R;+,-) gave rise to two positive Cotton effects with the second peak ($\sim$230 mμ) three or four times greater than the first ($\sim$270 mμ) (Fig. 1.I). The pairing (S,S;+,+) also gave rise to two positive Cotton effects; however, some minor peaks and troughs occurred between the first Cotton effect and the first extrema of the second Cotton effect (Fig. 1.II). Alkaloids of the berbamine class with asymmetric centres paired (R,R;-,-) or (S,S;+,+) gave rise to two negative and two positive Cotton effects respectively, the second peak being eight to ten times greater than the first (Fig. 1.III). Compounds of this class with centres paired (R,S;-,+) showed minor extrema between the two Cotton effects which were both positive (Fig. 1.IV).

Curine (1.XXVI) and its dimethyl ether (R,R;-,-) had similar negative rotatory dispersion curves, with the second Cotton effect three or four times as great as the first (Fig. 1.V). Chondrocurine (1.XXVII) and O,O-dimethylchondrocurine (S,R;+,-) gave similar positive curves but the magnitude of the Cotton effects was not as great



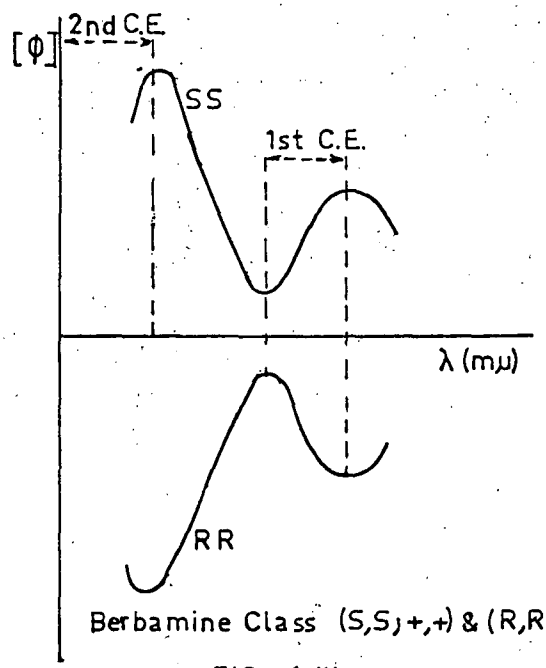
Oxyacanthine Class (S,R;+,-)

FIG. 1.I



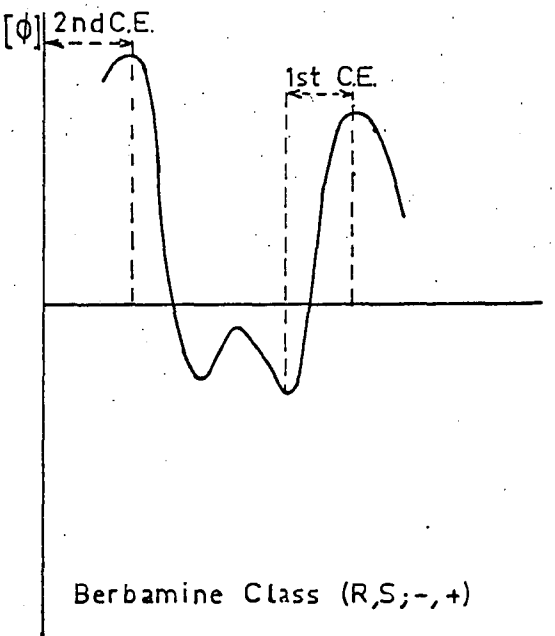
Oxyacanthine Class (S,S;+,+)

FIG. 1.II



Berbamine Class (S,S;+,+) & (R,R;-,-)

FIG. 1.III



Berbamine Class (R,S;-,+)

FIG. 1.IV

as for the (R,R) compounds.

It immediately becomes obvious that the O.R.D. curve and the n.m.r. spectrum of a bisbenzylisoquinoline constitute a powerful combination for structure determination. However, since the correlations

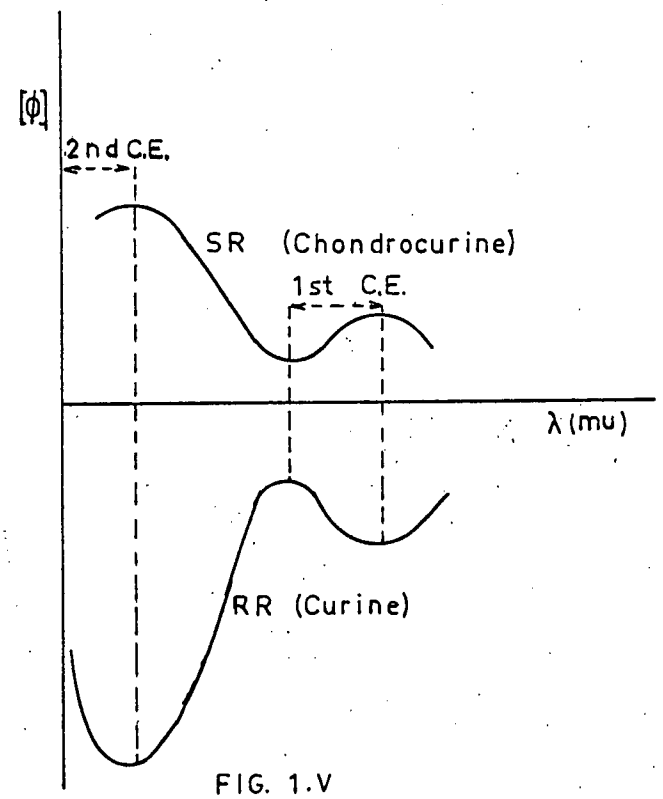
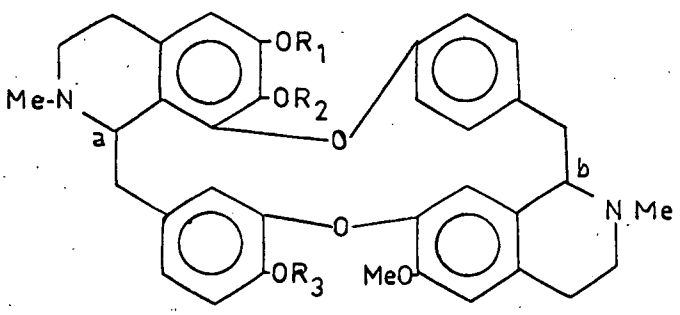


FIG. 1.V



1. XXVI R_1 = Me; R_3 = R_2 = H. (a,b; -,-)

1. XXVII R_1 = R_3 = H; R_2 = Me. (a,b; +,-)

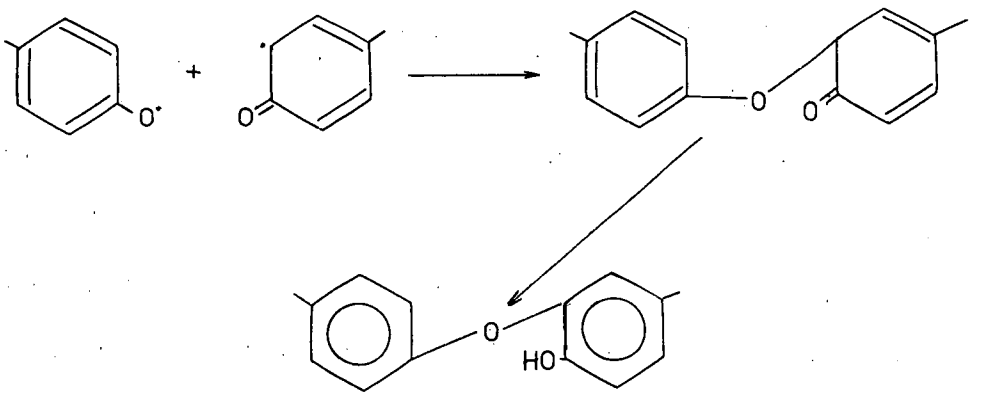
in both these measurements are empirical, chemical evidence is necessary to confirm any proposed structure.

Biosynthesis.

Much circumstantial evidence has accumulated that the bisbenzylisoquinoline molecule is formed in the plant by the manner originally suggested by Faltis[13], i.e. from two 1-(4-hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline molecules. Thus, most of the alkaloids which have so far been isolated may be accommodated in this scheme and monobenzylisoquinolines as well as bisbenzylisoquinolines have been found in the one plant.

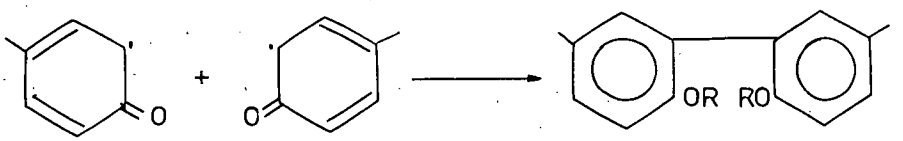
Barton and Cohen[14] discussed the formation of certain natural products in terms of a unifying reaction mechanism - the coupling of phenol radicals. The oxidation of phenols by one-electron transfer oxidising agents affords phenol radicals which are stabilised because of the spread of the odd electron over the ortho and para positions of the aromatic nucleus. Once the phenol radicals have been generated they may be converted to stable molecular products by several processes, one of which involves self-coupling with the formation of dimers. This may involve carbon-oxygen, carbon-carbon or oxygen-oxygen coupling. Carbon-carbon coupling may be ortho-ortho, ortho-para or para-para; the less common carbon-oxygen coupling involves the coupling of the oxygen atom of one phenol radical to nuclear positions ortho or para to the oxygen atom of a second phenol radical. If the method of formation of the diaryl ether linkages of bisbenzylisoquinolines is considered in the light of this mechanism it is readily seen how the benzylic moiety (or isoquinoline moiety) of, say, the oxyacanthine molecule may arise by phenol radical coupling

(Scheme 1.5).



Scheme 1.5

It can also be readily seen how the diaryl portion of tiliacorine may arise by carbon-carbon coupling of phenol radicals (Scheme 1.6).



Scheme 1.6

Experiments involving the use of ^{14}C tracers have been commenced at the University of Tasmania and the results of these experiments are awaited with great interest.

Alkaloids of the Australian Monimiaceae.

Plants of the family Monimiaceae include the endemic Doryphora, Daphnandra, Atherosperma and Dryadodaphne species, all of which are alkaloid containing. The first two genera have yielded only alkaloids of the bisbenzylisoquinoline type whilst Dryadodaphne novoguineensis (Perk.) A.C.Smith has yielded spermatheridine and bisbenzylisoquinoline bases. Atherosperma moschatum Labill. has yielded bases of the bisbenzylisoquinoline, spermatheridine, aporphine and 1-N,N-dimethylaminoethylphenanthrene types (Table 1.2).

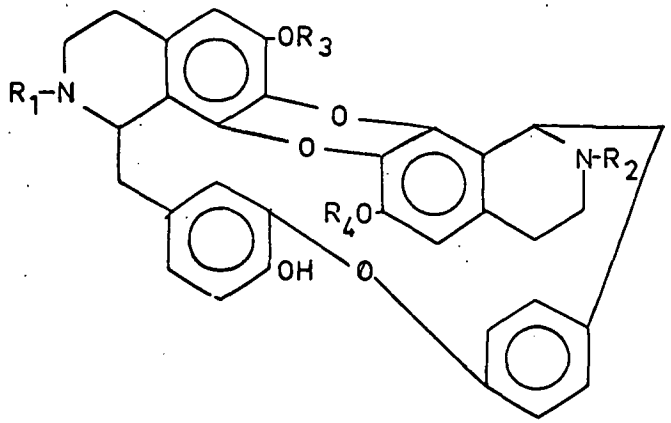
TABLE 1.2.

Alkaloids of the Australian Monimiaceae.

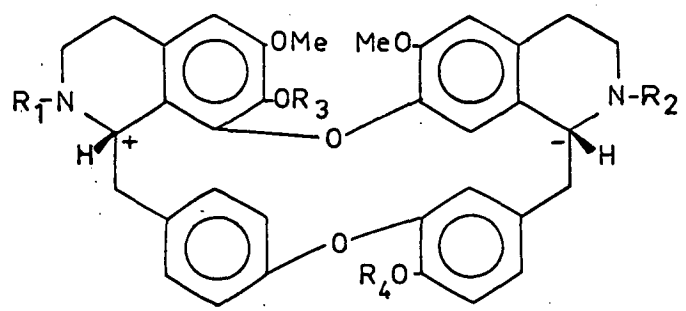
Plant	Alkaloids Isolated	Structure	Reference.
Genus: Daphnandra			
D.micrantha	Micranthine	1.XXVIII	15,16.
	Daphnoline	1.XXIX	17,18.
	Daphnandrine	1.XXX	
D.dielsii Perkins	Repanduline	Unknown	18,19.
	Repandinine	(±)-Tenuipine	
	O-Methylrepandine	1.XXXI	
	(-)-Tenuipine	1.XXXII	
D.repandula F.Muell	Repanduline	Unknown	20
	Repandine	1.XXXIII	
D.tenuipes (Whian Whian State Forest)	Repanduline	Unknown	18
	(-)-Tenuipine	1.XXXII	
	(-)-Nortenuipine	1.XXXIV	
	Aromoline	1.XXXVII	

Plant	Alkaloids Isolated	Structure	Reference
D. tenuipes (Oakes State Forest)	(+)-Tenuipine	1.XXXV	21
	(+)-Nortenuipine	1.XXXVI	
	Repandinine	(±)-Tenuipine	
Genus: *Doryphora*			
D.aromatica(Bail.)Smith	Daphnandrine	1.XXX	22
	Daphnoline	1.XXIX	
	Aromoline	1.XXXVII	
D.sassafras	Alkaloids present;	none isolated pure.	23
Genus: *Atherosperma*			
A.moschatum Labill.	Berbamine	1.XXXVIII	24,10
	Isotetrandrine	1.XXXIX	and this
	Atherospermoline	1.XL	thesis.
	Isocorydine	1.XLI	
	Spermatheridine	1.XLII	
	Atherospermidine	1.XLIII	
	Atherosperminine	1.XLIV	
	Methoxyathero-sperminine	1.XLV	
	Atheroline	1.XLVI	
	Moschatoline	1.XLVII(Tentative)	
	Alkaloid C	1.XLVIII(Tentative)	
Genus: *Dryadodaphne*			
D.novoguineensis (Perk.) A.C.Smith	Dryadine	Unknown	25 and
	Spermatheridine	1.XLII	this
	Alkaloid D	Unknown	thesis.
	Atheroline	1.XLVI	

The presence of alkaloids in the bark of trees of the *Daphnandra* species was first reported by Bancroft[26]. Much later Pyman[15] isolated



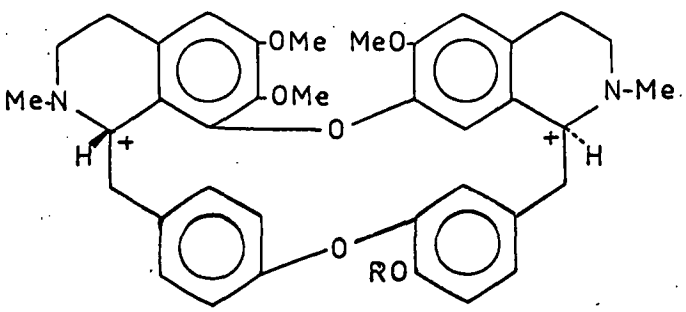
1. XXVIII $R_1 = H, R_2 = Me$ or vice versa.
$R_3 = H, R_4 = Me$ or vice versa.



1. XXIX $R_1 = Me, R_2 = R_3 = R_4 = H.$

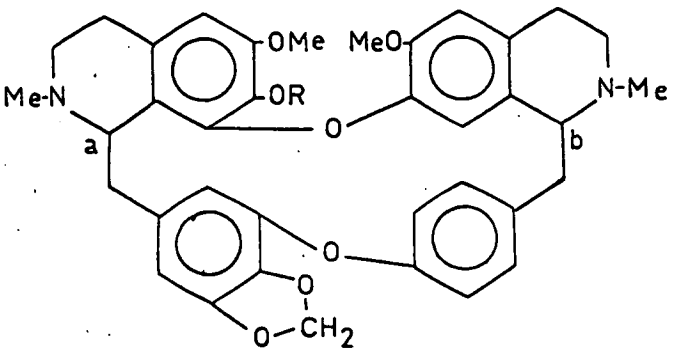
1. XXX $R_1 = R_4 = Me, R_2 = R_3 = H.$

1. XXXVII $R_3 = R_4 = H, R_1 = R_2 = Me.$



1. XXXI R = Me.

1. XXXIII R = H.

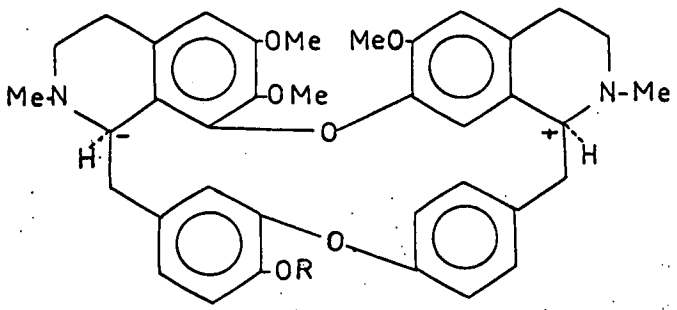


1. XXXII R = Me (a,b; −, −).

1. XXXV R = Me (a,b; +,+).

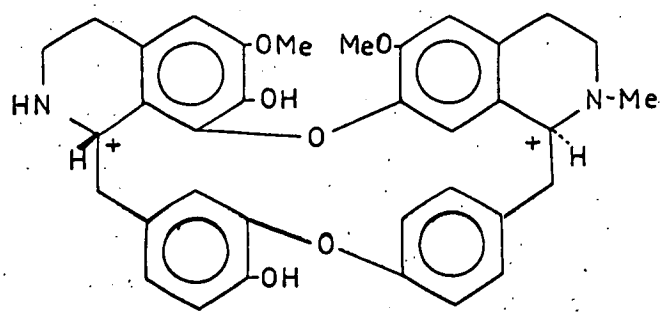
1. XXXIV R = H (a,b; −, −).

1. XXXVI R = H (a,b; +,+).

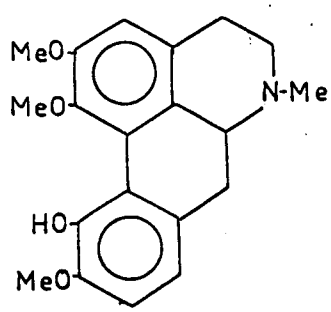


1. XXXVIII R = H.

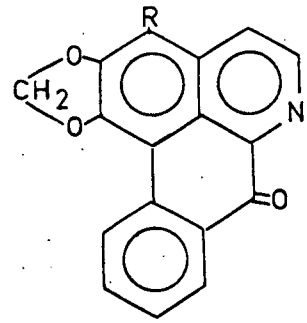
1. XXXIX R = Me.



1. XL

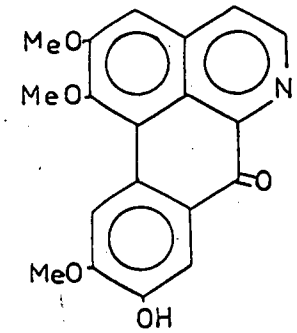


1. XLI

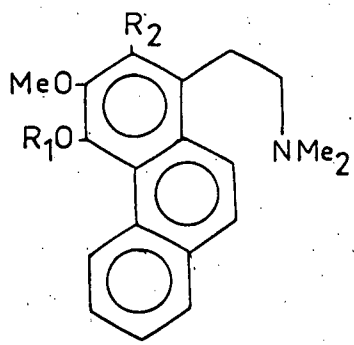


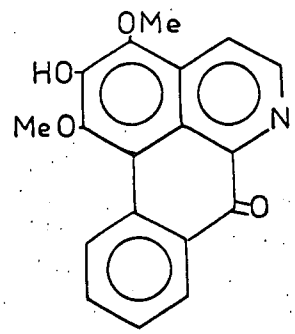
1. XLII R=H.

1. XLIII R=OMe.



1. XLVI





1. XLIV R_1=Me, R_2=H.

1. XLVIII R_1=R_2=H or vice versa.

1. XLV R_1=Me, R_2=OMe.

1. XLVII

three alkaloids from D.micrantha which he named daphnandrine, daphnoline and micranthine and suggested the molecular formulae $C_{36}H_{38}O_6N_2$, $C_{35}H_{36}O_6N_2$ and $C_{34}H_{34}O_6N_2$, respectively. He reported the physical constants of these bases but carried out no structural investigations. It is interesting to note that the dimeric formulae suggested by Pyman came some years before the existence of the bisbenzylisoquinoline molecule was realised.

The chemistry of the remaining Daphnandra species, as well as D.micrantha, have been studied by Bick and his colleagues who have also investigated the alkaloids of Atherosperma moschatum and Dryadodaphne novoguineensis.

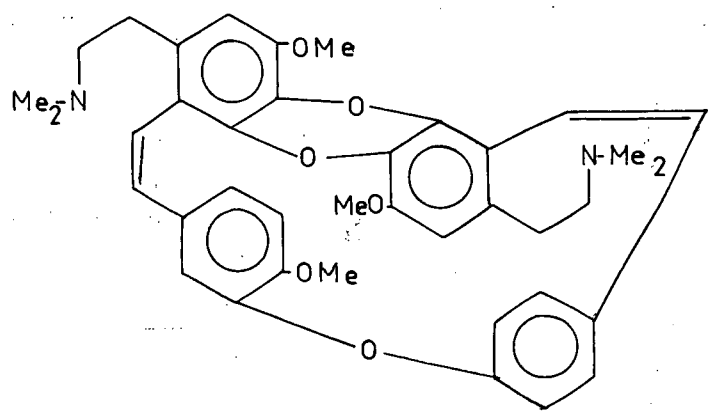
The alkaloidal content of Daphnandra micrantha was shown to vary considerably with the time and place of collection of the bark sample[16,18]; the three bases reported by Pyman were obtained only from a specimen of D.micrantha collected near Wauchope on the northern New South Wales coast. D.micrantha growing north of this point yielded less and less of daphnoline and daphnandrine until a specimen collected at Draper's Crossing (near Brisbane, Queensland) gave only micranthine.

Micranthine was shown to belong to the rather small class of bisbenzylisoquinoline alkaloids which possessed a dibenzo-1,4-dioxin system[17]. Analysis indicated the formula $C_{34}H_{36}O_6N_2$ (compare with $C_{36}H_{32}O_6N_2$ proposed by Pyman) with one methylimino group and two methoxyl groups. Two of the remaining oxygen atoms were assumed to be present as phenolic hydroxy groups and the third as a

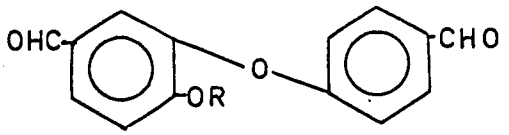
diaryl ether bridge.

Ozonolysis of O,O,N-trimethylmicranthine methine (1.XLIX) gave 5,4'-diformyl-2-methoxy-diphenylether (1.L) indicating head-to-tail linkage of the two benzylisoquinoline moieties. Hofmann degradation of a second product from the ozonolysis yielded a product which appeared to be a diformyldimethoxydivinyldibenzo-1,4-dioxin.

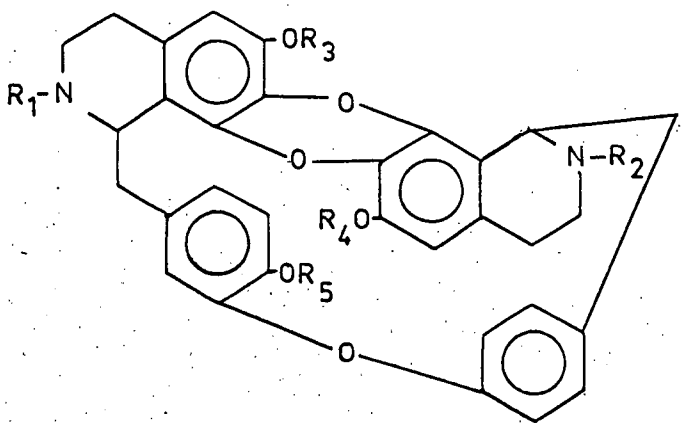
On biogenetic grounds the Cambridge authors suggested the possible bisbenzylisoquinolines containing a dibenzo-1,4-dioxin system linking the two isoquinoline portions were represented by 1.LI, 1.LII and 1.LIII since alkaloids containing oxy substituents on position 5 had not previously been reported and were unlikely to arise from 1-(4-hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline units.



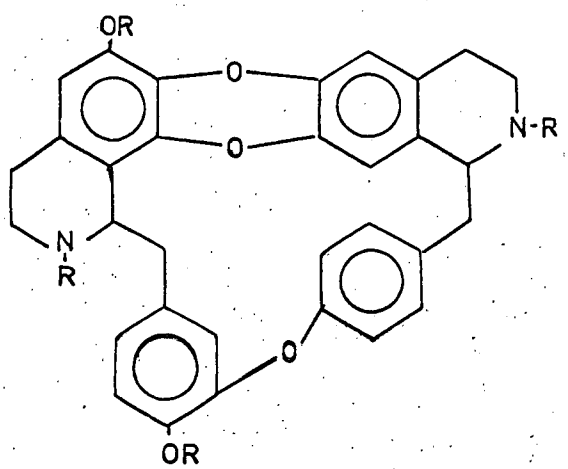
1.XLIX



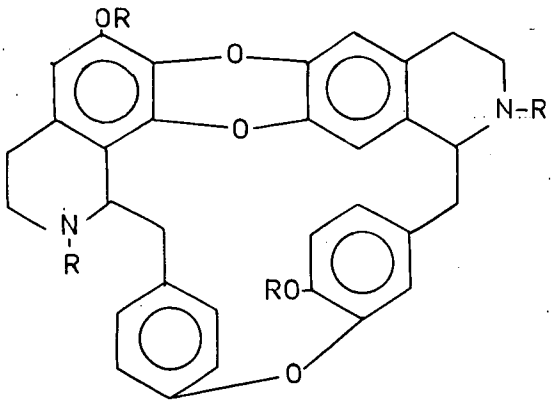
1.L R = Me
1.LIV R = Et



1.LI



1.LII



1. LIII R = H or Me

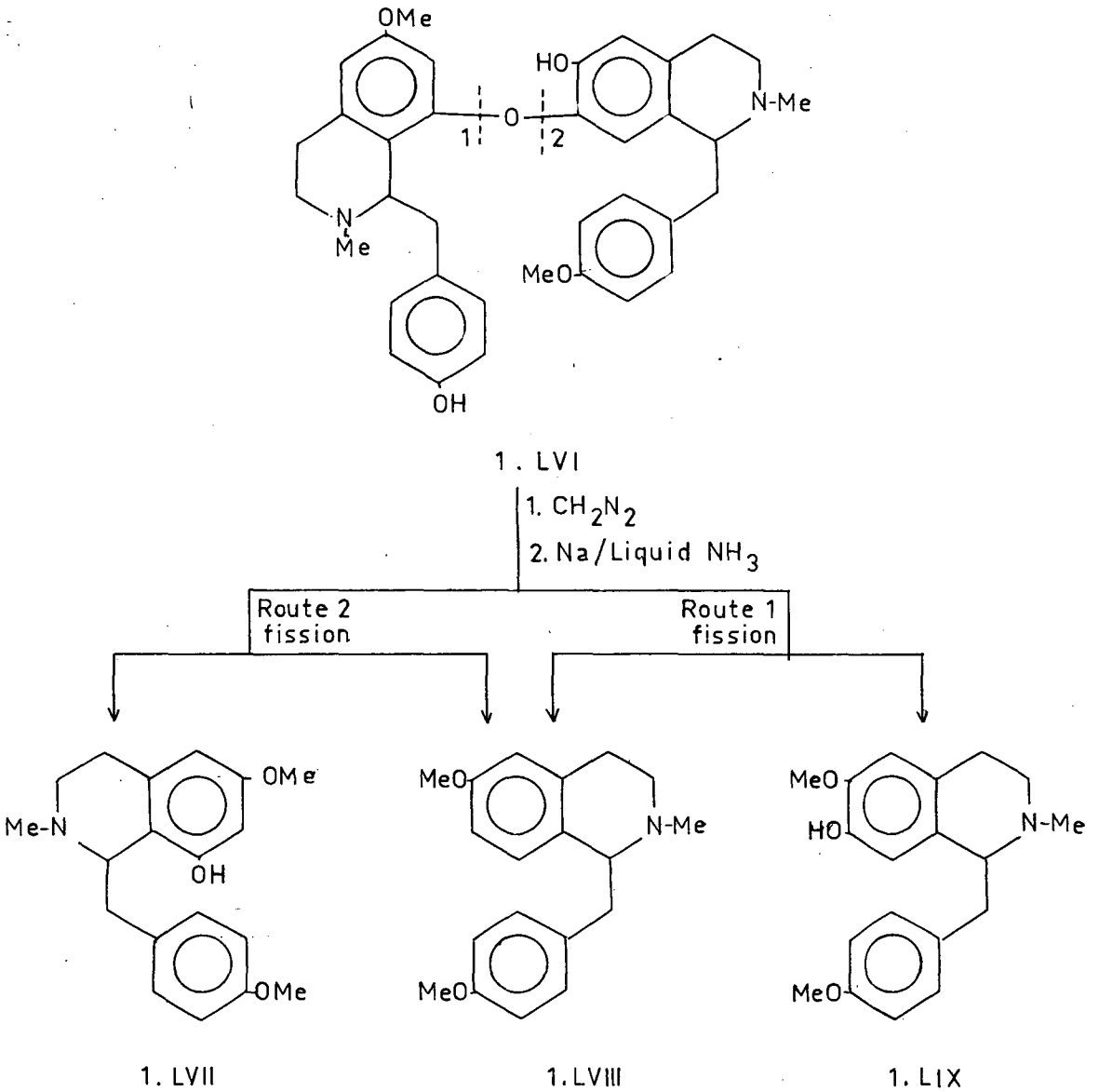
1. LV R = Me

Structure 1.LI was preferred for micranthine since 1.LII and 1.LIII would both yield a diformylmonomethoxydivinyldibenzo-1,4-dioxin.

The isolation of 5,4'-diformyl-2-ethoxydiphenylether (1.LIV) from O,O-diethylmicranthine dimethiodide by a sequence of reactions similar to that described above established the position of one of the phenolic hydroxy groups. The structure of micranthine was therefore proposed as 1.LI in which R_5 is H, of R_1 and R_2 one is H and the other Me, and of R_3 and R_4 one is H and the other is Me.

The application of the conventional sodium/liquid ammonia fission to alkaloids containing the dibenzo--1,4-dioxin system was not successful, probably because the intermediate product contained a phenolic group which was capable of forming an insoluble sodium salt. Recently, Inubushi, Wamura and Miyawaki[27] successfully cleaved isotrilobine (1.LV) in two stages. The first involved the use of sodium/sodium hydride in liquid ammonia and produced the

phenol 1.LVI which contained one diaryl ether linkage. Methylation of this product followed by sodium/liquid ammonia fission produced the three products 1.LVII, 1.LVIII and 1.LIX (Scheme 1.7) thus establishing the structure of isotrilobine.



Scheme 1.7

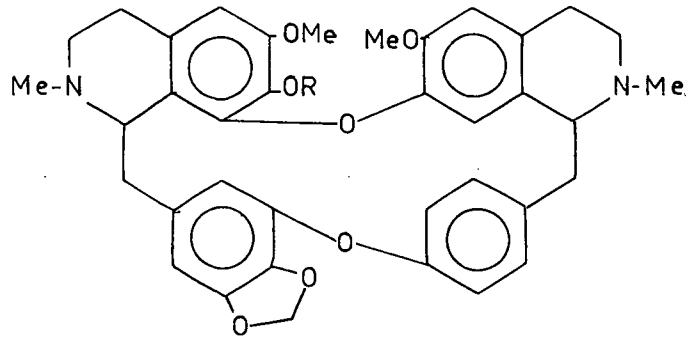
The application of this series of reactions to O,O,N-trimethylmiranthine is awaited with interest.

Daphnoline, daphnandrine and aromoline were all shown to be related to oxyacanthine[16], differing only in the degree of methylation. Their structures were established by Hofmann degradation and sodium in liquid ammonia fission of their O-ethyl ethers[28].

Tenuipine and nor-tenuipine.

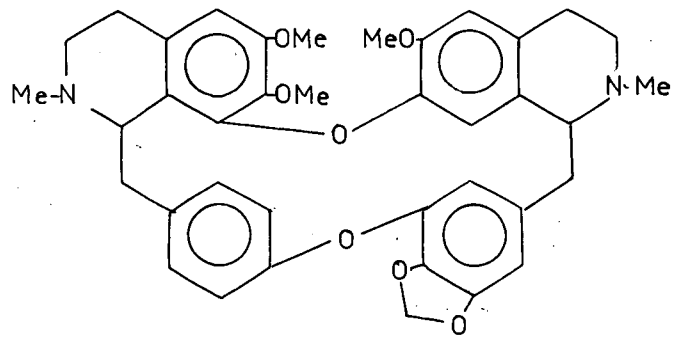
Tenuipine was first isolated as the laevorotatory isomer by Bick, Taylor and Todd from the bark of Daphnandra tenuipes growing in the Whian Whian State Forest, N.S.W.[18]. The leaves of the same tree yielded (-)-nortenuipine. Subsequently, (+)-tenuipine and (+)-nortenuipine were isolated from the bark of D.tenuipes growing in Oakes State Forest, N.S.W.[21]. The taxonomic implication of these results will be discussed later.

Tenuipine was shown to be a bisbenzylisoquinoline alkaloid[21] containing a methylenedioxy group, three methoxyl and two methylimino groups. Because of the occurrence of repanduline (which was known to contain a methylenedioxy group in the benzyl portion of the molecule[36]) in the same tree, Bick, Taylor and Todd[18] proposed that tenuipine was represented either by 1.LX or 1.LXI.



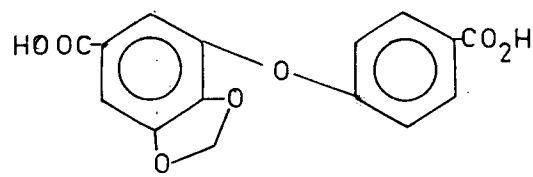
1.LX R = Me

1.LXIII R = H



1. LXI

This proposal was confirmed by Bick, Harley-Mason and Vernengo[21] who isolated repandulinic acid (1.LXII) as an oxidation product of (+)-tenuipine.



1. LXII

The n.m.r. spectrum of tenuipine[11] (Table 1.III) indicated that its structure was represented by 1.LX rather than by 1.LXI. The presence of two well-separated N-methyl resonances indicated that the alkaloid was of the berbamine class, and the high value of the 6'-methoxyl resonance suggested that the asymmetric centres were paired (+,+) or (-,-).

TABLE 1.III.

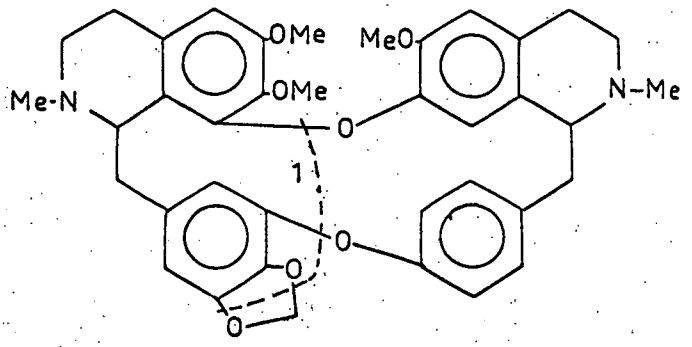
N.m.r. spectra of Tenuipine and Nor-tenuipine.

	Formula	OMe				NMe	
		4"	6	6'	7	2'	2
Tenuipine	1.LX	-	6.25	6.65	6.82	7.40	7.65
Nortenuipine	1.LXIII	-	6.22	6.67	-	7.37	7.68

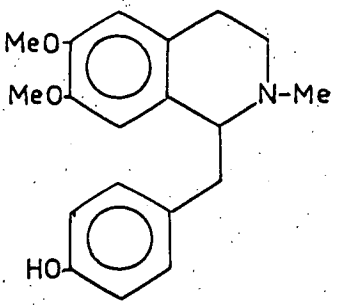
The O.R.D. curve of (+)-tenuipine[12] indicated that the two asymmetric centres were paired (S,S; +,+).

Nortenuipine contained two methoxyl groups and one phenolic hydroxy group. Methylation with diazomethane gave tenuipine. N.m.r. evidence[11] (Table 1.III) indicated that the phenolic hydroxy group was at position 7.

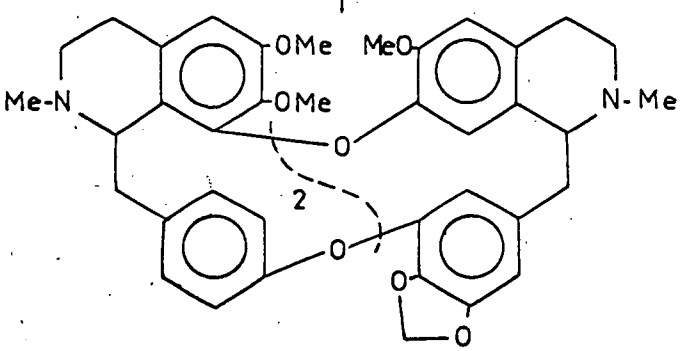
The presence of the methylenedioxy group complicated the cleavage of (+)-tenuipine with sodium in liquid ammonia[21]. Clayson[29] showed that hydrocotarnine (1.LXIV) furnished (1.LXV); treatment of (+)-tenuipine with sodium in liquid ammonia gave mostly phenolic material from which (+)-armepavine (1.LXVI) was isolated. However, this product could arise from 1.LXVII as a result of the cleavage



1. LXVII



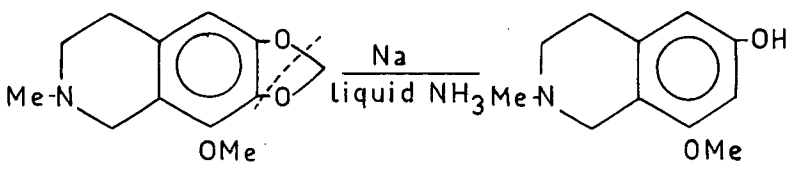
1. LXVI



1. LXVIII

Scheme .1. 8

of the methylenedioxy group (Pathway 1 in Scheme 1.8) or from (1.LXVIII) via pathway 2.



1.LXIV 1.LXV

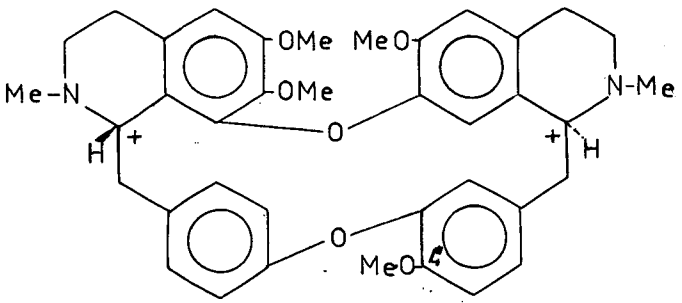
Attempts to replace the methylenedioxy group by two methoxyls produced only a small quantity of the required product.

Repandinine, isolated from D.tenuipes[21] (Oakes State Forest) and D.dielsii[18] was shown to be a racemic mixture of (+)-tenuipine and (-)-tenuipine[21].

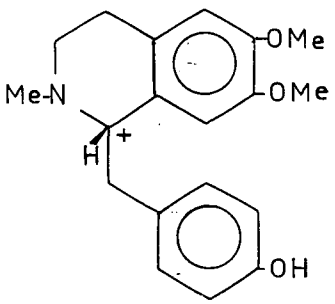
Repandine.

Repandine was first isolated by Bick and Whalley from Daphnandra repandula[20] and was subsequently shown by Bick and Todd[30] to be a stereoisomer of oxyacanthine. That the asymmetric centres were paired (S,S; +,+) was shown by the reduction of O-methylrepandine (1.LXIX) with sodium in liquid ammonia[31]. Two dextrorotatory fragments, (1.LXX) and (1.LXXI), were obtained thus establishing

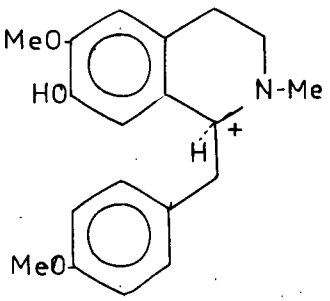
the structure of O-methylrepandine as (1.LXIX). The phenolic hydroxy group was placed at position 4" as a result of degradative experiments on O-ethylrepandine[30].



1. LXIX



1. LXX



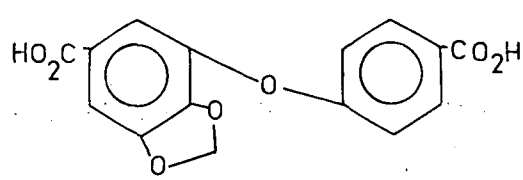
1. LXXI

In an attempt to prepare the monohydrochloride of oxyacanthine (isolated from a Berberis species), von Bruchhausen and Schulze[32] isolated a compound identical with repandine. The isolation of this base led von Bruchhausen[33] to suggest that it arose from oxyacanthine by a Walden inversion during treatment with hydrogen chloride. Bick

and Todd[30] were unable to repeat this work and concluded that repandine was not an artefact but that it actually occurred in small amounts along with oxyacanthine in at least one species of Berberis. However, the problem still remains unresolved for, although two further workers[34,35] have been unable to repeat the conversion of oxyacanthine into repandine, Tomita[31] has reported the conversion on a sample of oxyacanthine isolated from Berberis thunbergii.

Repanduline.

The novel yellow bisbenzylisoquinoline, repanduline, has been isolated from several Daphnandra species (see Table 1.II) the first of which was Daphnandra repandula[20]. The tenacious manner in which this base retained solvent of crystallisation hindered the correct interpretation of analytical data[36] until it was crystallised from carbon tetrachloride[37]. The amount of solvent of crystallisation in a sample thus crystallised was estimated by a chlorine analysis; the analytical data was consistent with the formula $C_{37}H_{40}O_7N_2 \cdot CCl_4$ with one methoxyl and two methylimino groups. This molecular

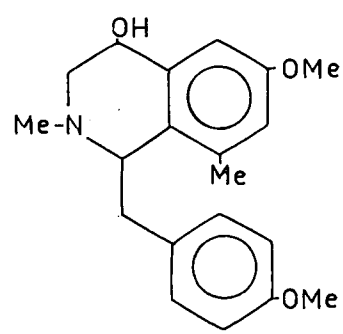


1 . LXXII

formula coupled with the isolation of repandulinic acid (1.LXXII) from the oxidation of repanduline[36] was strong evidence in favour of a bisbenzylisoquinoline skeleton.

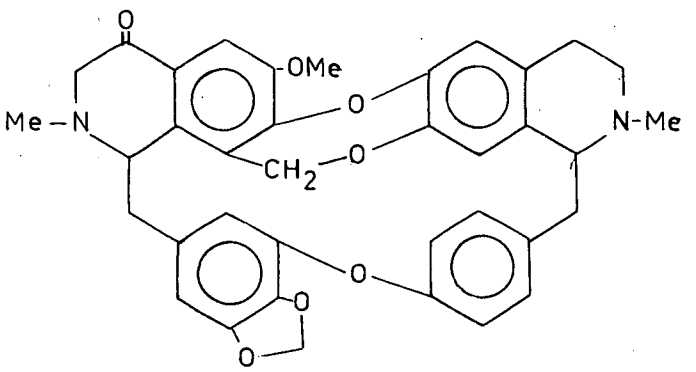
The presence of a carbonyl group in repanduline was indicated by a band at 1707 cm^{-1} in its i.r. spectrum & was confirmed by the reduction to a hydroxy group with lithium aluminium hydride, and the formation of a Grignard derivative[38].

Attempts to degrade repanduline by the Hofmann and Emde methods failed; reductive fission with sodium in liquid ammonia furnished in poor and variable yield a product, repandulol[34,37], which analysed for $C_{20}H_{25}O_3N$ with two methoxyls and an N-methyl group but no methylenedioxy or phenolic group. By a consideration of the analytical data and spectroscopic and chemical properties the tentative structure (1.LXXIII) was proposed for this compound.



1.LXXIII

The tentative structure 1.LXXIV was proposed for repanduline[37] on the basis of biogenetic considerations, N.M.R. data and the isolation of the above degradation products.



1. LXXiV

The study of the alkaloids of Atherosperma moschatum and of Dryadodaphne novoguineensis embodies the major portion of this thesis.

2. THE MINOR ALKALOIDS OF THE BARK OF ATHEROSPERMA MOSCHATUM LABILL.

Atherosperma moschatum Labill., commonly known as southern sassafras, occurs abundantly in rain-forest regions of Tasmania and Victoria. Endemic in Australia, it is a member of the family Monimiaceae to which also belong the endemic Daphnandra and Doryphora species of Queensland and northern New South Wales. Other representatives of the family Monimiaceae which have yielded alkaloids include the Laurelia[39] and Pneumus[40] species from which a number of aporphine alkaloids have been isolated.

The leaves and bark of sassafras have a marked aromatic odour; the essential oils were investigated by Scott[41] who reported the presence of safrole, eugenol methyl ether, pinene and camphor. It is likely that these oils contribute to the flavour of the beverages "sassafras tea" and "sassafras beer" which earlier settlers believed to have marked curative properties.

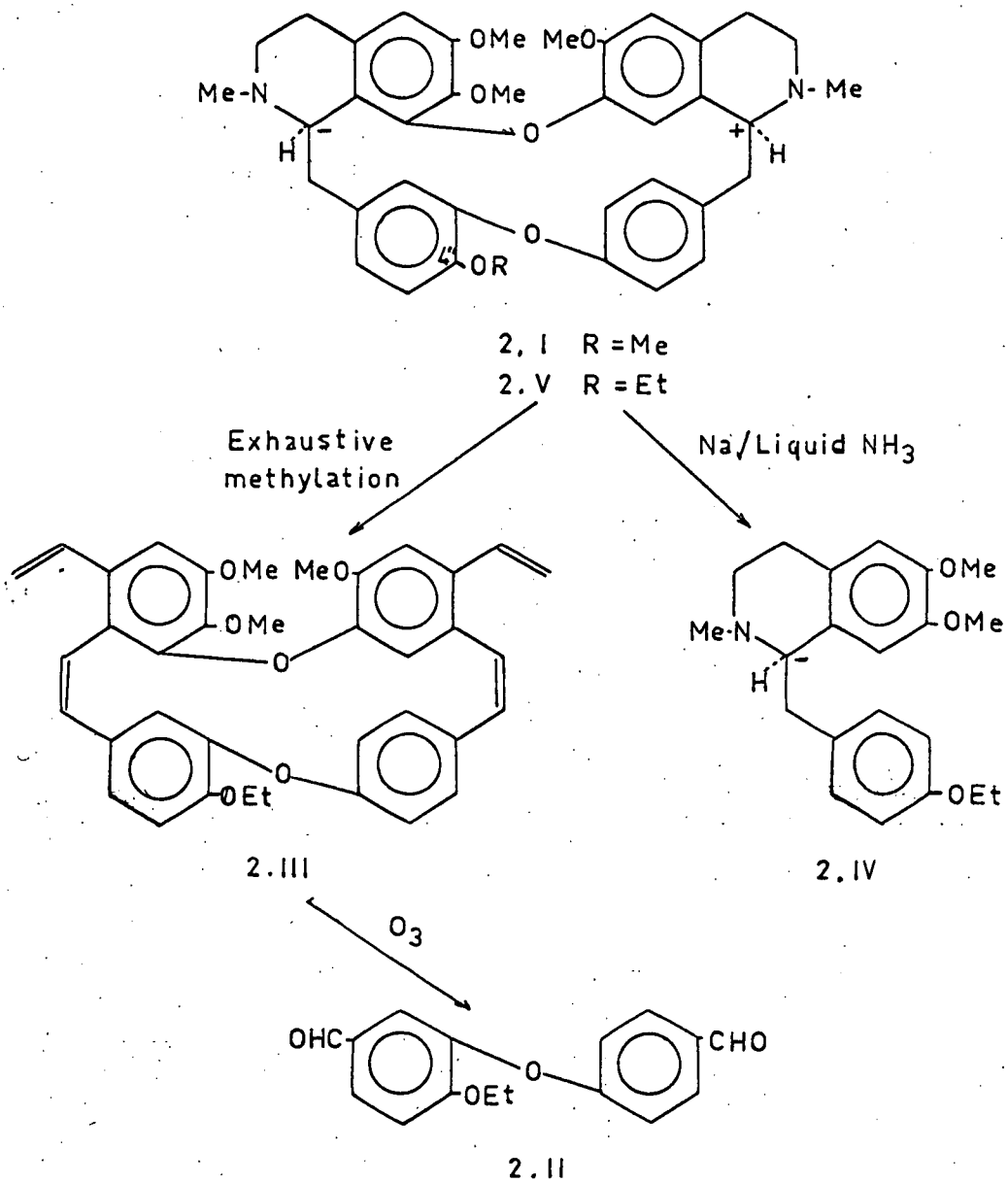
Economically, sassafras is of some importance in the manufacture of clothes pegs and paper pulp. It is particularly suitable to the former because of its low content of tannin and water-soluble, coloured material. Its use in the paper industry is, however, restricted by its yellow colour which is difficult to remove by bleaching. Extensive hypochlorite treatment does reduce the colour but higher costs make the process uneconomic. Under existing local conditions of manufacture not more than 5% of sassafras is incorporated in pulp which is comprised chiefly of the Eucalypt species E.regnans, E.delegatensis and E.obliqua. A discussion of

the possible source of this yellow colour will be reserved until later in this thesis.

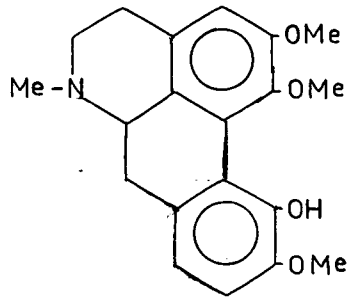
Sassafras is interesting in that it is probably the first Australian tree from which an alkaloid was isolated. In 1861 Zeyer[42] isolated a base, atherospermine, for which he recorded the melting point and suggested the formula $C_{30}H_{40}O_5N_2$; he carried out no chemical investigation of the compound. Almost one hundred years later, Bick, Clezy and Crow[10] showed atherospermine to be identical with berbamine which these authors isolated in 1.6% yield from the bark. O-Methylberbamine was known to have structure 2.I; the position of the phenolic hydroxyl group of berbamine had not been fixed but only assumed to be at position 4". This assumption was confirmed by Bick, Clezy and Crow who isolated 2-ethoxy-5,4'-diformyldiphenylether (2.II) from the ozonolysis of O-ethyl-de-N-berbamine (2.III) and (-)-O-ethylarmepavine (2.IV) as one of the products of cleavage of O-ethylberbamine (2.V) by sodium in liquid ammonia (Scheme 2.1).

Besides berbamine, the above authors reported the isolation of several minor alkaloids including the known bases isotetrandrine (2.I) and isocorydine (2.VI) and the new bases atherosperminine, spermatheridine and atherospermidine whose structures were not determined.

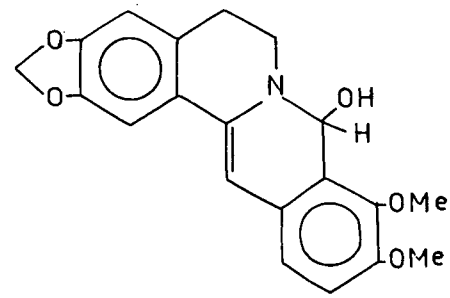
Both atherospermidine and spermatheridine were yellow but were readily reduced by zinc dust and acetic acid to colourless compounds. Analytical data indicated that atherospermidine was a methoxysperma-


OMe MeO
Me-N
OMe
N- Me
H
H
O
O
4'
OR
2, I R = Me
2.V R = Et
Exhaustive methylation
Na/Liquid NH3
OMe MeO
OMe
O
O
OEt
2.III
OMe
Me-N
OMe
H
OEt
2.IV
O3
OHC
CHO
O
OEt
2.II

Scheme 2.1



2.VI



2.VII

theridine and, on the assumption that they were isoquinoline alkaloids, the low C/H ratio led Bick, Clezy and Crow to suggest a type of structure similar to that of berberine (2.VII).

In the present investigation the three alkaloids of unknown structure were isolated and separated by a process similar to that previously described[10]; several other minor alkaloids were also obtained. Briefly, the isolation process was as follows: a chloroform solution of the alkaloid extract of sassafras bark was extracted with dilute alkali to remove phenolic bases. The chloroform layer was evaporated to dryness and the berbamine removed from the residue by fractional crystallisation from benzene; extraction of the benzene mother liquors with dilute alkali removed the cryptophenolic bases. Paper chromatography of the non-phenolic fraction indicated the presence of several alkaloids including one of R_f 0.83 and one of R_f 0.90; the former, whose R_f was the same as that reported for spermatheridine, showed a bright yellow fluorescence

under u.v. light whilst the latter exhibited a deep orange fluorescence and corresponded in R_f to atherospermidine. A third spot of R_f 0.74 (atherosperminine) showed a bright blue fluorescence under u.v. light. The three alkaloids were readily separated by Craig distribution in which the stationary phase was chloroform and the mobile phase was dilute hydrochloric acid, the concentration of which was steadily increased from 0.1 - 5%. Spermatheridine was the strongest base and distributed into 1% acid whilst atherospermidine was extracted by 5% acid; atherosperminine remained in the chloroform layer and was readily separated from a further colourless base, alkaloid B, by chromatography over alumina. The progress of the separation was readily followed because of the orange-yellow colour of spermatheridine in acid solution and the pink colour of atherospermidine.

Atherospermidine and spermatheridine were further purified by crystallisation from chloroform. Analytical data on the two alkaloids and the similarity of their u.v. and visible light absorption spectra (Table 2.I) confirmed the suggestion[10] that atherospermidine was a methoxyspermatheridine. Both bases gave a positive methylenedioxy-group test with chromotropic acid[43]; the presence of this group was also indicated by the i.r. spectra of the two bases[44] which showed absorption bands at 1420, 1365, 1120, 1055 and 960 cm^{-1}. A band at 1657 cm^{-1} was attributed to a highly-conjugated ketone group; the presence of the latter was confirmed when the two bases were converted to their respective oximes,

neither of which showed -OH absorption in their i.r. spectra. This lack of -OH absorption will be referred to later.

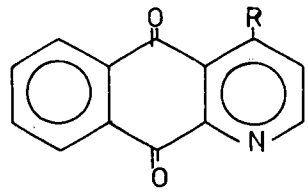
TABLE 2.I.

U.V. and Visible Light Absorption Spectra of Atherospermidine and Spermatheridine.

	In Ethanol		In 0.1N HCl	
	$\lambda^{m\mu}_{max}$	log ε_{max}	$\lambda^{m\mu}_{max}$	log ε_{max}
Spermatheridine	247.5	4.23	256.5	4.33
	269	4.16	280	4.25
	302	4.70	334	3.70
Atherospermidine	247	4.38	262.2	4.24
	281	4.52	283	4.16
	312 (inf)	3.95		

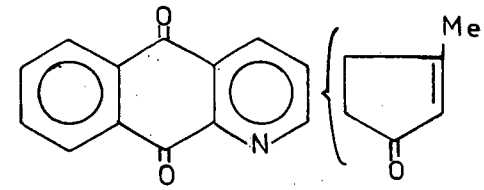
In 1960, Buchanan and Dickey[45] reported the presence of a yellow base, liriodenine, in the heartwood of the magnoliaceous tree Liriodendron tulipifera L. Liriodenine, which analysed for $C_{17}H_9O_3N$, was oxidised by chromic acid to 1-azaanthraquinone-4-carboxylic acid (2.VIII) which was readily decarboxylated to 1-azaanthraquinone (2.IX). These authors believed that portion of the skeleton of liriodenine was anthraquinonoid; the nature of the remaining ring remained undetermined but was inferred from the formation of an oxime, the suggested presence of a terminal methyl group (i.r. spectrum) and the lack of hydroxyl, methylene-dioxy or methoxyl groups in the molecule. On this basis Buchanan and Dickey suggested the structure (2.X) for liriodenine recognising,

however, that it was a rather unusual one for a natural product.



2.VIII R=CO_2H

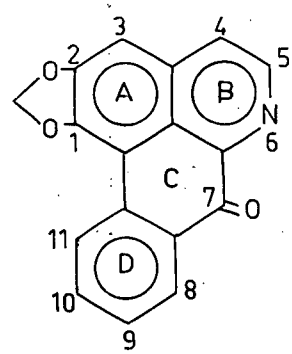
2.IX R=H



2.X

The presence of a methylenedioxy group in liriodenine was demonstrated by Taylor[46] who reinterpreted the experimental data and suggested structure (2.XI) for liriodenine. He confirmed this structural assignment through a facile and refined synthesis.

Working independently, two groups of workers in Taiwan[47,48] isolated a yellow base from Michelia compressa Maxim. var formosana and established its structure as (2.XI). Subsequently this base was directly compared with liriodenine and shown to be identical.



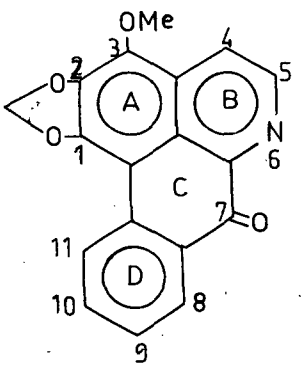
2.XI

The physical properties of spermatheridine were closely related to those of liriodenine; direct comparison of the two compounds

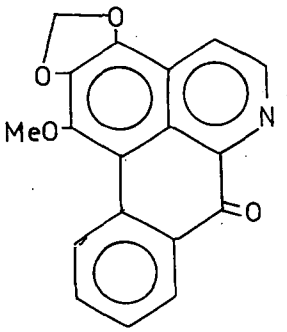
showed them to be identical. Under the name of liriodenine this base was obtained from Michelia champaca Linn.[48], from M.alba DC[49], and from Magnolia coco (Lour). DC[50] (as well as from Liriodendron tulipifera L.[45]); when isolated from Michelia compressa Maxim.[51] and from M.compressa Maxim. var formosana Kanehira it was called oxoushinsunine[47] and micheline B[47] respectively. The name spermatheridine is preferred for this alkaloid since it was the original term under which it was described.

Bick, Clezy and Crow[10] suggested the formula $C_{18}H_{13}O_4N$ for atherospermidine but the analytical data was also consistent with the formula $C_{18}H_{11}O_4N$; a similar analysis was obtained in the present work. Analysis also demonstrated the presence of a methoxy group, the position of which was indicated by a comparison of the i.r. and n.m.r. spectra of atherospermidine and spermatheridine. Both bases showed a strong band at 750 cm^{-1} in their i.r. spectra which was attributed to the C-H out-of-plane deformation of four adjacent aromatic protons[52]. However, a medium band at 861 cm^{-1} in the spectrum of spermatheridine (attributed to the C-H out-of-plane deformation of an isolated aromatic proton[52]) was missing from that of atherospermidine. This fact could only be accounted for if the methoxyl group of the latter was attached to ring A. Thus atherospermidine possessed either structure 2.XII or 2.XIII.

Because of the insolubility of atherospermidine and spermatheridine in the normal n.m.r. solvents their spectra were run in trifluoroacetic acid. Spermatheridine showed an absorption in the



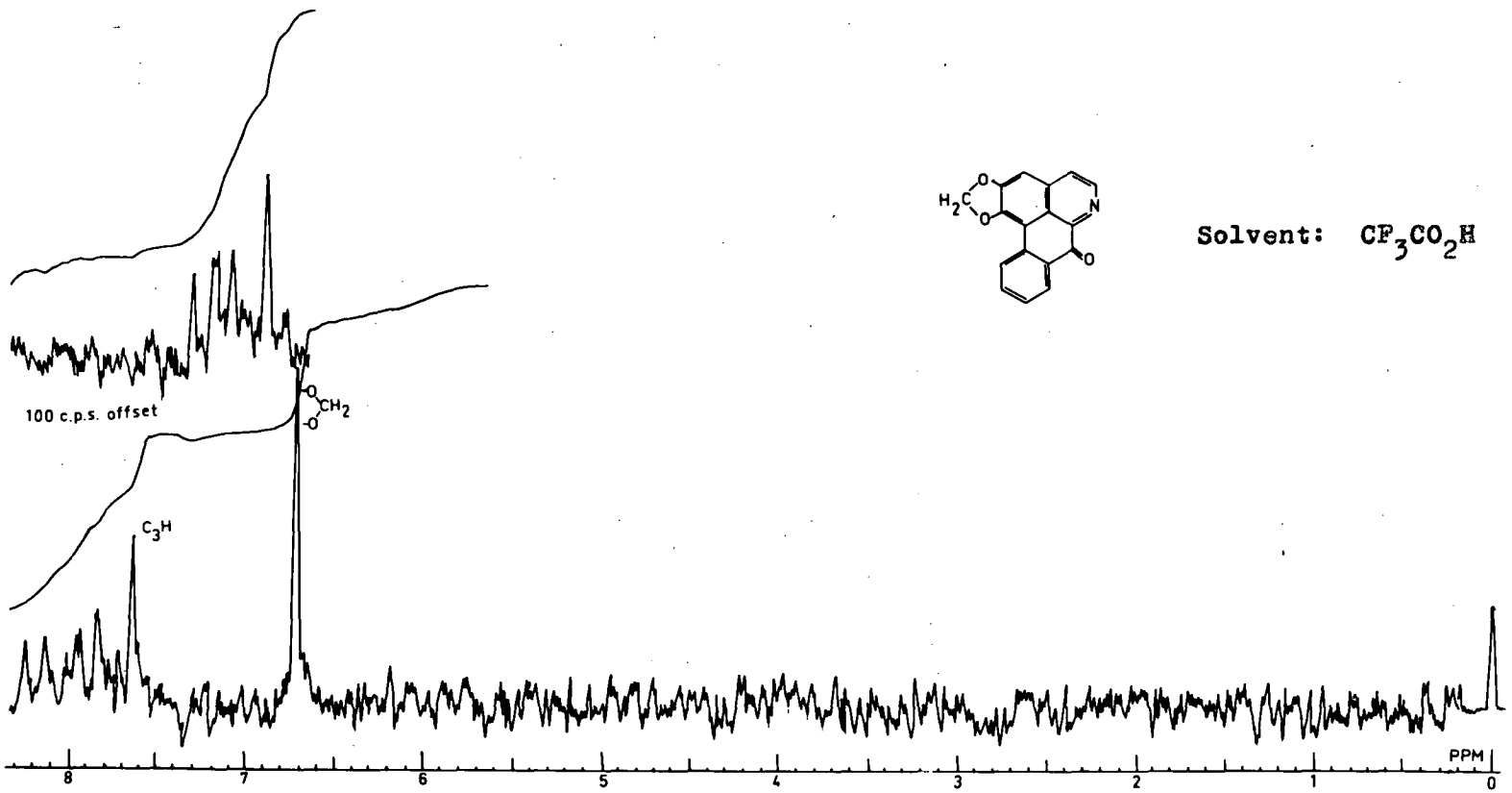
2 . XII



2.XIII

aromatic proton region of intensity one proton at relatively high field (δ7.63 ppm)(Fig.2.1). A high-field aromatic proton resonance in the spectra of aporphines (2.XIV) has been assigned[53] to the proton on C-3 and it seemed a reasonable conclusion that the resonance at δ 7.63 ppm in the spectrum of spermatheridine was also due to the proton attached to C-3. The spectrum of atherospermidine (Fig.2.2.) showed no resonance about δ 7.63 ppm which further indicated that the structure of this base was represented either by 2.XII or 2.XIII.

Since the proposed structures of atherospermidine carried no substituents on ring D, it would be expected that oxidation with chromic acid would yield a product identical with that from spermatheridine. Thus, atherospermidine yielded a carboxylic acid whose i.r. spectrum was identical with the published spectrum of 1-azaanthraquinone-4-carboxylic acid[45] (2.VIII); this acid was readily decarboxylated to 1-azaanthraquinone[45] (2.IX).



Solvent: CF_3CO_2H

Figure 2.1.

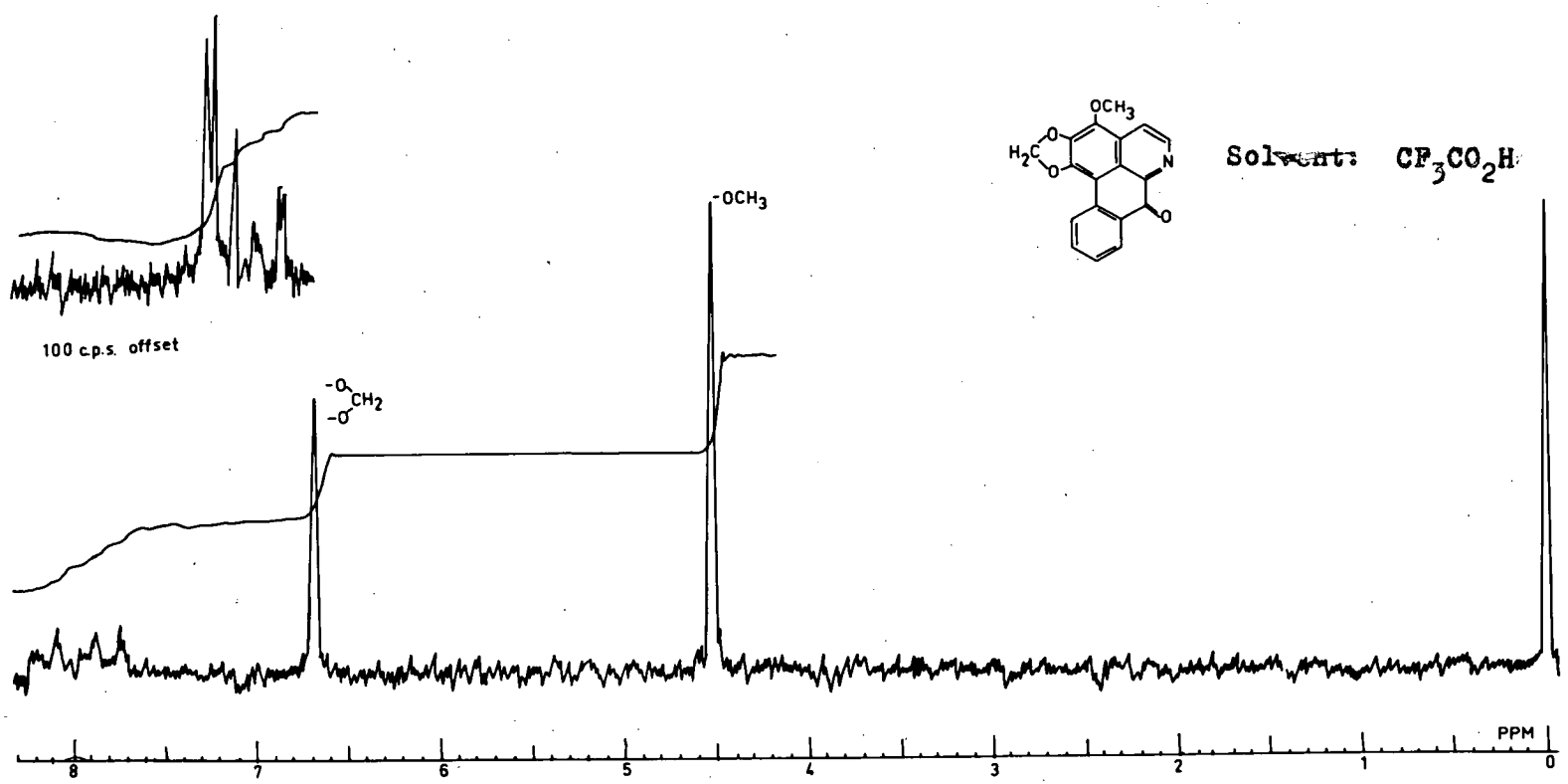
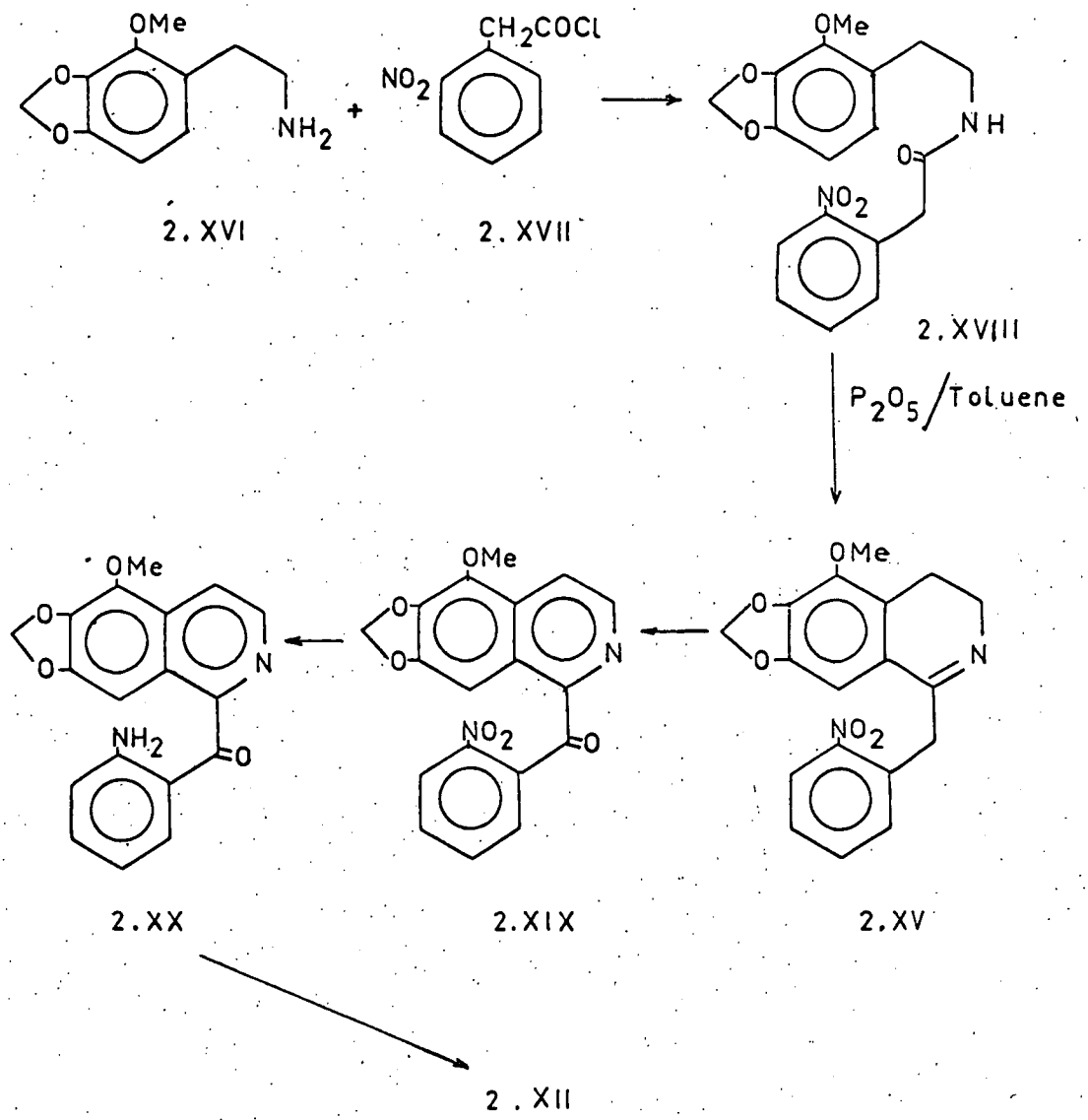

100 c.p.s. offset
-OCH3
-O
-O
CH2
OCH3
H2C
O
O
N
O
Solvent: CF3CO2H
PPM
8
7
6
5
4
3
2
1
0

Figure 2.2.

Proof that atherospermidine possessed structure 2.XII came when it was shown that its properties were identical with those of synthetic 1,2-methylenedioxy-3-methoxy-7-oxo-dibenzo-(de,g)-quinoline (2.XII). The synthesis (Scheme 2.2) involved the formation of 1-(O-nitrobenzyl)-5-methoxy-6,7-methylenedioxy-3,4-dihydroisoquinoline (2.XV). 2-Methoxy-3,4-methylenedioxy-β-phenethylamine[54] (2.XVI) was condensed with O-nitrophenylacetyl chloride (2.XVII) to yield the amide (2.XVIII) which readily underwent the Bischler-Napieralski reaction with phosphorus pentoxide in dry toluene with the formation of (2.XV). Oxidation of this product with selenium dioxide in acetic acid[55] yielded 1-(O-nitrobenzoyl)-5-methoxy-6,7-methylenedioxyisoquinoline (2.XIX). Catalytic reduction[46] of this compound gave the corresponding 1-(O-aminobenzoyl)-derivative (2.XX) which underwent the Pschorr cyclisation to yield 1,2-methylenedioxy-3-methoxy-7-oxo-dibenzo-(de,g)-quinoline (2.XII).

The structure thus demonstrated for atherospermidine was recently proposed independently for psilopine[56], an alkaloid from the anonaceous plant Guatteria psilopus. Through the kindness of Dr. Geissman the two bases were directly compared and shown to be identical.

Taylor[46] suggested that the absence of -OH absorption in the i.r. spectrum of liriodenine oxime (2.XXI) was due to hydrogen bonding between the hydroxyl group and the nitrogen atom of the isoquinoline ring; the absence of -OH absorption in the spectrum



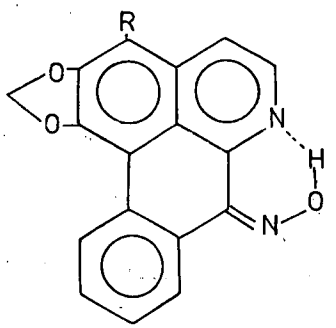
2.XVI 2.XVII 2.XVIII

2.XX 2.XIX 2.XV

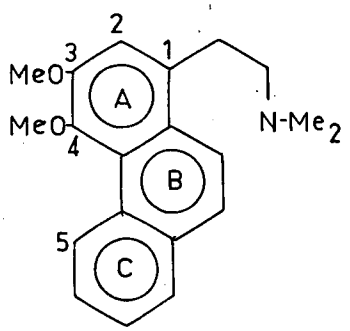
2.XII

Scheme 2.2

of atherospermidine oxime (2.XXII) can be regarded similarly.



2.XXI R=H
2.XXII R=OMe



2.XXIII

The third Atherosperma alkaloid, atherosperminine, failed to crystallise; it was isolated as a clear oil which rapidly darkened upon exposure to the atmosphere. It was purified via its picrate which crystallised as bright yellow needles analysing for $C_{20}H_{23}O_2N.C_6H_3O_7N_3$ with two methoxyl groups. The properties of this and other derivatives, in particular the u.v. spectrum of the methiodide, resembled those reported for 1-N,N-dimethylaminoethyl-3,4-dimethoxyphenanthrene (2.XXIII), an alkaloid isolated by Cook and Haynes from Cryptocarya angulata C.T.White[57] (see Table 2.2).

The n.m.r. spectrum of atherosperminine (Fig.2.3) was in accord with structure (2.XXIII), and showed a peak of intensity six protons at δ 2.25 ppm (NMe_2), a complex multiplet symmetrical about δ 2.80 ppm ($-CH_2-CH_2-$), and two peaks each of intensity three protons at δ 3.88 and δ3.91 ppm (2 x OMe). In the aromatic proton region a singlet of intensity one proton at δ 7.1 ppm was ascribed to the proton on C-2 and a complex multiplet of intensity

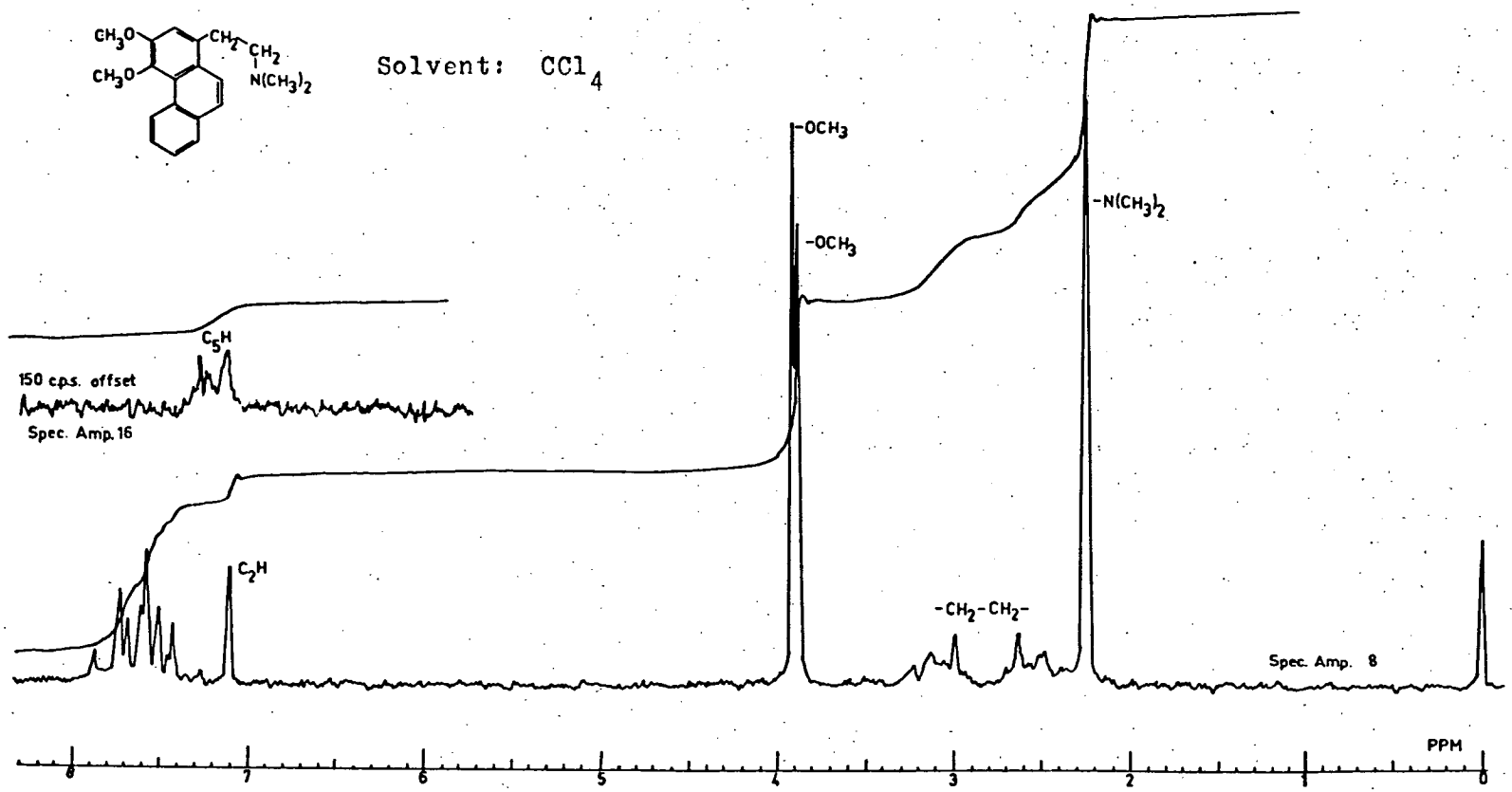

Solvent: CCl_4
CH_3O
CH_3O
CH_2
CH_2
$N(CH_3)_2$
$-OCH_3$
$-OCH_3$
$-N(CH_3)_2$
C_5H
150 c.p.s. offset
Spec. Amp. 16
C_2H
$-CH_2-CH_2-$
Spec. Amp. 8
PPM
8
7
6
5
4
3
2
1
0

Figure 2.3.

one proton at δ 9.67 ppm to that on C-5.

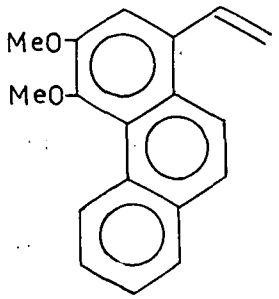
TABLE 2.2.

Physical Constants of Atherosperminine Derivatives and 1-N,N-Dimethylaminoethyl-3,4-dimethoxyphenanthrene Derivatives.

	Atherosperminine	1-N,N-dimethylaminoethyl-3,4-dimethoxyphenanthrene
Methiodide; m.p.	274.5 - 275.5	281 - 282
Methiodide; u.v. spectrum	312(4.08), 302(4.08) 279(4.08), 257(4.73) 251(4.69); inf).	312(4.09),304.5(4.09) 256.5(4.73),252.5(4.70 inf.)
Picrate; m.p.	188 - 189°	187.5 - 188

The identity of the two alkaloids was finally proved by a direct comparison of the picrates and hydriodides; the latter had identical i.r. spectra and showed no melting point depression upon admixture. Furthermore, Hofmann degradation of atherosperminine yielded a dimethoxyvinylphenanthrene, m.p. 80°, identical with the corresponding degradation product (2.XXIV) of 1-N,N-dimethylaminoethyl-3,4-dimethoxyphenanthrene.

Alkaloid B was similar to atherosperminine in that it failed to crystallise and was obtained as a colourless, viscous oil which darkened when exposed to the atmosphere. It was characterised as its picrate and methiodide. The latter analysed for $C_{21}H_{25}O_3N.CH_3I$ with three methoxy groups; its u.v. light absorption spectrum was



2.XXIV

closely related to that of atherosperminine methiodide (Table 2.3). The i.r. spectrum of the free base showed absorption maxima at 2810 and 2760 cm^{-1} attributed to a dimethylamino grouping[58]. Hofmann degradation produced a basic, ammoniacal-smelling gas, presumably trimethylamine, and a nitrogen-free compound whose i.r. spectrum showed the presence of a vinyl grouping. Oxidation of this compound gave a non-acidic product (2.XXVIII) which showed -OH absorption in its i.r. spectrum, analysed for $C_{19}H_{20}O_5$, and gave a positive glycol test[59].

It was evident that alkaloid B was a 1-N,N-dimethylaminoethyl-trimethoxyphenanthrene; the positions of the three methoxyl groups remained only to be determined.

If we assume that these phenanthrene derivatives are formed in nature from the corresponding aporphines, then, on the basis of biogenetic theory[60], positions 3 and 4 should carry oxy groups as in (2.XXIII). Of the possible locations for the third methoxyl

TABLE 2.3.

U.V. Absorption Spectrum of Alkaloid B.

λ_{max}^{EtOH}	log ε_{max}	λ_{max}^{EtOH}	log ε_{max}
308	4.11	259	4.80
295.5	4.05	217	4.52
284	4.03		

group in alkaloid B, i.r. and n.m.r. spectroscopy indicated that position 2 was the correct one.

In the C-H out-of-plane bending region, the i.r. spectrum of atherosperminine (2.XXIII) showed absorption maxima at 750 cm^{-1} (four adjacent aromatic protons[52]) and 860 cm^{-1} (isolated aromatic proton on C-2[52]). The first of these peaks appeared also in the spectrum of alkaloid B, but not the second. This indicated that ring A of alkaloid B was fully substituted and hence that all three methoxy groups were present in this particular ring.

A similar conclusion was reached upon examination of the n.m.r. spectrum of alkaloid B (Fig. 2.4) which resembled that of atherosperminine. It showed a resonance of intensity six protons at δ 2.38 ppm (NMe_2) and a complex multiplet symmetrical about δ 2.95 ppm ($-CH_2-CH_2-$). Three peaks, each of intensity three protons, occurred at δ 3.96, 4.00 and 4.05 ppm (3 x OMe), whilst in the aromatic proton region a series of peaks occurred between δ7.50 and 8.00 ppm, and a multiplet of intensity one proton at δ9.83 ppm (H_5). The absence of any resonance about δ 7.1 ppm

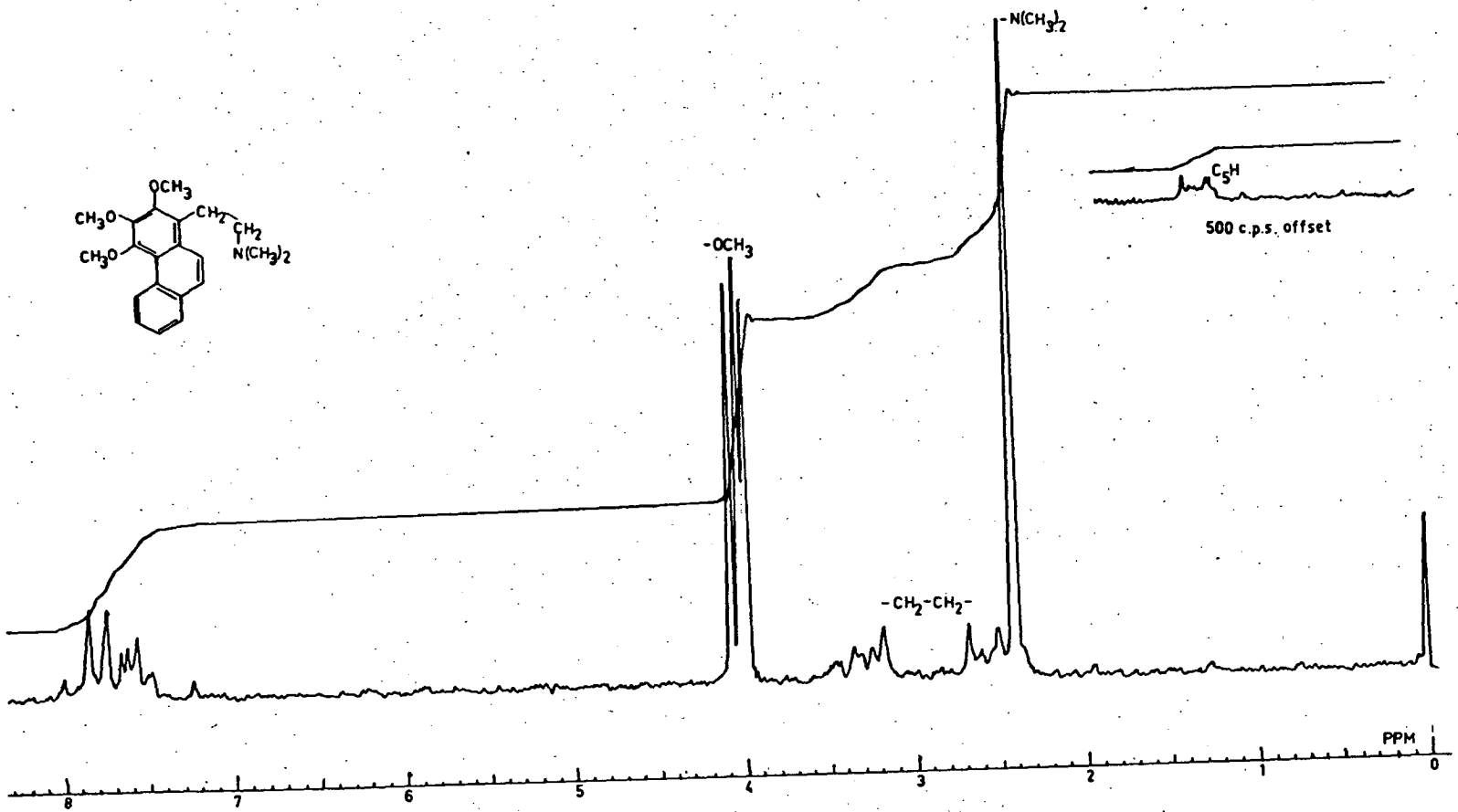
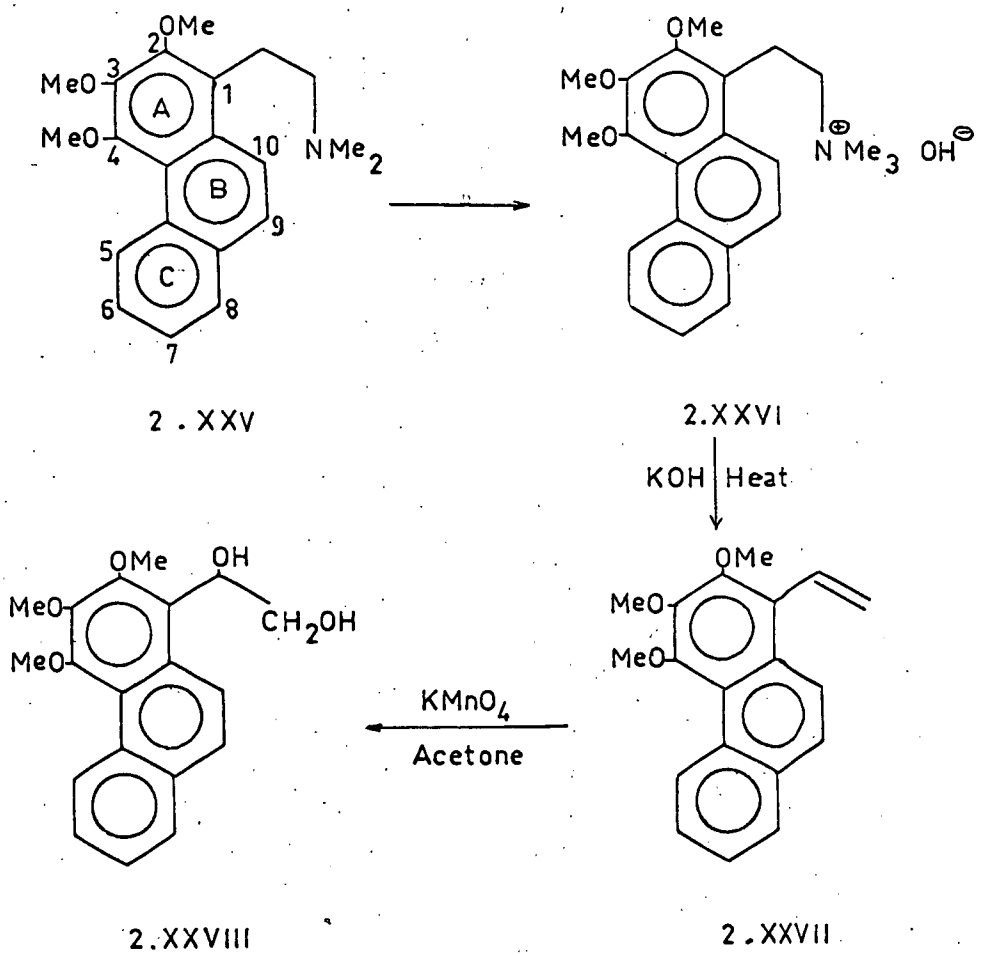

OCH3
CH3O
CH3O
CH2
CH2
N(CH3)2
-OCH3
-N(CH3)2
C5H
500 c.p.s. offset
-CH2-CH2-
PPM
8
7
6
5
4
3
2
1
0

Figure 2.4.

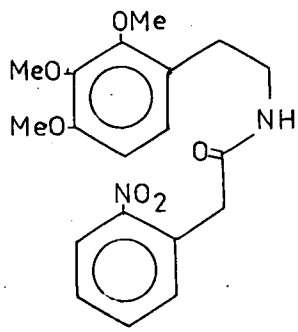
further indicated that C-2 carried the third methoxy group. Alkaloid B is thus a methoxyatherosperminine with structure (2.XXV). The degradation of alkaloid B must have followed the route shown in Scheme 2.3.



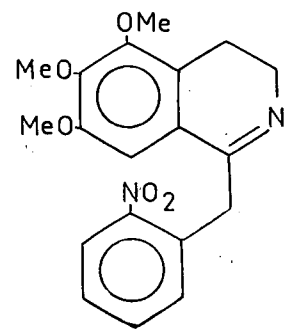
Scheme 2.3

The synthesis of 1-N,N-dimethylaminoethyl-2,3,4-trimethoxyphenanthrene (2.XXV) involved the formation of 1,2,3-trimethoxyaporphine (2.XXIX) by standard methods. The amide (2.XXX), formed from 2,3,4-trimethoxy-β-phenethylamine[61] and O-nitrophenylacetylchloride, underwent the Bischler-Napieralski reaction to form 1-(O-nitrobenzyl)-5,6,7-trimethoxy-3,4-dihydroisoquinoline (2.XXXI). The methiodide of this compound was reduced to 1-(O-aminobenzyl)-2-methyl-5,6,7-trimethoxy-1,2,3,4-tetrahydroisoquinoline (2.XXXII) which was converted to 1,2,3-trimethoxyaporphine by the Pschorr cyclisation. Hofmann degradation of this compound gave 1-N,N-dimethylaminoethyl-2,3,4-trimethoxyphenanthrene (2.XXV) which proved identical with alkaloid B from a comparison of the physical properties of the free bases and their derivatives. 1,2,3-Trimethoxyaporphine methiodide (2.XXXIII) crystallised from an acetone/benzene mixture and retained benzene of crystallisation quite tenaciously. Only after drying over phosphorus pentoxide for 48 hours at 100° and 0.03 mm Hg did the compound analyse for $C_{21}H_{26}O_3NI$. The presence of half a molecule of benzene in the analytical samples dried at 50° was supported by their n.m.r. spectra which showed a singlet of intensity three protons at δ 7.31 ppm.

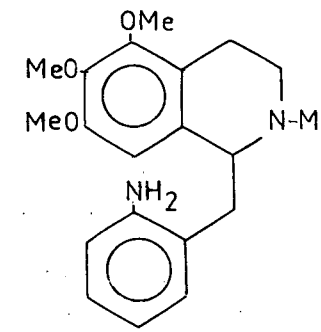
The ranunculaceous plant Thalictrum thunbergii D.C. contains the alkaloid thalicthuberine[62] (2.XXXIV), the only other recorded



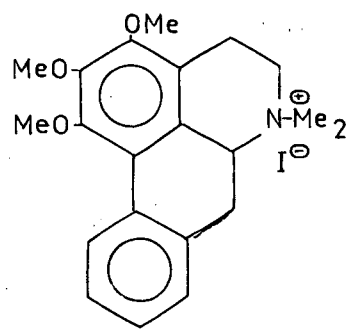
2. XXX



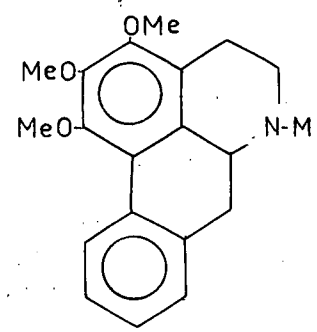
2. XXXI



2. XXXII



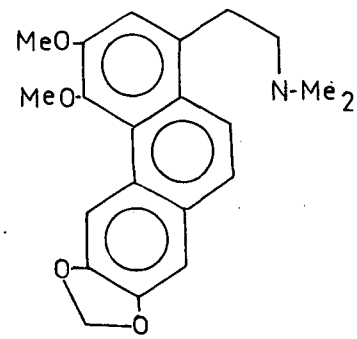
2. XXXIII



2. XXIX

alkaloid with the 1-aminoethylphenanthrene skeleton.

From the phenolic alkaloids of A.moschatum two further yellow alkaloids were obtained for which the names atheroline and moschatoline are suggested. They were separated by Craig distribution in a manner similar to that outlined for the non-phenolic fraction. Atheroline distributed into 0.1% acid whilst moschatoline was extracted by 0.5% acid.



2.XXXIV

Atheroline crystallised from chloroform/ethanol as orange prisms which melted with decomposition over a wide temperature range (250-260°). Its i.r. spectrum showed carbonyl absorption at 1639 cm^{-1}; its u.v. and visible light absorption spectra (Table 2.4) showed that it possessed a 7-oxo-dibenzo-(de,g)-quinoline skeleton.

A positive ferric chloride test and an absorption band at 3250 cm^{-1} in its i.r. spectrum indicated the presence of a phenolic hydroxy group. Treatment of atheroline with acetic anhydride in pyridine yielded O-acetylatheroline which crystallised from pyridine as yellow needles and analysed for $C_{21}H_{17}O_6N$ with one acetyl and three methoxy groups. In the aromatic proton region, the n.m.r. spectrum of O-acetylatheroline (Fig.2.5) showed three one-proton singlets at δ 7.11, 8.20 and 8.80 ppm, and two one-proton doublets (J = 7 cps) centred at δ 7.64 and 8.80 ppm respectively. These features were compatible with structure 2.XXXV, which had oxy

TABLE 2.4.

U.V. and Visible Light Apsorption Spectra of Atheroline.

In EtOH		In 0.05N HCl(EtOH/H_2O)		In 0.05N NaOH(EtOH/H_2O)	
$\lambda^{m\mu}_{max}$	log ε_{max}	$\lambda^{m\mu}_{max}$	log ε_{mex}	$\lambda^{m\mu}_{max}$	log ε_{max}
244	4.09	257	4.12	252	4.04
273	4.17	282	4.12	294	3.99
292(inf.)	3.96	-	-	320	3.98
355	3.90	-	-		
380(inf.)	3.83	385	4.05	390	3.74
435	3.62	500	3.38	535	3.46

substituents at positions 1,2,9 and 10. The singlet at high field was assigned to H_3[53], that at δ 8.20 ppm to H_8 and that at δ 8.80 ppm to H_{11}[53]. The doublets form an AB quartet arising from H_4 and H_5; by comparison with pyridine[63], H_5 would be expected at lower field than H_4, which was thus assigned the doublet around δ 8.80 ppm. The conversion of atheroline into the known alkaloid 1,2,9,10-tetramethoxy-7-oxo-dibenzo-(de,g)-quinoline[64](2.XXXVI) by methylation with methyl iodide and methanolic sodium methoxide confirmed the skeleton of atheroline and the location of oxy substituents at positions 1,2, 9 and 10.

At first, the structure 2.XXXVIII was proposed for atheroline on the basis of its n.m.r. and u.v. spectra[78]. In the methoxyl proton region of the spectrum of O-acetylatheroline (Fig.2.5) a peak of intensity six protons occurred at δ 4.01 ppm and one of intensity

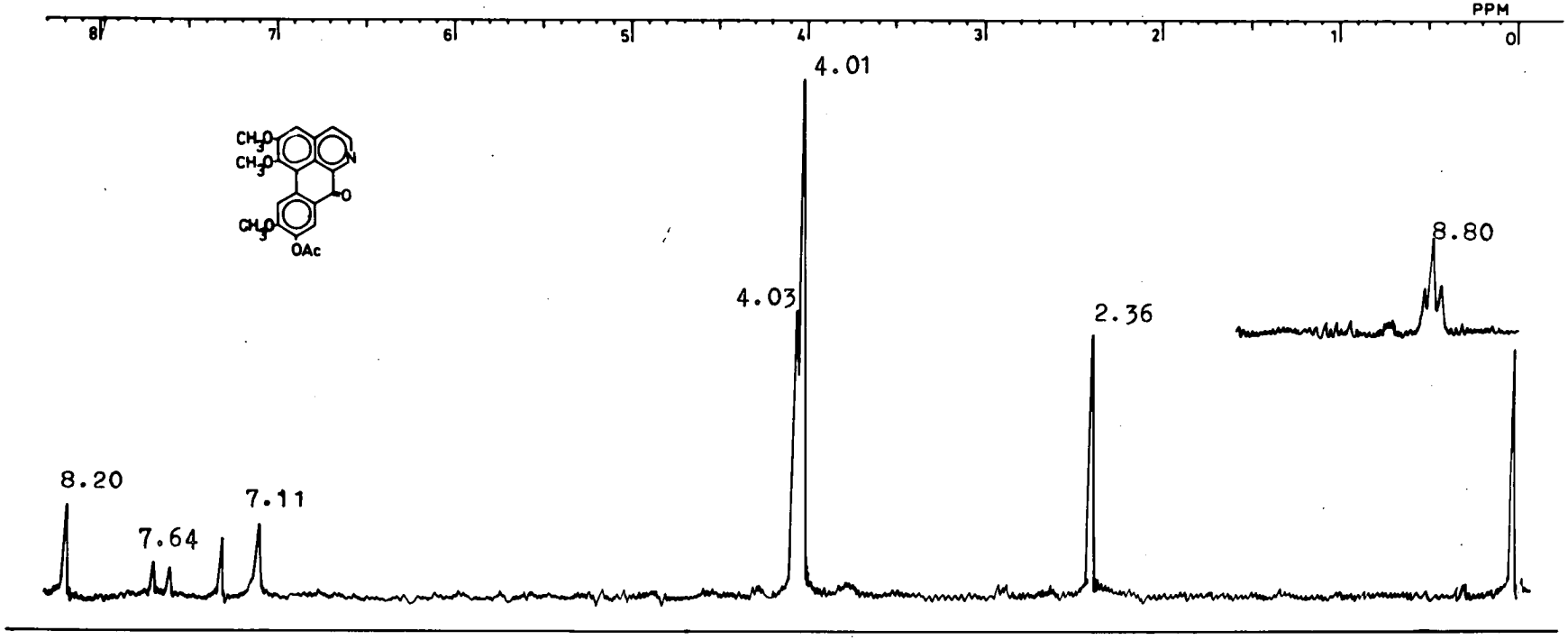

PPM
8
7
6
5
4
3
2
1
0
4.01
4.03
2.36
8.80
8.20
7.64
7.11
CH3O
CH3O
CH3O
OAc
N
O

Figure 2.5.

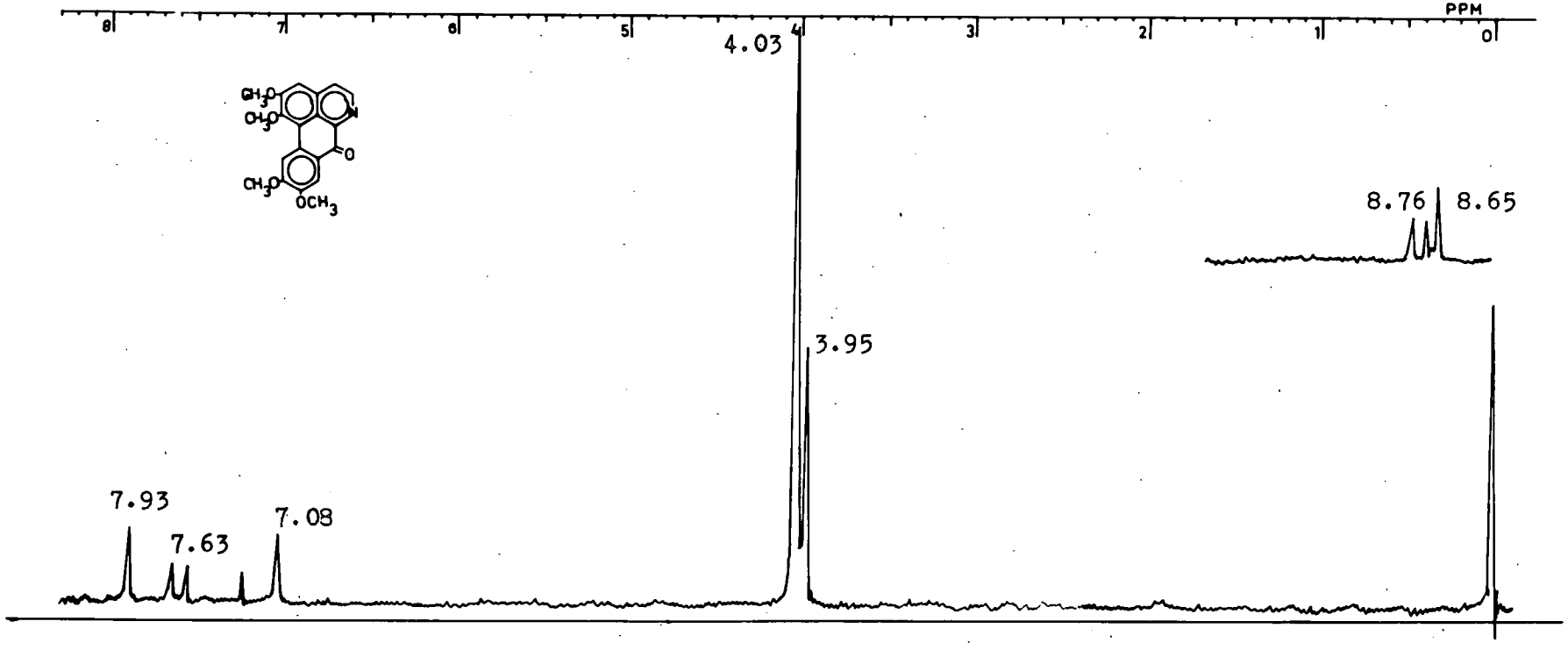
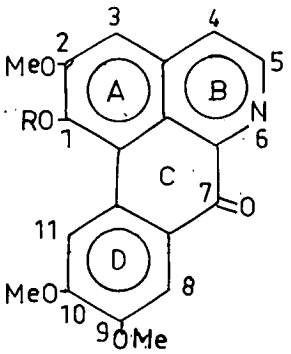


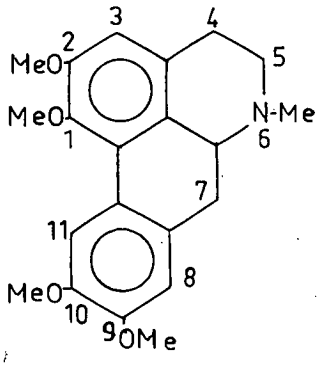
Figure 2.6.



2.XXXV (R = Ac)

2.XXXVI (R = Me)

2.XXXVIII (R = H)

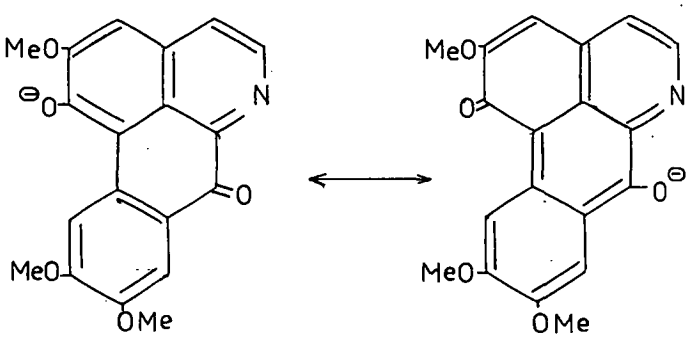


2.XXXVII

thee protons at δ 4.03 ppm. All three methoxyls thus gave almost indistinguishable proton absorptions; with O-methylatheroline (2.XXXVI) and glaucine[53] (2.XXXVII), however, three of the methoxyls absorbed at the same field, while the fourth absorbed at distinctly higher field, although the difference in the case of O-methylatheroline was far less marked than in the case of glaucine. The high-field methoxyl resonance in the spectrum of glaucine was assigned[53] to the methoxyl at position 1 since it was shielded by the adjacent aromatic ring D. The same consideration should hold good for O-methylatheroline (2.XXXVI) and thus the three proton peak at δ 3.95 ppm (Fig.2.6) was assigned to the methoxyl at position 1, and the nine-proton peak at δ 4.03 ppm to the remaining three methoxyls. The absence of a peak around δ 3.95 ppm in the spectrum of O-acetyl

atheroline indicated that this base had its acetoxy group located at position 1 and that it was represented by (2.XXXV), while atheroline was represented by (2.XXXVIII).

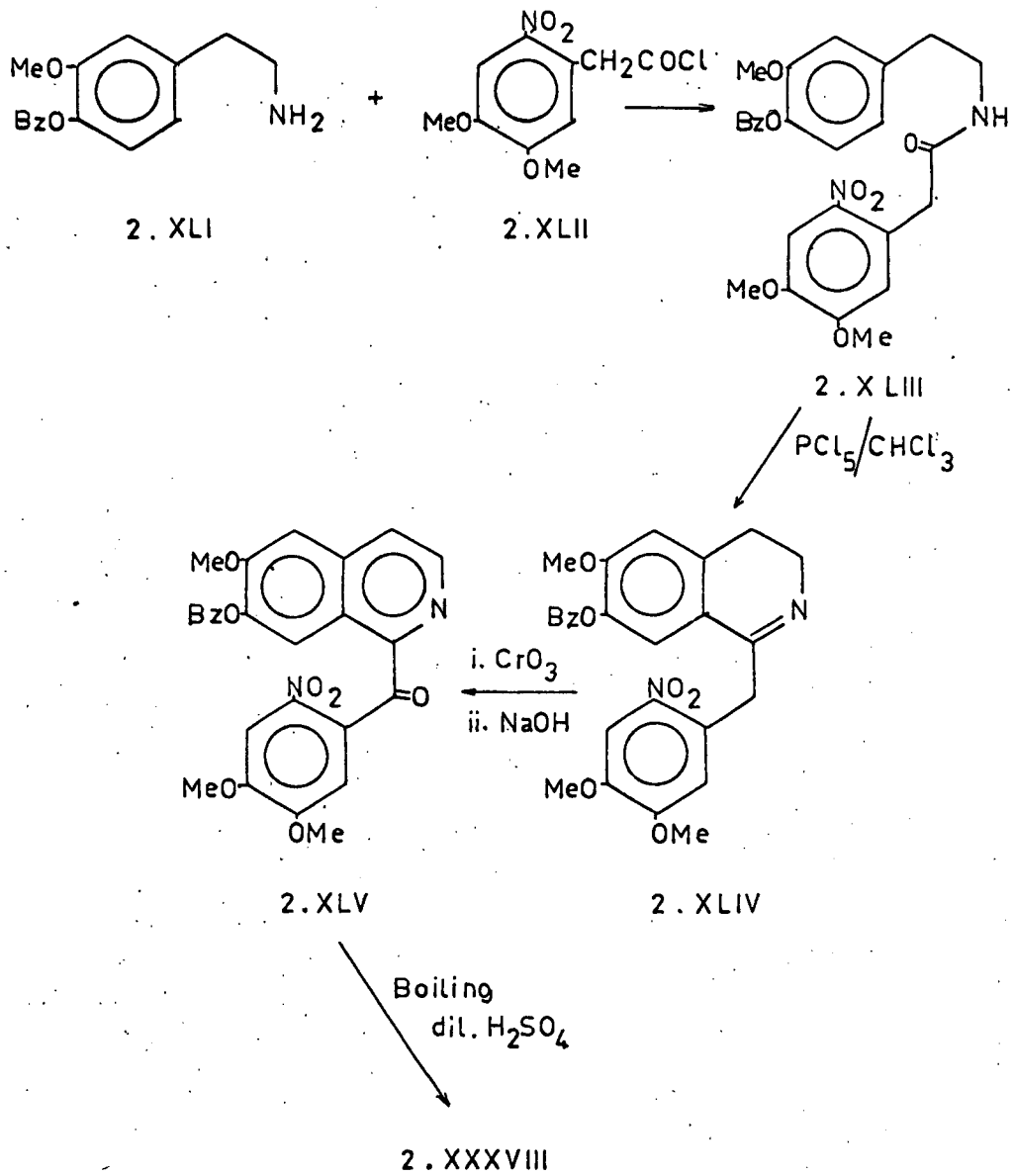
The large bathochromic shift of the absorption bands of the u.v. and visible light absorption spectra of atheroline in alkali as compared to those observed in neutral solution (Table 2.4.) was considered to support this proposal. This shift was ascribed to mesomerism of the anion (2.XXXIX) formed by ionization of the phenolic group. The contributing form (2.XL) to the resonance hybrid contains three aromatic rings as for (2.XXXIX) and should have comparable energy to the latter form. This fact, together with the distribution of charge between the two canonical forms, would be expected to produce a considerable deepening of colour of the anion as compared to the neutral molecule[65], greater than if the hydroxyl was located in any of the alternative positions 2,9 and 10, since none of these locations for the hydroxyl group would result in anions with contributing forms of so nearly equal energy.



2.XXXIX 2.XL

To confirm the proposed structure (2.XXXVIII) for atheroline, the total synthesis of 1-hydroxy-2,9,10-trimethoxy-7-oxo-dibenzo-(de,g)-quinoline was attempted, but failed at the penultimate stage. Throughout the synthesis the hydroxyl group was protected by benzylation; it was to be finally removed by hydrolytic cleavage with boiling dilute sulphuric acid[66]. The synthesis (Scheme 2.4) involved steps analogous to those outlined in the synthesis of atherospermidine. 1-(2'-Nitro-4',5'-dimethoxybenzoyl)-6,7-dimethoxyisoquinoline (2.XLV) was obtained in good yield but when it was shaken in a hydrogen atmosphere in the presence of a Raney Nickel catalyst and the reduction product subjected to a Pschorr cyclisation a considerable quantity of purple material was obtained and only a trace of yellow, non-phenolic product. The latter was heated under reflux in dilute sulphuric acid to yield a pink solution which gave a yellow compound upon neutralization. This product gave a blue colour in alkali - considerably deeper than the colour of atheroline in alkali. From these colour reactions it appeared that this small quantity of material was, in fact, the required product but its identity could not be confirmed because of insufficient material. Its deeper blue colour in alkali as compared to that of atheroline threw some doubt on the proposed structure (2.XXXVIII) for the latter.

Almost certainly the failure of the Pschorr cyclisation resulted from the debenzylation of (2.XLV) when the reduction of the nitro-group was attempted. Attempts to reduce this group using aqueous


MeO
BzO
NH2
+
NO2
CH2COCl
MeO
OMe
NH
O
2.XLI
2.XLII
2.XLIII
PCl5/CHCl3
i. CrO3
ii. NaOH
N
2.XLV
2.XLIV
Boiling
dil. H2SO4
2.XXXVIII

Scheme 2.4

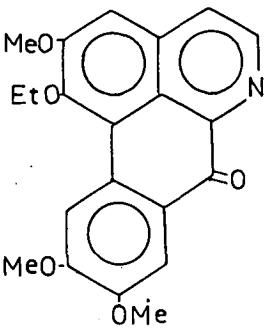
sodium sulphide[67] or iron filings in acetic acid[68] also failed.

An alternative means of confirming the proposed structure of atheroline involved the comparison of the properties of synthetic 1-ethoxy-2,9,10-trimethoxy-7-oxo-dibenzo-(de,g)-quinoline (2.XLVI) with those of O-ethylatheroline. The synthesis involved steps analogous to those outlined in Scheme 2.4; the reduction of the nitro-group of the compound corresponding to (2.XLV), and subsequent Pschorr cyclisation, proceeded smoothly and the required product (2.XLVI) was obtained.

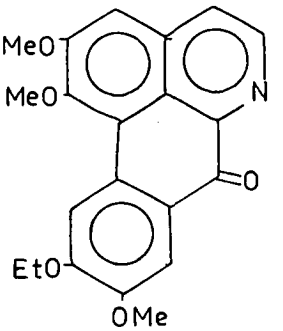
O-Ethylatheroline, prepared by ethylation of atheroline with ethyl iodide and sodium ethoxide, melted at 210-212° whilst the synthetic material (2.XLVI) melted at 196-198° ; a substantial depression in melting point resulted when the two compounds were mixed. The i.r. spectra of the two compounds were closely related but significant differences occurred in the 700-1000 cm^{-1} region.

On the basis of this evidence, structure (2.XLVI) for O-ethylatheroline was rejected and the proposed structure (2.XXXVIII) for atheroline proved to be incorrect. A further two isomers of 1-ethoxy-2,9,10-trimethoxy-7-oxo-dibenzo-(de,g)-quinoline (2.XLVI) were prepared and their properties compared with those of O-ethylatheroline. 1,2,9-Trimethoxy-10-ethoxy-7-oxo-dibenzo-(de,g)-quinoline (2.XLVII) depressed the melting point of O-ethylatheroline and the i.r. spectra of the two compounds, although closely related, were not identical. However, the i.r. spectra of 1,2,10-trimethoxy-9-ethoxy-7-oxo-dibenzo-(de,g)-quinoline (2.XLVIII) was identical with

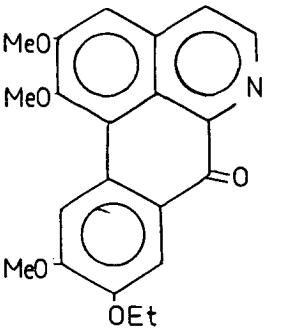
that of O-ethylatheroline and the two compounds showed no mixed-melting point depression. O-Ethylatheroline is therefore represented by structure 2.XLVIII and atheroline by 2.XLIX.



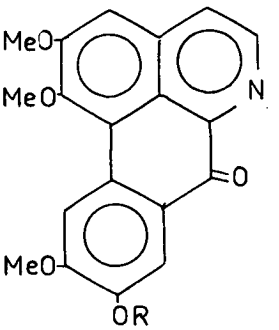
2.XLVI



2.XLVII



2.XLVIII



2.XLIX (R=H)

2.L (R=Ac)

A clue indicating the correct structure of atheroline, overlooked in the initial assessment of the n.m.r. evidence, could now be appreciated: whilst the aromatic proton resonances due to H_3, H_4 and H_5 (Figs.2.5 and 2.6) occurred at the same field for both O-acetylatheroline and O-methylatheroline, resonances due to H_8

(δ 8.20 ppm) and H_{11} (δ 8.80 ppm) in the spectrum of O-acetylatheroline occurred at rather lower field than the corresponding resonances in that of O-methylatheroline (δ 7.93 ppm and δ 8.65 ppm respectively.) Furthermore, the down-field shift of the H_8 resonance was greater than that of the H_{11} resonance.

It is well established that electron-withdrawing groups attached to an aromatic nucleus produce downfield shifts of the resonance positions of aromatic protons and this shift is greater in the ortho- than the meta- or para-positions[80]. Highet and Highet[69] have recently reported that such is the case for the aromatic protons of a phenol acetate as compared to those of the phenol whilst the resonance positions of the aromatic protons of the phenol remain unchanged upon the formation of the methyl ether. Taking these results into consideration, the acetyl group of O-acetylatheroline must be in ring D, ortho to H_8 and meta to H_{11} thus supporting the structure (2,L) for O-acetylatheroline and (2.XLIX) for atheroline.

Recently, atheroline was isolated from Nemuaron viellardii[70], a monimiaceous plant from New Caledonia.

Moschatoline failed to crystallise but formed a yellow crystalline acetate and a pink hydrochloride. Its u.v. and visible light absorption spectra (Table 2.5) clearly indicated that it possessed a 7-oxo-dibenzo-(de,g)-quinoline skeleton; this was supported by the i.r. spectrum of O-acetylmoschatoline which showed absorption bands at 1775 cm^{-1} and 1657 cm^{-1}. The former was assigned to the ester carbonyl. Moschatoline gave a negative methylenedioxy test and a

positive test with ferric chloride.

TABLE 2.5.

U.V. and Visible Light Absorption Spectra of Moschatoline.

In EtOH		In 0.05N HCl(EtOH/H_2O)		In 0.05N NaOH(EtOH/H_2O)	
$\lambda^{m\mu}_{max}$	log ε_{max}	$\lambda^{m\mu}_{max}$	log ε_{max}	$\lambda^{m\mu}_{max}$	log ε_{max}
237	4.47	246	4.37	247	4.42
272	4.41	281	4.40	283	4.31
315(inf.)	4.10	-	-	310	4.25
374	3.55	390	3.63	407	3.99
440	3.67	496	3.36	517	3.33

The n.m.r. spectrum of O-acetylmoschatoline (Fig.2.7) showed two methoxyl resonances (three-proton singlets at δ 3.91 and δ 4.11 ppm) and one acetyl group (three-proton singlet at δ 2.50 ppm). The absence of any one-proton singlet around δ 7.10 ppm in the aromatic proton region indicated that C-3 carried a substituent. Biogenetic theory[60] would predict that positions 1 and 2 would carry oxy substituents and hence ring A would be fully substituted. This conclusion was supported by the i.r. spectrum of O-acetylmoschatoline which showed a medium band at 760 cm^{-1} (C-H out-of-plane deformation of four adjacent aromatic protons[52]) and was confirmed when it was shown that the physical properties of O-methylmoschatoline were identical with those of synthetic 1,2,3-trimethoxy-7-oxo-dibenzo-(de,g)-quinoline (2.LI) prepared from 1-(O-nitrobenzyl)-5,6,7-trimethoxy-3,4-dihydroisoquinoline (2.LII)(Scheme 2.5).

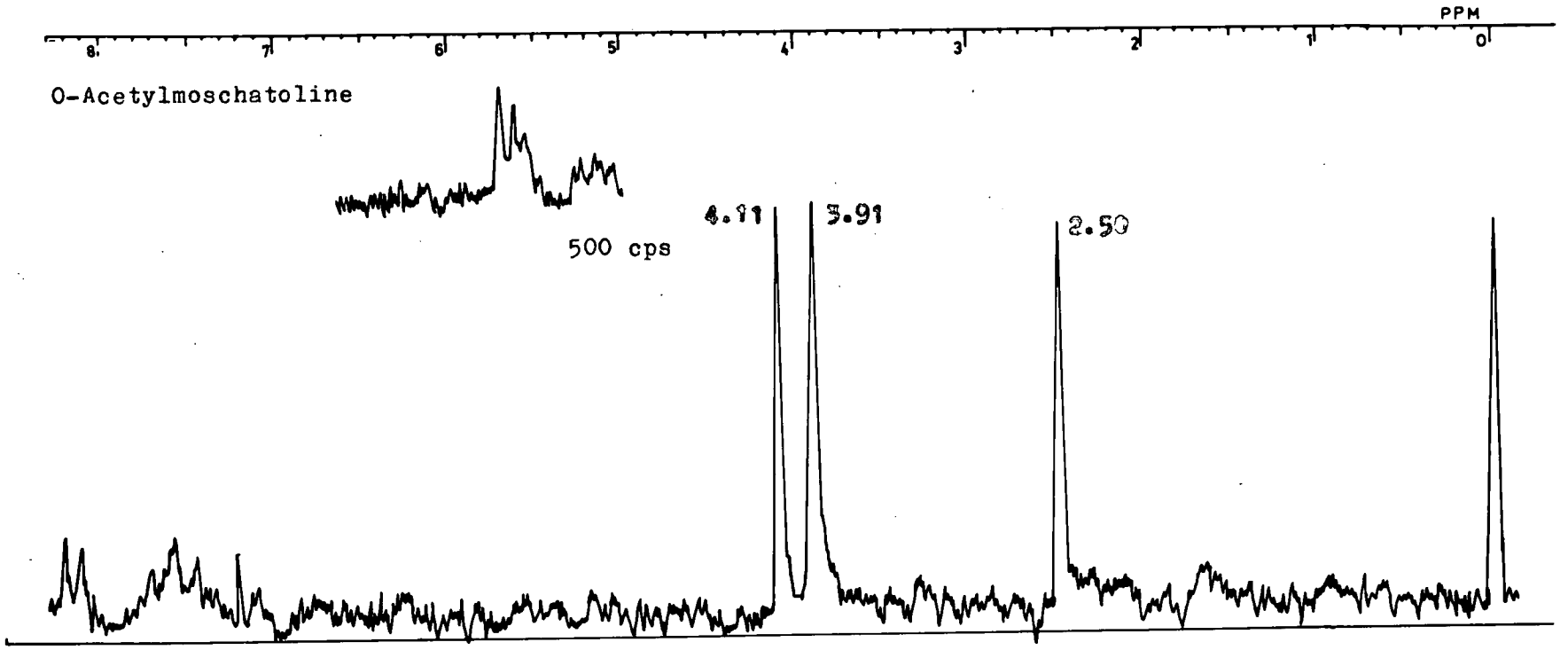


Figure 2.7.

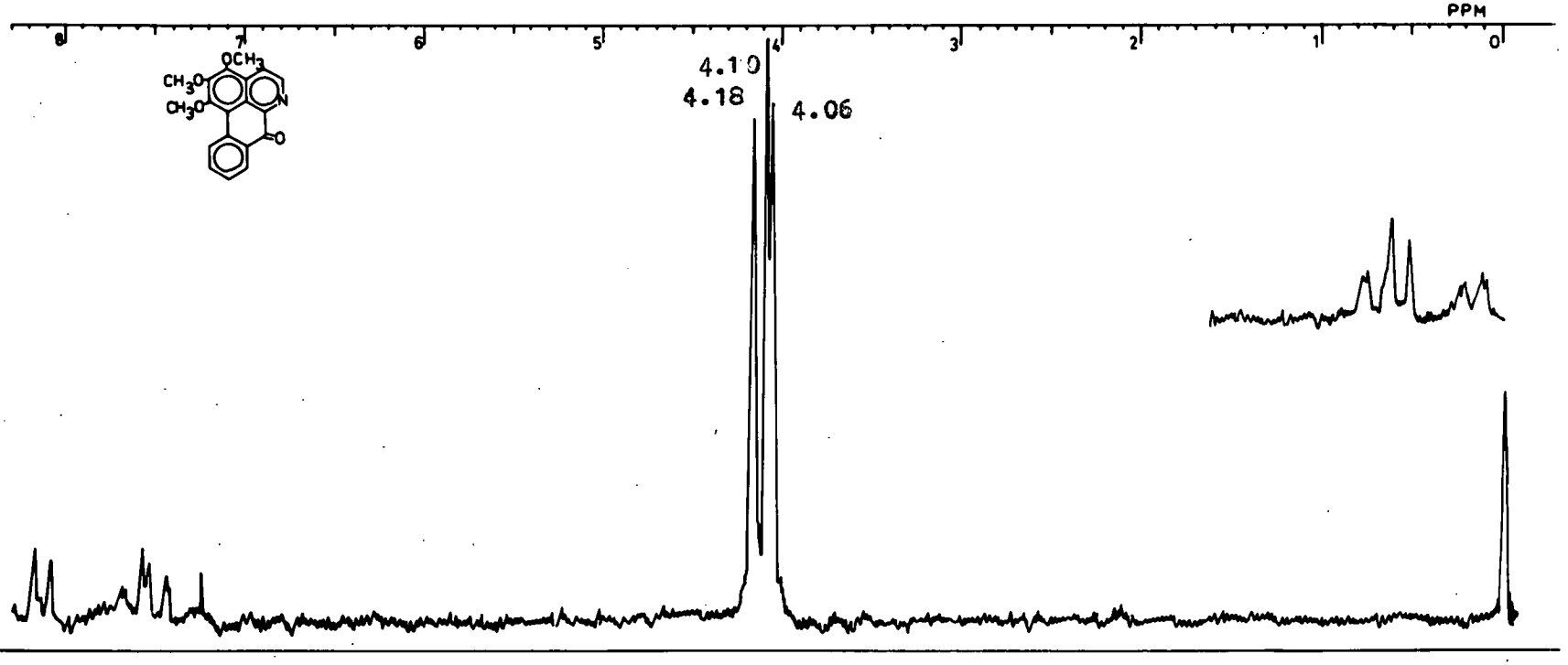
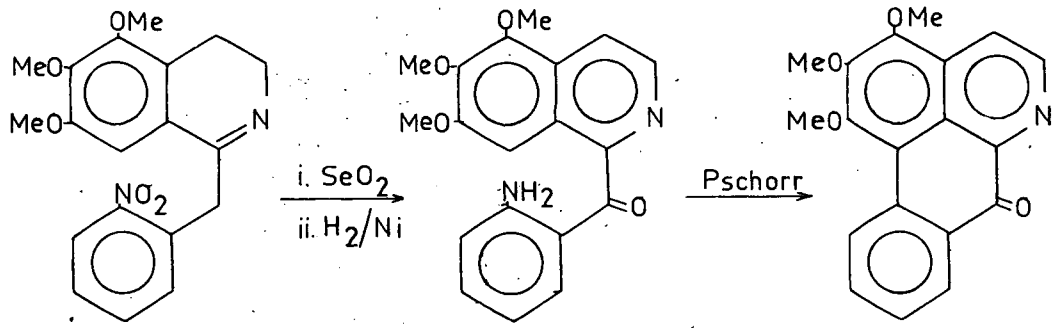


Figure 2.8.

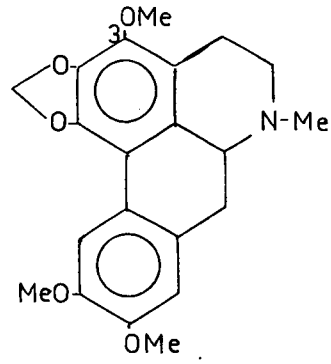


2.LII 2.LI

Scheme 2.5

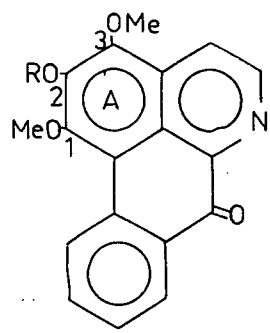
The n.m.r. spectrum of O-methylmoschatoline (Fig.2.8) showed three distinct methoxyl resonances at δ 4.06, 4.10 and 4.18 ppm. By comparison with the n.m.r. spectrum of the aporphine alkaloid ocoteine[71] (2.LIII) the low-field methoxyl resonance was assigned to the methoxyl group on C-3. The high-field resonance was assigned to the methoxyl on C-1[53]; the remaining resonance was due to that on C-2.

In the n.m.r. spectrum of O-acetylmoschatoline one of the methoxy groups resonated at considerably higher field (δ 3.91 ppm) than any of the methoxyl groups of O-methylmoschatoline. The presence of the acetyl group therefore had a pronounced effect on the position of the methoxy resonances thus enabling no correlation to be made between the resonance positions of the methoxyl groups



2.LIII

of O-methylmoschatoline with those of O-acetylmoschatoline. However, the wide separation of the methoxyl resonances in the latter leads one to suggest that these two groups are on positions 1 and 3. Thus O-acetylmoschatoline is assigned the tentative structure 2.LIV and moschatoline the structure 2.LV.



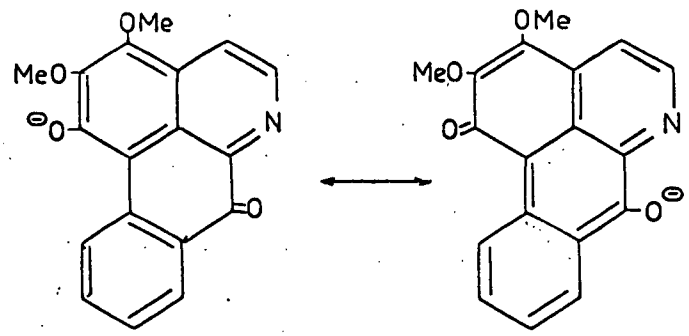
2. LIV (R = Ac)

2. LV (R = H)

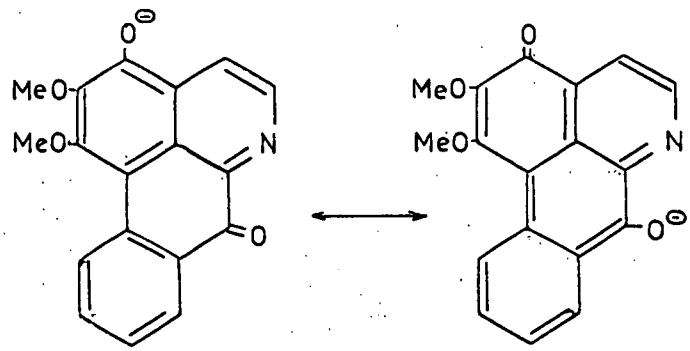
The u.v. and visible light absorption spectra of moschatoline (Table 2.5) lend support to this proposal. The absorption bands underwent a bathochromic shift in ethanolic alkali as compared to

those in ethanol; the magnitude of this shift was of the same order as that observed for atheroline and likewise was ascribed to mesomerism of the anion. If the phenol grouping was at position 1 or 3 the contributing structures (2.LVI) and (2.LVII) to the resonance hybrids of the respective anions (2.LVIII) and (2.LIX) would each contain three aromatic rings and thus have similar energies. Upon the addition of alkali, the u.v. and visible light absorption bands of both compounds would thus be expected to undergo bathochromic shifts of similar magnitude but these shifts would be greater than if the phenol group was at position 2. In the case of the latter compound the contributing structure (2.LX) to the resonance hybrid of the anion (2.LXI) contains only one aromatic ring and the energy difference between the two canonical forms would be expected to be far greater than if the phenol group was at either of the alternative positions 1 or 3, but only slightly less than that between the contributing structures (2.LXII) and (2.LXIII) to the resonance hybrid of the atheroline anion. Since the shift observed for moschatoline is of similar magnitude to that for atheroline the above considerations point to the phenol group of moschatoline being placed at position 2.

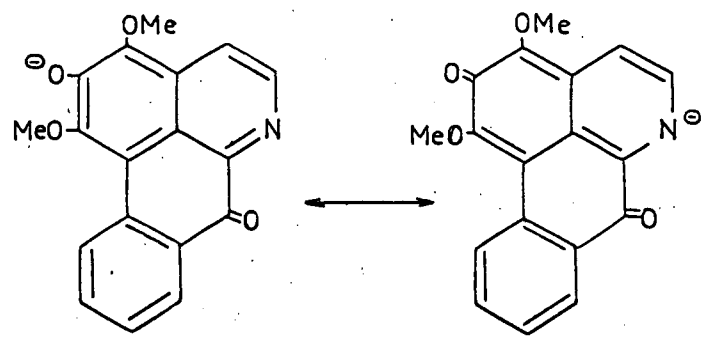
Thin-layer chromatography of the cryptophenolic bases of sassafras indicated that this fraction still contained considerable quantities of non-phenolic bases. Consequently they were redissolved in benzene and re-extracted with alkali. The alkali extracts were washed thoroughly with benzene, the washings combined



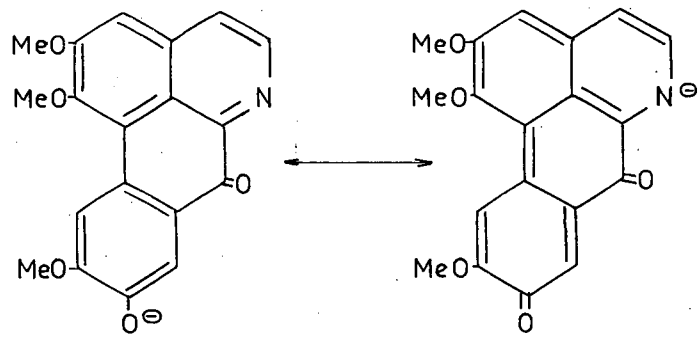
2.LVI 2.LVIII



2.LVII 2.LIX



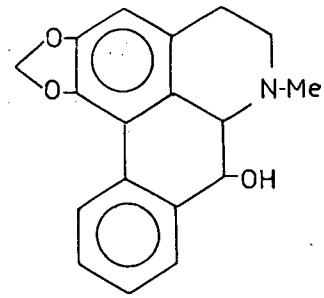
2.LXI 2.LX



2. LXII 2. LXIII

with the original benzene layer and the solvent removed under reduced pressure. The residual non-phenolic bases were separated by Craig distribution. The early fractions contained mainly one compound (A') but failed to crystallise; treatment with acetic anhydride in pyridine produced no crystalline material but thin-layer chromatography showed that a reaction must have occurred since the major component showed an R_f of considerably greater magnitude than for A'. It therefore seemed likely that an alcoholic hydroxy group was present in A'. Oxidation of A' with selenium dioxide in acetic acid produced a yellow, crystalline derivative identified as spermatheridine (2.XI). This evidence indicates that A' is identical with micheline A (2.LXIV) an alkaloid isolated from Michelia compressa var formosana[47].

An interesting biogenetic relationship (see Scheme 2.6) exists between the alkaloids isolated from Atherosperma moschatum. The spermatheridine types presumably arise by oxidation of the

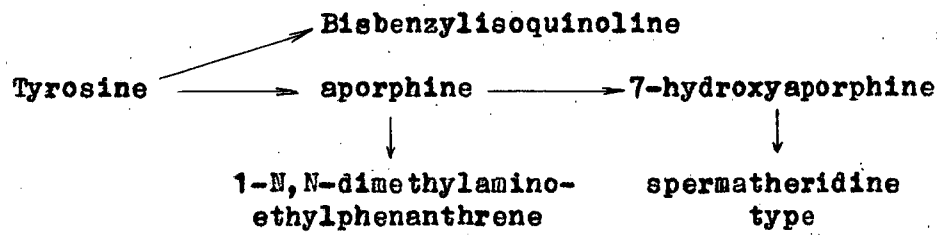


2.LXIV

corresponding aporphine, possibly by way of the 7-hydroxy-aporphine molecule; the 1-N,N-dimethylaminoethylphenanthrenes presumably arise from aporphines by a natural Hofmann degradation. Barton[72] et al. have shown that the methylenedioxy group arises in nature from a methoxy group ortho to a phenolic hydroxy. If the proposed structure for moschatoline proves to be correct then it is likely that this base is the direct biogenetic precursor of atherospermidine.

Scheme 2.6.

Possible Biogenetic Relationship of *Atherosperma* Alkaloids.



The application of tracer techniques to the biosynthesis of the Atherosperma alkaloids should yield interesting results.

EXPERIMENTAL.

Isolation and Separation of the Alkaloids.

A large scale extraction of the bark of A.moschatum (32 kg) was carried out by the Division of Organic Chemistry, C.S.I.R.O., and involved percolation with warm ethanol followed by concentration of the crude extract. This was poured into dilute sulphuric acid, the acid mixture filtered and the filtrate basified (NH_3; pH 9) and extracted with chloroform. The chloroform solution of crude alkaloid was extracted with aqueous sodium hydroxide (5%, 4 x 1½ l) to remove the phenolic alkaloids (175 g; Fraction A). A large quantity of dark, viscous material (Fraction B) which separated between the sodium hydroxide and chloroform layers was set aside. The chloroform was removed and the residual solid heated under reflux with benzene and filtered from insoluble material. When cooled, the filtrate deposited berbamine as colourless needles. Most of the berbamine was removed by repeated crystallisation. (Total yield 500 g; Fraction C.)

The combined benzene mother liquors were then extracted with aqueous sodium hydroxide (5%; 4 x 1½ l) and washed with water. The alkali extract and water washings were combined, neutralized and extracted with chloroform to give the cryptophenolic bases (Fraction D). Removal of the benzene gave a deep brown solution with a highly

aromatic odour similar to that of fresh sassafras bark. Since this solution evidently contained a large amount of essential oil which had not been removed by earlier processes, it was dissolved in chloroform and extracted with hydrochloric acid (5%; 5 x 2 l). The first hydrochloric acid extract (Fraction E; 275 g) was kept separate from the remaining extracts (114 g; Fraction F) because of the obviously higher concentration of atherospermidine and spermatheridine in the latter.

A partial separation of the components of portion of Fraction E (30 g) was achieved by Craig distribution in which chloroform was the stationary phase and the mobile phase was dilute hydrochloric acid, the concentration of which increased from 0.1% to 5%. (Table 2.6). Material which distributed into the 0.1% - 5% hydrochloric acid failed to crystallise, whilst the 1% acid extract gave spermatheridine (63 mg). Atherospermidine (50 mg) was obtained from the 5% acid extract.

TABLE 2.6.

Craig Distribution of Fraction E.

Transfer No.	Acid Concentration (%).
1-23	0.1
24-47	0.25
48-68	0.5
69-89	1.0
90-118	2.0
119-143	3.0
144-295	5.0

Chromatography on alumina of the material which remained in the chloroform layer gave atherosperminine (0.8 g) and alkaloid B (0.5 g), both of which were eluted by benzene and isolated as picrates.

Because of the limited solubility of the latter compounds in 5% hydrochloric acid the remaining portion of Fraction E was dissolved in chloroform and extracted with acid of this concentration (5 x 4 l). The acid layer gave 152 g of crude alkaloids which were separated by Craig distribution in a manner similar to that described above. The chloroform-soluble material (89 g) was chromatographed on alumina; the material eluted by chloroform (52 g) was combined and rechromatographed in benzene, the eluate from one column of alumina (1½ kg; 69 x 5.5 cm) passing on to the top of a second column (200 g; 2.4 x 48 cm). Fractions of 25 ml were collected; the content of each fraction was analysed by paper chromatography using the system butanol : acetic acid : water (80 : 3 : 17 $^{v}/_{v}$). Fractions 6-27 yielded alkaloid B (5.3 g) and fractions 28-615 yielded atherosperminine (31 g). Chloroform (30%) in benzene eluted isocorydine (2 g) m.p. 179-181° (acetone/pet. spirit).

Thin-layer chromatography of the cryptophenolic fraction (Fraction D) and the non-phenolic fraction (Fraction E) showed that complete separation of these two types of bases had not been achieved by the above process; although the cryptophenolic bases were concentrated into the cryptophenolic fraction considerable quantities

of non-phenolic bases were still present. Fraction D (100 g) was therefore re-dissolved in benzene and extracted with alkali (2%; 4 x 1 l). The alkali extract was washed thoroughly with benzene and the washings and original benzene solution evaporated to dryness. The residual non-phenolic material (21 g) was separated by Craig distribution. Colourless material (5 g; Fraction A') distributed into the 0.01% acid layer failed to crystallise. An attempt to form a crystalline acetate (acetic anhydride/pyridine) also failed but thin-layer chromatography on alumina (Fig.2.9) indicated that a reaction had occurred.

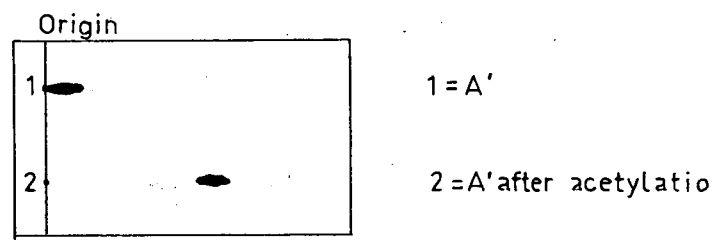


Fig. 2.9

A portion of fraction A' (1 g) in acetic acid (10 ml) was heated two hours on the water bath with selenium dioxide (0.5 g). The reaction mixture was poured into water, basified (NH_3) and extracted with chloroform. The chloroform extract was washed with water, dried, and chromatographed over alumina. Yellow material (0.25 g) was eluted which crystallised from chloroform as yellow needles (0.1 g), m.p. 278-279°, undepressed upon admixture with spermatheridine. Its i.r. spectrum was identical with that of spermatheridine.

The phenolic fraction (Fraction A) was separated by Craig

distribution. Colourless material which failed to crystallise was isolated from fractions 1-12 (Table 2.7). Atheroline (150 mg) was isolated from fractions 13-170 whilst moschatoline distributed into the stronger acid fractions 243-350.

TABLE 2.7.

Craig Distribution of Phenolic Bases.

Transfer No.	Acid Concentration (%).
1-146	0.1
147-220	0.25
221-371	0.5

Characterization and Degradation of the Alkaloids.

1. Spermatheridine.

Spermatheridine crystallised from chloroform as yellow needles, m.p. 276-278° (decomp.), undepressed upon admixture with authentic liriodenine. (Found: C, 73.9; H, 3.5. Calc. for $C_{17}H_9O_3N$: C, 74.0; H, 3.3%). Its u.v. absorption spectrum showed λ_{max}^{EtOH} (log ε_{max}) 247.5 (4.23), 269 (4.16), 302 mμ(3.70) and $\lambda_{max}^{0.1N\ HCl}$ (log ε_{max}) 256.5 (4.33) 280 (4.25) and 334 mμ (3.70). Its i.r. spectrum, which showed $\nu_{max}^{cm^{-1}}$ 1657 (>C=O) and 1480, 1420, 1365, 1119, 1052 and 962 (), was identical with that of liriodenine.

2. Atherospermidine.

Atherospermidine crystallised from chloroform as orange-yellow needles, m.p. 275-276° (decomp.). (Found: C, 70.9; H, 3.8. Calc. for $C_{18}H_{11}O_4N$: C, 70.8; H, 3.6%) Its u.v. absorption spectrum showed λ_{max}^{EtOH} (log ε_{max}) 247 (4.38), 281 (4.52) and 312 mμ

(inf.)(3.95) and $\lambda_{max}^{0.1N\ HCl}$ 262.2 (4.24) and 283 mμ (4.16). The hydrochloride crystallised as fine red needles, m.p. 256-258° (decomp.) and the oxime as yellow needles, m.p. 234-236.5°(decomp.). The i.r. spectrum of atherospermidine showed $\nu_{max}^{cm^{-1}}$ 1657 (>C=O), 1420, 1365, 1122, 1061 and 961 ($\overset{O-}{\underset{O-}{CH_2}}$), whilst that of the oxime showed neither carbonyl nor hydroxyl absorption.

Oxidation with Chromic Acid.

Atherospermidine (200 mg) in 25 ml of 1 : 1 (v/v) conc. sulphuric acid/water was heated for 1½ hours on the water bath with chromic oxide (0.4 g) dissolved in 2 ml of water and 8 ml of 1 : 1 (v/v) conc. sulphuric acid/water, then kept at room temperature for several hours. The solution was diluted with water and neutralized with sodium bicarbonate, filtered to remove some crystalline inorganic material, then extracted continuously with chloroform. Removal of the chloroform yielded a light yellow material which formed a red solution in ammonium hydroxide. Sublimation of this compound at 155°/5 x 10^{-3} mm Hg gave a product which decomposed at 300° and whose i.r. spectrum was identical with that recorded for 1-azaanthraquinone-4-carboxylic acid. When this acid was mixed with reprecipitated sodium carbonate and heated to 230° at atmospheric pressure a bright yellow sublimate was obtained, m.p. 274-277°, whose u.v. spectrum showed λ_{max}^{EtOH} log ε_{max} 326 (3.61) and 250 mμ (4.63), and whose i.r. spectrum was identical with the published spectrum of 1-azaanthraquinone.

3. Atherosperminine.

Atherosperminine picrate crystallised from acetone/methanol as yellow needles, m p. 186-188°. The methiodide melted at 274.5 - 276.5°)decomp.) and showed λ_{max}^{EtOH} (log ε_{max}) 312 (4.08), 302(4.08), 279 (4.08), 257 (4.73) and 251 mμ (inf.)(4.69). (Found: C, 55.6, H, 5.9. Calc. for $C_{20}H_{23}O_2N.CH_3I$: C, 55.9; H, 5.8%). The i.r. spectrum of the hydriodide, m.p. 227-230°, was identical with that of an authentic sample of 1-N,N-dimethylaminoethyl-3,4-dimethoxy-phenanthrene hydriodide, and the two compounds showed no mixed melting point depression. The picrates of the two compounds likewise showed no mixed melting point depression.

The free base was recovered as a brownish oil from the picrate by filtration of a chloroform solution through an alumina column.

4. Alkaloid B (Methoxyatherosperminine).

Alkaloid B failed to crystallise but formed a picrate, m.p. 161-162° (acetone/methanol). The methiodide melted at 243-245° (decomp.) and showed λ_{max}^{EtOH} (log ε_{max}) 308 (4.11), 295.5 (4.05), 284 (4.03), 259 (4.80) and 217 mμ (4.52). (Found: C, 55.1; H, 6.2; O, 9.7; N, 2.7; OMe, 18.6, after being dried at 50°/ 0.03 mm for 4 hours. Calc. for $C_{21}H_{25}O_3N.CH_3I$: C, 54.9; H, 5.8; O, 10.0; N, 2.9; 3 x OMe, 19.4%).

The free base was recovered from the picrate as a clear oil, rapidly darkening on exposure to the atmosphere, whose i.r. spectrum showed $\nu_{max}^{cm^{-1}}$ 2810, 2760 ($-N\begin{smallmatrix}CH_3\\CH_3\end{smallmatrix}$) and 750 (four adjacent aromatic protons).

Hofmann Degradation.

Alkaloid B methiodide (1.2 g) was dissolved in water (750 ml) and shaken with an excess of freshly prepared silver oxide for 1½ hours. The precipitated silver iodide and excess silver oxide of were removed by filtration (filter aid) and the filtrate evaporated to small bulk (30 ml). To this was added a solution of potassium hydroxide (5.25 g) in water (5 ml) and the resulting mixture was heated under reflux for 1 hour. Copious evolution of a basic, ammoniacal-smelling gas took place. The aqueous solution was cooled and the oil which had separated was extracted into chloroform. The chloroform solution was dried ($MgSO_4$) and evaporated to dryness. The resulting clear oil (0.5 g) showed absorption maxima at 3070, 1610, 1004, 913 cm^{-1} ($CH=CH_2$) and 745 cm^{-1} in its i.r. spectrum and yielded a picrate m.p. 100-101°.

This oil (0.5 g) in acetone (25 ml) at 40° was treated dropwise with a solution of potassium permanganate (1 g) in acetone (25 ml) and water (25 ml). The mixture was stirred at 40-42° for 3½ hours, then centrifuged to remove the manganese dioxide. Removal of the solvents in vacuo left a yellowish oil which was treated with dilute hydrochloric acid and extracted with chloroform. The chloroform layer was extracted with 10% sodium carbonate solution, washed with water, dried and evaporated to dryness to yield a product (393 mg) which crystallised from benzene, m.p. 87-89°. (Found: C, 69.6; H, 6.3. Calc. for $C_{19}H_{20}O_5$: C, 69.5; H, 6.1%). Its i.r. spectrum showed $\nu_{max}^{cm^{-1}}$ 3290 (-OH) and it gave a positive

glycol test with fuchsin/sulphurous acid.

The 10% sodium carbonate extract yielded only a trace of acidic material.

5. Atheroline.

Atheroline crystallised from a chloroform/ethanol mixture as orange prisms, m.p. 250-260° (decomp.) Its u.v. absorption spectrum (Table 2.4) was typical of the spermatheridine-type alkaloids and its i.r. spectrum showed $\nu_{max}^{cm^{-1}}$ 1639 (>C=O) and 3250 (-OH). It gave a positive test with ferric chloride but a negative test for the methylenedioxy group with chromotropic acid.

O-Acetylatheroline.

Atheroline (50 mg) in anhydrous pyridine (5 ml) was treated with acetic anhydride (2 ml), allowed to stand overnight and then methanol was added to decompose the excess of acetic anhydride. Evaporation of the solution to small bulk yielded O-acetylatheroline as yellow needles, m.p. 190-195°. (Found: C, 67.0; H, 4.7; OCH_3, 20.5. Calc. for $C_{21}H_{17}O_6N$; C, 66.5; H, 4.5; 3 x OMe 24.0 %).

O-Methylatheroline.

Atheroline (5 mg) in anhydrous methanol (15 ml) was heated under reflux for 1 hour with methyl iodide (0.5 ml) and methanolic sodium methoxide (0.02 g sodium in 0.5 ml methanol). A further addition of methyl iodide (0.5 ml) and sodium methoxide (0.5 ml) was made and the reaction mixture heated a further hour and then evaporated to dryness. Water was added and the mixture extracted with chloroform, the chloroform was washed with 5% aqueous sodium

hydroxide, then with water, dried and evaporated to dryness. The residue crystallised upon trituration with acetone, m.p. 235-236° undepressed upon admixture with an authentic sample of the second unnamed yellow pigment isolated from Liriodendron tulipifera L[45]. The i.r. spectra (in $CHCl_3$) of the two bases were identical.

O-Ethylatheroline.

To alkaloid F (30 mg) in refluxing ethanol (150 ml) was added an ethanolic solution of sodium ethoxide (0.2 ml; 0.6 g sodium in 15 ml ethanol) and ethyl iodide (0.2 ml). Upon the addition of base the solution took on a purplish colour which gradually gave way to yellow as the reaction proceeded. After one hour a further addition of sodium ethoxide (0.2 ml) and ethyl iodide (0.2 ml) was made and the mixture heated a further hour. The solvent was removed under reduced pressure and the residue treated in a similar manner to that described under O-methylatheroline to yield O-ethylatheroline (20 mg) which crystallised from acetone as yellow needles, m.p. 210-212°. Its u.v. and visible absorption spectra showed λ_{max}^{EtOH}(log ε_{max}) 243 (4.45), 273 (4.48) 290 (inf.)(4.22), 354 (3.92), 380 (inf.)(3.96) and 430 mμ (3.71), $\lambda_{max}^{0.05N\ HCl(EtOH/H_2O)}$ (log ε_{max}) 257 (4.51), 286 (4.46), 385 (4.10) and 520 mμ (3.34), typical of a spermatheridine-type base. Its i.r. spectrum showed $\nu_{max}^{cm^{-1}}$ 1638 cm^{-1} (>C=O).

6. Moschatoline.

Moschatoline failed to crystallise and was isolated as a greenish-yellow solid which turned pink in acid and purple in alkali. It

gave a positive test with ferric chloride but a negative test for the methylenedioxy group with chromotropic or gallic acids.

O-Acetylmoschatoline.

Crude moschatoline (0.025 g) in pyridine (4 ml) was treated with acetic anhydride (1 ml) and allowed to stand at room temperature for 2½ days. Methanol was added to decompose the excess of acetic anhydride and the solvents were then removed under reduced pressure; the last trace of pyridine was removed by azeotropic distillation with benzene. Upon trituration with acetone the residue crystallised as yellow prisms (17 mg), m.p. 190-200°, depressed upon admixture with O-acetylatheroline. Its i.r. spectrum showed $\nu_{max}^{cm^{-1}}$ 1775 (ester carbonyl), 1659 (conjugated carbonyl) and 760 cm^{-1} (four adjacent aromatic protons).

O-Methylmoschatoline.

Crude O-acetylmoschatoline (5 mg) in methanol was heated under reflux for ½ hour with methanolic sodium methoxide (0.02 g sodium in 0.5 ml methanol). Methyl iodide (1 ml) and further sodium methoxide (0.5 ml) were then added and the heating continued a further ½ hour. One further addition of sodium methoxide (0.5 ml) and methyl iodide (0.5 ml) was then made and the reaction mixture heated a further hour after which it was evaporated to dryness, dissolved in chloroform and extracted with 5% aqueous sodium hydroxide. The chloroform layer was washed with water, dried and evaporated to dryness. The residue was chromatographed over alumina; 15% chloroform/benzene eluted a small quantity of yellow material which crystallised

from acetone, m.p. 187-190°, undepressed upon admixture with a synthetic sample of 1,2,3-trimethoxy-7-oxo-dibenzo-(de,g)-quinoline. The i.r. spectra of the two compounds (in $CHCl_3$) were identical.

Synthetic Experiments.

1. Synthesis of Atherospermidine.

1-Methoxy-2,3-methylenedioxybenzene.

Prepared according to K.N.Campbell, R.F.Hopper and B.K.Campbell[73], m.p. 41°.

2-Methoxy-3,4-methylenedioxy-β-phenethylamine.

Prepared according to T.R.Govindachari, S.Rajadurai, C.V.Ramadas and N.Viswanathan[54].

B.P. 108-118°/0.5 mm (Lit. 180°/0.5 mm).
Picrate m.p.216-218 (Lit. 216-218°)

N-[β-(2-Methoxy-3,4-methylenedioxyphenyl)ethyl] -2'-nitrophenylacetamide.

O-Nitrophenylacetylchloride (0.816 g) in ether (6 ml) was added dropwise to a cold, stirred mixture of 2-methoxy-3,4-methylenedioxy-β-phenethylamine (0.984 g) in ether (6 ml) and 10% aqueous sodium hydroxide (6 ml). After this addition the reaction mixture was stirred during ½ hour at room temperature, then the product was filtered off, washed with dilute sulphuric acid, and crystallised from ethanol. It had m.p. 157-158° and its i.r. spectrum showed $\nu_{max}^{cm^{-1}}$ 3282 (-NH) and 1643 (amide $\geq C=O$). (Found: C, 60.6; H, 5.1. Calc. for $C_{18}H_{18}O_6N_2$; C, 60.3; H, 5.0 %)

1-(O-Nitrobenzyl)-5-methoxy-6,7-methylenedioxy-3,4-dihydroisoquinoline.

The above amide (2 g) in boiling toluene (500 ml) was treated portionwise with phosphorus pentoxide (20 g) during half an hour, then the mixture was heated for a further half hour and cooled. The toluene was decanted from the solid residue; the latter was dissolved in water (500 ml) and extracted with ether. The aqueous layer was basified (NH_3) and the oily precipitate extracted into ether. Removal of the ether left a red-brown residue (1 g) which formed prisms m.p. 136-138° (from ether). (Found: C, 63.6; H, 5.0. Calc. for $C_{18}H_{16}O_5N_2$: C, 63.5; H, 4.7 %). Its u.v. absorption spectrum showed λ_{max}^{EtOH} (log ε_{max}) 305 (inf.)(3.82) and 280 mμ(3.99), which underwent a bathochromic shift upon the addition of acid to $\lambda_{max}^{acid\ ethanol}$ 346 mμ.

1-(O-Nitrobenzoyl)-5-methoxy-6,7-methylenedioxy-isoquinoline.

The dihydroisoquinoline (2 g) in acetic acid (20 ml) was heated on the water bath with selenium dioxide (0.96 g) for 3 hours, then the solution was filtered and evaporated to dryness. The yellow residue (1.5 g) was chromatographed over alumina (60 g); a mixture of 5% chloroform/benzene eluted a yellow fraction (0.3 g) which crystallised from acetone as yellow prisms, m.p. 187-188°. Its u.v. absorption spectrum showed λ_{max}^{EtOH} (log ε_{max}) 270 (4.42) and 232 mμ (4.46), and its i.r. spectrum $\nu_{max}^{cm^{-1}}$ 1667 (>C=O), 1510 and 1359 ($-NO_2$).

1,2-Methylenedioxy-3-methoxy-7-oxo-dibenzo-(de,g)-quinoline[46].

The above isoquinoline (280 mg) was suspended in absolute alcohol and shaken for 19 hours with a Raney Nickel catalyst in an atmosphere of hydrogen at atmospheric pressure and room temperature. The catalyst was centrifuged off and the supernatant liquid evaporated to dryness. The residue (170 mg) in methanol (7 ml) and 10% sulphuric acid (7 ml) was cooled and diazotized with sodium nitrite solution (0.5 ml of N $NaNO_2$). The solution was allowed to stand in the cold during 15 minutes, then heated on the waterbath a further 30 minutes. Some red needles separated and the solution took on a deep red colour. It was cooled and basified (NH_3) and the yellow precipitate was extracted with chloroform. When the extract was evaporated to small bulk and cooled, fine yellow needles were obtained, m.p. 276-278° (decomp.). (Found: C, 71.0; H, 3.7. Calc. for $C_{18}H_{11}O_4N$: C, 70.8; H, 3.6 %). Its u.v. and visible light absorption spectrum showed λ_{max}^{EtOH} (log ε_{max}) 247 (4.33), 281 (4.46), 310 (inf.)(3.95), 380 (3.72) and 437 mμ (3.93). The i.r. spectrum of this material was identical with that of atherospermidine.

The natural and synthetic materials showed no mixed-melting point depression.

2. Synthesis of Alkaloid B.

2,3,4-Trimethoxy- β-phenethylamine.

This was prepared according to M.Erne and F.Ramirez[61]. Picrate m.p. 134-136° (Lit. 136-137°).

N-[β -(2,3,4-Trimethoxyphenyl)ethyl] -2'-nitrophenylacetamide.

O-Nitrophenylacetylchloride (0.816 g) in benzene (6 ml) was added dropwise over a period of 45 minutes to a cold, stirred mixture of 2,3,4-trimethoxy-β-phenethylamine(1.044 g) in benzene (6 ml) and 10% sodium hydroxide (6 ml). The product (900 mg) was filtered off and recrystallised from ether, m.p. 105-106°. (Found: C, 60.9; H, 5.8. Calc. for $C_{19}H_{22}O_6N_2$: C, 61.0; H, 5.9 %.) Its i.r. spectrum showed $\nu_{max}^{cm^{-1}}$ 3276 (-NH) and 1639 (amide carbonyl).

1,2,3-Trimethoxyaporphine.

Sodium borohydride (0.45 g) was carefully added to a stirred solution of the above dihydroisoquinoline methiodide (0.73 g) in methanol (10 ml) and water (1.0 ml). The mixture was stirred for 1 hour at room temperature after which zinc dust (0.9 g) and sulphuric acid (10 ml, 10%) were added. The resulting mixture was stirred and heated on the water bath for 15 minutes, the excess of zinc centrifuged off and the methanol removed under reduced pressure. The acid solution was basified (NH_3) and extracted with ether. Removal of the ether left a clear gum (460 mg) whose i.r. spectrum showed $\nu_{max}^{cm^{-1}}$ 3359 ($-NH_2$). This material (1.176 g) in methanol (12.5 ml) and 10% sulphuric acid (12.5 ml) was diazotized with a solution of sodium nitrite (0.2425 g) in water (1 ml). The reaction mixture was kept cold for 15 minutes and then heated for 30 minutes on the water bath. Zinc dust (0.7 g) and concentrated hydrochloric acid (1.8 ml) were then added and the mixture heated for a further 20 minutes to complete the reaction and remove the methanol. The acid solution was basified and extracted with

chloroform, the extract dried and evaporated to dryness to yield a clear oil (0.573 g) which was chromatographed over alumina. Benzene eluted material which crystallised from petroleum ether, m.p. 105-106°, λ_{max}^{EtOH} (log ε_{max}) 277 mμ (4.26). The methiodide melted at 214-216° (acetone/benzene). (Found, after being dried at 50° and 100° for 12 hours/0.03 mm: C, 56.6; H, 5.7. Calc. for $C_{21}H_{26}O_3NI.\frac{1}{2}C_6H_6$: C, 56.9; H, 5.7 %. Found, after being dried at 100°/0.03 mm for 48 hours: C, 54.2; H, 5.7. Calc. for $C_{21}H_{26}O_3NI$: C, 54.0; H, 5.6 %).

1-N,N-Dimethylaminoethyl-2,3,4-trimethoxyphenanthrene.

The above methiodide (96 mg) was dissolved in water and converted to the methohydroxide with freshly prepared silver oxide. The excess of silver oxide and precipitated silver iodide were then removed by filtration (filter-aid) and the filtrate reduced in volume to 3 ml. Potassium hydroxide (1.5 g) in water (2 ml) was added, the mixture heated under reflux for 1 hour, cooled and extracted with chloroform. The chloroform extract was dried and evaporated to dryness to yield a clear oil which formed a picrate, m.p. 161-164°, undepressed upon admixture with alkaloid B picrate. The methiodide, which melted at 248-250° (decomp.) showed no mixed-melting point depression with alkaloid B methiodide. (Found, after being dried at 50°/0.03 mm for 4 hours: C, 54.7; H, 5.9. Calc. for $C_{21}H_{25}O_3N.CH_3I$: C, 54.9; H, 5.8 %). Its u.v. absorption spectrum (λ_{max}^{EtOH} (log ε_{max}) 306 (4.13), 294 (4.09), 282 (4.09) and 259 mμ (4.80)) was identical with that of alkaloid B methiodide.

The i.r. spectrum of the free base was identical with the naturally occurring material.

3. Attempted Synthesis of 1-Hydroxy-2,9,10-trimethoxy-7-oxo-dibenzo-(de,g)-quinoline.

N-[β-(3-Methoxy-4-benzyloxyphenyl)ethyl]-2'-nitro-4',5'-dimethoxyphenylacetamide.

2-Nitro-4,5-dimethoxyphenylacetic acid[74] (1.31 g) was suspended in dry chloroform and phosphorus pentachloride (2.5 g) was gradually added[75]. After 20 minutes the acid and most of the phosphorus pentachloride had passed into solution which was then added dropwise to a cold, rapidly-stirred mixture of 3-methoxy-4-benzyloxy-β-phenethylamine (1.7 g) in chloroform (10 ml) and 5% aqueous sodium hydroxide (40 ml) diluted with 20 ml of water. After half an hour the chloroform layer was separated, the aqueous layer was washed with chloroform and the combined chloroform extracts were washed with acid, dried and evaporated to dryness. Trituration of the residual deep-brown residue with methanol gave colourless needles (0.8 g) which were removed and recrystallised from acetone, m.p. 155-156°. (Found: C,64.5; H,6.1 . Calc. for $C_{26}H_{28}O_7N_2$: C, 65.0; H, 5.8 %).

1-(2'-Nitro-4',5'-dimethoxybenzyl)-6-methoxy-7-benzyloxy-3,4-dihydroisoquinoline.

The above amide (0.78 g) in dry chloroform (10 ml) was treated with an ice-cold suspension of phosphorus pentachloride (2.5 g) in dry chloroform (10 mls), the reaction mixture was sealed in a tube

and allowed to stand seven days at room temperature. It was then poured into iced water, the chloroform layer was separated and the aqueous layer washed with chloroform. The chloroform extracts were combined, washed with water, dried and evaporated to dryness. The residue was exhaustively extracted with warm, dil. hydrochloric acid, the acid extract basified (NH_3) and extracted with chloroform. Removal of the chloroform gave a reddish residue which crystallised upon treatment with ether, m.p. 183-185°. (Found: C, 67.5; H, 5.6. Calc. for $C_{26}H_{26}O_6N_2$: C, 67.5; H, 5.6 %). When this dihydroisoquinoline (0.37 g) in acetic acid (4 ml) was heated 10 minutes on the water bath with selenium dioxide (0.14 g) an orange-yellow solution resulted which was allowed to cool, was centrifuged and poured into water. Extraction with chloroform gave a yellow solution from which was obtained a crystalline product as fine yellow needles, m.p. 210-211° and whose i.r. spectrum showed no carbonyl absorption. (Found: C, 69.7; H, 5.4; O, 6.3; N, 18.5. Calc. for $C_{26}H_{24}N_2O_5$: C, 70.1; H, 5.4; O, 6.3; N, 18.0 %).

1-(2'-Nitro-4',5'-dimethoxybenzoyl)-6-methoxy-7-benzyloxyisoquinoline[46].

The above dihydroisoquinoline (0.5 g) in acetic acid (10 ml) was heated with chromic oxide (0.5 g) on the water bath until an exothermic reaction began. After cooling, the reaction mixture was poured into water, basified (NH_3), and extracted with chloroform. The chloroform layer was washed with water, dried and evaporated to dryness to yield a reddish residue which was dissolved in hot ethan-

ol (1 ml) and treated with a couple of drops of 50% aqueous sodium hydroxide. The solution took on a deep red colour which gradually faded with the appearance of yellow needles. The heating was continued a further ten minutes, the reaction mixture allowed to cool and the product filtered off, m.p. 165-167°. (Found: C,65.9; H, 4.7. Calc. for $C_{26}H_{22}N_2O_7$: C, 65.8; H, 4.6 %).

Attempted Formation of 1-Hydroxy-2,9,10-trimethoxy-7-oxo-dibenzo-(de,g)-quinoline.

The above benzoylisoquinoline (400 mg) was suspended in ethanol and shaken 19 hours in an atmosphere of hydrogen at atmospheric pressure and room temperature in the presence of a Raney Nickel catalyst. The catalyst was removed by centrifugation and the ethanol solution was evaporated to dryness. The residue (270 mg) in methanol (6 ml) and 10% sulphuric acid (6 ml) was cooled and treated with N $NaNO_2$ (0,6 ml). The reaction mixture was allowed to stand in the refrigerator overnight, then brought to room temperature and heated under reflux for ½ hour. The methanol was removed under reduced pressure and the acid solution extracted with chloroform. The chloroform extract was washed with water, dried and evaporated to dryness. The residue (20 mg) was heated under reflux for ¾ hour with 15% hydrochloric acid. The pink solution was cooled, neutralized with sodium bicarbonate and extracted with chloroform. The chloroform layer was washed with water, dried and evaporated to dryness. The yellow residue was soluble in 5% sodium hydroxide to give a deep blue colour; it also formed a blue salt with sodium bicarbonate.

4. 1-Ethoxy-2,9,10-trimethoxy-7-oxo-dibenzo-(de,g)-quinoline.
1-(2'-Nitro-4',5'-dimethoxybenzyl)-6-methoxy-7-ethoxy-3,4-dihydro-isoquinoline.

Prepared according to R.H.F.Manske, E.H.Charlesworth and W.R. Ashford[76], m.p. 180-181°. (Lit. 183-184°).

1-(2'Nitro-4',5'-dimethoxybenzoyl)-6-methoxy-7-ethoxyisoquinoline[46].

The above dihydroisoquinoline (1 g) was added to chromic oxide (1 g) in acetic acid (25 mls) and the mixture heated on the steam bath until an exothermic reaction commenced. The reaction mixture was then removed from the bath and allowed to cool, poured into water, basified (NH_3) and extracted with chloroform. The chloroform extract was washed with water, dried and evaporated to dryness. The residue in ethanol (~15 ml) was treated with a few drops of 50% aqueous sodium hydroxide and heated on the water bath; the solution took on a deep red colour which gradually faded with the appearance of yellow prisms (200 mg), m.p. 164-166°. (Found: C, 61.5; H, 4.9; Calc. for $C_{21}H_{20}N_2O_7$: C, 61.2; H, 4.9 %). Its i.r. spectrum showed $\nu_{max}^{cm^{-1}}$ 1676 (C=O), 1520 and 1342 ($-NO_2$).

1-Ethoxy-2,9,10-trimethoxy-7-oxo-dibenzo-(de,g)-quinoline[46].

The above material in absolute ethanol was shaken at room temperature for twenty hours under one atmosphere of hydrogen in the presence of a Raney Nickel catalyst. The catalyst was removed by centrifugation, washed thoroughly with ethanol and the washings and original supernatant liquid were combined and evaporated to dryness. The residue (140 mg) was dissolved in methanol (7 ml) and 10% sulphuric acid (7 ml) and diazotized with N sodium nitrite (0.6 mls);

the solution was allowed to stand in the cold for ½ hour after which it was heated on the steam bath for ½ hour. The solution, which developed a deep red colour, was then cooled, basified (NH_3) and extracted with chloroform. The chloroform extract was washed with water and dried to give a yellow residue which crystallised from acetone as yellow needles (10 mg), m.p. 196-198°. Its u.v. spectrum showed λ_{max}^{EtOH} (log ε_{max}) 244 (4.45), 272.5 (4.49), 291 (4.22), 348 (4.03), 380 (inf.) (3.96) 427 mμ (3.70) and $\lambda_{max}^{0.05N\ HCl(EtOH/H_2O)}$ (log ε_{max}) 257 (4.52), 285 (4.46), 385 (4.09) and 500 mμ (3.33).

This material substantially depressed the melting point of O-ethylatheroline and the i.r. spectra of the two compounds differ noticeably in the 700-1000 cm^{-1} region.

5. 1,2,9-Trimethoxy-10-ethoxy-7-oxo-dibenzo-(de,g)-quinoline.
1-(2'-Nitro-4'-ethoxy-5'-methoxylbenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline.

Prepared according to G.Barger, J.Eisenbrand, L.Eisenbrand and E.Schlittler[77], m.p. 142-144° (Lit. 142-143°).

The experimental details for the remainder of the synthesis were essentially the same as described above for 1-ethoxy-2,9,10-trimethoxy-7-oxo-dibenzo-(de,g)-quinoline
1-(2'-Nitro-4'-ethoxy-5'-methoxybenzoyl)-6,7-dimethoxyisoquinoline.

M.p. 189-192°. (Found: C, 61.0; H, 5.0. Calc. for $C_{21}H_{20}N_2O_7$: C, 61.2; H, 4.9 %).

1,2,9-Trimethoxy-10-ethoxy-7-oxo-dibenzo-(de,g)-quinoline.

M.p. 220-221°. (Found: C, 68.7; H, 5.4. Calc. for $C_{21}H_{19}NO_5$: C, 69.0; H, 5.2 %). Its u.v. absorption spectrum showed λ_{max}^{EtOH} (log ε_{max}) 244 (4.45), 272.5 (4.48), 291 (4.23), 350 (4.03), 380 (inf.)(3.96) and 428 (3.70) and $\lambda_{max}^{0.05N\ HCl(EtOH/H_2O)}$ (log ε_{max}) 257 (4.51), 282 (4.47), 385 (4.12) and 500 mμ (3.36).

A substantial depression in melting point resulted upon admixture with O-ethylatheroline. The i.r. spectra of the two compounds were closely related but showed some differences about 1000 cm^{-1}.

6. 1,2,10-Trimethoxy-9-ethoxy-7-oxo-dibenzo-(de,g)-quinoline.

1-(2'-Nitro-4'-methoxy-5'-ethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline.

This was prepared according to G.Barger, J.Eisenbrand, L. Eisenbrand and E.Schlittler[77]. M.p. 170-172°. (Lit. m.p. 174-5°).

1-(2'-Nitro-4'-methoxy-5'-ethoxybenzoyl)-6,7-dimethoxyisoquinoline.

This was prepared from 1-(2'-nitro-4'-methoxy-5'-ethoxybenzyl)-3,4-dihydroisoquinoline in a manner similar to that described under 1-ethoxy-2,9,10-trimethoxy-7-oxo-dibenzo-(de,g)-quinoline. M.p. 186-188°. (Found: C, 59.7; H, 5.0. Calc. for $C_{21}H_{20}N_2O_7 \cdot \frac{1}{2}H_2O$ C, 61.2; H, 4.9 %).

1,2,10-Trimethoxy-9-ethoxy-7-oxo-dibenzo-(de,g)-quinoline.

This compound was prepared from the above 1-benzoylisoquinoline by reactions similar to those described for 1-ethoxy-2,9,10-

trimethoxy-7-oxo-dibenzo-(de,g)-quinoline. It crystallised from acetone as yellow needles, m.p. 210-212°, undepressed upon admixture with O-ethylatheroline. (Found: C,69.0; H, 5.3. Calc. for $C_{21}H_{19}NO_5$: C, 69.0; H, 5.2 %.) The i.r. spectra of the two compounds were identical as were their u.v. spectra.

7. 1,2,3-Trimethoxy-7-oxo-dibenzo-(de,g)-quinoline.

1-(O-Nitrobenzoyl)-5,6,7-trimethoxyisoquinoline.

1-(O-Nitrobenzyl)-5,6,7-trimethoxy-3,4-dihydroisoquinoline (0.5 g) in acetic acid (5 ml) was heated on the steam bath for 2 hours with selenium dioxide (0.24 g). The reaction mixture was allowed to cool, was centrifuged and evaporated to dryness. The deep yellow residue was chromatographed over alumina. 5% Chloroform/benzene eluted 200 mg of material which crystallised from ethanol as light yellow needles, m.p. 117-118°. (Found: C, 62.0; H, 4.5. Calc. for $C_{19}H_{15}O_6N_2$: C, 62.1, H, 4.1 %). Its i.r. spectrum showed $\nu_{max}^{cm^{-1}}$ 1687 (>C=O), 1524 and 1348 ($-NO_2$).

1,2,3-Trimethoxy-7-oxo-dibenzo-(de,g)-quinoline.

This was prepared in a similar manner to the corresponding synthesis described for 1-methoxy-2,3-methylenedioxy-7-oxo-dibenzo-(de,g)-quinoline. The product crystallised from acetone as orange plates, m.p. 187-190°. (Found: C, 71.2; H, 4.8. Calc. for $C_{19}H_{15}O_4N$: C, 71.0; H, 4.7 %). The i.r. spectrum of this material was identical with that of O-methylmoschatoline. Its u.v. absorption spectrum showed λ_{max}^{EtOH} (log ε_{max}) 234 (4.40),

272, (4.54), 310 (inf.)(3.75) and 435 mμ (3.90) and

$\lambda_{max}^{0.05N\ HCl(aq.EtOH)}$ (log ε_{max}) 236 (4.37), 280 (4.48) and

465 mμ (3.57).

3. THE ALKALOIDS OF THE LEAVES AND HEARTWOOD OF A.MOSCHATUM.

1. Alkaloids of the Leaves.

Bick, Clezy and Crow[10] isolated a small quantity of a cryptophenolic base from the leaves of A.moschatum which they named spermatherine. Spermatherine crystallised from acetone-petroleum as needles, m.p. 124-125°, which decomposed to a brown mass on exposure to light.

In an attempt to isolate further quantities of spermatherine the extraction of a large quantity of leaves was carried out and the alkaloids separated into phenolic, crypto-phenolic and non-phenolic frations. The cryptophenolic bases were separated by Craig distribution using chloroform as stationary phase and dilute hydrochloric acid as the mobile phase. A new base, atherosperm-oline, distributed into the 0.1% acid layer; the material which was extracted by 0.25% acid was combined and again separated by Craig distribution. This time, however, the strength of the acid phase was greatly reduced. Material which distributed into 0.001% acid was chromatographed over alumina. 15% Benzene/chloroform eluted an amorphous compound whose R_f (0.64) on paper chromato-graphy was identical with that published for spermatherine and, like the latter, the spot developed a brown colour after exposure to iodine vapour. On this evidence the two compounds were thought to be identical. The spermatherine which was isolated in this work rapidly developed a brown colour upon exposure to the atmos-phere. It formed a crystalline picrate and a crystalline

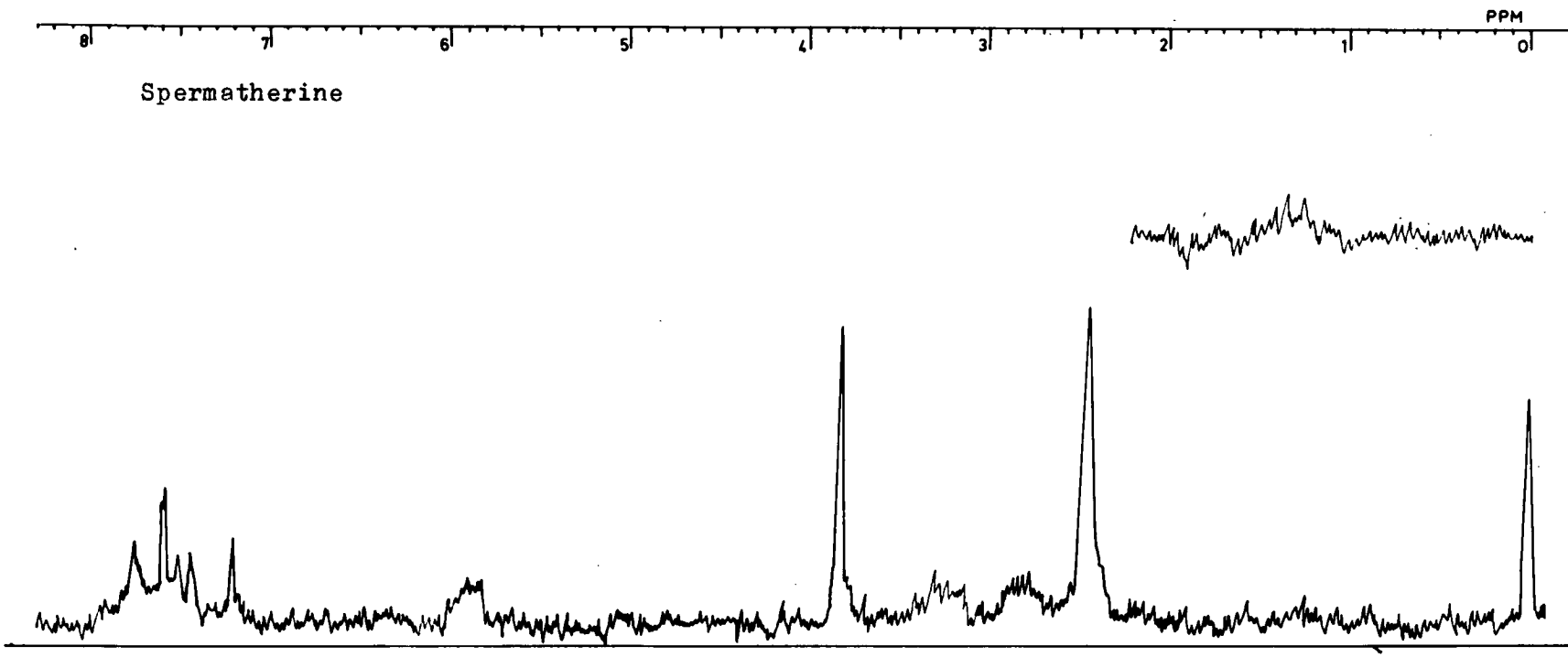
methiodide which analysed for $C_{19}H_{21}O_2N.CH_3I.H_2O$ with one methoxyl group. The u.v. absorption spectrum of the methiodide (Table 3.1) showed that it possessed a phenanthrene nucleus.

TABLE 3.1.

U.V. Absorption Spectrum of Spermatherine Methiodide.

λ_{max}^{EtOH}	log ε_{max}	λ_{max}^{EtOH}	log ε_{max}
365	3.34	278	4.02
346	3.35	255.5	4.62
312.5	4.02	250	4.59
303	3.99		

The n.m.r. spectrum of the free base (Fig. 3.I) confirmed the presence of one methoxyl group (three-proton singlet at δ 3.83 ppm); a resonance of intensity six protons at δ 2.45 ppm was assigned to a dimethylamino grouping and a complex series of peaks symmetrical about δ 3.07 ppm to a $-CH_2-CH_2-$grouping. Thus spermatherine possessed a 1-N,N-dimethylaminoethylphenanthrene skeleton. A broad resonance at δ 5.91 ppm in its n.m.r. spectrum was assigned to a phenolic hydroxy group (although the i.r. spectrum of spermatherine showed only very weak hydroxyl absorption). A singlet at δ 7.25 ppm coincided with the resonance due to the chloroform proton but its intensity (integration 1.5 protons) was far greater than that observed in previous spectra and was thought to be due to H_2 as well as to $CHCl_3$ (Fig. 3.I). A low-field multiplet of intensity one proton (δ 9.67 ppm) was



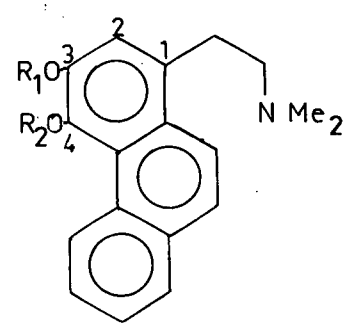
Spermatherine

Figure 3.1.

assigned to H_5.

If, as before, the assumption is made that this class of alkaloids arises from the corresponding aporphine, then spermatherine would carry oxy substituents at C-3 and C-4[60]. This conclusion was supported by its i.r. spectrum which showed a strong band at 750 cm^{-1} (C-H out-of-plane bending of four adjacent aromatic protons[52]) and a weak band at 865 cm^{-1} (C-H out-of-plane bending of an isolated aromatic proton[52]).

The foregoing evidence leads to the assignment of the tentative structure (3.I) for spermatherine.



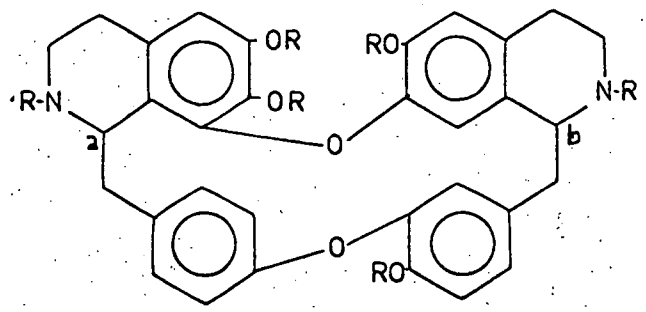
3.I R_1=H,R_2=Me or vice versa

Atherospermoline crystallised from chloroform as colourless prisms, m.p. 183-188°, $[\alpha]_D^{18}$ + 202° (C, 0.15 in $CHCl_3$). The analytical data pointed to the formula $C_{35}H_{36}O_6N_2 \cdot CHCl_3$ with one methylimino and two methoxyl groups. This formula, together with the ultra-violet absorption spectrum[12] (λ_{max}^{EtOH} 284 mμ ; log ε_{max} 3.97) indicated that the alkaloid belonged to the bisbenzylisoquinoline series. The distinct phenolic properties of athero -

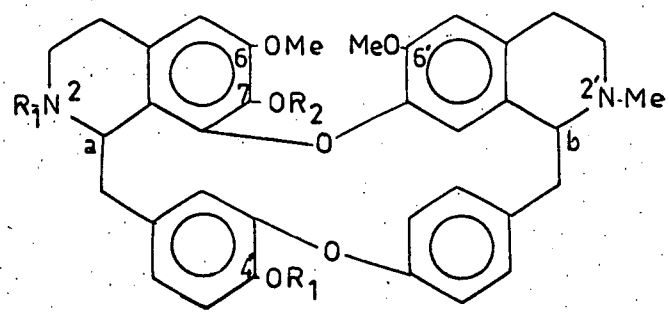
spermoline were consistent with the presence of two phenolic groups, since alkaloids of this series with one such group were usually cryptophenolic[10,1]. The isolation of atherospermoline from the cryptophenolic fraction is not readily explained. The two remaining oxygen atoms presumably link the two halves of the molecule together and atherospermoline would thus appear to belong to one of the types of bisbenzylisoquinoline bases with two diaryl bridges.

As outlined in the Introduction, the principal structural types of these bases (3.II - 3.V) were shown[12] to give rise to broadly similar O.R.D. curves with two exceptions - type 3.II alkaloids in which the asymmetric centres (marked a and b) were paired (S,S; +,+) or (R,R; -,-) and type 3.III with the pairing (R,S; -,+) or S,R; +,-). The O.R.D. curve for atherospermoline was of the general pattern (Fig. 3.2) so that the above configurational sub-types which gave rise to the exceptional and more complex O.R.D. curves were excluded for this base. Furthermore, since its O.R.D. curve showed two positive Cotton effects, the configurations (R,S; -,+) of type 3.II and (R,R; -,-) of type 3.III, which would give rise to negative curves, were likewise excluded.

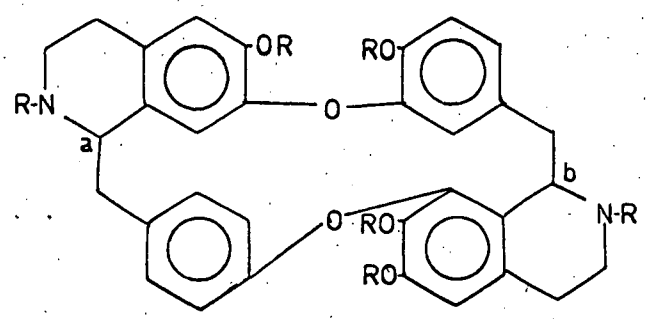
In the n.m.r. spectrum of atherospermoline, the N-methyl resonance at δ 2.62 ppm fell within the range found[11] for those bases of type 3.II which normally had two methylimino groups absorbing near δ 2.55 ppm, and also for those of type 3.III, in which the two methylimino peaks were well separated and appeared around δ 2.3 ppm


OR
RO
R-N
OR
N-R
a
b
O
O
RO

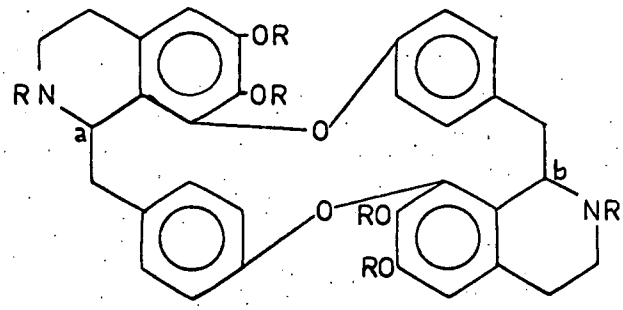
3.II


OMe
MeO
6
6'
R1N 2
7
OR2
2'N-Me
a
b
O
O
4
OR1

3.III


OR
RO
R-N
O
a
b
O
RO
N-R
RO

3.IV



3.V

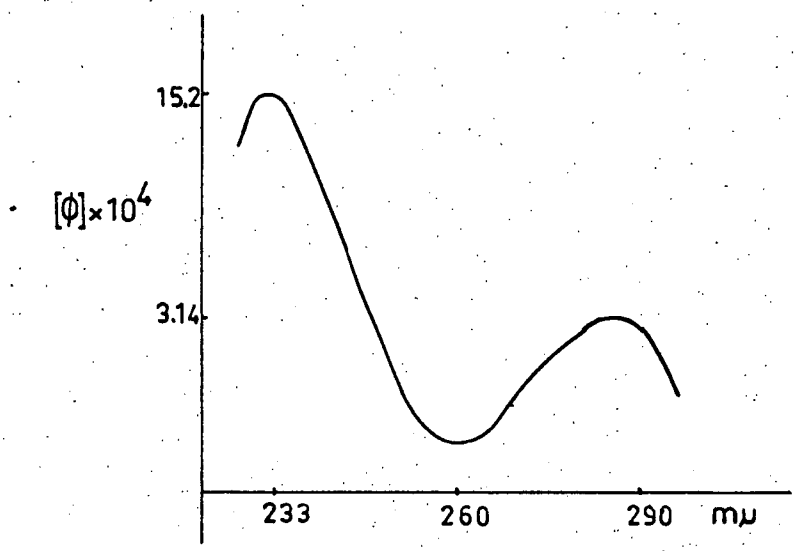


Fig. 3.2

and δ 2.6 ppm, but not for those of types 3.IV and 3.V. Taken in conjunction, the n.m.r. and O.R.D. evidence thus pointed either to structural type 3.II with the configuration (S,R; +,-) or to type 3.III with the configuration (S,S; +,+).

For alkaloids of type 3.II and 3.III it was shown[11] that methoxyls at positions 4", 6 and 7 absorbed around δ 3.9, 3.75 and 3.15 ppm, respectively, while ones at position 6' absorbed near

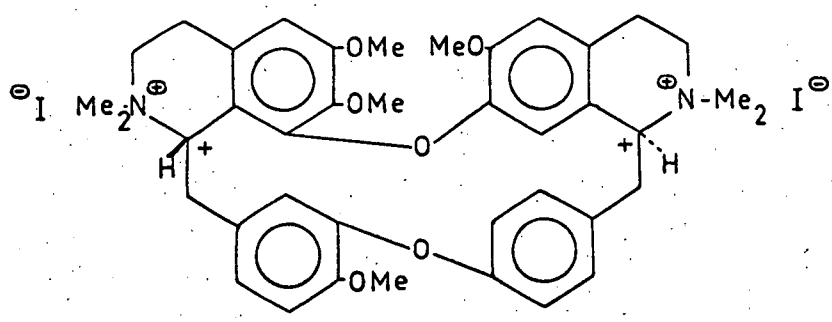
δ 3.35 or δ 3.6 ppm depending on whether the asymmetric centres a and b had the same or opposite sign. The atherospermoline spectrum showed two resonances, each of intensity three protons, at δ 3.76 and δ 3.30 ppm. These were assigned to methoxyls at positions 6 and 6' respectively, and furthermore, the latter was situated in a molecule with asymmetric centres of the same sign. The structure of atherospermoline was thus limited to type 3.III and configuration (S,S; +,+), with methoxyls at positions 6 and 6', while positions 7 and 4" were presumably occupied by two phenolic hydroxyls.

Of the two methylimino resonances around δ 2.3 and δ 2.6 ppm normally shown by type 3.III alkaloids, the former was assigned[11] to the N-methyl at position 2 and the latter to that at position 2'. Atherospermoline had only one N-methyl group which absorbed at δ 2.62 ppm and was thus located at position 2'; the nitrogen at position 2 was presumably in a secondary amino group.

The foregoing evidence can be expressed by the tentative structure and configuration (3.III, $R^1=R^2=R^3=H$; a,b; +,+) for atherospermoline.

Support for the above assignment came when atherospermoline was converted into O,O-dimethylatherospermoline dimethiodide whose i.r. spectrum was identical with that of tetrandrine dimethiodide (3.VI).

O-Ethylatheroline was obtained as a non-crystalline gum by treatment of atheroline with a large excess of diazoethane.


OMe
MeO
OMe
$^{\ominus}I$ $Me_2N^{\oplus}$
$^{\oplus}N\text{-}Me_2$ $I^{\ominus}$
H
+
O
+
H
O
OMe

3.VI

EXPERIMENTAL.

Leaves of Atherosperma moschatum (53.5 Kg; dry weight) were collected at Maydena (Tas.), dried and extracted by warm methanol percolation (C.S.I.R.O.). The extract was concentrated and shaken with sulphuric acid (0.5%, 15 l.), the acid layer filtered from insoluble material and basified with ammonia (pH 9). A flocculent precipitate formed which was allowed to settle in the cold room overnight; the dark supernatant liquid was decanted and extracted with chloroform. This extract was combined with a chloroform/methanol solution of the residue and extracted with aqueous sodium hydroxide (1%, 10 l) to remove the phenolic bases (10 g). The organic layer was evaporated to dryness and the residue was dissolved in benzene; the resulting solution was extracted with aqueous sodium hydroxide (2%, 2 x 1.5 l). During this process a third, syrupy layer formed which was kept separate. The alkali layer was washed with benzene, made acid with conc. HCl and then basified with ammonia (pH 9). The resulting mixture was extracted with chloroform and the chloroform layer was washed with water, dried and evaporated to dryness to yield the cryptophenolic bases (47 g). The benzene layer contained a considerable quantity of essential oil which was separated from the alkaloids by extracting the benzene solution with dil. hydrochloric acid (5%, 3 x 1 l; 0.5%, 3 x 1 l.). The acid layer yielded the non-phenolic alkaloids (28 g).

The cryptophenolic bases were separated on a Craig machine us-

ing chloroform as the stationary phase and increasing concentrations of dil. hydrochloric acid as the mobile phase. Fractions 9-23 (0.1% hydrochloric acid) yielded atherospermoline (0.5 g). Fractions 24-89 (0.1 - 0.25% HCl) were combined and the counter-current distribution ropeated using hydrochloric acid of a lower concentration. Fractions 19-99 (0.001% hydrochloric acid), which all showed spots of R_f 0.64 and 0.30 on paper chromatography, were combined, dissolved in chloroform, and extracted with alkali. The chloroform soluble material (4 g) was chromatographed over alumina (120 g; acid washed, activity ~ III). 15% Benzene/chloroform eluted spermatherine as a brown, amorphous compound which was purified as its picrate, m.p. 221-223° (acetone/methanol). It also formed a methiodide which crystallised from acetone/pet. spirit, m.p. 209-212° (decomp.). (Found: C, 53.2; H, 5.9; OCH_3, 6.9. Calc. for $C_{19}H_{21}O_2N.CH_3I.H_2O$: C, 52.8; H, 5.7; 1 x OCH_3, 6.8%).

Atherospermoline crystallised from chloroform as colourless prisms, m.p. 183-188°, $[\alpha]_D^{18}$ + 202° (C = 0.15; calc. for base with $CHCl_3$ of crystallisation). (Found: C, 61.9; H, 5.2; N, 3.8; O, 14.2; OCH_3, 8.9; NCH_3, 2.2; Cl, 15.7. Calc. for $C_{35}H_{36}O_7N_2.CHCl_3$: C, 61.7; H, 5.3; N, 4.0; O, 13.7; 2 x OCH_3, 8.9; 1 x NCH_3, 2.1; Cl, 15.2). It gave a strong positive ferric chloride test and a negative methylenedioxy group test. The hydrochloride, m.p. 268-274° (decomp.), crystallised as fine, colourless needles.

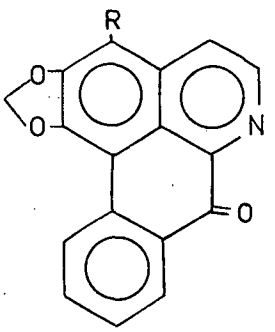
0,O,N-Trimethylatherospermoline dimethiodide.

Atherospermoline (0.05 g) was dissolved in boiling methanol (10 ml) and methyl iodide (0.15 ml) was added followed by methanolic sodium methoxide (0.006 g sodium in 0.15 ml methanol). The mixture was heated under reflux and similar quantities of sodium methoxide and methyl iodide were added at intervals of six hours until, in all, six such additions had been made. The solvent was then removed and the residue dissolved in hot water. As it cooled, the solution deposited a white amorphous solid which was filtered off, redissolved in hot water, boiled with copper turnings for 10 minutes and then filtered. The water was removed under reduced pressure and the residue crystallised from ethanol, m.p. 236-240° (decomp.).

Tetrandrine dimethiodide, prepared in a similar manner, had m.p. 238-243° (decomp.); its i.r. spectrum was identical with that of O,O,N-trimethylatherospermoline dimethiodide.

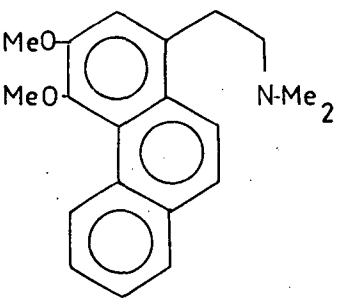
(ii) Alkaloids of the Heartwood.

As mentioned earlier, Atherosperma moschatum is of limited use in the paper-pulp industry because of its yellow colour. Experiments performed on the heartwood showed that the yellow colour was extractable with methanol and, moreover, was associated with the basic fraction of the extract. The yellow constituents of this fraction were separated on a Craig machine into two highly coloured substances which were further purified by crystallisation from chloroform. They proved identical with the alkaloids spermatheridine (3.VII) and atherospermidine (3.VIII). No bisbenzylisoquinoline alkaloids were found in the heartwood; the only other alkaloid obtained therefrom was atherosperminine (3.IX).

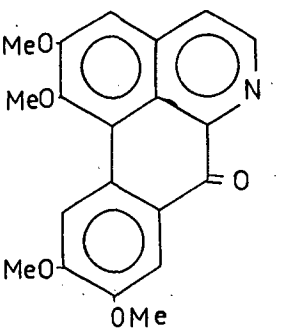


3.VII R=H

3.VIII R=OMe



3.IX



3.X

The presence of spermatheridine (3.VII) and another unnamed alkaloid (3.X) of similar structure in tulipwood[45] (Liriodendron tulipifera L.) limits the use of this tree for papermaking in America; a recent Russian report[79], however, indicates the successful use of tulipwood for making pulp by the calcium bisulphite process.

EXPERIMENTAL.

Finely ground sassafras heartwood (5.5 Kg) was exhaustively extracted by cold percolation with methanol. The volume of the extract was reduced to 4 l *in vacuo*, the temperature being kept below 45°. The concentrate was acidified with concentrated hydrochloric acid (5 ml) and the remainder of the methanol removed *in vacuo*. The non-basic material was filtered off and the acid filtrate, which contained the bulk of the coloured material, was made alkaline with ammonia (pH 9). The yellow-brown precipitate was extracted into chloroform, and the extract washed with aqueous alkali to remove phenolic bases. Evaporation of the chloroform extract left a yellow-brown residue (4.3 *g*); chromatography failed to separate the yellow constituents. They were eventually separated by Craig distribution between chloroform and dilute hydrochloric acid. The separation could be followed by the two pink bands moving progressively along the tubes of the Craig machine. Spermatheridine (28 mg) distributed into acid of lower strength (1%) than atherospermidine (11 mg; 5%). The yellow bases were further purified by crystallisation from chloroform and proved identical (m.p., mixed m.p., i.r. spectra) with samples of spermatheridine and atherospermidine isolated from the bark of *A.moschatum*. When evaporated to dryness, the chloroform layers of tubes 26-50 of the Craig machine gave a yellowish oil which was chromatographed over alumina; 15% chloroform/benzene eluted atherosperminine, isolated as its picrate (30 mg), m.p. 188-189°.

4. ALKALOIDS OF THE BARK OF DRYADODAPHNE NOVOGUINEENSIS.

Dryadodaphne novoguineensis (Perk.) A.C.Smith [native name "anonya" (Gasup dialect)] grows at Aiyura in the Eastern Highlands of New Guinea. Taylor[25] reported the isolation from this plant of a bisbenzylisoquinoline alkaloid for which the name dryadine was suggested. He also isolated[25] dryedine from the bark of a tree growing in the Atherton area of North Queensland. This tree was morphologically similar to Daphnandra aromatica and was assigned the provisional name Daphnandra new species affiliated to aromatica[82]. Subsequent investigation showed[83] that this plant was a Dryadodaphne species allied to Dryadodaphne novoguineensis. (The genus Dryadodaphne is at present being reclassified and extended.)[83]

Taylor summarised his findings as follows:

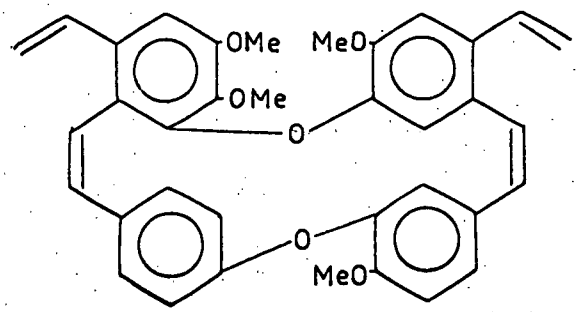
"Dryadine analysed for $C_{36}H_{38}O_6N_2$, contained two methoxyl and two methylimino groups, gave one active hydrogen in the Zerewitinoff estimation and was soluble in the Claisen cryptophenol reagent. The absorption maximum at 285 mμ (ε = 8270) in its u.v. spectrum was of the correct order for a bisbenzylisoquinoline alkaloid. It gave negative reactions for a secondary amine and the phenodioxin ring system.

"Analyses on dryadine still left one oxygen function unaccounted for and if we assumed two diphenylether linkages, two methoxyl and one phenolic group, it was assumed that this extra oxygen function was present as a highly hindered phenolic group.

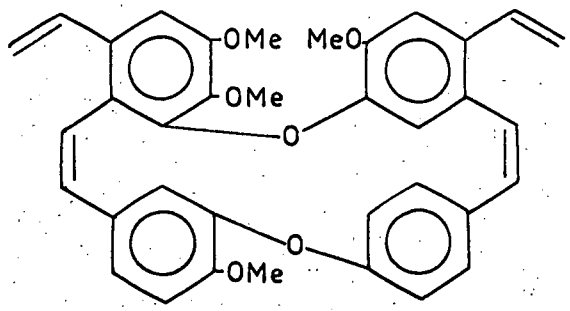
Dryadine was unaffected by diazomethane and experiments towards fully methylating the base were unsuccessful because no crystalline products were obtained. It was then decided to methylate with dimethyl sulphate and alkali and carry out the Hofmann degradation without isolating the intermediate methosulphate. This was done but no optically inactive methine base was formed (compare with O-methyloxyacanthine[84]). Therefore the crude methine bases were methylated and a second Hofmann degradation carried out to yield a crystalline O-methyl-de-N-dryadine, $C_{36}H_{32}O_6$, which was isomeric but not identical with the de-N-compounds derived from oxyacanthine (4.I)[16], berbamine (4.II)[85], and berbeerine (4.III)[86], but like them it was optically inactive and crystallised with chloroform of crystallisation.

"Proof that dryadine was a member of the bisbenzylisoquinoline class of compounds with head-to-head and tail-to-tail ether bridges came from the oxidation of O-methyl-de-N-dryadine to 5,4'-dicarboxy-2-methoxydiphenylether (4.IV) and a second acid isolated as its methyl ester (A), m.p. 215°. Analyses on (A) were not good but showed that it must be $C_{23}H_{24}O_{12}$ and therefore isomeric but not identical with 5,6,4',5'-tetracarbomethoxy-2,3,2'-trimethoxydiphenylether (4.V)[87], m.p. 152°, which can be derived from either the oxyacanthine or berbamine class of alkaloids.

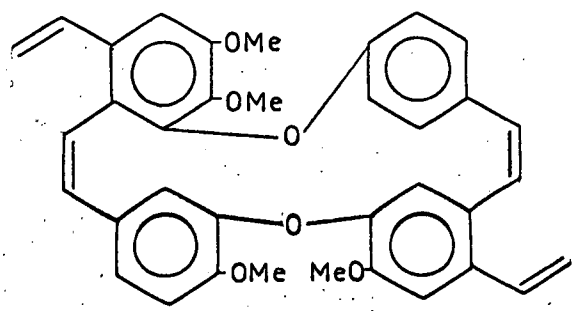
"Dryadine cannot be regarded as arising from diphenylether formation in the system (4.VII) since such derived compounds



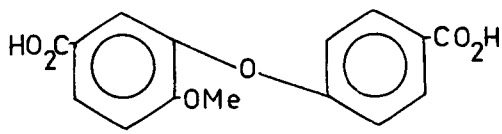
4.I



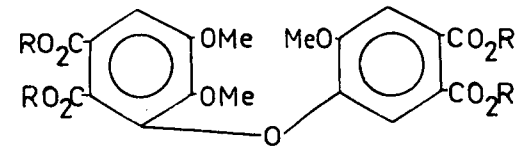
4.II



4.III



4.IV

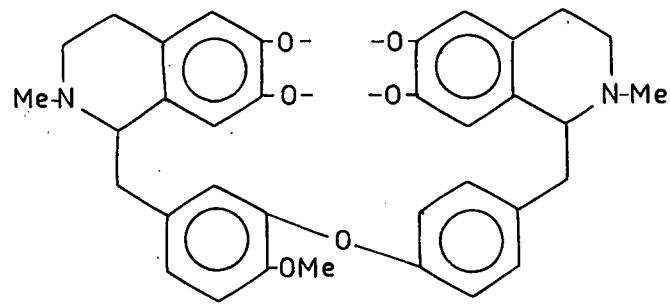


4.V R=Me

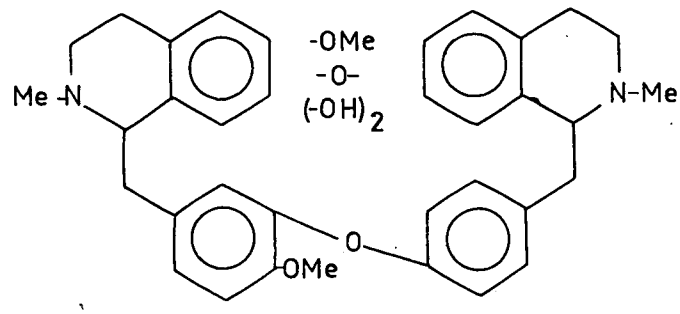
4.VI R=H

after O-methylation would yield 5,6,4',5'-tetracarboxy-2,3,2'-trimethoxydiphenylether (4.VI) on oxidation; therefore eight possible bisbenzylisoquinoline skeletal structures are eliminated. Dryadine can therefore be represented by the partial formula (4.VIII) ".

The interpretation of the analytical data for dryadine proved difficult because of the tenacious manner in which this compound retained solvent of crystallisation. However, analytical data on material crystallised from a variety of solvents, reinforced by n.m.r. and mass spectral evidence, established the formula $C_{37}H_{40}O_6N_2$ for dryadine and indicated the presence of two methylimino groups and three methoxyl groups rather than two methylimino and two methoxyl groups as suggested by Taylor. Thus analyses on dryadine crystallised from chloroform/methanol were consistent with the formulae $C_{37}H_{40}O_6N_2 \cdot H_2O$ or $C_{36}H_{38}O_6N_2 \cdot CH_3OH$ with two methylimino and three methoxyl groups. At first the latter formula was thought to be correct, because the n.m.r. spectrum of dryadine crystallised from chloroform/methanol showed a three-proton singlet at δ 3.40 ppm; this was believed to arise from



4.VII



4.VIII

methanol of crystallisation, since this chemical shift was the same as that for methanol. However, the n.m.r. spectrum of a sample of dryadine crystallised from chloroform/isopropanol still showed this three-proton resonance thus indicating that it was due to a methoxyl group in the dryadine molecule. Had dryadine retained a molecule of isopropanol when thus crystallised, this would have been readily detected by the appearance of two three-

proton resonances around δ 1.20 ppm[88] and the absence of a three-proton singlet around δ 3.40 ppm.

Analyses on dryadine crystallised from benzene were consistent with the formula $C_{37}H_{40}O_6N_2 . 1/3\ C_6H_6$ with three methoxyl groups whilst material crystallised from acetone analysed for $C_{37}H_{40}O_6N_2 . 1/2\ CH_3COCH_3$ with three methoxyl groups. The presence of half a molecule of acetone of crystallisation in the latter sample was supported by its n.m.r. spectrum which showed a resonance of intensity three protons at δ 2.13 ppm[89] as well as resonances attributed to three methoxyl and two methylimino groups.

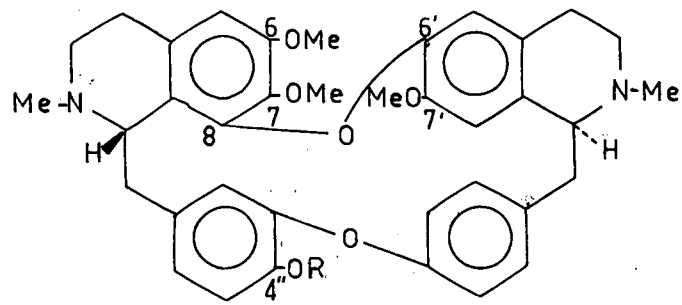
The foregoing evidence suggested that dryadine, $C_{37}H_{40}O_6N_2$, was isomeric with oxyacanthine and berbamine. This was supported by its mass spectrum which showed a parent peak of $^m/_e$ 608.

Dryadine was methylated in methanol with methyl iodide and sodium methoxide to yield a fully methylated product which was degraded by the Hofmann method to a mixture of optically active O-methyldryadine methines; further Hofmann degradation gave O-methyl-de-N-dryadine, $C_{36}H_{32}O_6$. The n.m.r. spectrum of this compound showed, as expected, the presence of four methoxyl groups. Oxidation with permanganate gave 5,4'-dicarboxy-2-methoxy-diphenyl-ether (4.IV); no product was obtained which corresponded to the tetracarboxylic acid isolated by Taylor.

The three methoxyl groups of dryadine account for three oxygen atoms, and if a further two are involved in ether linkages, then the sixth must be present in the form of a phenolic hydroxy group. This is consistent with the cryptophenolic nature of dryadine. Furthermore, since O-methyl-de-N-dryadine is isomeric but not

identical with either of the corresponding O-methyl-de-N-compounds derived from oxyacanthine or berbamine it is concluded that dryadine differs from oxyacanthine (4.XII) and berbamine (4.XI) either in the position of the oxy functions or the position of the ether linkage between the isoquinoline residues.

Recently, Fujita and Tomimatsu[90] established the structures of thalicberine (4.IX) and O-methylthalicberine (4.X). These two compounds contained the unique 8-6' ether bridge.



4 IX R=H

4 X R=Me

The n.m.r. spectrum of O-methylthalicberine[91] (Table 4.1) showed two almost coincident methoxyl resonances at δ 3.88 and δ 3.85 ppm. The former was assigned to the methoxyl on position 4" and the latter to that on position 6. No other type of bis-benzylisoquinoline showed methoxyl resonances which were nearly so close to each other[11].

The n.m.r. spectrum of dryadine (Table 4.1) showed two coincident methoxyl resonances at δ 3.87 ppm.

TABLE 4.1.

N.m.r. Spectra of Thalicberine, O-Methylthalicberine and Dryadine. (PPM, δ)

Name	Formula	OMe				NMe	
		4'	6	7	7'	2'	2
Thalicberine	4.IX	-	3.85	3.75	3.62	2.55	2.09
O-Methyl-thalicberine	4.X	3.88	3.85	3.75	3.64	2.55	2.10
Dryadine	(Tentative)	3.87	3.87	-	3.40	2.63	2.20

The wide separation of the N-methyl resonances[11] in the n.m.r. spectrum of dryadine leads one to suggest that in the arrangement of its diphenylether linkages, dryadine, like thalicberine, is an analogue of berbamine (4.XI; a,b; -,+) rather than oxyacanthine (4.XII; a,b; +,-). The similarity to the berbamine type is indicated also by the behaviour on Hofmann degradation[85]; none of the optically inactive distilbene type of methine is obtained, in contrast to the behaviour of oxyacanthine[84] and repandine[30]. The same skeleton as thalicberine (4.IX) can be thus inferred for dryadine, although this assignment is made with some reservation in the absence of more model compounds. If it is correct, O-methyldryadine and O-methylthalicberine must be diasteriomeric to account for the considerable difference in specific rotation of dryadine on the one hand (+404°) and thalicberine (+231°) and O-methylthalicberine (+266°) on the other; also to account for the difference in chemical shifts of the 7' methoxyl (δ3.40, 3.62

and 3.64 ppm, respectively). This difference may be compared with that found[11] for the 7-methoxyls of the diastereomeric pairs oxyacanthine (4.XII; a,b; +,-) and repandine (4.XII; a,b; +,+) and also tetrandrine (4.XIII; a,b; +,+) and isotetrandrine (4.XIII; a,b; -,+).

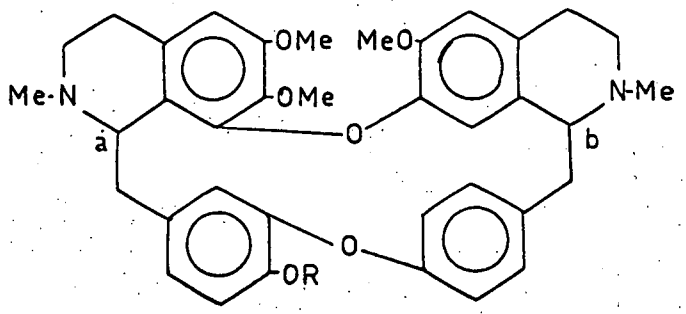
The O.R.D. curve of dryadine (Fig.4. I) was of normal pattern and showed two positive Cotton effects.

If dryadine is assigned the thalicberine skeleton, then the tetracarboxylic acid arising from the oxidation of O-methyl-de-N-dryadine would be identical with that (4.VI) from O-methyl-de-N-berbamine. Taylor found a considerably higher melting point for the former compound as compared to the melting point reported in the literature for the latter; this may not be sufficient evidence to exclude the possibility of their identity.

The mass spectrum of dryadine showed daughter peaks at $^{m}/_{e}$ 226 and $^{m}/_{e}$ 382 which were believed to arise by cracking via pathway 1 in Scheme 4.1. These peaks indicated that position 4" carried a methoxyl group and that the phenolic hydroxy group was attached to either of the isoquinoline moieties of dryadine, and thus lent partial support to the n.m.r. assignments.

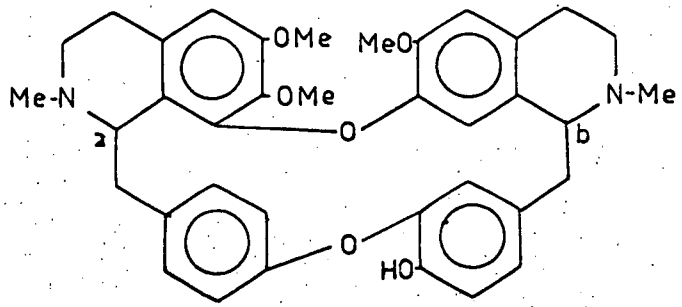
The foregoing evidence leads to the assignment of the tentative structure and stereochemistry (4.XIV; S,R; +,-) to dryadine.

Additional information would no doubt be obtained by cleavage of the diphenyl ether bonds with sodium in liquid ammonia[5]. How-



4.XI R=H

4.XIII R=Me



4.XII

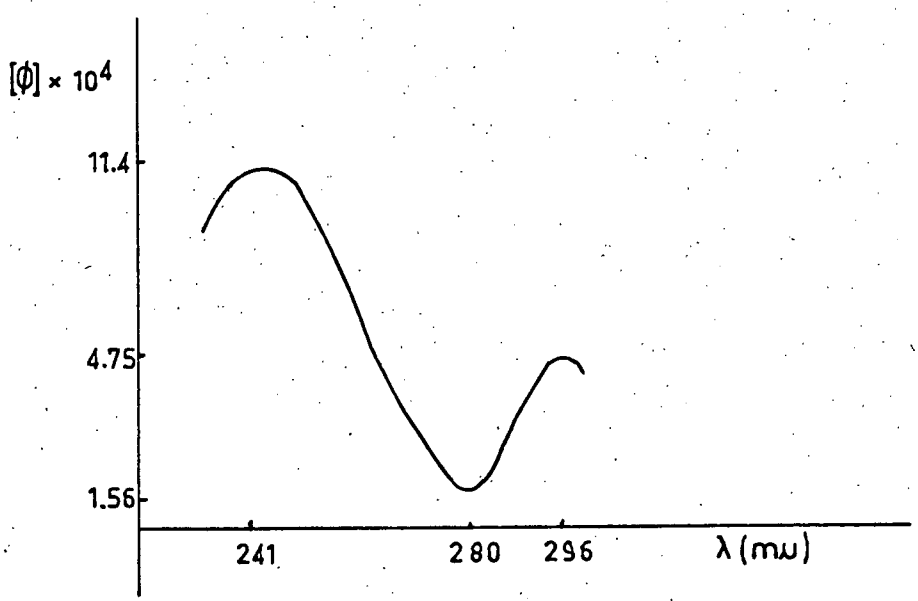
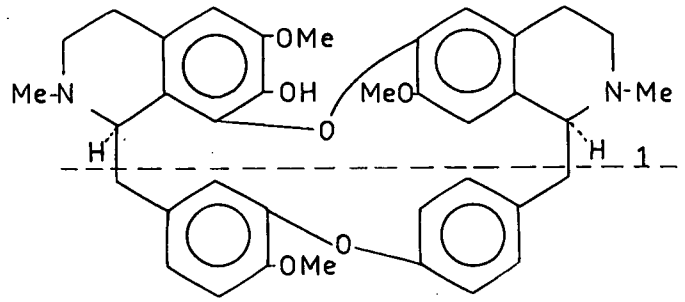


Fig. 4.1



4.XIV

Scheme 4.1

ever, the phenolic group must first be alkylated before the reaction will proceed satisfactorily. This group is cryptophenolic and unreactive towards diazomethane, as found by Taylor. Attempts to form O-methyldryadine by the dequaternization of O-methyldryadine dimethiodide with ethanolamine[95] resulted in a mixture of bases from which only a trace of crystalline material was obtained. A similar dequaternization of O-ethylberbamine dimethiodide afforded O-ethylberbamine in high yield.

The cryptophenolic fraction of the alkaloid extract of Dryadodaphne novoguineensis yielded a small quantity of a yellow, optically inactive base, alkaloid D, which exhibited a bright, yellow-green fluorescence under u.v. light. Alkaloid D crystallised from acetone/petroleum spirit as yellow prisms, m.p. 198-201°, whose u.v. and visible light absorption bands underwent a marked hypsochromic shift in acid as compared to those in neutral solution (Table 4.2); this was manifested in the formation of a colourless

hydrochloride. The i.r. spectrum of alkaloid D showed maxima at 3570 cm^{-1} and 3380 cm^{-1} and these, coupled with a positive ferric chloride test, indicated the presence of at least one phenolic hydroxy group. A band at 1633 cm^{-1} was attributed to a hydrogen-bonded or amide carbonyl.

TABLE 4.2.

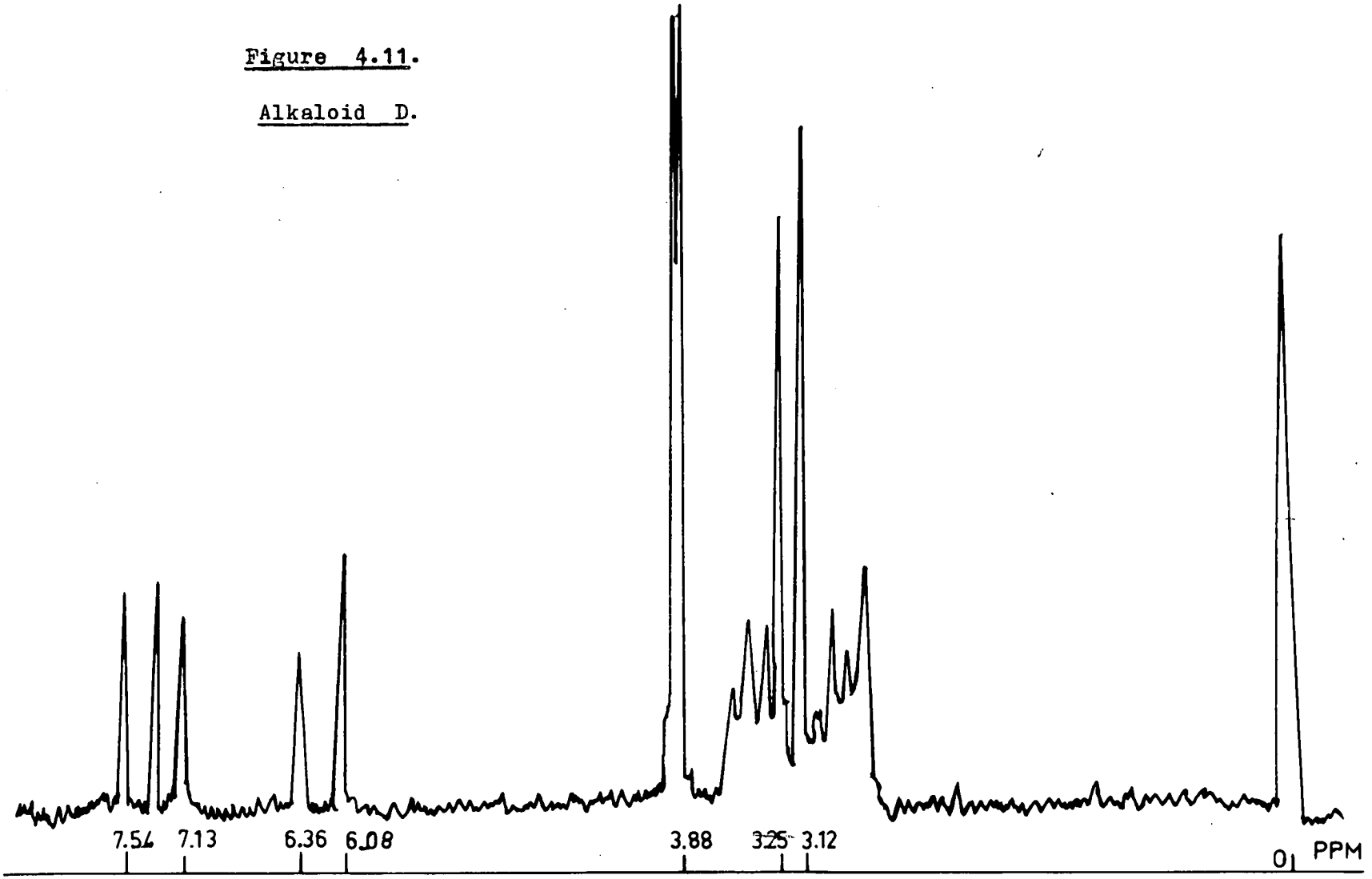
U.v. and Visible Light Absorption Spectrum of Alkaloid D.

λ_{max}^{EtOH}, mμ	$\lambda_{max}^{acidic\ EtOH}$, mμ
270	251
278 (inf.)	-
300 (inf.)	270 (inf.)
-	308
407	352

Alkaloid D analysed for $C_{22}H_{28}O_7N_2$ and contained two methoxy and two methylimino groups. The presence of these groupings was confirmed by the n.m.r. spectrum (Fig.4.II). Also in the n.m.r. spectrum there was a complex splitting pattern between δ 2.56 ppm and δ 3.64 ppm; this was attributed to a $-CH_2-CH_2-$ grouping. The integration showed fourteen protons between these frequencies; six are accounted for by the two methylimino groups, and the remaining eight protons presumably result from two $-CH_2-CH_2-$ groupings. Since all alkaloids so far isolated from monimiaceous plants belong to the isoquinoline group it seems likely that Alkaloid D would be an isoquinoline alkaloid. To account for two N-methyl and two

Figure 4.11.

Alkaloid D.



$-CH_2-CH_2-$ groupings the alkaloid must contain two tetrahydroisoquinoline units.

Alkaloid D gave a negative test for the methylenedioxy group[43]. Treatment of an ethanol solution of the base with ferric chloride gave a purple colour which gradually faded with the appearance of yellow needles. These were removed and recrystallised from ethanol. They analysed for $C_{22}H_{28}O_7N_2.FeCl_3.H_2O$ and melted at 170° with decomposition.

The i.r. spectrum of alkaloid D hydrochloride showed an absorption maximum at 1655 cm^{-1} as well as at 1633 cm^{-1}. Thus we have a decrease in colour associated with the formation of a carbonyl group.

Insufficient of Alkaloid D was isolated to perform degradative experiments but the evidence gathered so far indicates a unique structure.

The non-phenolic alkaloid fraction of D.novoguineensis yielded a trace of spermatheridine whilst the phenolic fraction gave atheroline.

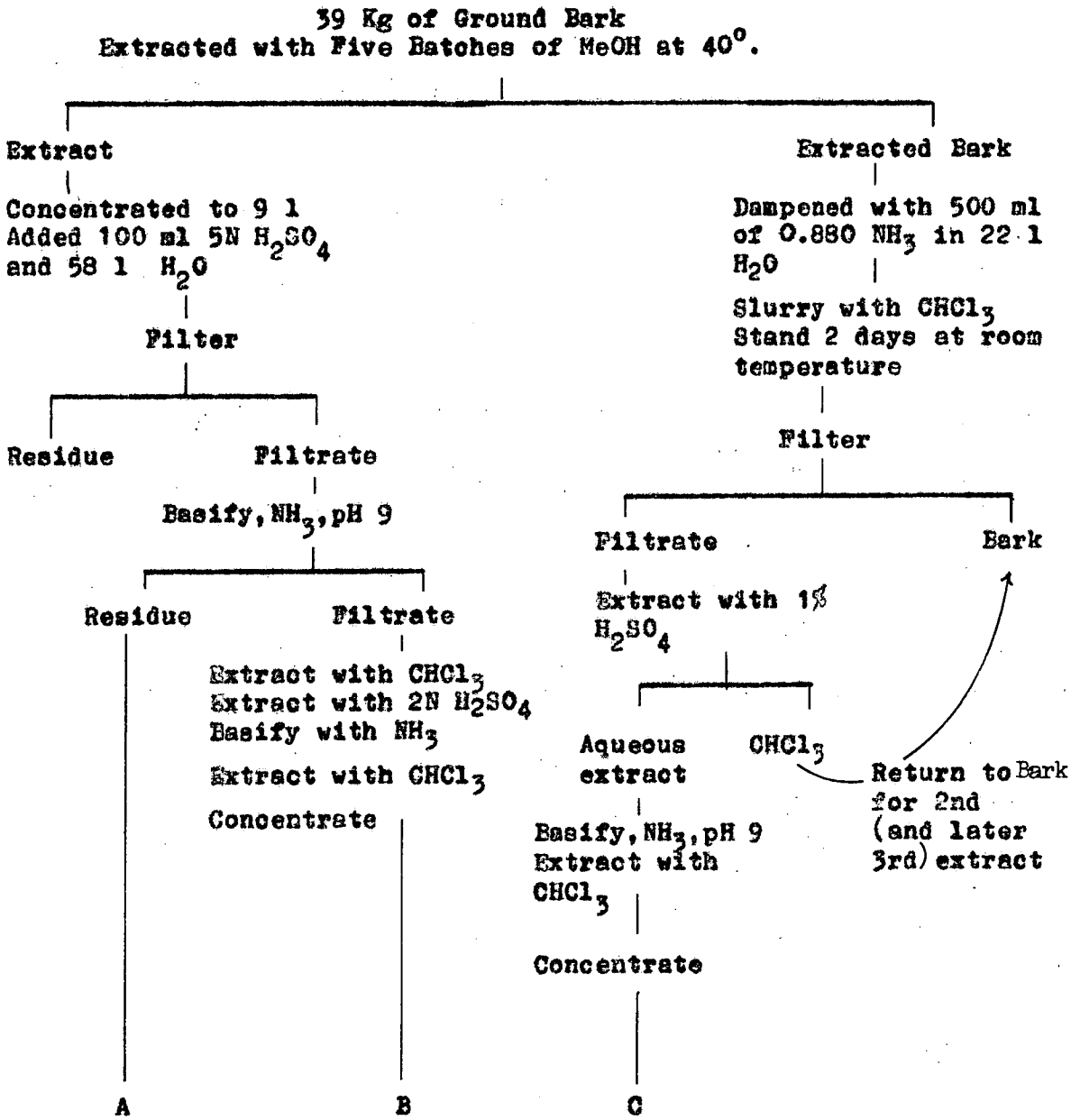
EXPERIMENTAL.

Isolation and Separation of the Alkaloids.

A crude alkaloid extraction of *D.novoguineensis* was carried out by C.S.I.R.O. according to Scheme 4.2.

The combined fractions A, B and C (201 g; contained occluded chloroform) were extracted with chloroform in a Soxhlet extractor. The chloroform solution was extracted with sodium hydroxide (2%; 2 x 2 l), washed with water and dried. Most of the chloroform was removed under reduced pressure leaving a deep-brown residue (Fraction D). The alkali extract was made acid and allowed to stand. Some brown, gummy material which separated was removed by filtration and the filtrate was neutralized with ammonia and extracted with chloroform. The chloroform extract was washed, dried and evaporated to dryness to yield a deep brown residue of phenolic alkaloid (60 g; Fraction E).

Fraction D was dissolved in benzene and extracted with sodium hydroxide (2%; 2 x 2 l). The alkali extract was neutralized and extracted with chloroform; the latter was washed with water, dried and evaporated to dryness to yield the cryptophenolic alkaloids (27 g; Fraction F). Evaporation of the benzene layer to small bulk yielded dryadine as a colourless, crystalline compound (10 g) which was removed. The benzene mother liquors yielded 27.5 g of non-phenolic alkaloid which was chromatographed over alumina. Chloroform/benzene (35%) eluted spermatheridine (50 mg) whilst chloroform eluted dryadine (1 g).


39 Kg of Ground Bark
Extracted with Five Batches of MeOH at 40°.
Extract
Concentrated to 9 l
Added 100 ml 5N H_2SO_4
and 58 l H_2O
Filter
Residue
Filtrate
Basify, NH_3, pH 9
Residue
Filtrate
Extract with $CHCl_3$
Extract with 2N H_2SO_4
Basify with NH_3
Extract with $CHCl_3$
Concentrate
A
B
Extracted Bark
Dampened with 500 ml of 0.880 NH_3 in 22 l H_2O
Slurry with $CHCl_3$
Stand 2 days at room temperature
Filter
Filtrate
Bark
Extract with 1% H_2SO_4
Aqueous extract
$CHCl_3$
Return to Bark for 2nd (and later 3rd) extract
Basify, NH_3, pH 9
Extract with $CHCl_3$
Concentrate
C

Scheme 4.2.

The cryptophenolic bases were separated by Craig distribution in which chloroform was the stationary phase and the mobile phase was hydrochloric acid, the strength of which was gradually increased from 0.001% to 1.0% (Table 4.3).

TABLE 4.3.

Transfer No.	Strength of HCl (%).
0 - 74	0.001
75 - 148	0.0025
149 - 395	0.01
396 - 550	0.025
551 - 600	0.05
601 - 650	0.1
651 - 750	0.5
751 - 851	1.0

The first ninety fractions contained very little alkaloid and were combined. Every fourth fraction of the remainder was analysed by thin-layer chromatography. Fractions 91-190 (1.6 g) all showed a bright yellow spot (R_f 0.4; 1% MeOH/$CHCl_3$), exhibiting a bright yellow-green fluorescence under u.v. light. The combined fractions were chromatographed over alumina (55 g). Chloroform eluted a yellow fraction which crystallised from acetone/petroleum spirit as yellow prisms (150 mg; alkaloid D).

The phenolic alkaloids (27 g) were dissolved in 2% sodium hydroxide and extracted continuously with chloroform for 24 hours. The chloroform-soluble material was then extracted with ether in a

Soxhlet extractor; the ether-soluble material (17.5 g) was kept separate.

Upon acidification of the sodium hydroxide layer some brown material precipitated and was centrifuged off. The acid layer was neutralized ($NaHCO_3$) and extracted with chloroform to yield material (8 g) which was separated by Craig distribution in the usual manner (Table 4.4).

TABLE 4.4.

Transfer No.	Strength of HCl (%).
1 - 49	0.01
50 - 195	0.05
196 - end	0.1

Fractions 76-188 yielded atheroline (150 mg).

Characterization and Degradation of the Alkaloids.

1. Dryadine.

Dryadine crystallised from chloroform/methanol, acetone, benzene and chloroform/isopropanol as needles m.p. 249-251° (decomp.), $[\alpha]_D^{15}$ + 404°(C, 1.51 in $CHCl_3$). (Found for dryadine crystallised from chloroform/methanol: C, 70.6; H, 6.7; N, 4.4; OMe, 14.7; NMe, 4.7%. Calc. for $C_{37}H_{40}O_6N_2.H_2O$: C, 70.9; H, 6.7; N, 4.5; 3 x OMe, 14.8; 2 x NMe, 4.8%. Found for dryadine crystallised from benzene: C, 73.7; H, 6.6; OMe, 14.7%. Calc. for $C_{37}H_{40}O_6N_2.1/3\ C_6H_6$: C, 73.8; H, 6.6, 3 x OMe, 14.7%. Found for dryadine crystallised from acetone: C, 72.5; H, 6.6;

OMe, 14.8. Calc. for $C_{37}H_{40}O_6N_2 \cdot 1/2\ CH_3COCH_3$: C, 72.5; H, 6.6; 3 x OMe, 14.6)

Dryadine was soluble in Claisen's cryptophenol reagent and gave negative tests for the methylenedioxy group and the dibenzodioxin system.

O-Methyl-de-N-dryadine.

Dryadine (1.25 g) was extracted by Soxhlet extraction into a mixture of methanol (500 ml) methyl iodide (5 ml) and methanolic sodium methoxide (3.75 ml; 3 g of sodium in 75 ml of methanol). The reaction mixture was heated for six hours under reflux and a further addition of methyl iodide (5 ml) and sodium methoxide (3.75 ml) was made. The refluxing was continued and the addition of methyl iodide and sodium methoxide was repeated at six-hourly intervals until, in all, six such additions had been made. The solvent and excess of methyl iodide were then removed under reduced pressure and the residue was dissolved in boiling water, heated for 10 minutes with copper powder, filtered and stored in the refrigerator. The amorphous O-methyldryadine dimethiodide which separated was removed by filtration, redissolved in warm water and shaken with freshly prepared silver oxide for 2 hours. The excess of silver oxide and precipitated silver iodide were then removed by filtration (supercel) and the volume of the filtrate reduced to 30 ml. Potassium hydroxide (5.25 g) in water (5 ml) was then added and the solution heated under reflux for 1 hour. The O-methyldryadine methines which separated upon cooling were remov-

ed by filtration and converted to methiodides (0.82 g). A second Hofmann degradation on this material gave O-methyl-de-N-dryadine (0.33 g) which crystallised from chloroform/acetic acid, m.p. 203-207°. (Found: C, 76.2; H, 5.7; O, 18.5; OMe, 23.1%. Calc. for $C_{36}H_{32}O_6 \cdot 1/2\ H_2O$: C, 75.8; H, 5.8; O, 18.2; 4 x OMe 21.7%.)

Oxidation of O-Methyl-de-N-dryadine.

O-Methyl-de-N-dryadine (0.8 g) in acetone (30 ml) was treated at room temperature with a solution of potassium permanganate (2 g) in acetone (90 ml). After being stirred for 2 hours at room temperature the reaction mixture still maintained its pink colouration and was subsequently heated 4 hours at 40° on the water bath. The precipitated manganese dioxide was removed by centrifugation and washed thoroughly with sodium bicarbonate. These washings were acidified and extracted with chloroform. The latter was washed with water, dried and evaporated to dryness. The residue (0.5 g) was dissolved in acetone; upon standing in the refrigerator this solution deposited white crystalline material which was removed by filtration and sublimed at 200°/ 1.0 x 10^{-5} mm Hg to yield a colourless sublimate of 5,4'-dicarboxy-2-methoxydiphenyl-ether (30 mg), m.p. 310° (decomp). Lit. m.p. 315°(decomp).

The acetone filtrate was evaporated to dryness and methylated in methanol with diazomethane. The mixture of esters was chromatographed over alumina; no crystalline material was obtained.

Dequaternization of O-Ethylberbamine Dimethiodide.

O-Ethylberbamine dimethiodide (1 g) was heated at 165° with ethanolamine (1.1 ml) for 20 minutes. The resulting mixture was treated with 5% hydrochloric acid, basified (NH_3) and extracted with chloroform. The chloroform layer was washed with water, dried and evaporated to dryness to yield a colourless residue which crystallised from methanol as colourless prisms (0.8 g) m.p. 186-188°.

Attempted Dequaternization of O-Methyldryadine Dimethiodide.

Similar treatment of O-methyldryadine dimethiodide (1.25 g) gave a brown, amorphous residue (1.0 g) which was chromatographed over alumina (40 g) in 25% chloroform/benzene. The earlier fractions (0.7 g) failed to crystallise (thin-layer chromatography indicated that they were mixtures) whilst the later fractions crystallised from ether m.p. 168-170°, λ_{max}^{EtOH} 280 mμ.

2. Spermatheridine.

Spermatheridine crystallised from chloroform as yellow needles, m.p. 271-275° (decomp.), undepressed upon admixture with spermatheridine from A.moschatum. The two compounds showed identical i.r. and u.v. spectra.

3. Alkaloid D.

Alkaloid D crystallised from acetone/petroleum spirit (60°-80°) as yellow prisms m.p. 198-201°. (Found: C, 60.6; H, 6.4; N, 6.3; OMe, 14.2; NMe, 6.3%. Calc. for $C_{22}H_{28}O_7N_2$: C, 61.1; H, 6.5; N, 6.5; 2 x OMe 14.3; 2 x NMe, 6.9%.)

It formed a colourless hydrochloride, m.p. 260-261° and was soluble in warm 5% aqueous sodium hydroxide.

Treatment of an ethanol solution of Alkaloid D with ferric chloride solution produced a purple colour which, upon standing, turned green with the appearance of yellow needles; these were filtered off and recrystallised from ethanol, m.p. 170-175° (decomp.). (Found: C, 43.3; H, 4.3; OMe, 10.1; NMe, 4.5; Ash, 13%. Calc. for $C_{22}H_{28}O_7N_2.FeCl_3.H_2O$: C, 42.9; H, 4.9; 2 x OMe, 10.1; 2 x NMe, 4.9%.)

Alkaloid D gave a negative test for the methylenedioxy group.

4. Atheroline.

Atheroline crystallised from chloroform/ethanol as orange prisms, m.p. 250-260° (decomp.). Its i.r. spectrum was identical with that of atheroline isolated from A.moschatum.

5. TAXONOMIC PROBLEMS ASSOCIATED WITH THE NEW SOUTH WALES DAPHNANDRA SPECIES.

The problem of the taxonomy of the N.S.W. Daphnandra species arose when Bick, Harley-Mason and Vernengo[21] isolated (+)-tenuipine (1.XXXV), (+)-nortenuipine (1.XXXVI) and repandinine (tenuipine racemate) from the bark of a tree described as D.tenuipes from which Bick, Taylor and Todd[18] had previously isolated repanduline (1.LXXIV), (-)-tenuipine (1.XXXII) and aromoline (1.XXXVII). The latter authors also isolated (-)-nortenuipine (1.XXXIV) from the leaves of the plant. The different chemical constituents may have arisen as a result of different environmental conditions but Bick suspected that the two plants were distinct species.

Recently R.R.Schodde (Division of Land Research and Regional Survey, C.S.I.R.O.) undertook a botanical survey of the Australian Monimiaceae and in particular the taxonomy of the N.S.W. Daphnandra species. To obtain support for his taxonomic analysis he forwarded to these laboratories for alkaloid analysis portions of twenty herbarium specimens which had been collected by several other botanists as well as himself. It is to be emphasised that these samples were merely labelled 1-20 and we were unaware of Schodde's opinion on the taxa.

The samples were extracted with Prolius' solution and the alkaloids were isolated and separated into non-phenolic and phenolic fractions. The former were analysed by thin-layer chromatography on alumina (Fig.5.1.); the results of these analyses (together

TABLE 5.1.

Sample No.[a]	Identity (Schodde)	Collection Reference	Alkaloid Analysis[b] (See Fig.5.1)	Group (According to alkaloid analysis)
1	D.tenuipes Perk.	Floyd & Hayes, 2 May 1957 ex Coff's	(-)Nortenuipine, tenuipine	A
18	" "	W.T.Jones, BRI05799-802	" "	
7	D.micrantha(Tul.)Benth.	J.L.Boorman, N.S.W. 69730	" "	
17	" " "	F.A.Bailey, N.S.W. 67927	" "	
2	D.tenuipes Perk.	Betche; N.S.W., 67922; TYPE	Insufficient alkaloid isolated	
3(bark)	D.johnsonii Schodde	Schodde 3475	Tenuipine, Unknowns Rf 0.41, 0.15 nortenuipine.	B
3	" "	" 3475	Tenuipine, nortenuipine, Unknown, R_f 0.15, Unknown, R_f 0.8	
15	" "	Johnson & Constable; TYPE	Tenuipine, nortenuipine Unknown, R_f 0.15, Unknown, R_f 0.8	
4	D.crypta Schodde	Schodde 3375	Micranthine, Unknowns R_f 0.65, 0.75, 0.85	C
5	" "	Schodde 3543	" " " 0.65, 0.75, 0.85	
6	" "	Story 6930	" " " 0.65, 0.75, 0.85	
19	" "	C.T.White 6884	" " " 0.65, 0.75, 0.85	
20	" "	Schodde 3540	" " " 0.65, 0.75, 0.85	

TABLE 5.1.

Sample No.[a]	Identity (Schodde)	Collection Reference	Alkaloid Analysis[b] (See Fig. 5.1)	Group (According to alkaloid analysis)
8	Daphnandra aff. crypta or Nos. 11,13	W. Forsyth, N.S.W. 67893	Tenuipine	D
9 (bark)	Daphnandra aff. micrantha (i.e. 7 & 17)	S.T. Blake 15898	Tenuipine, unknown, R_f 0.15	
9	" " " (i.e. 7 & 17)	" 15898	" " " 0.15	
10	" " " (i.e. 7 & 17)	N.S.W. 67896	" " " 0.15	
11 (bark)	Daphnandra spec. nov. or aff. crypta	Schodde 3575	Tenuipine, nortenuipine, unknown, R_f 0.77	E
11	" " " " "	Schodde 3575	Unknown R_f 0.14	
13 (bark)	" " " " "	Schodde 3525	(+)-Tenuipine, nortenuipine, unknown, R_f 0.77	
13	" " " " "	Schodde 3525	Unknown, R_f, 0.14.	
16	Daphnandra intermediate between tenuipes and species = samp. 11,13	Jones 2332	Unknown, R_f, 0.14.	
12	(?) Daphnandra, spec. nov. or aff. crypta (i.e. = samples 11,13)	Schodde 3579	Unknown, R_f 0.14, nortenuipine, tenuipine	Either B or E
14	" " " " " "	Schodde 3574	Unknown, R_f 0.14, " "	

[a]: All extractions were on leaf samples unless otherwise stated.

[b]: Indicates major alkaloid.

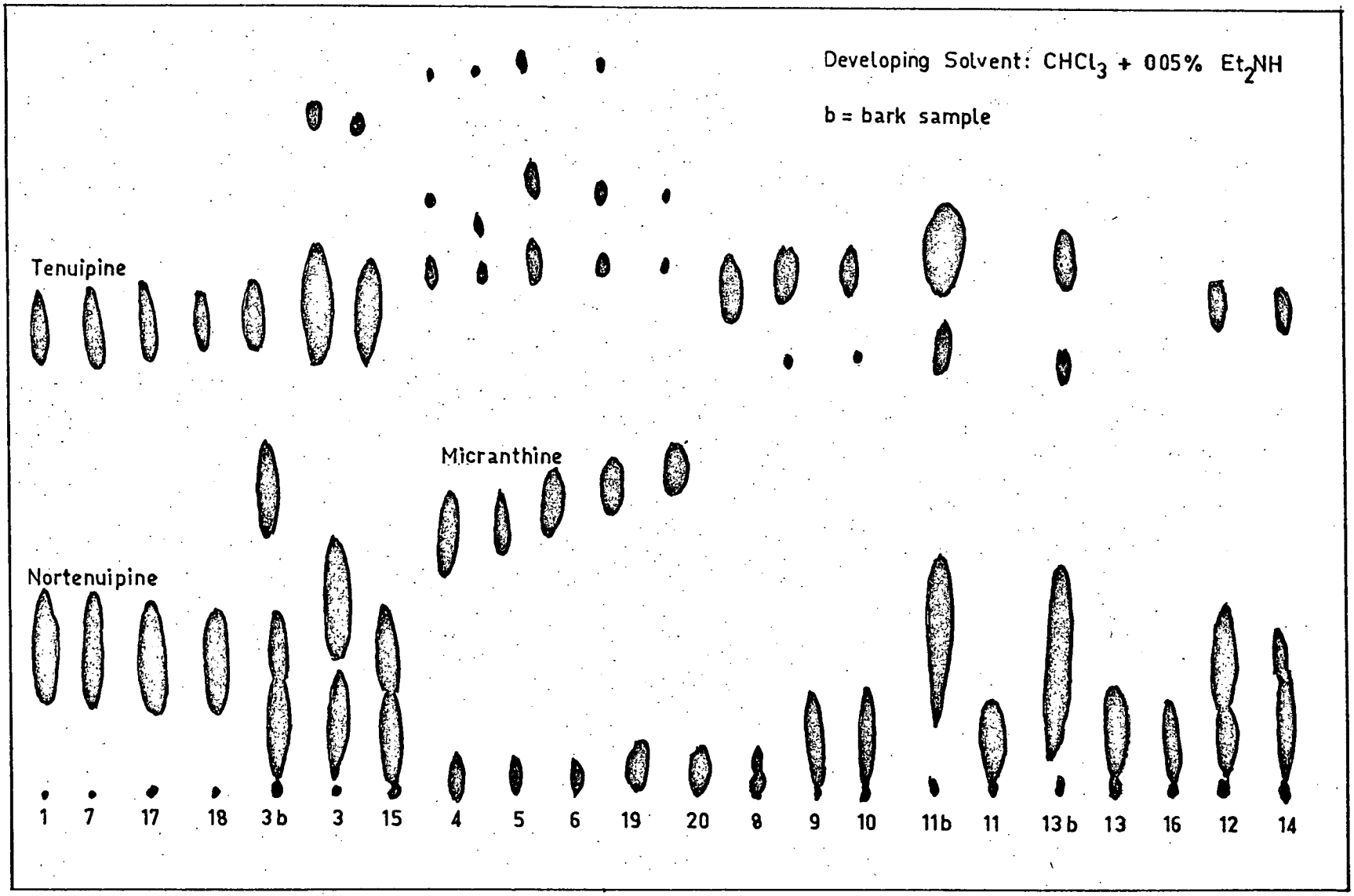


Fig.5.1 TLC of Samples 1-20

with Schodde's identity of samples 1-20) are summarised in Table 5.1.

Possibly the most exciting feature is the agreement between the taxonomic analysis on the one hand and the chemical analysis on the other.

Previous chemical investigations [15,16,17,18] of material described as Daphnandra micrantha have yielded micranthine (1.XXVIII) as the major alkaloid; according to Schodde, the type specimen of the species micrantha originally described by Tulasne (1857) belongs to a distinct morphological species found only about Port Macquarie, Taree and Wingham, and represented by samples 7 and 17. These two samples contained only tenuipine and nortenuipine. The widespread species which gives rise to micranthine (represented by samples 4,5,6,19,20), customarily known as D.micrantha, has been given the name D.crypta Schodde.

Sample 1 yielded (-)-nortenuipine in high yield and since this is the only leaf sample with nortenuipine as the major alkaloid, it is almost certainly the same as that extracted by Bick, Taylor and Todd. Chemically, it is indistinguishable from D.micrantha (Tul.) Benth. (Samples 7 and 17).

The presence of (+)-tenuipine and nortenuipine in sample 13 (bark)(identical with 11) indicated that it was the same as that extracted by Bick, Harley-Mason and Vernengo. This form or species morphologically, comes close to the new D.crypta and Schodde was uncertain whether to regard it as specifically distinct. However, it

shows no relationship to D.crypta in alkaloid make-up and, on this basis, should be regarded as a distinct species. Sample 16 contained the same unknown alkaloid as samples 11 (leaf) and 13 (leaf) and showed, contrary to Schodde's analysis, no intermediacy in alkaloid content between 11 and true D.tenuipes (Sample 1).

Samples 8,9 and 10 were chemically indistinguishable although Schodde believed that sample 8 belonged either to group E (samples 11,13,16) or is a hybrid between it and D.crypta.

The new species, D.johnsonii Schodde, from the Illawara district of N.S.W., is quite distinct morphologically and this is reflected in its alkaloid properties. It is allied distantly to D.tenuipes and is the only species of the genus found (and confined) south of Sydney.

Schodde concluded that there were two morphological groupings of the species of Daphnandra in New South Wales and this is certainly supported by their alkaloid properties. Thus we have the crypta group with only one widespread species, containing micranthine as major alkaloid, and the tenuipes group with apparently three locally occurring allopatric species, tenuipes, micrantha and johnsonii; all contain tenuipine and/or nortenuipine.

A third species group may be represented by Samples 11, 13 and 16, with again only one species.

Thus chemotaxonomy is a valuable tool in the classification of the Daphnandra species. Schodde has also investigated the taxonomy of the Dryadodaphne species[83]. He concluded that the specimen

originally described by Webb[82] as *Daphnandra new species affiliated with aromatica* was a *Dryadodaphne* species (which he has given the provisional name *Dryadodaphne gracilis* Schodde). This conclusion was supported by Taylor[25] who isolated dryadine from Webb's material as well as from *Dryadodaphne novoguineensis* (Perk.) Smith.

EXPERIMENTAL.

Samples of the 20 Daphnandra species were extracted with Prolius' solution (2 x 160 ml) and the extracts evaporated to dryness. The residue in methanol was poured into a rapidly stirred solution of sulphuric acid (5%; 200 ml). The acid solution was filtered of non-basic material and the filtrate was basified (NH_3; pH 9) and extracted with chloroform. The chloroform solution was extracted with aqueous sodium hydroxide (2%, 30 ml), washed with water, dried and evaporated to dryness (Table 5.2). The residual alkaloid was analysed by thin-layer chromatography on layers of alumina (0.025 cm thick) which had been activated by heating in an oven at 75° for 1½ hours. The compounds were visualised as brown spots by exposure to iodine vapour; a spray of conc. sulphuric acid containing a trace of conc. nitric acid revealed micranthine as a blue-green spot[17] and the remaining alkaloids as brown spots.

Sample 1 crystallised from benzene as needles which were identified as nortenuipine by thin-layer chromatography; the material was laevorotatory.

Sample 13 was chromatographed on alumina in 10% chloroform/benzene. The earlier fractions yielded tenuipine (38 mg), $[\alpha]_D^{18}$, + 215° (C, 0.76 in $CHCl_3$).

Chromatography of Sample 11 (leaf) gave a small quantity of crystalline material (ex acetone) which gave a strong positive methylenedioxy test.

TABLE 5.2.

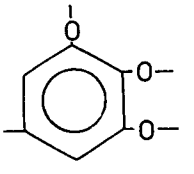
Sample No.*	Mass of Sample(g).	Mass of Non-phenolic Alkaloid (mg).
1	2.0	47.0
2	0.5	18
3(bark)	1.5	39
3	4.5	72
4	2.2	18
5	2.7	34
6	2.0	59
7	2.0	31
8	1.5	38
9(bark)	0.3	10
9	1.3	39
10	2.5	28
11(bark)	2.1	156
11	2.5	84
12	7.0	260
13(bark)	7.0	276
13	2.0	37
14	2.3	55
15	0.5	27
16	0.3	16
17	1.7	26
18	0.5	18
19	0.6	28
20	2.3	77

* All were leaf samples unless stated otherwise.

APPENDIX.

THE ISOLATION OF LIRIODENDRINE FROM A.MOSCHATUM.

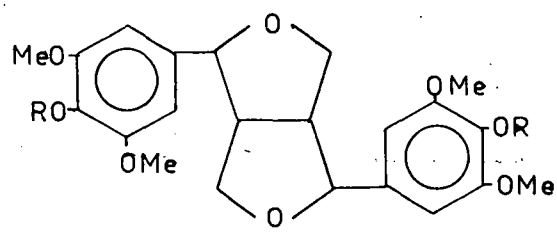
Evaporation of a Prolius' solution extract of A.moschatum yielded considerable quantities of a colourless, crystalline material which did not possess alkaloid properties. The material was not soluble to any appreciable extent in the normal organic solvents or water but it was readily soluble in 50% aqueous ethanol from which it crystallised as colourless needles, m.p. 261-262°. A positive Molisch test and a positive test for a glycol grouping indicated the presence of a sugar residue. The compound showed intense hydroxy absorption in its i.r. spectrum and readily formed an acetate, m.p. 124-125°, upon treatment with acetic anhydride in pyridine. The acetate analysed for $C_{50}H_{26}O_6$ with four methoxyl and eight acetoxy groups; this analysis was compatible with its n.m.r. spectrum (Fig.A.1). The acetate gave a positive Maüle test[92] (as did the free alcohol) which indicated the presence of a syringyl residue (A.I).



A.I

These properties were closely related to those of the lignan glucoside, liriodendrine (A.II) which Pearl, Beyer and Dickey[93] had isolated from Liriodendron tulipifera L. Through the courtesy of Dr.E.E.Dickey an authentic sample of liriodendrine was

obtained and its i.r. spectrum was identical with that of the material isolated from Atherosperma moschatum; the two compounds showed no melting point depression upon admixture.



A.II. R = glucose

Dickey et al.[93] were able to hydrolyse liriodendrine to three stereoisomeric aglycones which they named lirioresinol A, B and C, m.ps. 210-211°, 172-177° and 185-186°, respectively. Dilute acid hydrolysis of the glycoside from A.moschatum gave crystalline material, m.p. 200-210°, whose i.r. spectrum was identical with the published spectrum of lirioresinol A. Paper chromatography of the aqueous acid solution indicated the presence of glucose.

The comparatively strain-free cis- form (A.III) of the central tetrahydrofurofuran nucleus on which these lignans are built is preferred to the puckered and strained trans- form (A.IV). When the cis- form is diagonally substituted four asymmetric centres result which furnish three dl- pairs (A.V, A.VI and A.VII).

Recently, Gurevich, Kolosov and Shemyakin[94] deduced that liriodendrine possessed the configuration (A.VII). The n.m.r. spectrum of octa-O-acetylliriodendrine (Fig.A.1) lends partial support for

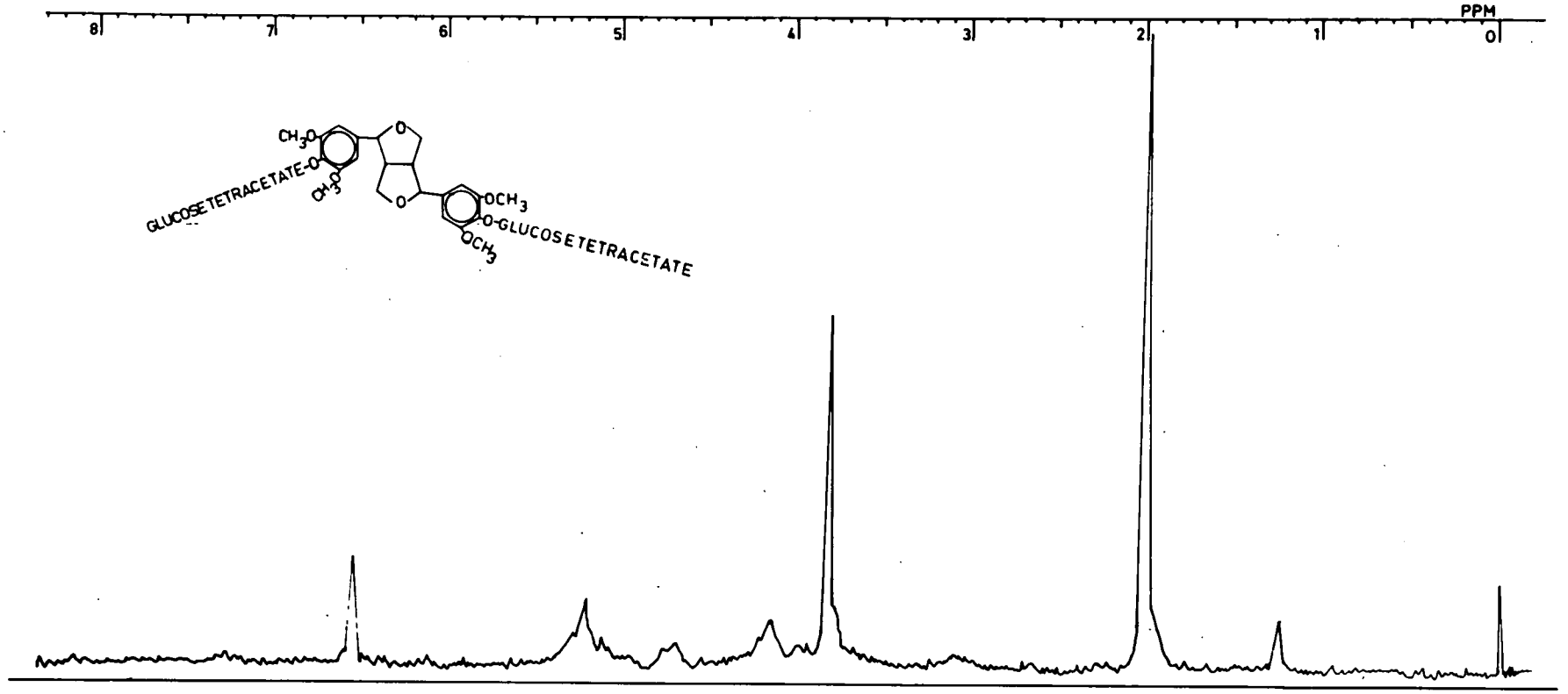
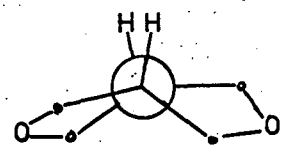
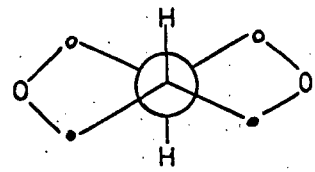

PPM
8
7
6
5
4
3
2
1
0
CH3O
GLUCOSETETRACETATE-O
CH3O
O
O
OCH3
O-GLUCOSETETRACETATE
OCH3

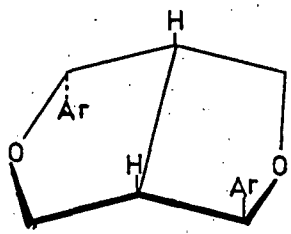
Figure A.1.



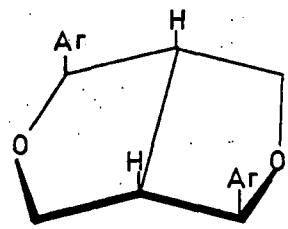
A.III. cis-form



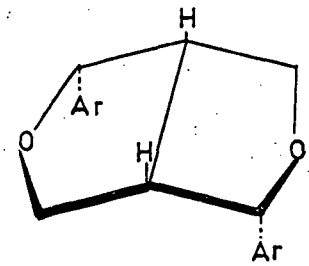
A.IV. trans-form



A.VI



A.V



A.VII

this deduction, at least in the sense that configuration (A.VI) can be rejected. Thus all four aromatic protons of the octa-O-acetylliriodendrine resonate at exactly the same field which indicates that they are held in precisely the same chemical environment. Molecular models revealed that these conditions were satisfied by the configurations (A.V) or (A.VII) but not by (A.VI).

EXPERIMENTAL.

Paper chromatograms were developed with the system ethyl/acetate/acetic acid/water (9:2:2; v/v). The syringyl substances were located by placing air-dried chromatograms in a chlorine atmosphere for 10 minutes after which they were sprayed with 10% aqueous sodium sulphite (Maüle test).

Wet bark of A.moschatum (1.6 Kg) was ground and extracted with Prolius' solution (2 x 4.2 l). Concentration of the second extract gave 4 g of crystalline material which was removed by filtration and recrystallised from 50% aqueous ethanol, m.p. 261-262°, R_f 0.2. It gave a positive Molisch test, a positive glycol test with fuchsin/sulphurous acid and a positive Maüle test. Its melting point was undepressed upon admixture with liriodendrine and the two compounds had identical i.r. spectra.

The acetate melted at 124-125°. (Found: C, 55.7; H, 5.7; OMe, 11.1; $COCH_3$, 33.8. Calc. for $C_{50}H_{62}O_{26}$: C, 55.7; H, 5.8; 4 x OMe, 11.5; 8 x $COCH_3$, 31.9 %). Its u.v. absorption spectrum showed λ_{max}^{EtOH} (log ε_{max}) 272, (3.41), 230 mμ (4.20).

Hydrolysis with Dilute Acid.

The glycoside (0.528 g) in hot water (40 ml) was heated on the steam bath for 2 hours with 1N hydrochloric acid (8 ml). A brownish deposit was then filtered off and the filtrate allowed to stand overnight. A deposit of colourless, crystalline material was removed by filtration and recrystallised from ethanol, m.p. 200-210°. Its i.r. spectrum was identical with the published spectrum of lirio-

resinol A[93].

The sugar was located on a paper chromatogram with aniline hydrogen phthalate and had an R_f identical with that of glucose.

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