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The alkaloids of some Australian and New Guinea plants

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THE ALKALOIDS OF SOME AUSTRALIAN AND NEW GUINEA PLANTS

A THESIS

submitted in fulfilment of the
requirements for admittance to
the degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF NEW SOUTH WALES

by

ROGER EVERETT SUMMONS

Wollongong University College,
The University of N.S.W.,
OCTOBER, 1971

SUMMARY

S U M M A R Y

Four plants belonging to three genera, Cinnamomum (Lauraceae), Mitrella (Annonaceae) and Marsdenia (Asclepiadaceae), have been examined and their main alkaloid constituents identified.

The bark of an unnamed New Guinea Cinnamomum species, designated by the herbarium voucher number T.G.H. 13,077, yielded two new 1-benzyltetrahydroisoquinoline alkaloids, cinnamolaurine and norcinnamolaurine, together with the known alkaloids corydine, norisocorydine and reticuline. The leaves of the same species were found to contain corydine and reticuline as the major constituents together with traces of cinnamolaurine. Camphor was identified as a neutral component of the leaves.

The structure 1-(4^1-hydroxybenzyl)-6,7-methylenedioxy-2-methyl-1,2,3,4-tetrahydroisoquinoline was assigned to cinnamolaurine on the basis of spectroscopic evidence and this was confirmed by synthesis of the racemate. The structure of norcinnamolaurine was deduced from spectroscopic evidence and was confirmed by the formation of cinnamolaurine on N-methylation and also by synthesis of its racemate. Both cinnamolaurine and norcinnamolaurine were shown to belong to the D series of benzylisoquinoline alkaloids.

Cinnamomum laubattii F. Muell, a Queensland species, was found to contain reticuline as the only major alkaloid. This may be of chemotaxonomic interest since *C. camphora*, the only other species of the genus which has been examined for alkaloids, also yielded reticuline as a major constituent.

Mitrella kentii (Bl.) Miq., a New Guinea species belonging to the family Annonaceae, appears to be the first of its genus to be investigated for alkaloids. The known compounds anonaine, asimilobine and liriodenine were identified as the main constituents while a minor one was shown to be 3,9-dihydroxy-2,10-dimethoxytetrahydroprotoberberine, a structure that has not been reported previously.

Marsdenia rostrata R.Br., the fourth plant studied, originated from two different collection localities. A sample obtained from the Toonumbar State Forest in Northern N.S.W. was found to contain the known alkaloid anabasine and a new basic ester aglycone which has been identified as a mixture of O-acetyl-O-nicotinoyl sarcostin and the corresponding 5α-dihydrosarcostin derivative. A sample collected from the South Coast of N.S.W. yielded the above ester aglycone mixture and a number of neutral pregnane aglycones, one of which has been tentatively identified as metaplexigenin.

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I N T R O D U C T I O N

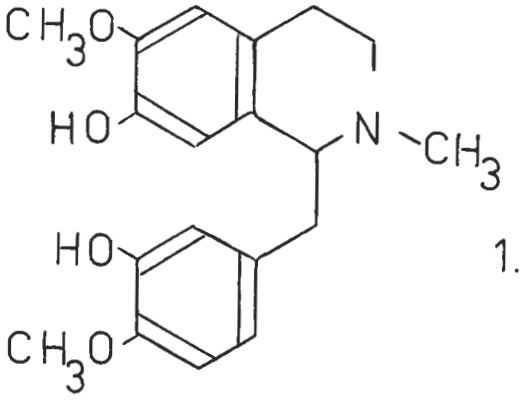
A large scale systematic survey of the chemical constituents of Australian flora began in 1944 as an extension of a war-time project aimed at locating sources of drugs in short supply. This expanded project, under the direction of Dr. C. Barnard of the Council for Scientific and Industrial Research (later the C.S.I.R.O.), became known as the Australian Phytochemical Survey. Dr. Barnard and Dr. L. J. Webb of the Division of Plant Industry, C.S.I.R.O., carried out most of the original plant screening and Dr. J. R. Price of the Division of Industrial Chemistry supervised the original chemical studies. As the survey progressed chemical investigations were carried out by the C.S.I.R.O. and interested university departments while pharmacological testing was undertaken by various university departments and pharmaceutical companies.

Queensland was chosen as the first State for intensive searching since it offered diverse plant systems and large areas of tropical rain forest. These investigations were summarised in the "Guide to the Medicinal and Poisonous Plants of Queensland"[1] compiled by Dr. Webb in 1948. This publication was followed by supplementary bulletins[2,3,4] which listed numerous

species which gave positive field tests for alkaloids and other classes of natural products. In 1955 Webb published his original New Guinea findings in "A Preliminary Phytochemical Survey of Papua and New Guinea",[5] and from then until the present time the work there has been continued by the C.S.I.R.O. and by botanists of the Department of Forests.

During the course of the survey, many hundreds of Australian and New Guinea plants have been intensively studied and the resulting new compounds discovered would be numbered in their thousands. Apart from the wealth of chemical information produced, many of the compounds found have shown interesting physiological activity and quite a few have reached the stage of clinical testing. Although many plants have been fully investigated, there remain a number which are known to contain alkaloids, or other classes of natural products, which have not been examined in detail. The present study involved a detailed examination of a number of these species.

The first two plants examined by us were a Cinnamomum species designated by the herbarium voucher number T.G.H. 13,077 from New Guinea and Cinnamomum laubattii F. Muell. from Northern Queensland. The genus Cinnamomum belongs to the family Lauraceae and alkaloid containing members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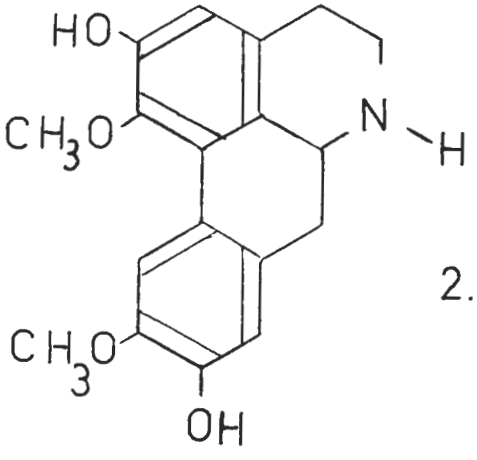
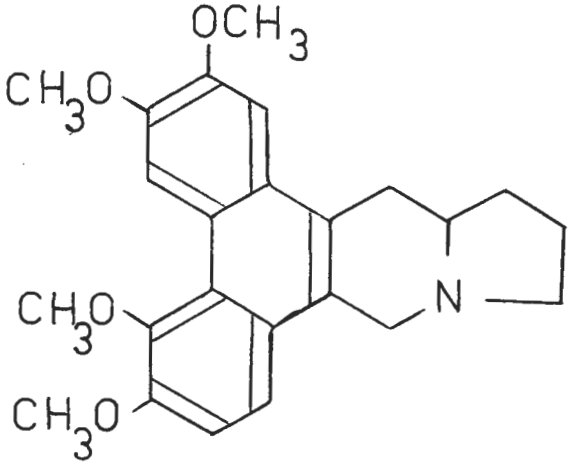
HO
N
CH3
HO
CH3O

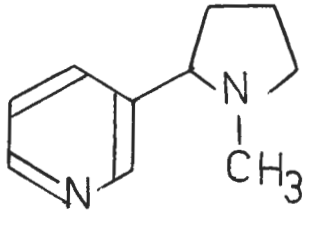
1.


HO
CH3O
N
H
CH3O
OH

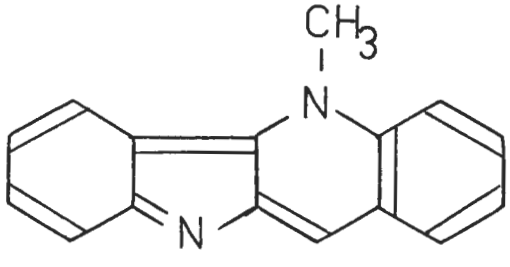
2.


OCH3
CH3O
CH3O
CH3O
N

3.


N
CH3
N

4.


CH3
N
N

5.

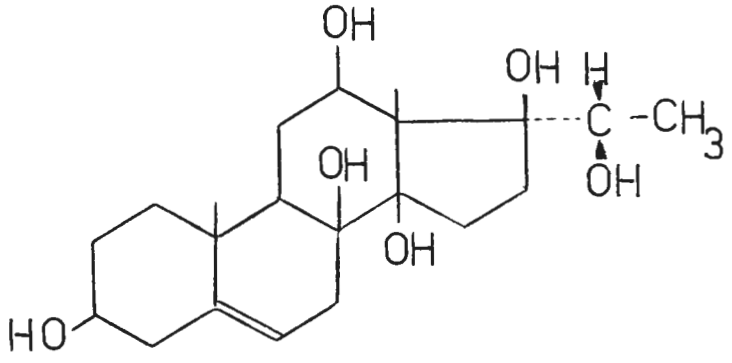
of this family have previously yielded alkaloids of the isoquinoline type - mainly 1-benzylisoquinolines, bisbenzylisoquinolines, and aporphines. Some members of the genus Cryptocarya of the family Lauraceae have yielded alkaloids of the smaller but related groups of phenanthroquinolizidines and dibenzopyrrocolines.[6] Within the genus Cinnamomum itself members have previously been known for their essential oil content especially C. camphora (Camphor laurel) which is used as a commercial source of camphor. C. camphora is also the only species of the genus which has been examined for alkaloids. Tomita and Kozuka[7] isolated reticuline (1) and laurolitsine (2) from this plant and therefore it would not be unreasonable to expect the two species reported here to contain compounds similar to these.

A third plant investigated was Mitrella kentii (Bl.) Miq. belonging to the family Annonaceae. Members of this family also afford isoquinoline alkaloids, principally aporphines, oxoaporphines, and protoberberines, but it appears that no Mitrella species have previously been examined. M. kentii is a New Guinea species and is designated by the herbarium voucher number T.G.H. 11,488.

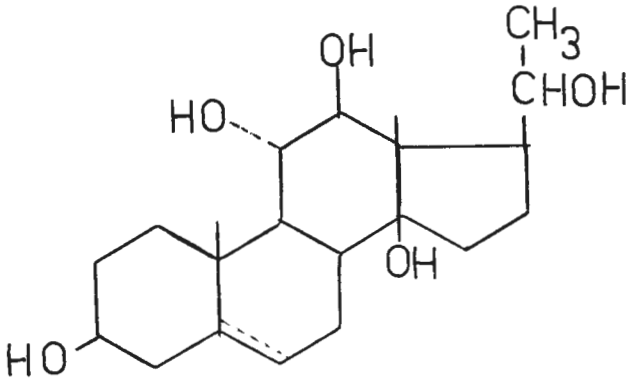
A fourth plant examined was Marsdenia rostrata R.Br. of the family Asclepiadaceae. Plants belonging to the genera Tylophora,[8] Vincetoxicum,[9] Antitoxicum[10] and

Cynanchum,[11] of this family are known to contain alkaloids belonging to the small group of phenanthroindolizidines (e.g. tylocrebrine (3)), while other groups occurring in the family are simple pyridine bases such as nicotine (4) from Asclepias syriaca[12] and the much rarer δ-carboline type represented by cryptolepine (5) which has been isolated from Cryptolepis triangularis[13] and C. sanguinolenta.[14] There does not appear to be any record of an alkaloid isolation from a Marsdenia species although there are several reports of positive field tests for a number of species.[1,2,3].

The family Asclepiadaceae has also been an important source of triterpenoid and steroid glycosides. The genera Asclepias, Cynanchum, Gongronema, Gymnema, Marsdenia, Metaplexis and Sarcostemma have all afforded compounds of this type. Within the genus Marsdenia itself M. condurango is best known for its use in medicine.[15] The dried stem bark, which contains a mixture of glycosides loosely named condurangin, has been used as a gastric sedative and also as an aromatic bitter. The glycosides of Marsdenia condurango have been studied independently by several groups. In Japan, Mitsuhashi and co-workers[16] obtained a crude glycoside mixture from commercially available cortex condurango which, when submitted to mild acid hydrolysis, yielded a mixture of ester aglycones and the sugars oleandrose, cymarose and others. Alkaline hydrolysis

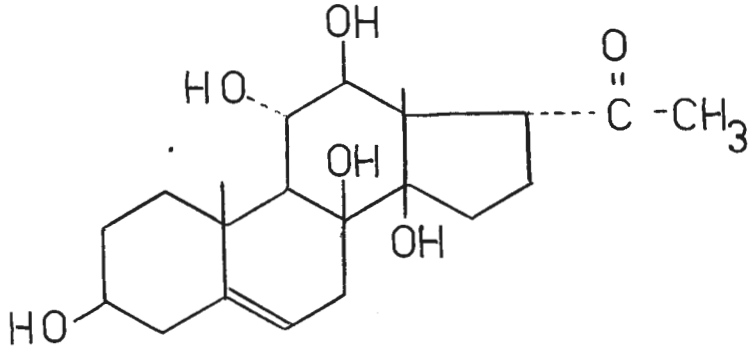


6.



7. Δ^5

8. 5α-H



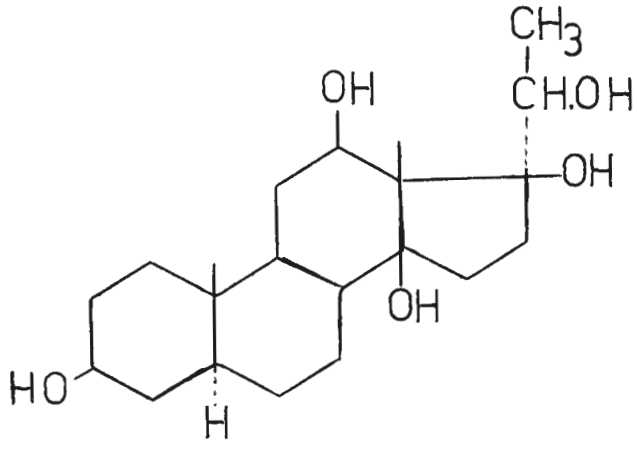
9.

of the aglycone mixture gave the polyhydroxy pregnane derivatives sarcostin (6), drevogenin-D (7), dihydro-drevogenin-D (8), and marsdenin (9) as well as cinnamic and acetic acids. Analysis of the ester aglycones themselves showed a mixture of the above deacyl genins which were esterified with cinnamic and acetic acids at some hydroxy groups. Tschesche and co-workers[17] have identified essentially the same aglycones and sugars as well as a number of the complete glycosides.

Mitsuhashi et al have also isolated the deacyl genins sarcostin and tomentogenin (10) from Marsdenia tomentosa Decne.[18] Reichstein and co-workers have studied the components of Marsdenia erecta R.Br.[19] from which they isolated an aglycone mixture containing the pregnane derivatives marsdenin, drevogenin-D, and marsectohexol (11) which were partially esterified with acetic, benzoic, and tiglic acids.

In the present study, the section on the components of Marsdenia rostrata began as an investigation of the alkaloid content of the plant since the initial extraction yielded a considerable quantity of basic material. When it was found that the principal "base" was in fact a pregnane ester aglycone, a second collection and extraction was made so that other aglycones lost in

9.



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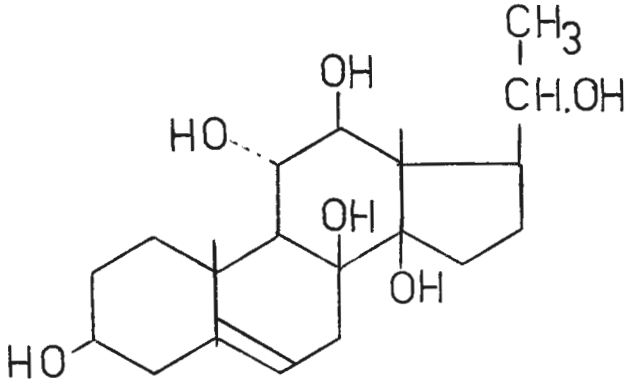


11.

the original work-up could be identified. No attempt has been made to isolate any of the original glycosides or any of the sugars produced by acid hydrolysis of the glycoside mixture.

D I S C U S S I O N

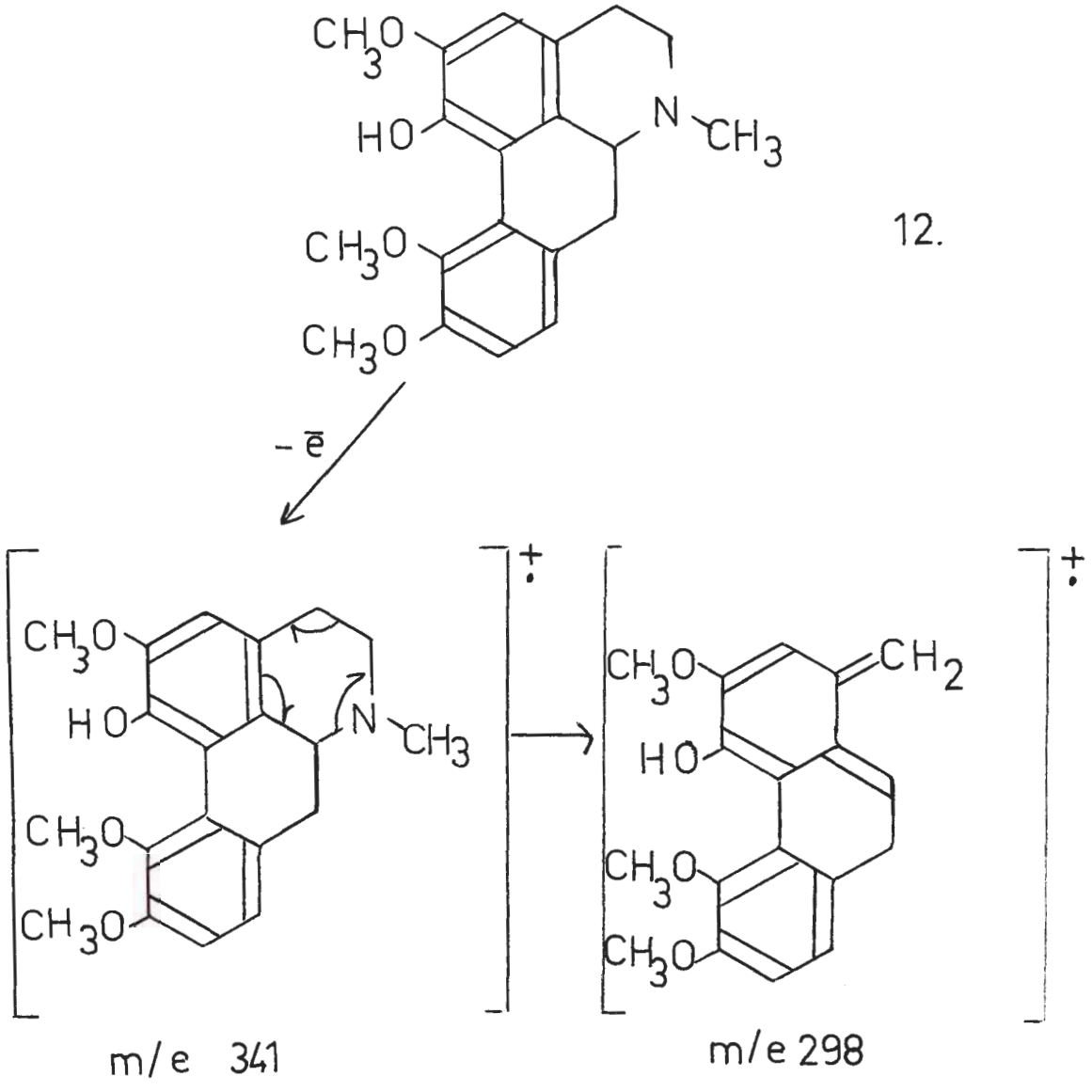
I. ALKALOIDS OF CINNAMOMUM sp. T.G.H. 13,077

Bark and leaves of Cinnamomum species T.G.H. 13,077 were collected by Mr. J. S. Womersley near Gairana in the Morobe district of New Guinea. The procedure for the isolation of the crude alkaloid mixture was the same in this and the following investigations and consisted of extraction of the dried milled plant material by percolation with cold methanol followed by treatment of the concentrated extract with dilute acid. The filtered acid solution was then basified with ammonia and extracted with chloroform until the aqueous phase gave no further Mayers test. The chloroform solution was then dried and evaporated to yield the crude alkaloid mixture. In most cases further purification could be achieved by extracting the alkaloids from chloroform or ether solution into dilute acid and then re-extracting them into chloroform.

Alkaloids of the Bark

The crude alkaloid mixture, obtained in 0.055% yield, was fractionated by chromatography on silica gel. Further chromatography on silica and alumina was required to separate the components of some fractions. The alkaloids corydine, cinnamolaurine, norcinnamolaurine, reticuline and norisocorydine were identified as follows:

FIGURE 1.


CH3O
HO
N
CH3
CH3O
CH3O
12.
- ē
CH3O
HO
N
CH3
CH3O
CH3O
+·
m/e 341
CH2
CH3O
HO
CH3O
CH3O
+·
m/e 298
+ CH2= N-CH3

Corydine (12)

Corydine was obtained as colourless needles m.p. 151°, $[\alpha]_D^{24}$ + 204°. Its ultraviolet absorption spectrum was consistent with it being a 1,2,10,11-tetrasubstituted aporphine while a bathochromic shift with increased intensity of absorption in the presence of alkali indicated that it was phenolic. The n.m.r. spectrum showed signals for an N-methyl group, three methoxyl groups, and three aromatic protons (a one proton singlet and a two proton AB quartet) and except for the N-methyl signal was almost identical with a published spectrum for norcorydine[20]. The identity of the alkaloid with corydine was also indicated by its mass spectrum which, at 12 e.v., showed a strong parent and base peak at m/e 341 with practically no other significant signals. At 70 e.v. the spectrum showed strong peaks at m/e 326 ($M-CH_3$), 310($M-OCH_3$) and 298 ($M-CH_2 = N-CH_3$). The peak at m/e 298 arises through the opening of ring B as in figure 1 and is typical of aporphines[21].

The identification of the compound as corydine was confirmed by comparison with an authentic specimen. The i.r. spectra and chromatographic behaviour of the two samples were identical and there was no depression of m.p. in a mixed m.p. determination.

F I G U R E 2.

MASS SPECTRUM — **CINNAMOLAURINE**

Inlet temp. 160° — Electron Voltage 70 e.v.

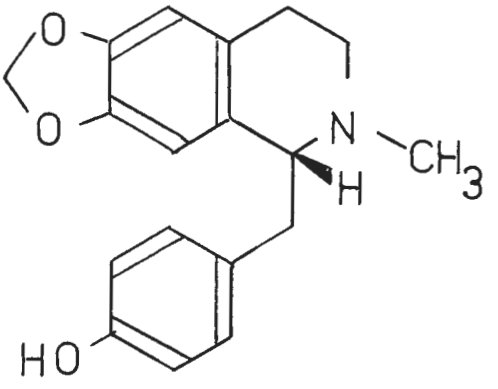
M/E	R.I.	M/E	R.I.
297	0.10	174	0.50
296	0.50	161	0.63
295	0.11	160	1.38
294	0.11	150	0.46
280	0.19	149	4.00
279	0.69	132	1.76
256	0.32	131	1.50
191	17.50	107	2.13
190	100.00	91	1.13
177	0.25	81	1.63
176	0.31	65	0.66
175	1.50		

Cinnamolaurine (13).

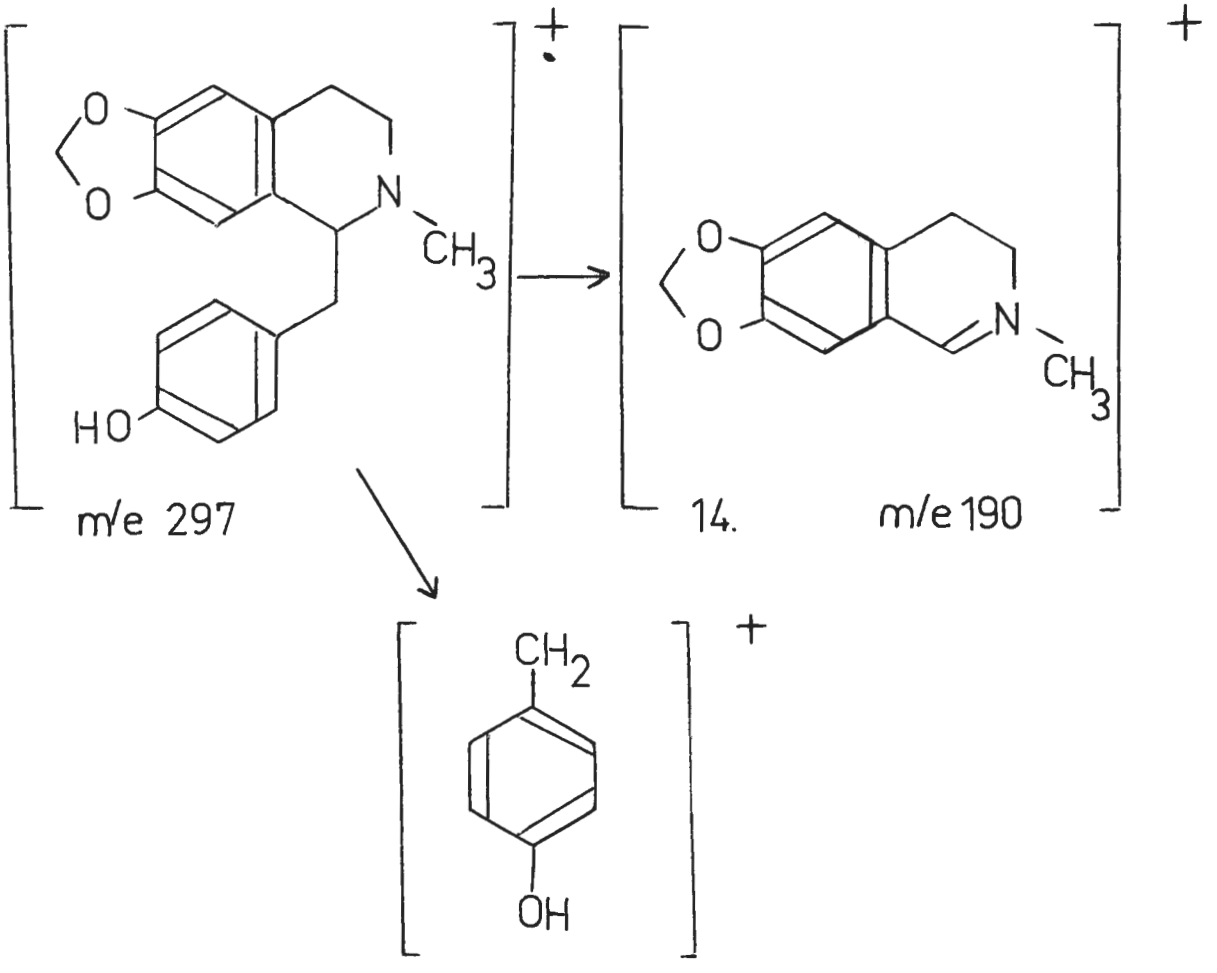
A second alkaloid, which we have named cinnamolaurine[23], was isolated as colourless prisms m.p. 212-213° (dec.), $[\alpha]_D^{25}$ - 100°. The formula $C_{18}H_{19}NO_3$ was established by microanalysis and by the presence of a weak molecular ion signal at m/e 297 in the mass spectrum. The compound showed a maximum at 287 nm (log ϵ 3.72) in its u.v. spectrum - consistent with it being a 1-benzyl-1,2,3,4-tetrahydroisoquinoline type[24]. The presence of a phenolic hydroxyl group was demonstrated by a positive ferric chloride test and by a bathochromic shift of the u.v. maximum on the addition of alkali. On the basis of the spectroscopic evidence given below, the structure (-)-1-(4^1-hydroxybenzyl)-6,7-methylenedioxy-2-methyl-1,2,3,4-tetrahydroisoquinoline was assigned to cinnamolaurine.

An n.m.r. spectrum of cinnamolaurine showed signals for an N-methyl group (singlet at δ 2.3), a methylenedioxy group (singlet at δ 5.85), two aromatic protons (singlets at δ 6.45 and δ 6.57) and four more aromatic protons which appeared as an A_2B_2 quartet centred at δ 6.56 and δ 6.92 (J = 8.0 Hz). The aromatic proton signals at δ 6.45 and δ 6.57 can be assigned to C8-H and C5-H respectively while the quartet arises from $C2^1$-H, $C3^1$-H, $C5^1$-H and $C6^1$-H, the protons of the benzyl portion of the molecule.

FIGURE 3.



13.

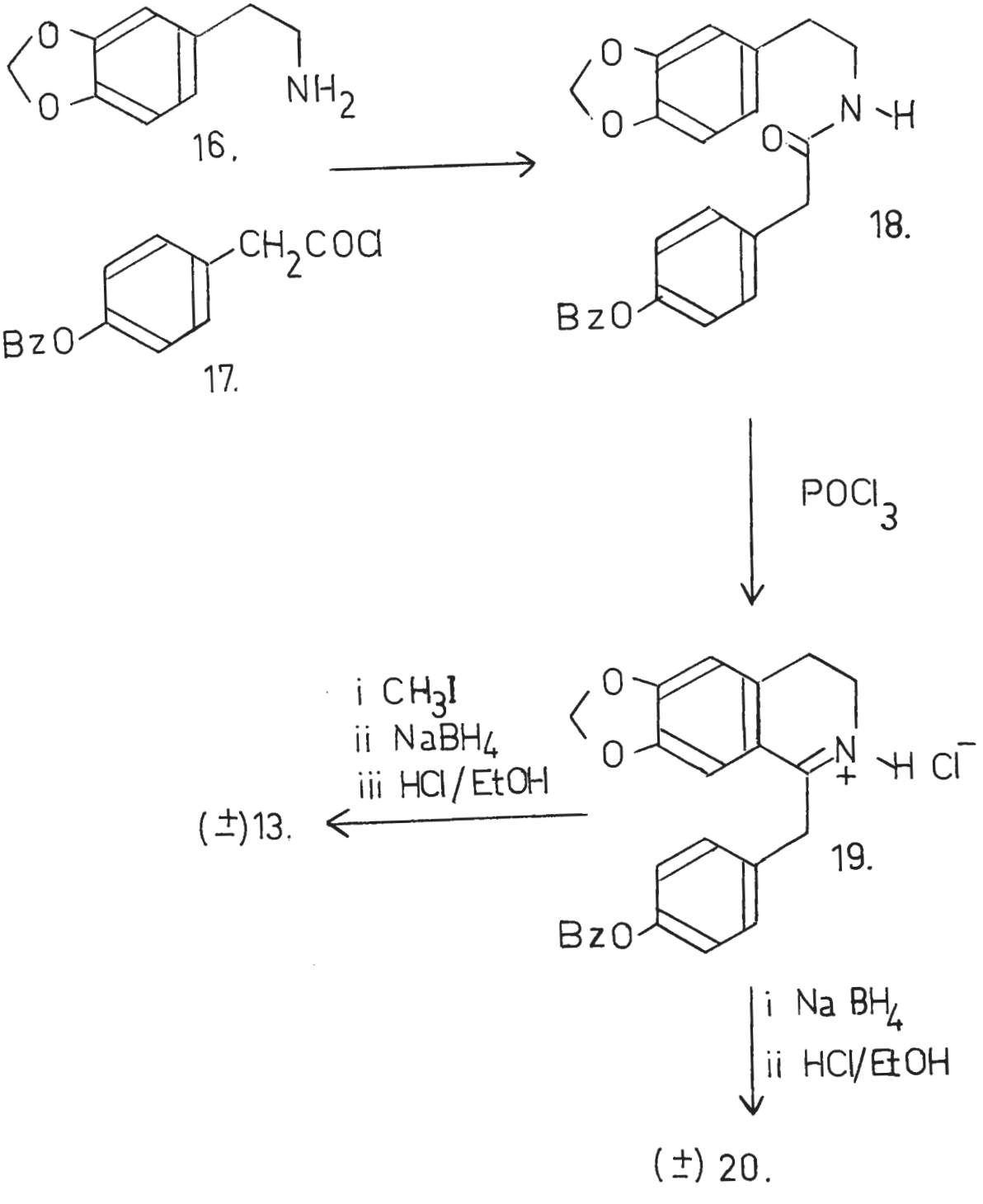


m/e 297

14. m/e 190

15. m/e 107

FIGURE 4


16.
17.
18.
BzO
CH_2COCl
NH_2
$POCl_3$
i CH_3I
ii $NaBH_4$
iii HCl/EtOH
(±)13.
19.
$H\ Cl^-$
i $NaBH_4$
ii HCl/EtOH
(±) 20.

In addition to the molecular ion signal at m/e 297, the mass spectrum (figure 2) showed strong signals at m/e 190 and m/e 107. These can be assigned to the breakdown fragments (14) and (15) respectively shown in figure 3. The base peak at m/e 190 fixes the isoquinoline portion of the molecule as carrying methylenedioxy and N-methyl substituents because those are the only groups which can satisfy the mass requirement, while the hydroxybenzyl fragment gives rise to the signal at m/e 107.

That the structure of cinnamolaurine is correctly represented by (13) was confirmed by synthesis of its racemate using the standard Bischler-Napieralski method (figure 4). 3,4-Methylenedioxyphenylethylamine (16) was condensed with 4-benzyloxyphenylacetyl chloride (17) to give the amide (18) which cyclised smoothly in the presence of phosphorus oxychloride affording the amine hydrochloride (19). The free base of (19) was converted to its methiodide which was reduced with sodium borohydride and then hydrolysed by refluxing in a mixture of ethanol and hydrochloric acid. The product had u.v., i.r. and mass spectra and chromatographic behaviour identical with those of the natural product.

The absolute stereochemistry of cinnamolaurine was established by comparison of its o.r.d. spectrum with

spectra of structurally related compounds whose stereochemistry is known. It has been established[22,58,59] that 1-benzylisoquinolines of the D-(or R) series exhibit three negative (and their L-(or S) enantiomers three positive) Cotton Effects in the 200-320 nm region of the spectrum. The o.r.d. spectrum of cinnamolaurine showed a negative Cotton Effect at about 280 nm and a much more intense peak in the region of 200-240 nm (apparently two unresolved peaks).** This evidence is sufficient to assign cinnamolaurine to the D-series of benzylisoquinoline alkaloids.

Norcinnamolaurine (20)

Another new alkaloid isolated was norcinnamolaurine[25] which was obtained as colourless needles of m.p. 197-198°, $[\alpha]_D^{25}$ + 55°. The formula $C_{17}H_{17}NO_3$ was established by microanalysis and by the presence of a molecular ion signal at m/e 283 in the mass spectrum. The compound was recognised as a phenolic 1-benzyl-1,2,3,4-tetrahydroisoquinoline by its u.v. spectrum which was almost identical with that of cinnamolaurine. An n.m.r. spectrum was also almost identical with that of cinnamolaurine except

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** Cinnamolaurine and norcinnamolaurine have been submitted to Professor J. C. Craig for a more accurate determination of the o.r.d. spectra, but the results have not arrived in time for inclusion in this thesis.

F I G U R E 5.

MASS SPECTRUM

Inlet temp. 170°

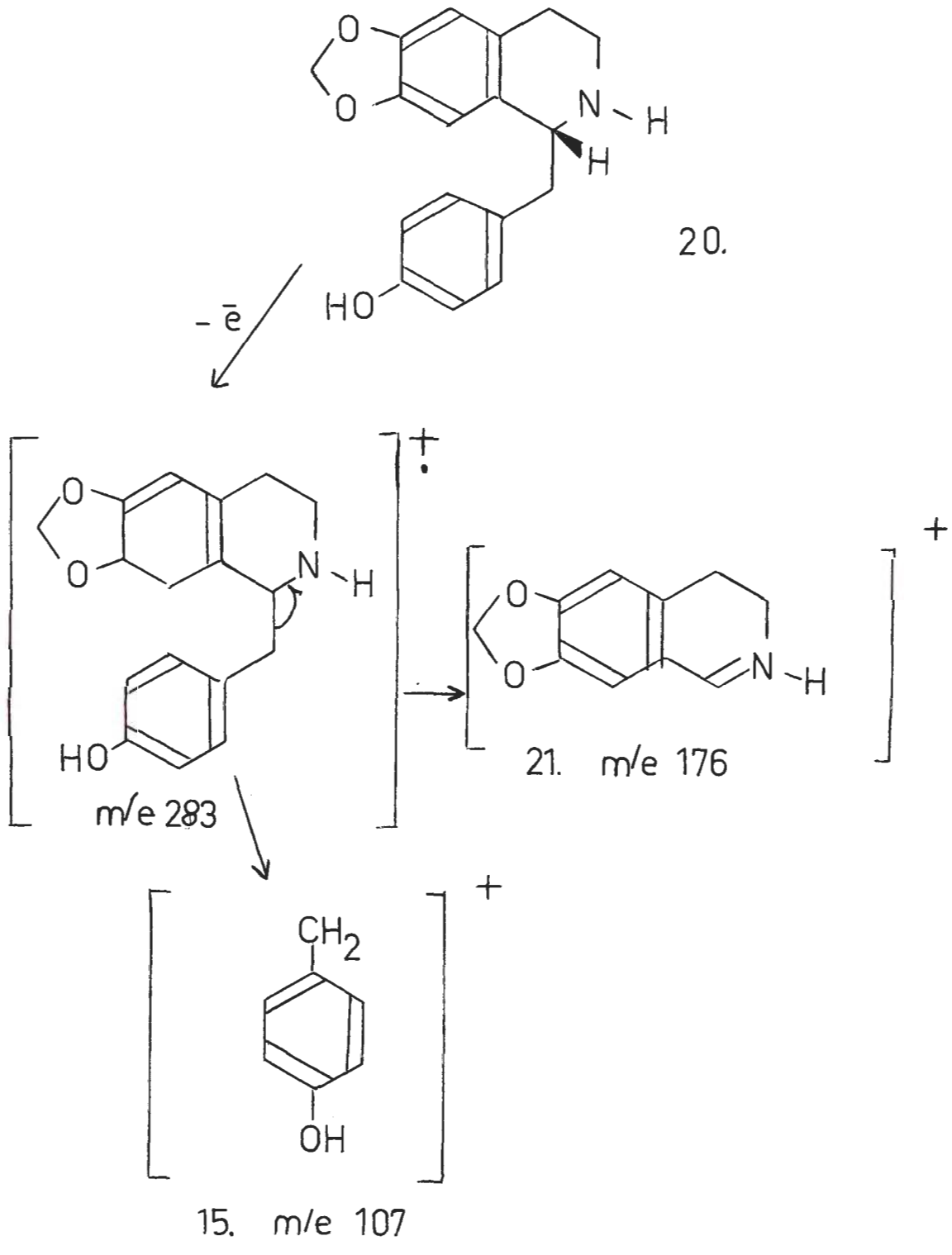
M/E	R.I.
283	0.13
282	0.66
281	0.28
280	0.57
279	0.12
178	1.34
177	11.80
176	100.00
175	2.34
174	3.14

NORCINNAMOLAURINE

Electron Voltage 70 e.v.

M/E	R.I.
161	1.34
149	1.50
107	3.73
91	2.58
90	0.84
89	1.64
78	0.73
77	2.24
65	1.64

FIGURE 6



for the absence of an N-methyl signal. It showed a two proton singlet at δ 5.9, indicative of a methylenedioxy group, two aromatic proton singlets at δ 6.71 and δ 6.57 which can be assigned to C5-H and C8-H respectively and the four peaks of an A_2B_2 system of aromatic protons centred at δ 6.67 and δ 7.05 (J = 8.0 Hz) which can be assigned to $C2^1$-H, $C3^1$-H, $C5^1$-H and $C6^1$-H, the four protons of the benzyl portion of the molecule.

In addition to the molecular ion signal, the mass spectrum (figure 5) showed strong peaks at m/e 176 (base peak) and at m/e 107 which can be interpreted as belonging to the fragments (21) and (15) of figure 6.

From this evidence the structure of the alkaloid can be assigned as (+)-1-(4^1-hydroxybenzyl)-6,7-methylene-dioxy-1,2,3,4-tetrahydroisoquinoline or (+)-norcinnamolaurine. This structure was proven correct by N-methylation of the compound to give (-)-cinnamolaurine. The methylation also confirmed that, although the two alkaloids have optical rotations of opposite sign at the sodium D-line, they both possess the same absolute configuration. Norcinnamolaurine has since been isolated from Lindera glauca by Kozuka and its structure determined independently by him as the N-nor derivative of cinnamolaurine[26]. A comparison carried out by Prof. Kozuka confirmed the identity of the two alkaloids.

The racemate of norcinnamolaurine was prepared using the same sequence of reactions (figure 4) as for the synthesis of cinnamolaurine but with the intermediate quaternisation step omitted. The synthetic racemate was indistinguishable from the natural product in its i.r. spectrum and t.l.c. behaviour while its N-methylation product was shown to be identical with (±)-cinnamolaurine.

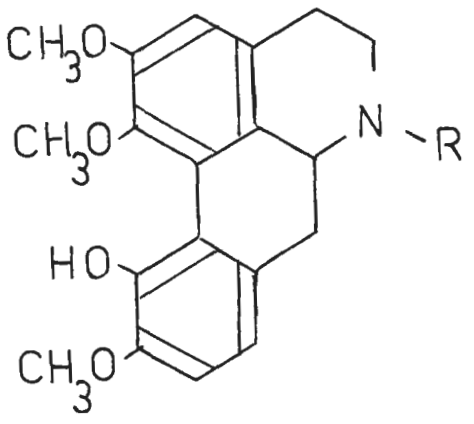
Reticuline (1)

Reticuline was obtained as a colourless gum which could not be induced to crystallise. Its u.v. spectrum indicated that it was a phenolic 1-benzyl-1,2,3,4-tetrahydroisoquinoline type[24]. An n.m.r. spectrum showed signals for an N-methyl group at δ 2.43, two methoxyl groups as a six proton singlet at δ 3.78, two hydroxyl groups as a broad singlet at δ 5.3, exchangeable with deuterium oxide and five aromatic protons as a complex series of multiplets between δ 6.25 and δ 6.70. This spectrum was very similar to a literature spectrum for reticuline[27]. Reticuline perchlorate m.p. 202°, $[\alpha]_D^{25}$ + 75° was prepared from the gum according to the method given in the literature[28] and was found to be identical (mixed m.p. and i.r. spectrum) with an authentic specimen of reticuline perchlorate.

Norisocorydine (22)

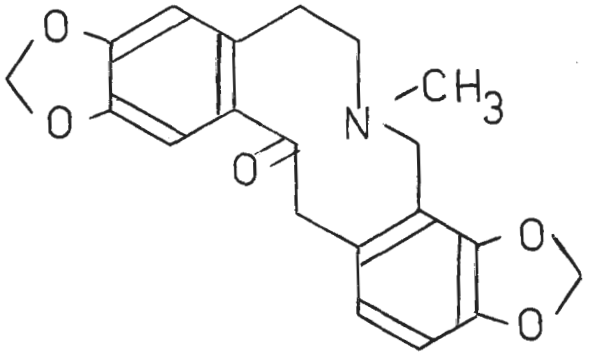
A minor, and unstable component of the alkaloid mixture was isolated as a crystalline hydrobromide salt m.p. 203-207°, $[\alpha]_D^{24}$ + 200°. These physical constants corresponded fairly closely to the m.p. 203-205°, $[\alpha]_D$ + 159° reported for the hydrobromide salt of the alkaloid norisocorydine which has previously been isolated from Hernandia catalpifolia[29] and from Peumus boldus[30].

The u.v. spectrum of the alkaloid was almost identical with the spectrum of corydine while an n.m.r. spectrum was very similar, except for the absence of an N-methyl signal, to a reported n.m.r. spectrum of isocorydine (23)[31]. It showed a three proton singlet at δ 3.71 which can be assigned to the C1 methoxyl group and a six proton singlet at δ 3.90 which can be assigned to the C2 and C10 methoxyls. The aromatic proton signals were interesting in that one proton (C3-H) appeared as a singlet at δ 6.71 as expected but the C8 and C9 protons occurred as a two proton singlet at δ 6.83. In the corresponding spectra of corydine[32] and norcorydine[20] it was observed that the C8 and C9 protons resonated as an AB pair of doublets centred at δ 6.87 and δ 7.10 (J = 8.0 Hz). This constitutes an example of a case where the coincident chemical shift of two aromatic ortho protons leads to the collapse of the usual AB quartet and the phenomenon appears common to those aporphines



22. R=H

23. R=CH_3



24.

which have C11 hydroxyl and C10 methoxyl substitution[31]. It has also been observed in the spectrum of protopine (24) where the two ortho protons are adjacent to a methylenedioxy group[31].

Since a sample of norisocorydine could not be obtained for comparison purposes, the alkaloid was methylated using the formaldehyde-sodium borohydride method and the resulting isocorydine converted to its methiodide. This was then compared with an authentic specimen of isocorydine methiodide and the two compounds were found to be identical.

Alkaloids of the Leaves

The dried, crushed leaves of Cinnamomum sp. T.G.H. 13,077 were extracted with methanol and the extract worked up by a similar procedure to that used for the bark. The process, however, was made quite difficult by the presence of a considerable amount of essential oil in the extract. The crude alkaloids were obtained in 0.008% yield and were fractionated by chromatography on deactivated neutral alumina. The following three alkaloids were identified:-

Corydine

T.l.c. comparison with the bark alkaloids indicated that a major component of the leaf alkaloid mixture was

corydine. Accordingly, fractions which contained this compound were combined and crystallised and the product shown, by mixed m.p. determination and comparison of i.r. spectra, to be identical with the corydine isolated from the bark.

Reticuline

The other major component of the alkaloid mixture was shown by t.l.c. comparison to be reticuline. This was confirmed by conversion of the compound to the crystalline perchlorate salt and comparison with the reticuline perchlorate isolated from the bark.

Cinnamolaurine

A very minor component of the leaf alkaloid mixture, which could not be separated from the complex mixture of minor alkaloids, was shown by t.l.c. to be cinnamolaurine, but this was not confirmed by a direct comparison.

Identification of Camphor as a non-basic constituent of the Leaves

During the work-up of the methanol extract of the leaves, a colourless, neutral, semi-crystalline compound with the distinctive odour of camphor was isolated. This was purified by repeated sublimation to give colourless needles m.p. 174^{o}. The reported m.p. for camphor is 176^{o} [33].

The identification of the compound as camphor was confirmed by a mixed m.p. determination, and by comparison of its i.r. spectrum with that of an authentic specimen of camphor.

II. ALKALOIDS OF CINNAMOMUM LAUBATTII

Bark of *Cinnamomum laubattii* F. Muell. was collected by the C.S.I.R.O. on the Atherton Tableland in Northern Queensland. The dried, milled bark was worked up in the usual manner to yield the crude alkaloid mixture in 0.01% yield.

Thin-layer chromatography indicated that the mixture consisted of several components but that one compound, reticuline, constituted the bulk of the material. The alkaloids were fractionated by chromatography on alumina and fractions which showed a single component by t.l.c. were combined and converted to a crystalline perchlorate m.p. 201°, $[\alpha]_D^{25}$ + 80°. Comparison with an authentic sample of reticuline perchlorate by means of i.r. spectra and mixed m.p. determination confirmed that the compound was reticuline.

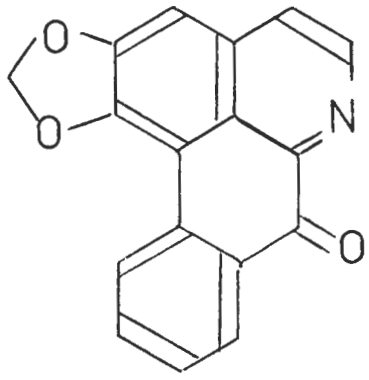
The isolation of reticuline from the plant is interesting from a chemotaxonomic viewpoint since this alkaloid has now been identified as a major constituent of *Cinnamomum* sp. T.G.H. 13,077, *C. camphora* and *C. laubattii*, the three species of this genus which have so far yielded alkaloids.

III. ALKALOIDS OF MITRELLA KENTII

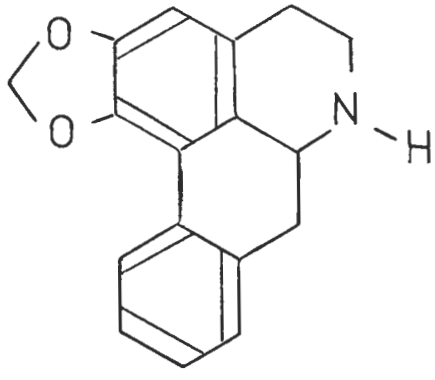
Bark of *Mitrella kentii* (Bl.) Miq. (Annonaceae), an "oak-forest" liana, was collected and identified by Dr. T. G. Hartley near Wau in the Morobe District of New Guinea. The sample was designated by the herbarium voucher number T.G.H. 11,488. After the usual extraction and work-up, the crude alkaloid mixture was obtained in 0.06% yield and was fractionated by chromatography on alumina.

As the separation of the alkaloids progressed, it was observed that the composition of the mixture bore a marked resemblance to that of the mixture obtained from *Schefferomitra subaequalis* (Scheff.) Diels. (Annonaceae), a rain-forest liana, also from New Guinea, which was concurrently being investigated by Mr. R. Rudzats in this laboratory[34]. *S. subaequalis* had previously been examined by Lamberton and co-workers[35], and was found to contain at least eight constituents but only two of these, anonaine and liriodenine, were fully characterised.

The three major constituents of *M. kentii* have been shown by direct comparison with the *S. subaequalis* alkaloids to be liriodenine, anonaine and asimilobine. A fourth and minor component has been shown by t.l.c. and u.v. and mass spectral comparison to be identical with another of the



25.



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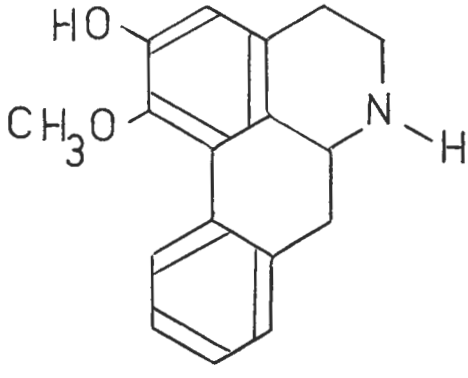


27.

Schefferomitra alkaloids, 3,9-dihydroxy-2,10-dimethoxy-tetrahydroprotoberberine, a structure that has not been reported previously.

Liriodenine (25)

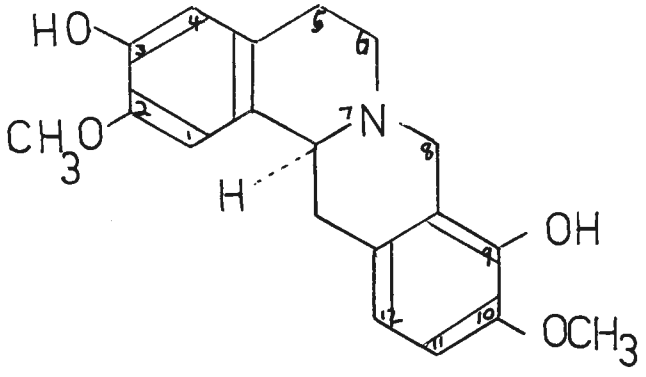
Liriodenine was obtained as bright yellow needles m.p. 281-4° and its u.v. spectrum indicated that it was a 1,2-disubstituted oxoaporphine type[24]. An n.m.r. spectrum was similar to a published spectrum for liriodenine[36] and showed a two proton methylenedioxy singlet at δ 6.65 and a complex multiplet of aromatic protons between δ 7.6 and δ 8.9. The identification of the compound as liriodenine was confirmed by i.r. spectra, t.l.c. comparison and by a mixed m.p. determination with an authentic sample.

Anonaine (26)

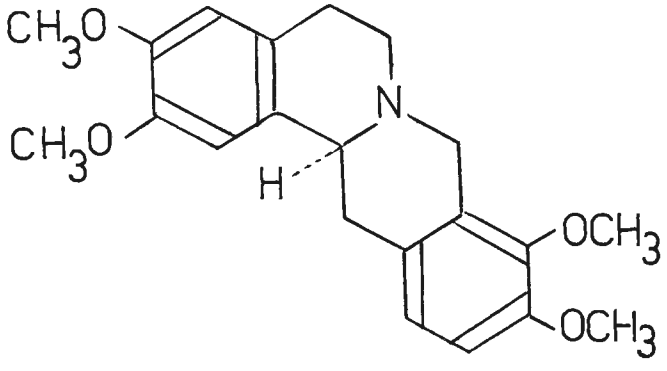
Anonaine, shown by t.l.c. to be identical with that alkaloid isolated from S. subaequalis, was obtained crystalline as its hydrochloride salt m.p. 270-5° (dec.), $[\alpha]_D^{26}$ - 80°. The identification was confirmed by comparison of its i.r. spectrum and by a mixed m.p. determination with an authentic sample of anonaine hydrochloride.

Asimilobine (27)

The t.l.c. behaviour of the remaining major alkaloid



28.



29.

showed that it was asimilobine. The n.m.r. spectrum of the compound showed one methoxyl singlet at δ3.62, one aromatic proton singlet at δ6.74 (C3-H) and a complex multiplet between δ 7.2 and δ 7.4 for the remaining four aromatic protons and was very similar to a published spectrum of asimilobine[37]. The compound was crystallised as the hydrochloride salt m.p. 244-246° and was found to be identical (mixed m.p. and i.r. spectrum) with an authentic specimen of asimilobine hydrochloride.

Alkaloid A (28)

The t.l.c. behaviour of a very small fraction which followed asimilobine from the alumina column showed that its main constituent was probably the same as alkaloid A isolated from Schefferomitra subaequalis. Although the fraction was rechromatographed, the base could not be obtained crystalline. On treatment with hydrochloric acid the fraction deposited a few small crystals of the hydrochloride salt but because of a slight amount of impurity and its tendency to decompose above 220° a reliable m.p. could not be determined. The u.v. and mass spectra of the compound were, however, very similar to those measured for the Schefferomitra alkaloid.

The u.v. spectrum showed maxima at 209 and 284 nm

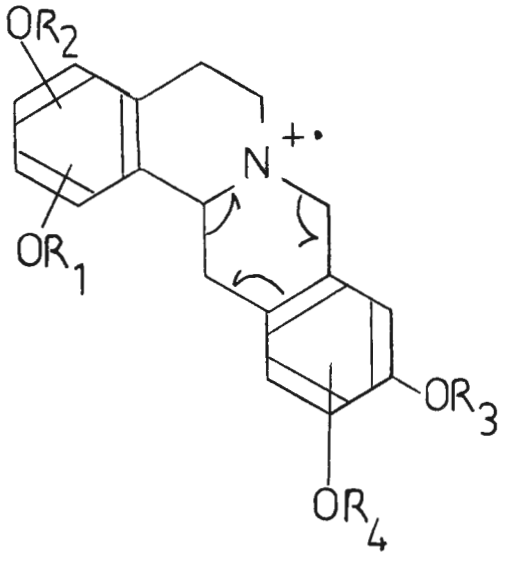
F I G U R E 7.

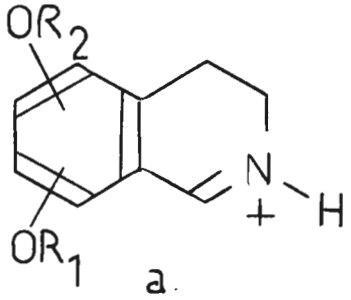
MASS SPECTRUM — **ALKALOID A**

Inlet temp. 250° — Electron Voltage 70 e.v.

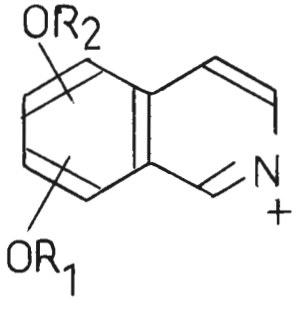
M/E	R.I.	M/E	R.I.
328	7.0	151	39.0
327	28.0	150	73.0
326	20.0	149	25.0
312	5.0	136	12.0
297	7.0	135	42.0
179	17.0	134	11.0
178	100.0	133	20.0
177	25.0	132	27.0
176	45.0	131	37.0
163	18.0	130	28.0
		107	45.0

FIGURE 8

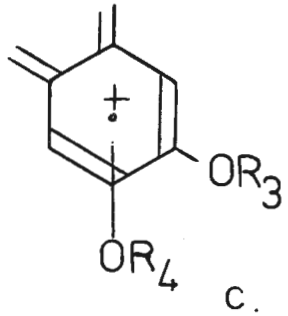




a.



b.



c. $\xrightarrow{-R_3^{\bullet}}$ d.

if $R_1, R_3 = CH_3$

and $R_2, R_4 = H$

then parent m/e = 341

a. m/e = 178 $\xrightarrow{-CH_3\cdot}$ m/e 163

c. m/e = 150 $\xrightarrow{-CH_3\cdot}$ d. m/e = 135

(log ϵ 4.5 and 3.7) and a shoulder at 223 nm (log ϵ 4.1). All moved to longer wavelength and had increased intensity of absorption in the presence of alkali. These characteristics could indicate either a phenolic tetrahydroprotoberberine or a phenolic 1-benzyl-tetrahydroisoquinoline alkaloid[24]. The mass spectrum (figure 7) was however that of a tetrahydroprotoberberine with hydroxyl and methoxyl substitution in both ring A and ring D, and had peaks at m/e 327, 312, 178 (base peak), 163, 150 and 135. Figure 8 shows the main decomposition fragments given by the tetrahydroprotoberberine type alkaloids[21,38] with the predominant decomposition being the retro Diels-Alder opening of ring C. This produces an ion of the type (c) with subsequent loss of one of its R (H or CH_3) groups to give fragment (d). There are two possible fragmentation pathways for the neutral nitrogen containing fragment, depending on the substitution in ring D:

(i) If ring D contains a hydroxyl substituent then there is a hydrogen transfer - presumably from ring C or D - to give an ion of the type (a).

(ii) If ring D contains no hydroxyl substituent the fragment loses a single hydrogen to give an ion of the type (b).

In collaboration with Mr. Rudzats in the elucidation of the structure of alkaloid as 3,9-dihydroxy-2,10-

dimethoxytetrahydroprotoberberine the following additional information was used[34].

(i) Complete O-methylation of the compound gave (-) -tetrahydropalmatine (29) thereby establishing the 2,3,9,10-substitution pattern and also the absolute stereochemistry. (-)-Tetrahydropalmatine has the *L*-configuration[57,58].

(ii) The measured physical constants of the alkaloid (m.p. 232°, $[\alpha]_D^{25}$ - 283°) differed from those reported for (-)-scoulerine[39] (m.p. 204°, $[\alpha]_D$ - 70°) and for (-)-stepholidine[40] (m.p. 158-160°, $[\alpha]_D$ - 311°), the two isomers with 2-hydroxy-3-methoxy substitution in ring A. Alkaloid A, therefore, must have the 3-hydroxy-2-methoxy pattern.

(iii) The remaining two possibilities can be distinguished from each other by the mass spectrum. Chen and MacLean[38] have shown that 9-hydroxy-10-methoxy compounds expel a methyl group from ion (c) to give an ion at m/e 135 whereas the 10-hydroxy-9-methoxy compounds preferentially lose a hydrogen atom to give an ion at m/e 149. The presence of the strong signal at m/e 135 provides firm evidence that the compound has the former pattern.

(iv) The n.m.r. spectrum of the alkaloid showed two three proton methoxyl signals at δ 3.74 and δ 3.77, a broad two proton peak at δ 8.80 which disappeared on exchange

with deuterium oxide, and signals for four aromatic protons which appeared as two one proton singlets at δ 6.48 and δ 6.80 and a two proton singlet at δ 6.68. There was also half of an AB pair of doublets at δ 4.02 (J = 16.0Hz), the high field half being obscured by the methoxyl signals. These peaks can be assigned to the C8 axial and equatorial protons and it has been shown[38,60] that in tetrahydroprotoberberines with 9,10-substitution these appear as an AB pair of doublets while in those alkaloids with 10,11-substitution they appear as a broad singlet. In the latter case the two protons apparently have the same chemical shift whereas the introduction of an oxygen substituent at C9 causes a downfield shift of the equatorial proton and an upfield shift of the axial proton and thus an AB quartet is observed.

Although it has not been possible to compare alkaloid A obtained from *M. kentii* with that obtained from *S. subaequalis* by i.r. spectra or mixed m.p., the t.l.c. behaviour in five different solvent-adsorbent systems together with the u.v. and mass spectral correlation strongly indicate that the two compounds are one and the same.

IV. ALKALOIDS OF MARSDENIA ROSTRATA

The constituents of Marsdenia rostrata are of interest because of the well documented physiological activity of M. condurango[15] and also because there are several reports in the literature of the unfortunate results which can arise when it is ingested by grazing stock[1,56]. As mentioned previously, the examination of this plant was commenced because field tests[1,2,3] and small scale laboratory extraction indicated a high alkaloid content. When the major base was found to be a pregnane aglycone it was decided to investigate the composition of the neutral aglycone mixture as well. This section deals with the identification of the basic constituents and the following section summarises the progress made so far in the separation and identification of the neutral aglycones of the plant.

There are considerable difficulties associated with the identification of steroid aglycones if a wide range of comparison compounds and spectra are not available. They are often difficult to separate because structural and stereochemical differences are only small. Even though crystalline compounds can be obtained, a closer examination frequently reveals that they are, in fact, mixtures. As these problems were also encountered in our attempts to

F I G U R E 9.

MASS SPECTRUM

ANABASINE

Inlet temp. 100°

Electron Voltage 70 e.v.

M/E	R.I.
163	13.9
162	76.0
161	44.5
160	2.7
147	7.0
135	1.0
134	13.9
133	76.0
132	6.3
130	3.2
121	7.6

M/E	R.I.
120	32.0
119	57.0
118	1.1
107	1.1
106	63.0
105	75.0
104	13.9
93	5.5
92	29.0
85	9.7
84	100.0
83	8.1

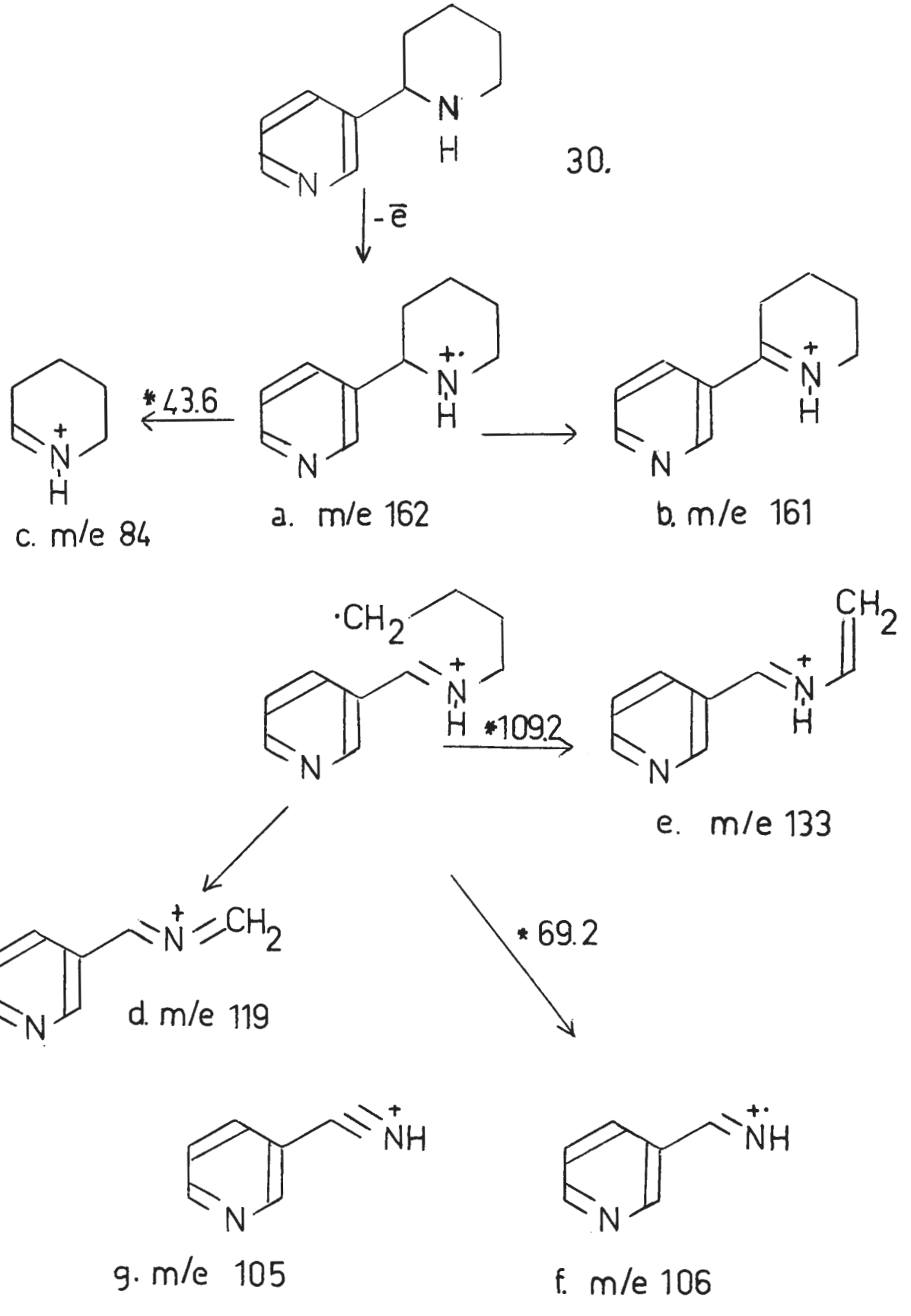
isolate and identify the components of the aglycone mixture, the investigation was found to be very time consuming and consequently it is not as yet complete. The section is included as a guide to the completion of the study.

Marsdenia rostrata R.Br. a robust liana, was collected by the late Mr. W. T. Jones (C.S.I.R.O.) in the Toonumbar State Forest in Northern N.S.W. The dried milled whole plant was extracted and worked up in the usual manner to give the crude base mixture in 0.45% yield, and this was fractionated by chromatography on alumina. Two major components were isolated and identified.

Anabasine (30)

Anabasine was obtained as a mobile brown oil. A crystalline hydrochloride could be prepared but was found to be extremely hygroscopic and as a result its physical constants were not determined. The identification of the compound was made partly on the basis of its u.v. spectrum which was characteristic of a simple pyridine derivative[24] (λmax. 257sh, 261 and 267sh nm; logϵ 3.45, 3.42 and 3.38 resp.) but mainly by the mass spectrum which is shown in figure 9. There was an intense parent peak at m/e 162 (a) and a large M-1 peak at m/e 161 (b). Other intense and diagnostic signals were at m/e 133 (e), 119 (d), 106 (f), 105 (g) and 84 (c, base peak). The

FIGURE 10


30.
-ē
*43.6
a. m/e 162
b. m/e 161
c. m/e 84
·CH2
*109.2
e. m/e 133
d. m/e 119
* 69.2
g. m/e 105
f. m/e 106

spectrum also showed a number of metastable ion signals and those at m/e 43.6, 69.2 and 109.2 were readily assigned to the known breakdown pathways for anabasine[41,42] which are shown in figure 10. In contrast to this, anabasine's more common isomer, nicotine, exhibits a much simpler spectrum with the main peaks occurring at m/e 162, 161, 133 and 84[42].

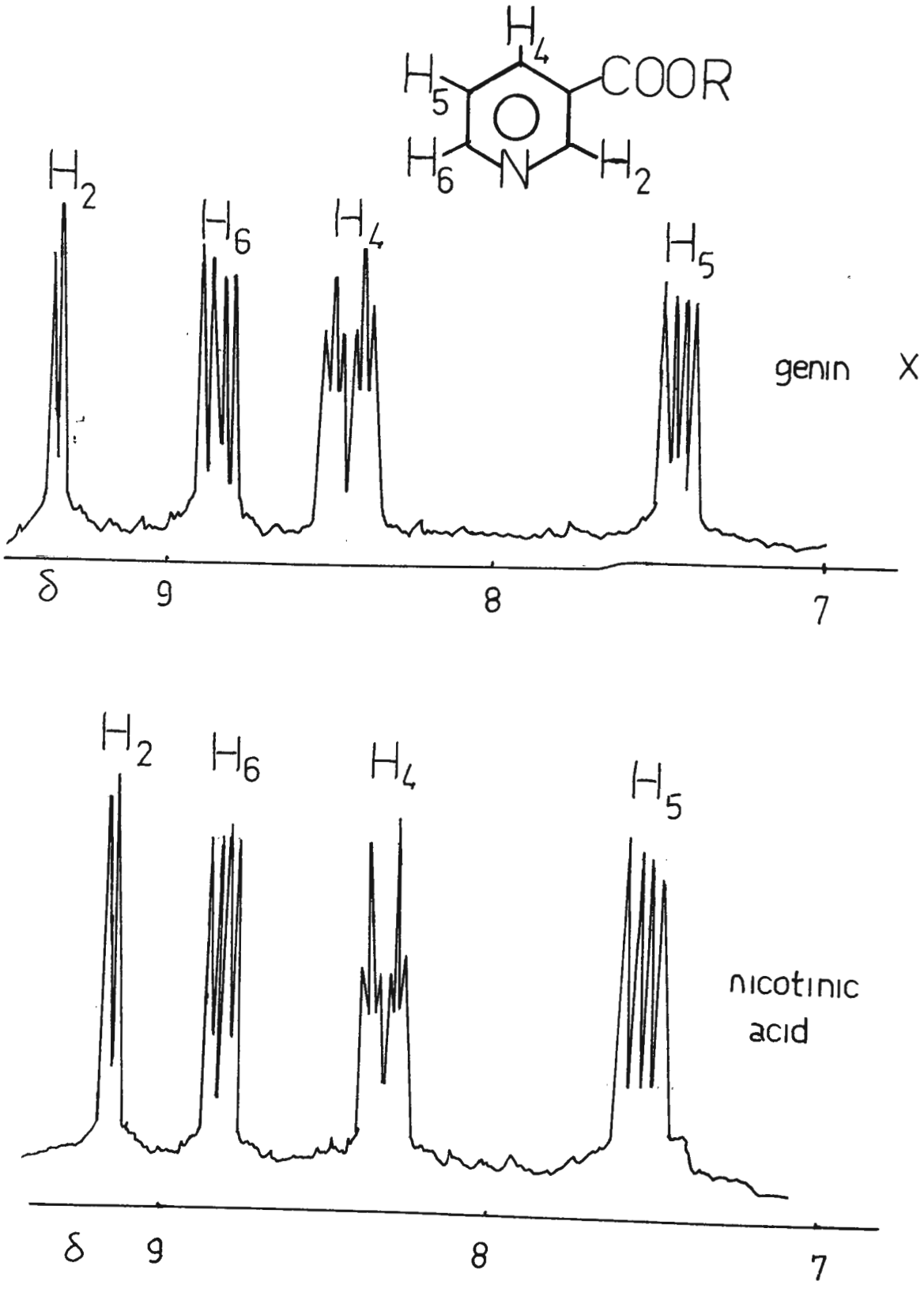
Further evidence of the identity of the alkaloid was supplied by its n.m.r. spectrum and this showed the characteristic pattern of aromatic proton signals of the 3-substituted pyridine system, similar to that of nicotinic acid and genin X (figure 11).

Neither the free base nor the picrate prepared from it showed any measurable optical rotation. This is apparently a common observation for anabasine. The proton of the chiral centre of anabasine is fairly labile and previous experience has shown that unless the work-up of the alkaloids is carried out under very mild conditions only a racemate or partly racemised compound will be isolated.[43]

The identification of this alkaloid as anabasine was confirmed by comparison of its picrate with an authentic sample of (-) anabasine picrate. The i.r. spectra of the two compounds were identical and the m.p. 209-211° of the

FIGURE 11

Comparison of Low Field n.m.r. Signals



(±) anabasine picrate was very close to the measured m.p. 205-207° of the authentic (-) anabasine picrate. In a mixed m.p. determination there was no depression and the m.p. was between the two ranges quoted above.

Genin X

The genin X was crystallised from acetone as colourless prisms m.p. 264°, $[\alpha]_D^{25}$ + 18°. It gave a blue Liebermann-Burchard[54] reaction and crystallisation to constant m.p. and the t.l.c. behaviour indicated that it was homogeneous. The mass spectral and elemental analysis evidence, however, was suggestive of the compound being a mixture of two components of molecular formulae $C_{29}H_{39}NO_8$ and $C_{29}H_{41}NO_8$.

An ultraviolet spectrum of the genin demonstrated the presence of a pyridine moiety in the molecule. There was a peak at 220 nm (log ∈ 3.93) and another at 263 nm (log ∈ 3.45) with shoulders at 258 and 270 nm (log ∈ 3.42 and 3.38 resp.). An i.r. spectrum showed hydroxyl absorption at 3460 and 3520 cm^{-1} together with ester carbonyl peaks at 1715 and 1730 cm^{-1}, and an aromatic double bond peak at 1600 cm^{-1}.

The low-field region of the n.m.r. spectrum (figure 11) showed four aromatic proton signals in a similar pattern to those of nicotinic acid. There was a quartet at

F I G U R E 12.

HIGH RESOLUTION MASS SPECTRUM — GENIN X

* Indicates composition confirmed by peak matching.

M/E		Composition		R.I.
514.24727		$C_{28}H_{36}NO_8$	*	
511.25742		$C_{29}H_{37}NO_7$	*	
471.26586		$C_{27}H_{37}NO_6$		0.6
469.24975		$C_{27}H_{35}NO_6$	*	1.7
454.22342		$C_{26}H_{32}NO_6$	*	1.2
436.21203		$C_{26}H_{30}NO_5$	*	1.0
418.20044		$C_{23}H_{28}NO_4$		3.1
400.19178		$C_{26}H_{26}NO_3$	*	1.5
392.17173		$C_{20}H_{26}NO_7$	*	1.2
373.15292		$C_{20}H_{23}NO_6$	*	8.8
346.21390		$C_{21}H_{30}O_4$	*	9.4
313.18042		$C_{20}H_{25}O_3$	*	6.9
295.16284		$C_{20}H_{23}O_2$		3.5
276.12622		$C_{15}H_{18}NO_4$		3.0
	or	$C_{12}H_{20}O_7$		
208.10423		$C_{12}H_{16}O_3$		8.6
141.08018		$C_8H_{13}O_2$		20.2
138.10356		$C_9H_{14}O$		12.4
124.03995		$C_6H_6NO_2$		71.3
123.03271		$C_6H_5NO_2$		37.5
123.08008		$C_8H_{11}O$		11.4
106.03052		C_6H_4NO		44.6
105.07144		C_8H_9		23.9
105.02138		C_6H_3NO		20.0
43.00897		C_2H_3O		100.0

F I G U R E 13.

MASS SPECTRUM METASTABLE MODE RESULTS

GENIN X

Daughter	Measured Parent	Assigned Parent	Neutral Fragment Mass	Neutral Fragment Comp.
469	530.0	529	60	$C_2H_4O_2$
454	469.4	469	15	CH_3
	515.3	514	60	
436	453.4	454	18	H_2O
	496.6	496	60	
	469.6	469	33	
	513.8	514	78	
418	434.2	433	15	
	452.3	451	33	
	479.0	478	60	
	495.3	496	78	
400	416.0	415	15	
	432.4	433	33	
	460.6	460	60	
373	387.2	388	15	
	495.0	496	123	$C_6H_5NO_2$
	511.8	511	138	$C_9H_{14}O$
	528.9	529	156	
346	363.6	364	18	
	387.9	388	42	
	406.9	406	60	
	469.9	469	123	
	530.1	529	183	

δ7.5 (J = 4.5 and 8.0 Hz) for C5-H, a doublet at δ 8.3 (J = 8.0 Hz - partly resolved into a pair of triplets) for C4-H, a quartet at δ8.8 (J = 4.5 and 1.5 Hz) for C6-H, and a doublet at δ9.3 (J = 1.5 Hz) for C2-H. The high-field region of the spectrum (figure 16) will be discussed below but one readily assignable signal was a three proton acetyl methyl signal at δ1.96.

Both high and low resolution mass spectrometry first indicated that the compound was possibly a mixture. There was a parent peak at m/e 529 together with an unusually large p+2 peak at m/e 531. The ratio of peak intensities indicated that the proportion of unsaturated to saturated component was about 2:1. These two signals were too weak for accurate mass measurement but their composition of $C_{29}H_{39}NO_8$ and $C_{29}H_{41}NO_8$ could be inferred from the prominent M-60 (acetic acid) peaks at m/e 469.24658 ($C_{27}H_{35}NO_6$) and m/e 471.2658 ($C_{27}H_{37}NO_6$). The complete spectrum is shown in figure 12, and metastable mode results (peaks resulting from the parent at m/e 529) are given in figure 13. Even with this information it is not easy to propose an accurate decomposition scheme since several pathways are obviously operating simultaneously. It can be noted however that the numerous losses of $CH_3^{\cdot}$ and H_2O are characteristic of polyhydroxy pregnane derivatives[44,45,46] while the predominant losses of neutral fragments of masses

F I G U R E 14.

MASS SPECTRUM

Inlet temp. 200°

M/E	R.I.
384	Not observed
382	Not observed
367	1.0
366	3.4
365	1.4
364	6.4
349	0.8
348	2.4
347	3.4
346	9.0
340	1.4
339	8.0
338	8.6
337	36.0

HYDROLYSIS PRODUCT OF GENIN X SARCOSTIN + DIHYDROSARCOSTIN

Electron Voltage 70 e.v.

M/E	R.I.
366	5.4
321	10.4
320	16.0
319	30.0
305	4.0
304	14.6
303	11.2
302	30.0
301	18.0
286	3.4
285	8.0
284	8.6
283	18.0
43	100.0

High Resolution

M/E	Composition
366.2387	$C_{21}H_{34}O_5$
364.2233	$C_{21}H_{32}O_5$

60 and 123 indicate the presence of acetyl and nicotinoyl ester substituents[47,50].

The identity of the deacyl genin was ascertained by alkaline hydrolysis of genin X. The neutral hydrolysis product had m.p. 230-250° and microanalyses indicated the formula $C_{21}H_{34}O_6$. The mass spectrum (figure 14) showed no parent but there was an M-18 (H_2O) peak at m/e 364.2250 ($C_{21}H_{32}O_5$) which was used to infer the formula of the parent as $C_{21}H_{34}O_6$. The spectrum also showed a prominent peak at m/e 366.2387 ($C_{21}H_{34}O_5$) which can only be explained by the presence of an impurity of molecular formula $C_{21}H_{36}O_6$. Other peaks in the spectrum were at m/e 337 (M-45 = CH_3CHOH), 328 (M-54 = $3xH_2O$), 320 (364-44 = acetaldahyde), 319 (364-45), 302 (320-H_2O) and 301 (321-H_2O). Each of the peaks mentioned had a corresponding p + 2 peak of about 1/2 to 1/3 intensity. The main peaks however followed exactly the pattern given in the literature[44] for sarcostin (6) and it therefore appeared very likely that the deacyl genin was a mixture of sarcostin and 5α-dihydro-sarcostin (31).

Final proof of the identity of the deacyl genin was obtained by catalytic hydrogenation of the hydrolysis product to give 5α-dihydrosarcostin which after several recrystallisations had m.p. 264-266° (undepressed on admixture with an authentic specimen), $[\alpha]_D^{25}$ + 47.5°

F I G U R E 15.

MASS SPECTRUM		DIHYDROSARCOSTIN	
Inlet Temp. 250°		Electron Voltage 14 e.v.	
M/E	R.I.	M/E	R.I.
384	Not observed	322	4.8
366	1.0	321	7.2
349	0.7	315	3.0
348	2.8	314	3.2
340	0.8	313	1.9
339	3.0	312	6.8
331	1.5	305	6.0
330	4.8	304	40.0
323	0.8	43	100.0

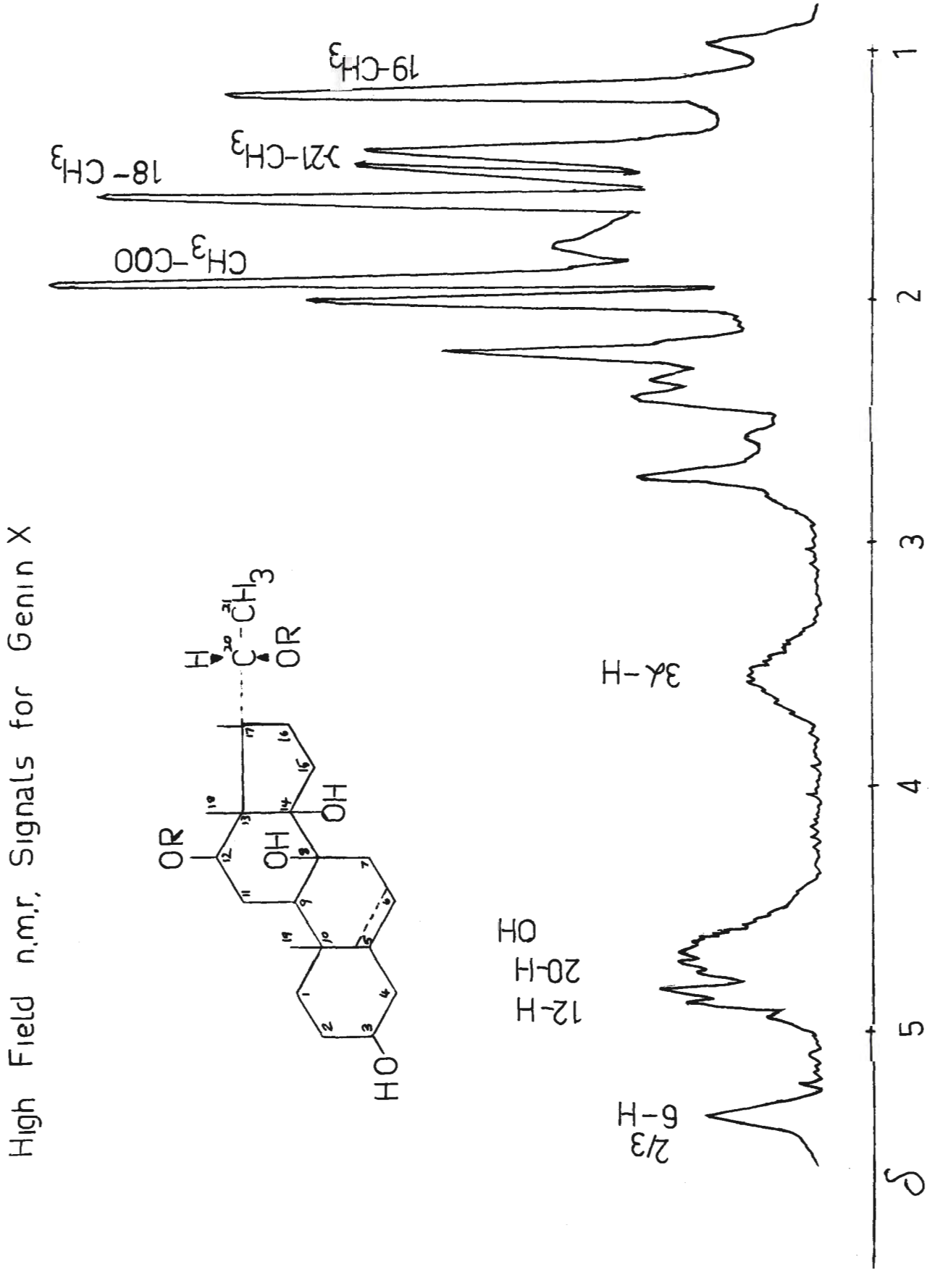
(lit.[48,49] $[\alpha]_D^{25}$ + 50.5°). The identity of the 5α-dihydrosarcostin with the authentic sample was also confirmed by comparison of the i.r. and mass spectra (figure 15) and by the t.l.c. behaviour.

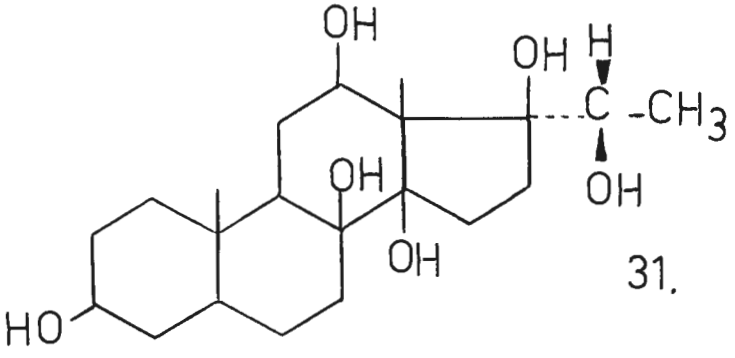
An acidic product of the hydrolysis of genin X was identified as nicotinic acid by direct comparison of i.r. and mass spectra, mixed m.p. and t.l.c. behaviour with an authentic sample.

From the above evidence the structure O-acetyl-O-nicotinoyl sarcostin- 5α-dihydrosarcostin (32) was proposed for genin X. It remained to fix the positions of the nicotinoyl and acetyl ester groupings on the sarcostin nucleus and this can be done partly by reference to the n.m.r. spectrum (figure 16) and by analogy to known compounds. It seems probable that the ester groups are located on the hydroxyls of carbon atoms 12 and 20 but the exact position of each has not been determined.

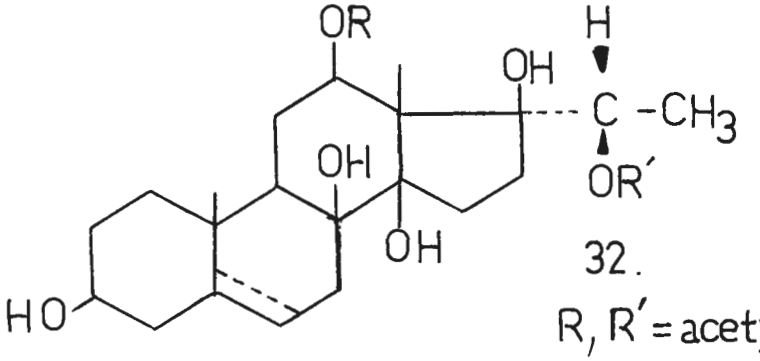
Comparison with known compounds of a similar type shows that the most likely sites for esterification are the secondary hydroxyl groups on carbons 12 and 20[47,50,51,52]. Carbon 3 hydroxyl is unlikely since these steroid derivatives usually occur in plants as glycosides in which the sugar residue is attached to the 3 -OH group. The closest analogous structure recorded in the literature is mono-O-

FIGURE 16


High Field n.m.r. Signals for Genin X
δ
1
2
3
4
5
2/3 6-H
12-H
20-H
OH
3α-H
CH3-COO
18-CH3
21-CH3
19-CH3
HO
OH
OR
OR
H
OH

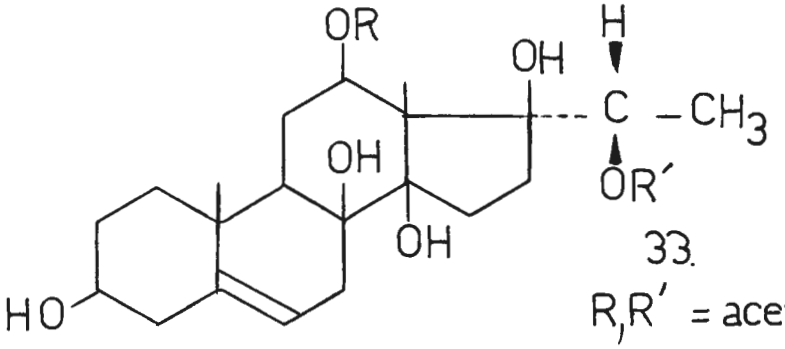


31.



32.

R, R' = acetyl, nicotinoyl.



33.

R, R' = acetyl, benzoyl.

acetyl-mono-O-benzoyl sarcostin (33), isolated by Reichstein and co-workers from Sarcostemma viminale[51] and from Asclepias lilacina[48,52]. Here the ester groups were assigned to C12-OH and C20-OH by the presence in its n.m.r. spectrum of two one proton quartet signals at δ 4.9 and δ4.6 for C12-H and C20-H. In sarcostin these signals occur as quartets at δ3.98 and δ4.44. The C12-H quartet arises from the splitting by the C11 protons (J = 5.0 and 11.0 Hz) while the C20-H signal is a quartet because of the adjacent C21 methyl group. On esterification of C12-OH and C20-OH the C12 and C20 protons are moved downfield to between δ4.5 and δ5.0.

Although there is some overlapping of signals and interference from an exchangeable hydrogen in the same region, genin X displays two one proton signals δ 4.90 (quartet J = 5.0 and 11.0 Hz) and δ 4.76 (partly obscured quartet) which can be explained in terms of esterified C12 and C20 hydroxyl groups. Also the very broad characteristic signal for the C3 -α proton is observed at its usual position at δ 3.55 showing that C3-OH is not esterified.

The remainder of the spectrum is consistent with the proposed structure and bears a very close resemblance to the literature spectrum[51] of Reichstein's compound. The signal at δ 5.38 is due to the vinylic C6 proton and this signal only integrates to about 2/3 of one proton (based

FIGURE 17

N.M.R. SPECTRA OF PREGNANE AND PREGNANE ESTER DERIVATIVES

COMPOUND	δ-VALUES RELATIVE TO TETRAMETHYLSILANE							
	3α-H	12α-H	20-H	6-H	19-CH_3	18-CH_3	21-CH_3	Acetyl Group
Sarcostin[51]	3.9 *	3.98 *	4.44 q	5.47 m	1.47 s	1.94 s	1.52 s	-
5α-Dihydro-sarcostin[51]	3.8 *	3.88 q	4.40 q	-	1.26 s	1.90 s	1.47 d;6.5Hz	-
Genin X	3.55 m	4.90 q	4.76 q*	5.38	1.16 s	1.56 s	1.42 d;6.0Hz	1.96 s
0-Acetyl-0-benzoyl sarcostin[51]	3.5 m	4.9 *	4.6 *	5.32 m	1.14 s	1.68 s	1.15 d;6.0Hz	1.90 s
Lineolon[53]	3.5 *	3.8 *	-	-	1.42 s	1.93 s	2.40 s	-
3-0-Acetyl lineolon[53]	5.39 m	3.80 q	-	-	-	-	2.24 s	2.1 s
12-0-Nicotinoyl lineolon[50]	3.50 m	5.0 q	-	5.4 m	1.25 s	1.40 s	2.24 s	-

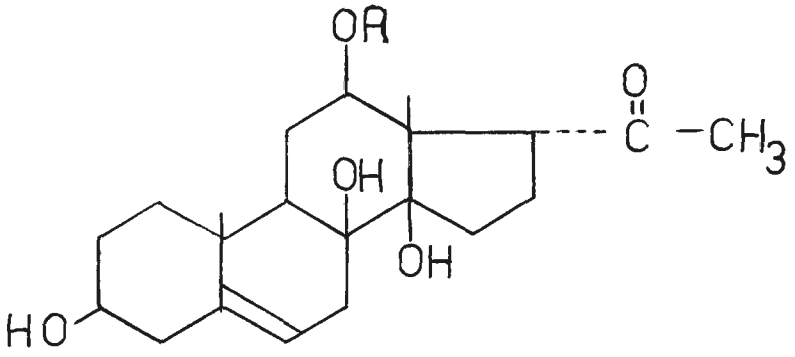
* -Indicates position of signal not clear because of overlapping

s -Singlet

d -Doublet

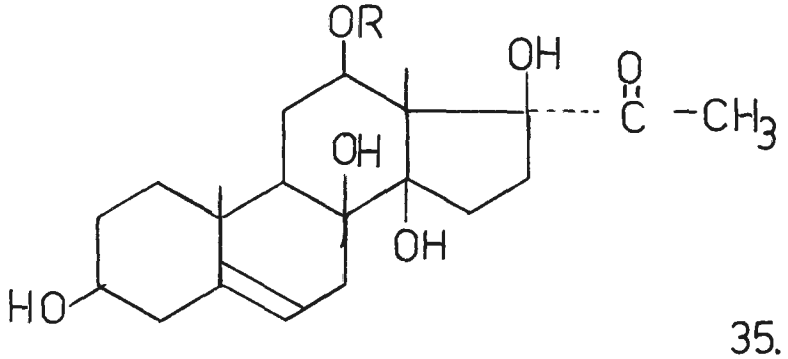
q -Quartet

m -Multiplet



34.

R = nicotinoyl



35. R = Ac

36. R = H

on total integral of aromatic protons as 4H) as one would expect for this mixture. The methyl signals at δ 1.16 (singlet), δ 1.42 (doublet J = 6.0 Hz) and δ 1.56 (singlet) can be assigned to the C19-CH_3, C21-CH_3 and C18-CH_3 by analogy to the corresponding signals of O-acetyl-O-benzoyl sarcostin.

There appears to be only one other example in the literature of the isolation of a plant steroid conjugated with an amino acid. In 1969 Mitsuhashi and co-workers[50] isolated a compound $C_{27}H_{35}NO_6$ to which they assigned the structure 12-O-nicotinoyl lineolon (34). The n.m.r. spectral characteristics of this compound are useful in providing additional confirmatory evidence for the structure proposed for the Marsdenia ester. In this case the C12-OH was assigned as the site of esterification because of the shift in the C12-H quartet signal to δ 5.0 from its position at about δ 3.8 in lineolon. Also, the C3-OH could be discounted because the broad multiplet for C3-H occurred at δ 3.5. If C3-OH was esterified the C3-H signal would occur at about δ 4.6 as it does in lineolon-3-acetate[53]. Figure 17 shows the comparison of n.m.r. signals for sarcostin, 5α-dihydrosarcostin, genin X, O-acetyl-O-benzoyl sarcostin, lineolon, 3-O-acetyl lineolon and 12-O-nicotinoyl lineolon.

V. THE NEUTRAL AGLYCONES OF MARSDENIA ROSTRATA

For this investigation a second collection of plant material was made by the author from the top of the escarpment at Mt. Keira, near Wollongong, N.S.W. *Marsdenia rostrata* R.Br. is easily distinguished from the other species, *M. flavescens* A. Cunn.[80], common to the area, by the shape and appearance of the leaves. *M. rostrata* has leaves which are glabrous and ovate to almost orbicular. *M. flavescens* has leaves which are slightly yellowish and pubescent underneath and are elliptical to oblong-elliptical in shape. The identification of the material as *M. rostrata* was confirmed by the National Herbarium, Sydney[79].

The plant material was air dried, milled and extracted in the usual manner and the concentrated extract warmed with dilute sulphuric acid to hydrolyse the glycosides. The resulting aglycones were separated into basic and neutral fractions. The basic fraction was chromatographed and found to contain O-acetyl-O-nicotinoyl sarcostin-dihydrosarcostin mixture identical with that obtained from the C.S.I.R.O. collection. Anabasine, however, was absent but it is not uncommon for plants collected from different localities to exhibit variations in the composition of the alkaloid content or even the disappearance of alkaloids altogether[3].

FIGURE 18.

COMPARISON OF *MARSDENIA* AGLYCONES

Genin	m.p.	$[\alpha]_D$	Rf.	I.R. Spectrum	Mass Spectrum
1	235-237°	-	0.28	3460, 3390, 1700, 1290, 1175, 1155, 1060, 1047 and 1002 cm^{-1}	m/e 348, 330, 323, 315, 305, 302, 287, 269 and 262
1A	255-259°	-	0.41	3410, 3300, 1240, 1230, 1042 and 994cm^{-1}	m/e 364, 346, 337, 332, 323, 314, 305, 288, 271, 269 and 262
2	199-200°	+12.4°	0.49	3460, 3430, 3320, 3230 and 1595, 1242, 1212, 1040 and 991cm^{-1}	m/e 362, 348, 330, 319, 312, 305, 287, 269 and 262
3 Metaplexigenin	270-272°	-12.0°	0.61	3510, 3450, 3340, 1740, 1735, 1710, 1240, 1049, 1040, 1015 and 985cm^{-1}	Figure 19.

The neutral aglycone mixture was fractionated by chromatography on alumina and rechromatography of certain fractions gave the four most polar aglycones (genins 1, 1A, 2 and 3) in crystalline form. Genins 1, 1A and 2 have been partly characterised as shown in figure 18 while genin 3 has tentatively been identified as metaplexigenin (35)[55]. The presence of at least four other aglycones (genins 4, 5, 6 and 7) has been demonstrated by t.l.c. but these compounds have so far resisted all attempts to separate them.

Metaplexigenin

Genin 3, metaplexigenin, was obtained as colourless needles from acetone m.p. 270-2° (lit. m.p. 268-75°)[51,55]. An i.r. spectrum showed the presence of hydroxyl groups (3320, 3455, 3510 cm^{-1}), a side chain ketone (1710 cm^{-1}) - and an ester function (1730 cm^{-1}) - and was identical in every detail with a published spectrum of metaplexigenin[51]. The mass and n.m.r. spectra were also identical to published spectra for this compound[51]. The mass spectrum, shown in figure 19, showed the following signals:- m/e 422 (parent), 404 (M-H_2O), 379 (M-CH_3CO), 362 (M-AcOH), 344 (M-AcOH-H_2O), 319 (379-AcOH), 301 (319-H_2O), 283 (301-H_2O) and 265 (M-CH_3CO-AcOH-$3H_2O$). An authentic sample of metaplexigenin is not yet available and therefore a mixed m.p. determination and t.l.c. comparison has not been made.

F I G U R E 19.

MASS SPECTRUM — GENIN 3 — METAPLEXIGENIN

Inlet temp. 270°

Electron Voltage 12 ev.

M/E	R.I.
422	0.05
404	0.05
380	0.4
379	2.5
362	0.4
344	0.8
320	2.5
319	15.0

M/E	R.I.
302	2.5
301	15.0
284	3.0
283	17.5
266	1.5
265	6.0
43	100.0

Hydrolysis of genin 3, using refluxing methanolic potassium hydroxide, gave a neutral product which had m.p. and i.r. and mass spectra identical with those given in the literature for deacylmetaplexigenin (36)[51].

E X P E R I M E N T A L

G E N E R A L

Melting points were determined using a hot stage micro-melting point apparatus and are uncorrected. Optical rotations were measured on a Hilger instrument using an 0.5 dm, 5 ml polarimeter tube.

Microanalyses were carried out by Dr. E. Challen of the University of N.S.W. and by the C.S.I.R.O. Microanalytical Laboratory, Melbourne.

Infrared spectra were recorded in nujol mull using a Perkin-Elmer 237 Grating Infrared Spectrophotometer and Ultraviolet spectra were recorded in ethanol using a Perkin-Elmer 137 Ultraviolet-Visible Spectrophotometer. N.m.r. spectra were determined using Jeolco JNM-4H-100 and Varian HA-100 instruments with tetramethylsilane as an internal reference. Abreviations used to describe the spectra are: s, singlet; d, doublet; q, quartet and m, multiplet.

Low resolution mass spectra were run on an E.A.I. MS.902 instrument at the University of Sydney or on an E.A.I. Quad 300D mass spectrometer at Wollongong University College. High resolution mass spectra were determined by Mr. C. G. MacDonald, Division of Entomology, C.S.I.R.O., Canberra using an E.A.I. MS.9 instrument.

Column chromatography was carried out using mainly "Merck Kieselgel" or "Merck Aluminium Oxide, Grade II-III, Neutral".

Thin-layer chromatography was carried out mainly on plates prepared from "Merck Kieselgel G. nach Stahl" developed in the solvent system chloroform-methanol (9:1) and Rf data quoted refer to this system unless otherwise stated. The alkaloids were visualised by exposure to iodine vapour and gave characteristic colours on standing in the atmosphere. The Marsdenia aglycones were visualised by spraying with a mixture of sulphuric acid and acetic anhydride (1:1) and warming the plate to 90°. These compounds also gave characteristic colour reactions.

Light petroleum refers to the fraction b.p. 60-80°.

Combined organic phases from extractions were dried with anhydrous magnesium sulphate unless otherwise specified.

I. ALKALOIDS OF CINNAMOMUM SP. T.G.H. 13,077

Bark and leaves of Cinnamomum species (Herbarium Voucher No. T.G.H. 13,077) were originally collected by Dr. T. G. Hartley about 15 miles south-east of Gairana in the Morobe district of New Guinea. It has since been collected by Mr. J. S. Womersley from the same locality[61].

(i) Identification of Alkaloids of the Bark.

Dried milled bark (8.0 kg) was extracted by percolation with cold methanol. Most of the methanol was removed by distillation to give a dark syrupy residue which was acidified with 2N hydrochloric acid, diluted with water, allowed to stand for 24 hours and filtered. The aqueous acid solution was basified with conc. ammonia and extracted exhaustively with chloroform. The chloroform extract was washed with water, concentrated to a small volume and re-extracted with 0.5N sulphuric acid. The acid solution was basified with conc. ammonia, extracted with chloroform, the chloroform solution dried, and the chloroform removed by distillation under reduced pressure to give 4.4g. (0.055%) of the crude alkaloid mixture as a brown foam.

The crude alkaloids were fractionated by chromatography on a silica column using chloroform-methanol (19:1) as eluant. The proportion of methanol in the eluant was

gradually increased to 20% to speed elution of the final fractions. The composition of the individual fractions was examined by t.l.c.

Corydine

First fractions from the silica column showed a single brown spot Rf 0.87 which gradually faded to green on exposure to the atmosphere. The combined fractions were dissolved in ethanol and deposited a crystalline hydrochloride on addition of conc. hydrochloric acid. The salt was recrystallised from ethanol and the free base recovered and crystallised from ether to give corydine (270 mg) m.p. 151°, $[\alpha]_D^{24}$ + 204° (c, 0.86 in $CHCl_3$) (lit.[6,62] m.p. 149°, $[\alpha]_D$ + 205°); λ max. 221, 267sh, 272 and 307 nm (log ϵ 4.42, 3.89, 3.90 and 3.59); n.m.r. spectrum ($CDCl_3$): δ 2.57 (3H, s, NCH_3), 3.73 (3H, s, OCH_3), 3.90 (6H, s, 2xOCH_3), 6.69 (1H, s, C3-H), 6.87 and 7.10 (2H, pair of doublets J = 8.0 Hz, C9-H and C8-H). Mass spectrum (12 e.v.): m/e 341 (parent, base peak), (70 e.v.): m/e 341 (100%), 326 (71%), 310 (76%) and 298 (17%).

The alkaloid was identical with an authentic specimen of corydine (i.r., t.l.c., mixed m.p.).

Cinnamolaurine

After corydine had been eluted from the column the next fractions consisted mainly of two components. These were rechromatographed on an alumina column using chloroform-methanol (19:1) as the solvent. The first fractions from this column contained corydine together with some minor components and were set aside for further chromatography (cf below). They were followed by fractions showing a single brown spot, Rf 0.46, which gradually faded to yellow on standing. These fractions were combined and crystallised from ethanol to give colourless prisms (260 mg) of cinnamolaurine m.p. 212-213° (dec.), $[\alpha]_D^{25}$ -100° (c, 0.75 in EtOH) (Found: C, 72.7; H, 6.5; N, 5.0; O, 15.8%. $C_{18}H_{19}NO_3$ requires: C, 72.7; H, 6.4; N, 4.7; O, 16.1%), λ max. 287 nm (log ϵ 3.72), hydrochloride m.p. 230-233° (dec.); n.m.r. spectrum (CD_3-SO-CD_3): δ 2,3 (3H, s, NCH_3), 5.85 (2H, s, O-CH_2-O), 6.45 (1H, s, C8-H), 6.57 (1H, s, C5-H), 6.56 and 6.92 (4H, pair of doublets J = 8.0 Hz, C2'-H, C3'-H, C5'-H and C6'-H). The mass spectrum is shown in figure 2.

Norcinnamolaurine

A number of the fractions following cinnamolaurine from the alumina column showed one main yellow spot, Rf 0.41, in t.l.c. These fractions were combined and crystallised from ethanol as colourless needles (280 mg)

of <u>norcinnamolaurine</u> m.p. 197-198°, $[\alpha]_D^{25}$ + 55° (<u>c</u>, 0.4 in EtOH) (Found: C, 71.9; H, 6.1; N, 4.8%, $C_{17}H_{17}NO_3$ requires: C, 72.0; H, 6.1; N, 4.9%), λ max. 287 nm (log ϵ 3.78), <u>hydrochloride</u> m.p. 231-232°; n.m.r. spectrum (CD_3-SO-CD_3): δ 5.9 (2H, s, O-CH_2-O), 6.57 (1H, s, C8-H), 6.77 (1H, s, C5-H), 6.67 and 7.05 (4H, pair of doublets J = 8.0 Hz, C2'-H, C3'-H, C5'-H and C6'-H). The mass spectrum is shown in figure 5.

Reticuline

The final fractions (390 mg) from the silica column showed one main spot, Rf 0.3, which gradually faded to pink on standing. These fractions were combined and a portion was rechromatographed on preparative thin-layer plates developed in chloroform-methanol (9:1). Extraction of the appropriate zone yielded a colourless gum (80 mg) which could not be induced to crystallise. The gum had $[\alpha]_D^{28}$ + 81° (<u>c</u>, 0.62 in EtOH), λ max. 285 nm (log ϵ 3.7); n.m.r. spectrum ($CDCl_3$): δ 2.43 (3H, s, NCH_3), 3.78 (6H, s, 2xOCH_3), 5.30 (exchangeable with D_2O, 2H, broad s, 2xOH), 6.25-6.70 (5H, m, Ar-H).

The gum was taken up in absolute ethanol (10 ml), perchloric acid (2 drops) and benzene (10 ml) added and the solvent distilled off under vacuum. Further portions of absolute ethanol and benzene were added and again

distilled off under vacuum. On cooling the oil remaining deposited colourless prisms which were recrystallised from absolute ethanol to give reticuline perchlorate m.p. 202°, $[\alpha]_D^{25}$ + 75° (c, 0.2 in EtOH) (lit.[27,28] m.p. 202-204°, $[\alpha]_D^{25}$ + 87°). The compound was identical with an authentic specimen of reticuline perchlorate (i.r., mixed m.p.).

Norisocorydine

As noted above, fractions from the alumina column between corydine and cinnamolaurine contained a number of minor components as shown by t.l.c. Fractions showing a brown spot, Rf 0.60, (gradually fading to green), were rechromatographed on Kiesel gel G preparative plates developed in the solvent chloroform-methanol (19:1). Extraction of the appropriate zone yielded a gum (120 mg) which appeared to be unstable and developed a red colouration on standing. The gum had n.m.r. spectrum ($CDCl_3$): δ 3.71 (3H, s, C1-OCH_3), 3.90 (6H, s, C2 and C10-OCH_3), 6.71 (1H, s, C3-H) and 6.83 (3H, s, C8-H and C9-H). When treated with hydrobromic acid the gum deposited crystalline norisocorydine hydrobromide m.p. 203-207°, $[\alpha]_D^{24}$ + 200° (c, 0.13 in EtOH) (lit.[29,30] m.p. 203-205°, $[\alpha]_D$ + 159°), λ max. 222, 267 and 305 nm (log ϵ 4.4, 3.95 and 3.94) λ infl 272 nm (log ϵ 3.94).

The free base (105 mg) in methanol (5 ml) was treated with formalin (6.8 ml) and then sodium borohydride (600 mg). The solution was diluted with water, basified with ammonia and extracted with chloroform. The crude product obtained from evaporation of the chloroform extract was chromatographed on preparative alumina plates developed in the solvent chloroform-methanol (98:2). Extraction of a zone which moved almost with the solvent front gave an oil (30 mg) which when treated with acetone (1 ml) and methyl iodide (0.1 ml) formed prisms of isocorydine methiodide m.p. 228° (lit.[6,63] m.p. 225°) identical with (mixed m.p., i.r. spectrum) an authentic sample of isocorydine methiodide.

(ii) N-Methylation of (+)-Norcinnamolaurine

A mixture of (+)-norcinnamolaurine (100 mg), formic acid (1.0 ml) and formalin solution (0.6 ml) was heated on a water bath for 6 hours. The solution was cooled, diluted with water (30 ml), acidified with hydrochloric acid and extracted several times with ether. The aqueous acidic solution was basified with ammonia, extracted with chloroform, the chloroform extract dried and the chloroform removed by distillation. The oil remaining crystallised

on trituration with ethanol to give colourless prisms of cinnamolaurine m.p. 212-213° (dec.), $[\alpha]_D^{25}$ - 105° (c, 0.2 in EtOH). The i.r. spectrum and t.l.c. behaviour of the compound were identical with those of natural cinnamolaurine and there was no depression of m.p. in a mixed m.p. determination.

(iii) Synthesis of (±)-Cinnamolaurine

(a) 3,4-Methylenedioxyphenylethylamine

A solution of 3,4-methylenedioxybenzaldehyde (21.9g) and nitromethane (9.0 ml) in methanol (60 ml) was treated with a solution of methylamine (0.7 g) in methanol (10 ml). The mixture was left standing for four days, during which time the product, 3,4 methylenedioxy-ω-nitrostyrene crystallised as bright yellow needles. These were filtered and washed with methanol. The yield was 26.5g (95%) m.p. 162° (lit.[64] m.p. 161.5°).

The above nitrostyrene (9.6g) in tetrahydrofuran (100 ml) was added dropwise to a suspension of ether (300 ml) and lithium aluminium hydride[65,66,67]. The solution was stirred for one hour after the addition was complete and then the excess hydride destroyed by careful addition of small volumes of water and 5N sodium hydroxide

solution. The ether was decanted and the precipitate washed several times with ether. The combined ether solutions were dried and concentrated to a small volume by distillation. The resulting solution was saturated with dry HCl gas and deposited the 3,4-methylenedioxy-phenylethylamine hydrochloride (5.4g, 55%) as colourless needles m.p. 210° (lit.[68] m.p. 214°).

(b) N-(3,4-methylenedioxyphenylethyl)-p-benzyloxy-phenyl acetamide[69].

The above phenylethylamine hydrochloride (4.0g) was suspended in ether (50 ml) and 5N sodium hydroxide (5 ml) solution added. The mixture was cooled to 0° and to it was added an ether solution containing p-benzyloxyphenylacetyl chloride[70] (from 5.0g of acid). The mixture was stirred for one hour after the addition and the resulting precipitate filtered, dried and recrystallised from benzene-light petroleum to give the amide (6.5g, 85%) m.p. 132° (lit.[69] m.p. 133°).

(c) 1-(p-Benzyloxybenzyl)-6,7-methylenedioxy-3,4-dihydroisoquinoline[71,72].

The above amide (6.5g) was dissolved in toluene (100 ml) and the solution refluxed with 15 ml phosphorus oxychloride for two hours. On cooling and addition of light petroleum the solution deposited an oil which gradually crystallised as yellow prisms of the

dihydroisoquinoline hydrochloride, m.p. 208°.

(d) (±) Cinnamolaurine

The above dihydroisoquinoline hydrochloride (1.5g) was converted to the free base, dissolved in methanol, and allowed to stand at room temperature, under nitrogen with methyl iodide (5.0 ml). After two hours the solvent was removed by distillation and the oily residue dissolved in 20 ml of methanol and treated with 3.0g of sodium borohydride. The mixture was stirred for two hours and then poured into 300 ml 0.1N HCl. The solution was basified with ammonia, extracted with chloroform, the chloroform extract dried and the chloroform removed by distillation. The residue was refluxed for two hours in a solution of ethanol-hydrochloric acid (1:1, 100 ml) and the resulting solution concentrated by distillation under reduced pressure to about 50 ml. The concentrate was poured into water, extracted with ether and the aqueous phase made alkaline with dilute sodium hydroxide and extracted again with ether. The aqueous alkaline solution was neutralised by addition of solid carbon dioxide and extracted with chloroform. The dried, evaporated chloroform extract, when treated with ethanol gave colourless needles (0.7g) of (±) cinnamolaurine m.p. 184-186° (dec.) (Found: C, 72.7; H, 6.4; N, 4.9% $C_{18}H_{19}NO_3$ requires: C, 72.7;

H, 6.4; N, 4.7%), hydrochloride m.p. 230° (dec.). The racemate was identical (i.r., u.v. and mass spectra and t.l.c.) with the natural product.

(iv) Synthesis of (±) Norcinnamolaurine

1-(p-Benzyloxybenzyl)-6,7-methylenedioxy-3,4-dihydroisoquinoline (4.6g) was dissolved in methanol (100 ml) and treated with sodium borohydride (9.0g) over a period of two hours. The mixture was stirred for a further 24 hours at room temperature. The methanol was removed by distillation and the residue taken up in 0.5N hydrochloric acid. The solution was basified with ammonia and extracted with chloroform. The chloroform was removed by distillation and the residue dissolved in ethanol and treated with a few drops of conc. hydrochloric acid. The salt which formed was recrystallised from ethanol to give colourless needles (3.7g) of O-benzylnorcinnamolaurine hydrochloride m.p. 209° (Found: C, 70.6; H, 6.1; N, 3.6%. $C_{24}H_{24}ClNO_3$ requires: C, 70.3; H, 5.9; N, 3.4%). The hydrochloride (0.5g) was hydrolysed by refluxing in ethanol-hydrochloric acid (1:1, 25 ml). After the usual work-up the product, (±)-norcinnamolaurine was obtained as colourless plates (0.29g), m.p. 207-208° (Found: C, 71.8; H, 6.0; N, 4.8%. $C_{17}H_{17}NO_3$ requires: C, 72.1; H, 6.1; N, 4.9%), hydrochloride m.p. 248-250°. The i.r. and mass

spectra and t.l.c. behaviour of the racemate were indistinguishable from those of natural (+) norcinnamolaurine.

(v) N-Methylation of (±) Norcinnamolaurine

(±) Norcinnamolaurine (0.1g) was methylated using the formalin-sodium borohydride method. (±) Cinnamolaurine was obtained as colourless prisms (60 mg) m.p. 184-186° (dec.), hydrochloride m.p. 228-230° (dec.). The i.r. spectra and the t.l.c. behaviour of the racemate showed that it was identical with natural (±) cinnamolaurine and the synthetic racemate prepared above.

(vi) Identification of Alkaloids of the Leaves

Dried crushed leaves (27kg) were extracted by percolation with cold methanol. The methanol extract was evaporated to a syrupy consistency, acidified with 2N sulphuric acid and diluted with water. The insoluble material formed a suspension of oil droplets in the aqueous solution and this was partially clarified by extraction with petrol. After two days standing the supernatant aqueous solution was fairly clear and was decanted. The strong aromatic odour of the essential oil content was still persistent so a portion of the solution was evaporated

under reduced pressure to remove any methanol which would be increasing the solubility of the oily components. As the evaporation proceeded a quantity of colourless, semicrystalline material, later identified as camphor, accumulated in the condenser of the evaporator. Because of the difficulties involved in treating the whole of the aqueous solution in this way, the solution was basified with ammonia and exhaustively extracted with chloroform. The chloroform extracts were combined, dried and evaporated to give a brown viscous oil which was distributed between 1N sulphuric acid and ether. The aqueous acid layer was basified, extracted with chloroform and the chloroform extract dried and evaporated to give an oil, upon which the above process was repeated. The crude alkaloids were finally obtained as a brown foam (2.07g, 0.008%). This mixture was fractionated by chromatography on deactivated neutral alumina using chloroform with increasing methanol content (0-20%) as solvent.

Corydine

First fractions from the alumina column were shown by t.l.c. to consist essentially of corydine (brown spot, fading to green, Rf 0.86). These fractions were combined and crystallised from ether to give colourless needles (700 mg) of corydine m.p. 151°, identical with (mixed m.p. and i.r. spectrum) an authentic sample of corydine.

Reticuline

Final fractions from the column (950mg) were shown by t.l.c. to consist mainly of reticuline (brown spot - fading to pink, Rf 0.3). These fractions were combined, converted to the perchlorate salt and crystallised from absolute ethanol to give colourless prisms m.p. 201°, identical with (i.r., mixed m.p.) an authentic sample of reticuline perchlorate.

Cinnamolaurine

Intermediate fractions from the column were small (120 mg) and were shown by t.l.c. to consist of several components, one of which appeared to be cinnamolaurine (brown spot - fading to yellow Rf 0.46). Further t.l.c. in a number of solvent/adsorbent systems showed that the mixture would be very difficult to separate and therefore a positive identification of cinnamolaurine was not made.

(vii) Identification of Camphor

The semicrystalline material obtained during the isolation of the crude alkaloid mixture was purified by sublimation under vacuum to give needles of m.p. 174°, undepressed on admixture with an authentic specimen of camphor.

II. ALKALOIDS OF CINNAMOMUM LAUBATTII

Bark of C. laubattii F. Muell. was collected at McKeller Road, Atherton Tableland, Northern Queensland, by botanists of the C.S.I.R.O. The sample was designated by the collection number SN8816[73].

Dried, milled bark (22.0 kg) was extracted by percolation with cold methanol. After the usual work-up the extract yielded the crude alkaloids as a brown foam (2.3g, 0.01%). The mixture was fractionated by chromatography on alumina using chloroform-methanol (0-5%) as eluant. Individual fractions were examined by t.l.c. and were combined according to their composition. Four main fractions resulted:

Fraction	Weight (mg)	Composition acc. to t.l.c.
1	900	Mainly non-alkaloid
2	100	Component 1 + several minor
3	220	Component 1 only
4	810	Component 1 + several minor

T.l.c. comparison with the alkaloid isolated from Cinnamomum sp. T.G.H. 13,077 indicated that component 1 was reticuline. It gave a brown spot (Rf 0.3) which gradually faded to pink on standing. Although fractions 2 and 4 contained compounds other than reticuline the small amount of material available prohibited further investigation.

Reticuline

Fraction 3 in absolute ethanol was treated with several drops of perchloric acid. Benzene was added and the solvent removed by distillation under vacuum. The residual gum was treated with further portions of benzene and absolute ethanol and the evaporation repeated. On cooling and trituration with absolute ethanol the gum deposited a crop of colourless prisms of reticuline perchlorate m.p. 201°, $[\alpha]_D^{25}$ + 80° (c, 0.2 in EtOH) (lit.[27,28] m.p. 203-204°, $[\alpha]_D^{25}$ + 87°). Comparison with an authentic sample of reticuline perchlorate (i.r. spectrum and mixed m.p.) confirmed the identity of the compound.

III. ALKALOIDS OF MITRELLA KENTII

Bark of Mitrella kentii (Bl) Miq. (Herbarium voucher No. T.G.H. 11,488) was collected and identified by Dr. T. G. Hartley at Kuali Creek, about 5 miles south of Wau in the Morobe district of New Guinea.

Dried milled bark (2.7 kg) was extracted by percolation with cold methanol and the extract worked up in the usual manner to yield the crude alkaloids (1.7g, 0.063%) as a brown foam. The alkaloid mixture was fractionated by chromatography on an alumina column using chloroform with increasing methanol content as eluant. Individual fractions were examined by t.l.c. and combined according to their composition.

Fraction	Weight (mg)	Composition acc. to t.l.c.
1	210	Non-alkaloid
2	490	2 components, 1 yellowRf 0.95, 1 green Rf 0.53
3	100	Complex mixture
4	90	Complex mixture
5	180	1 component, purple spot Rf 0.28
6	160	Complex mixture
7	120	Complex mixture
8	50	1 component - yellow-green spot, Rf 0.68

Liriodenine

Fraction 2 deposited yellow-orange needles on trituration with acetone. Recrystallisation from chloroform afforded liriodenine as yellow needles m.p. 281-284° (lit.[74,75] m.p. 285-6°). λ max. 207, 247, 270, 310 and 413 nm (log ϵ 4.43, 4.24, 4.14, 3.60 and 3.80), identical with the recorded spectrum[76]; n.m.r. spectrum (CD_3-SO-CD_3) δ 6.56 (2H, s, O-CH_2-O), 7.8-8.9 (9H, m, Ar-H). Comparison with an authentic specimen (i.r. spectrum, t.l.c., mixed m.p.) confirmed that the compound was liriodenine.

Anonaine

The mother liquors from the crystallisation of liriodenine were rechromatographed on a silica column using chloroform-methanol (98:2) as eluant. A further quantity of liriodenine was obtained from the first fractions and this was followed by a mixture of liriodenine and the second component. Final fractions contained the second component alone - yellow spot (Rf 0.53) fading to yellow-green on standing. T.l.c. comparison with anonaine isolated from Schefferomitra subaequalis indicated that the alkaloid was anonaine. The compound was converted to crystalline anonaine hydrochloride m.p. 270-275° (dec.), $[\alpha]_D^{26}$ - 80° (c, 0.1 in EtOH) (lit.[77] m.p. 277°, $[\alpha]_D$ - 74°). The identity of the compound was confirmed

by comparison (i.r. spectrum, mixed m.p.) with an authentic specimen of anonaine hydrochloride.

Asimilobine

T.l.c. comparison with asimilobine isolated from Schefferomitra subaequalis showed that the major constituent of fraction 5 (brown spot Rf 0.28 gradually fading to purple) was probably asimilobine. N.m.r. spectrum ($CDCl_3$): δ3.62 (3H, s, OCH_3), 6.74 (1H, s, C3-H), 7.2-7.4 (4H, m, C8-H, C9-H, C10-H and C11-H). The compound was converted to a crystalline hydrochloride m.p. 244-246° (lit.[78] m.p. 242-244°). Comparison with an authentic sample of asimilobine hydrochloride (i.r. spectrum, mixed m.p.) confirmed the identity of the compound.

Alkaloid A.

Fraction 8 was rechromatographed on a preparative thin-layer silica plate developed in chloroform-methanol (9:1). Extraction of the appropriate zone gave about 5 mg. of an oil which could not be induced to crystallise. It was treated with one drop of hydrochloric acid and the excess removed by evaporation. Trituration with methanol gave a few crystals of the hydrochloride salt m.p. $>$ 220° (dec.), λ max. 209, 223 sh and 284 nm (log ϵ approx. 4.5, 4.1 and 3.7). A mass spectrum is shown in figure 7.

T.l.c. comparison in a number of solvent-adsorbent systems indicated that the alkaloid was the same as alkaloid A isolated from Schefferomitra subaequalis i.e. 3,9-dihydroxy-2,10-dimethoxytetrahydroprotoberberine. The systems were:

Silica:	Chloroform-methanol	9:1	Rf 0.68
	Butanol-acetic acid-water	5:3:2	Rf 0.32
	Butanol-acetic acid-water	12:3:5	Rf 0.59
Alumina:	Chloroform-methanol	19:1	Rf 0.39
	Benzene-methanol	19:1	Rf 0.15

IV. ALKALOIDS OF MARSDENIA ROSTRATA

Marsdenia rostrata R.Br. was collected by the late Mr. W. T. Jones of the C.S.I.R.O. in the Toonumbar State Forest, Northern N.S.W. Dried, milled whole plant (4.0 kg) was extracted by percolation with cold methanol and the extract worked-up using the procedure given before. The crude alkaloid mixture was obtained as a brown foam (18g, 0.45%). A portion of the foam (4.1g) was submitted to chromatography on alumina using chloroform as the initial solvent and later gradually increasing the methanol content from 0 to 5%. Individual fractions were examined by t.l.c.

First fractions eluted contained an oily material (0.45g). T.l.c. showed a number of spots which gave only a transient colour reaction with iodine and an intense purple colour with the sulphuric acid - acetic anhydride reagent. A Mayer's test was negative and the material was discarded.

Intermediate fractions contained an oil (1.0g) which showed a number of iodine sensitive spots in t.l.c. The material was acid soluble and gave a positive Mayer's test.

Final fractions (1.6g, foam), when examined by t.l.c.,

gave only a weak colouration with iodine but an intense blue spot with the sulphuric acid - acetic anhydride reagent. The material was acid soluble and a Mayer's test on the acid solution was positive.

Anabasine

A portion of the intermediate fraction was rechromatographed on preparative thin-layer Kieselgel G plates developed in chloroform-methanol (19:1). Extraction of a well defined zone yielded a brown oil which could not be induced to crystallise. The oil was treated with conc. hydrochloric acid and all traces of water removed by addition of absolute ethanol and benzene and distillation of the solvent under vacuum. On cooling, the hydrochloride crystallised as colourless needles. Attempts to recover the crystals were unsuccessful because of the deliquescent nature of the compound and consequently the free base was recovered.

The free base had $[\alpha]_D^{25}$ 0° (c, 0.6 m EtOH), λ max. 261 nm (log ϵ 3.45), λ infl 257 and 267 nm (log ϵ 3.42 and 3.38); n.m.r. spectrum ($CDCl_3$): δ 8.56 (1H, d J= 2.0Hz, Ar-H2), 8.45 (1H, q J= 5.0 and 2.0 Hz, Ar-H6), 7.78 (1H, d J= 8.0 Hz (partly resolved pair of triplets), Ar-H4), and 7.72 (1H, q J= 8.0 and 5.0 Hz, Ar-H5). The

mass spectrum is given in figure 9.

A picrate m.p. 209-211° was prepared and was identical (i.r. spectrum, mixed m.p.) with an authentic specimen of (-)-anabasine picrate m.p. 205-207°. The t.l.c. behaviour of the free bases confirmed the identification.

Genin X

Final fractions from the alumina column crystallised from acetone as colourless prisms of genin X m.p. 264°, $[\alpha]_D^{25}$ + 18° (c, 1.0 in MeOH), λ max. 220 and 263 nm (log ϵ 3.93 and 3.45), λ infl 258 and 270 nm (log ϵ 3.42 and 3.38) (Found: C, 65.7; H, 7.5; N, 2.3; O, 23.6%, $C_{29}H_{39}NO_8$ requires: C, 65.8; H, 7.4; N, 2.6; O, 24.2%, $C_{29}H_{41}NO_8$ requires: C, 65.5; H, 7.8; N, 2.6; O, 24.1%), ν max 3520, 3460 cm^{-1} (OH), 1730, 1715 cm^{-1} (ester carbonyl) and 1600 cm^{-1} (Aromatic C=C). The mass and n.m.r. spectra are shown in figures 11, 12, 13 and 16.

Genin X (1.0g) in methanol 50 ml and 5N potassium hydroxide (2 ml) were refluxed for five hours. The mixture was diluted with water, the methanol removed by distillation under reduced pressure, and then allowed to stand. Colourless needles (250 mg) of the product were formed. Repeated extraction of the mother liquors with ether yielded

a further 300 mg of the product. The total yield was recrystallised from acetone to give colourless needles m.p. 235-239°, ν max 3560, 3460, 3400 and 3340 cm^{-1} (OH). (Found: C, 66.0; H, 9.1%, Calc. for $C_{21}H_{34}O_6$: C, 65.9; H, 9.0%, Calc. for $C_{21}H_{36}O_6$: C, 65.6; H, 9.4%).

After isolation of the neutral hydrolysis product the aqueous mother liquors were acidified with hydrochloric acid, concentrated to about 20 ml and placed on a column of Zeo-Karb 225 ion exchange resin in H^+ form (bed volume 20 ml). The column was then washed with water (200 ml) and then with 2N ammonia solution (100 ml). The ammonia washings were concentrated to 5 ml and the pH adjusted to 4.5 by careful addition of hydrochloric acid. The colourless crystals of nicotinic acid which formed had m.p. 230° (lit.[33] m.p. 230°) and was identical (mixed m.p., i.r. spectrum and t.l.c.) with an authentic specimen of nicotinic acid.

The neutral product of the hydrolysis of genin X (700 mg) was dissolved in glacial acetic acid (30 ml) and was hydrogenated over platinium oxide for four hours. The catalyst was removed by filtration and the solvent by distillation under vacuum. On trituration with water the product crystallised as colourless needles. Recrystallisation from acetone to constant m.p. gave

5 α-dihydrosarcostin (360 mg) m.p. 264-266°, $[\alpha]_D^{25}$ + 47.5° (c, 0.8 in MeOH) (lit.[48,49] m.p. 267-269°, $[\alpha]_D^{25}$ + 50.5°). The compound was identical (mixed m.p., t.l.c., i.r. and mass spectra) with an authentic specimen of 5α-dihydrosarcostin.

V. THE NEUTRAL AGLYCONES OF MARSDENIA ROSTRATA

Marsdenia rostrata R.Br, was collected by the author from the top of the escarpment (elevation 1400') at Mt. Keira near Wollongong, N.S.W. and the identification confirmed by Dr. J. S. Beard, Director and Chief Botanist, National Herbarium, Sydney[79,80].

Dried, milled plant material (4.5 kg) was extracted by percolation with cold methanol and the extract (12ℓ) concentrated to about 1.5ℓ. The concentrated extract was exhaustively extracted with light petroleum and 0.5ℓ 2N sulphuric acid added. The solution was warmed to about 40° and then allowed to stand for three days. The supernatant liquor was decanted from the black tar which had formed and was concentrated under reduced pressure until it became turbid. The concentrate was then diluted with water (3ℓ) and extracted with chloroform. The chloroform extract was washed several times with 1N sulphuric acid, once with dil. sodium bicarbonate and then several times with water. The chloroform solution was dried and the chloroform removed by distillation under reduced pressure to give the neutral aglycones (115g) as a viscous yellow oil.

The aqueous acid solution and acid washings were

combined, basified with ammonia and extracted with chloroform. This chloroform extract yielded the bases as a brown oil (13.0g). Examination of the oil by t.l.c. revealed that a major constituent was genin X and that anabasine was absent. The bases were fractionated by chromatography on alumina and the genin X obtained was shown by t.l.c. and i.r. spectral comparison and mixed m.p. determination to be identical with that obtained from the Marsdenia rostrata collected by the C.S.I.R.O.

T.l.c. examination of the neutral aglycone fraction revealed the presence of at least 8 constituents which gave well defined coloured spots with the sulphuric acid - acetic anhydride reagent. A portion of the mixture (55 g) was fractionated by chromatography on alumina with chloroform-methanol (0-10%) as solvent. The various fractions were examined by t.l.c. and their composition is indicated below:

Fraction	Weight (g)	Composition indicated by t.l.c.
1	23.0	Oil
2	4.0	Oil + genins 4,6,7.
3	3.0	genins 4,6,7.
4	2.1	genins 4,5,6,7.
5	8.1	genins 3,4,5,6,7.
6	3.6	genins 3,5.
7	1.3	genin 3 (crystallised)
8	1.9	genins 2,3.
9	2.5	genin 2 (crystallised)
10	0.8	genins 1, 1A, 2.

(i) Analysis of the Fractions.

Fractions 2, 3, 4, 5 and 6 all contained compounds which showed only small Rf differences in all solvent-adsorbent systems examined. As their separation appeared to involve a very lengthy process these fractions have been reserved for a later investigation.

Fraction 7 crystallised on trituration with acetone to give colourless needles of genin 3.

Fraction 9 crystallised on trituration with acetone to give colourless plates of genin 2.

Fractions 8 and 10 and the mother liquors from fractions 7 and 9 were combined and rechromatographed on a silica column using chloroform-methanol (2%-5%) as solvent. Further quantities of genins 2 and 3 were obtained together with two more crystalline compounds, genin 1 and genin 1A.

The physical data for the above crystalline compounds are tabulated below. The corresponding data for the hydrolysis product of genin 3, genin X and 5α-dihydro-sarcostin are included for comparison.

Genin	Weight(mg)	m.p.	Rf	Colour reaction H_2SO_4 - Ac_2O
1	20	235-7°	0.28	Yellow → brown
1A	25	255-9°	0.41	Blue → brown grey
2	900	199-200°	0.49	Yellow → orange → brown
3	1100	270-2°	0.61	Blue
3 Hyd.Prod.		215-20°	0.49	Green
X		264°	0.60	Blue
Dihydrosarcostin		264-6°	0.41	Blue → brown grey

(ii) Identification of Genin 3 as Metaplexigenin

Genin 3 crystallised as colourless needles m.p. 270-272° (subl.), $[\alpha]_D^{25}$ - 12° (C, 1.0 in MeOH) (lit.[51] m.p. 268-275°, $[\alpha]_D^{25}$ - 22°); n.m.r. spectrum (C_5D_5N): δ 5.75 (1H, s, C6-H), 4.80 (1H, q J = 11.0 and 5.0 Hz, C12 -H), 3.75 (1H, m, C3 -H), 2.40 (3H, s, CH_3CO), 2.0 (3H, s, CH_3CO), 1.85 (3H, s, C18-CH_3), 1.33 (3H, s, C19-CH_3) and was almost identical to a literature spectrum of metaplexigenin[51]. The mass (figure 19) and i.r. spectra were also identical with literature spectra for this compound[51]. A direct comparison has not yet been made. (Found: C, 65.6; H, 8.1%. Calc. for $C_{23}H_{34}O_7$: C, 65.4; H, 8.1%.)

Genin 3 (50 mg) was refluxed for five hours in 5% methanolic potassium hydroxide solution. The reaction mixture was diluted with water, extracted with chloroform, the solvent removed by distillation and the residue crystallised from acetone. The product, deacetylmetaplexigenin (25 mg) m.p. 215-220° (lit.[51] m.p. 218-223°) had i.r. and mass spectra identical with literature spectra for this compound.

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