THE CHEMISTRY OF THE *VINCA* ALKALOIDS SITSIRIKINE, CATHARANTHINE, AND THEIR DERIVATIVES

by

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B.A., Oxford University, 1960
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FACULTY OF GRADUATE STUDIES

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THE CHEMISTRY OF THE VINCA ALKALOIDS

SITSIRIKINE, CATHARANTHINE, AND THEIR DERIVATIVES

ABSTRACT

In part I of this thesis are described the structural determinations of sitsirikine, dihydrositsirikine and isositsirikine, three new alkaloids from Vinca rosea Linn.

Sitsirikine, $C_{21}H_{26}O_3N_2$, and dihydrositsirikine, $C_{21}H_{28}O_3N_2$, were isolated as an inseparable mixture, which was shown by hydrogenation studies to be comprised of an olefin and its dihydro derivative. The formation of formaldehyde upon ozonisation of the mixture, and of propionic acid in a modified Kuhn-Roth oxidation of dihydrositsirikine demonstrated that sitsirikine possessed a vinyl group.

Both sitsirikine and dihydrositsirikine gave monoacetates, and the N.M.R. data indicated that primary hydroxyl groups were present in the original alkaloids. A methyl ester function suggested by spectral evidence was established by hydride reduction of dihydrositsirikine to a diol. Since the diol yielded an acetonide, it was inferred that dihydrositsirikine possessed a β-hydroxy-ester unit.

The U.V. spectrum of dihydrositsirikine was characteristic of an indole chromophore, which the mass spectrum showed to be part of a tetrahydro-β-carboline system. Dehydrogenation afforded a compound with a flavocoryline-type U.V. spectrum, and this suggested that sitsirikine was a relative of the tetracyclic corynantheine class of alkaloids. This was confirmed by conversion of dihydrocorynantheine into dihydrositsirikine.

The structure of the related indole alkaloid isositsirikine, $C_{21}H_{26}O_3N_2$, was determined by a similar series of reactions. Ozonolysis yielded acetaldehyde, which authenticated the ethylidene group indicated by the N.M.R. spectrum. Acetylation afforded a mono-acetate, whose N.M.R. spectrum suggested that isositsirikine had a primary hydroxyl function. A methyl ester was established by hydride reduction to a diol, which formed an acetonide and hence showed the presence of a β-hydroxy-ester unit in the original alkaloid. Since dehydrogenation of dihydro-isositsirikine yielded flavocoryline, a tetracyclic structure very similar to that of sitsirikine

could be postulated for isositsirikine.

Part II is concerned with the chemistry of cleavamine, a scission product of the Vinca alkaloid catharanthine.

Treatment of catharanthine with aqueous acid in the presence of a reducing agent led to the isolation of descarbomethoxycatharanthine, cleavamine and two epimeric dihydrocleavamines. A tentative mechanism for the reaction is proposed, which can account for the formation of these compounds.

Reduction of catharanthine in glacial acetic acid provided carbomethoxy-dihydrocleavamine. Mercuric acetate oxidised this compound to a mixture of two immonium ions, both of which underwent transannular cyclisations. One of the ions gave the known Iboga alkaloids coronaridine and dihydrocatharanthine, whereas the other afforded pseudo-vincadifformine - a synthetic analogue of the known Vinca alkaloid vincadifformine.

The structure of pseudo-vincadifformine was determined by conversion into compounds which had U.V., I.R., N.M.R. and mass spectra completely analogous to the corresponding derivatives of vincadifformine.

Similar transannular cyclisations to the above are postulated in the scheme advanced by Wenkert for the biogenesis of Iboga and Aspidosperma alkaloids, and the significance of our results with regard to this theory is duscussed. The formation of coronaridine and dihydrocatharanthine in the reaction constituted partial syntheses of these alkaloids, and the potential use of transannular cyclisations in laboratory syntheses of Iboga and Aspidosperma alkaloids is also considered.

GRADUATE STUDIES

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PUBLICATIONS

- J.P. Kutney and R.T. Brown,

  The Structure of Sitsirikine - A new Alkaloid from Vinca rosea Linn,

  Tetrahedron Letters, No. 26, 1815 (1963)

- J.P. Kutney, R.T. Brown and E. Piers,

  The Synthesis of a Vincadifformine-Type Skeleton via a Novel Transannular Cyclization Reaction,

  J. Am. Chem. Soc., 86, 2286 (1964)

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Abstract

In part I of this thesis are described the structural determinations of sitsirikine, dihydrositsirikine and isositsirikine, three new alkaloids from Vinca rosea Linn.

Sitsirikine, $C_{21}H_{26}O_3N_2$, and dihydrositsirikine, $C_{21}H_{28}O_3N_2$, were isolated as an inseparable mixture, which was shown by hydrogenation studies to be comprised of an olefin and its dihydro derivative. The formation of formaldehyde upon ozonisation of the mixture and of propionic acid in a modified Kuhn-Roth oxidation of dihydrositsirikine demonstrated that sitsirikine possessed a vinyl group.

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The structure of pseudo-vincadifformine was determined by conversion into compounds which had U.V., I.R., N.M.R. and mass spectra completely analogous to the corresponding derivatives of vincadifformine.

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GENERAL INTRODUCTION

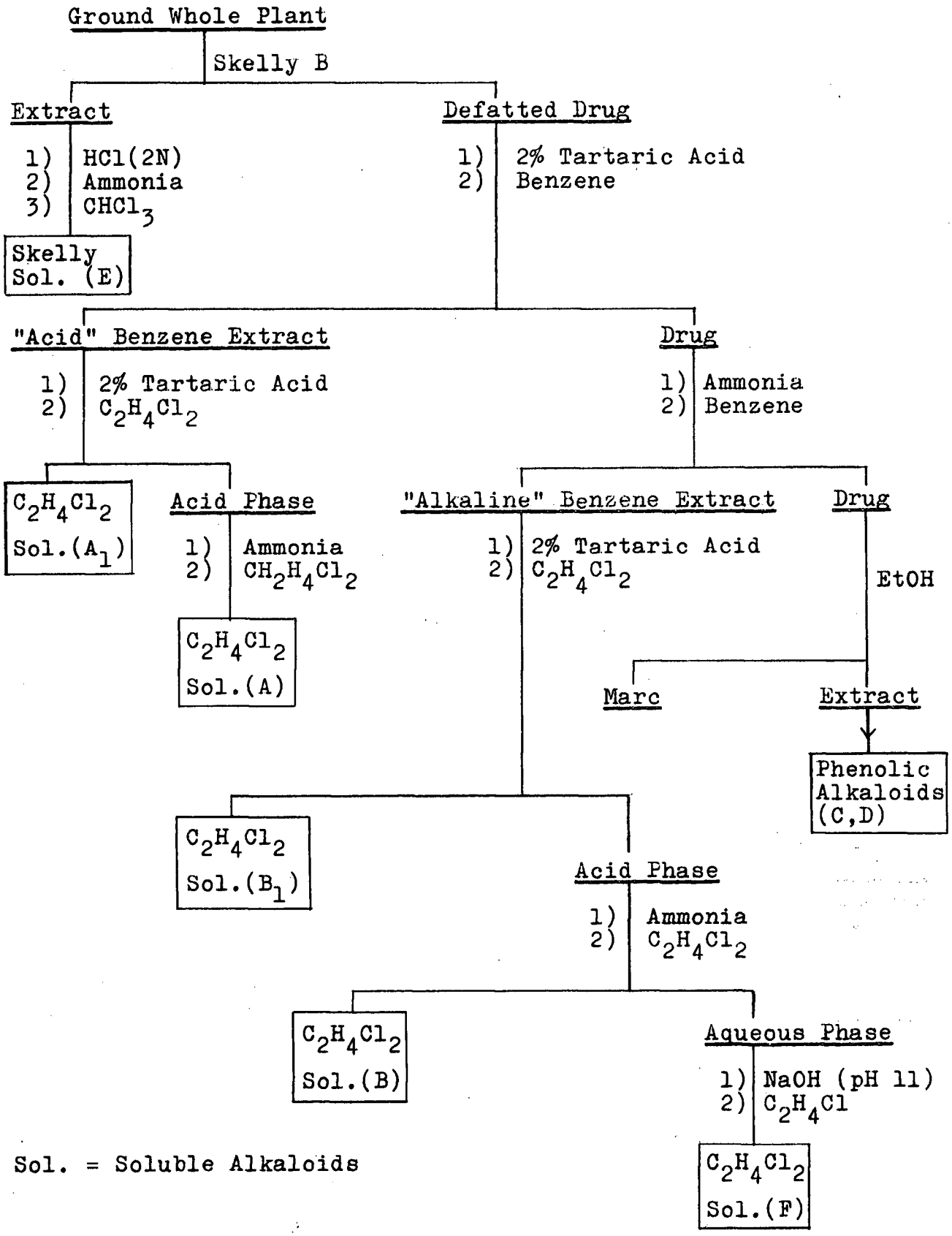
Interest in the Apocynaceous plant Vinca rosea Linn. (Lochnera Reichb. or Catharanthus roseus G.Don) was stimulated by the observation that certain preparations of the plant exhibited a remarkable anti-tumour activity[1,2]. This discovery prompted a systematic fractionation of extracts in a search for the active principles free of extraneous substances. Initial testing indicated that the biologically active entities were confined to the complex alkaloidal portion of the plant constituents, and a separation scheme was then devised which permitted the isolation of four active alkaloids — vincaleukoblastine (VLB) leurosine, leurocristine and leurosidine — in addition to numerous other alkaloids of unknown structure[3,4]. The experimental anti-tumour activity of these four alkaloids against the transplanted P-1534 leukemia in mice has been reported by the Canadian group of Noble, Beer and Cutts[1,5,6] and by workers at the Lilly laboratories[7,8]. It was also found that VLB produced severe leukopenia in rats[1,5], and, moreover, markedly inhibited the growth of a transplanted human carcinoma in the hamster cheek pouch[9]. Reports on clinical trials of VLB and leurocristine have been presented by several groups[10,11,12,13].

The earliest chemical investigation of the plant was performed in the late 19th century by Greshoff[14], who was only able to demonstrate the presence of alkaloidal material.

Cowley and Bennett[15] in 1928 succeeded in isolating two crystalline sulphates and a tartrate, but did not describe any chemical or physical properties, and in 1953 French workers[16] reported an unidentified crystalline alkaloid. More recently, several groups have obtained known alkaloids: ajmalicine[17], serpentine[17,18], akuammicine[17], tetrahydroalstonine[18], lochnerine[19], and reserpine[20]. In 1958 Kawat and coworkers[21] isolated two crystalline and two amorphous alkaloids, and Noble, Beer and Cutts described VLB[1].

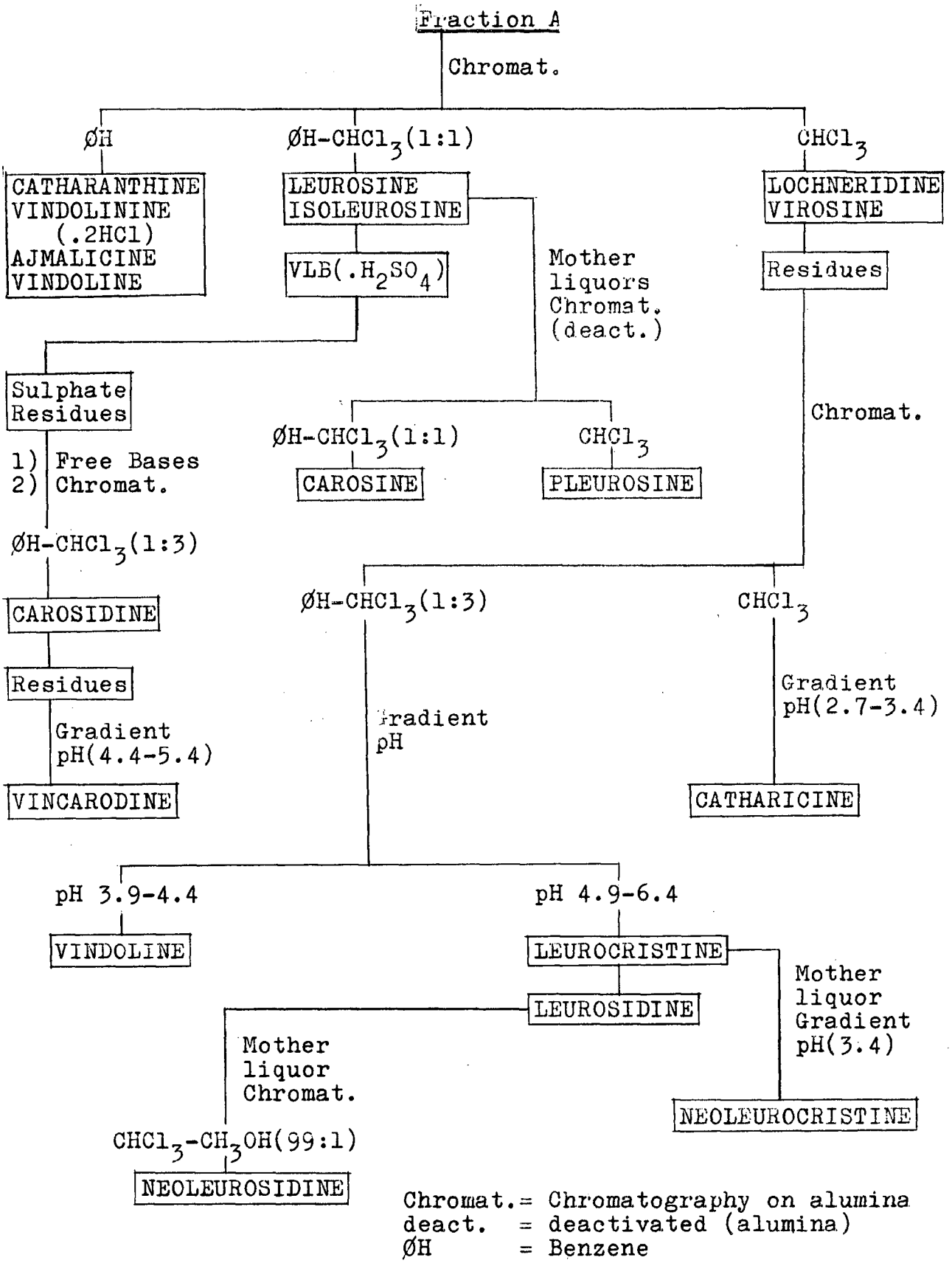
A major advance in the phytochemistry of Vinca rosea Linn. was made when the Lilly group[3,4] devised an extraction scheme capable of separating the large number of alkaloids present in the plant. The process consists essentially of separating the alkaloid tartrates which are soluble in organic solvents from those which are insoluble; a brief outline of the procedure is given in Scheme 1. The constituents of each fraction were further separated by chromatography on alumina and gradient pH extraction, as shown in Schemes 2 and 3.

Scheme 1 - Extraction



Sol. = Soluble Alkaloids

Scheme 2 - Fraction A



Chromat. = Chromatography on alumina
deact. = deactivated (alumina)
ØH = Benzene

Scheme 3 - Fractions A_1, (A+B), B_1, B, F and E.

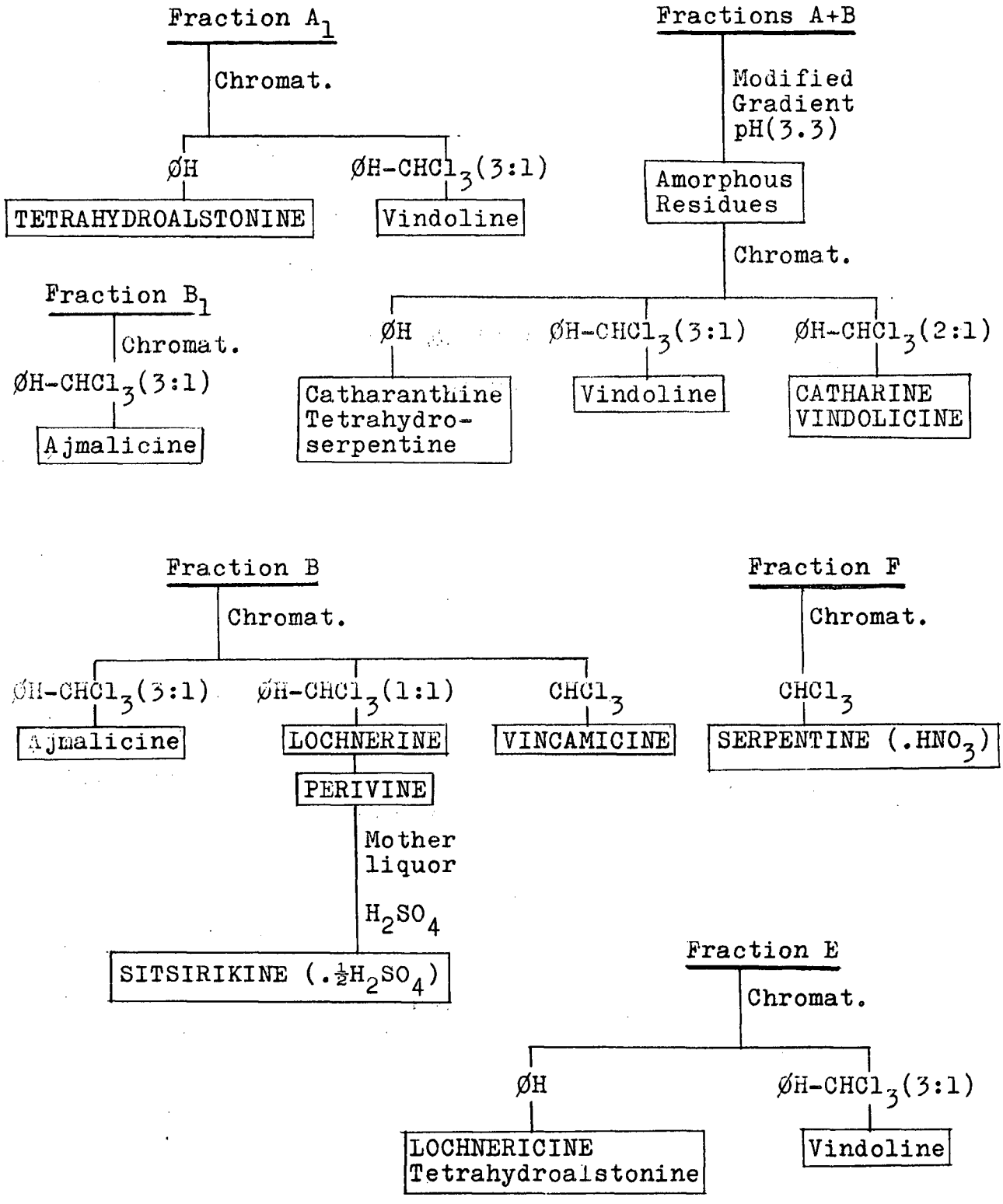


Table 1

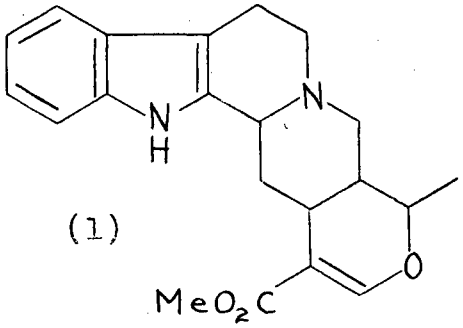
Name	Formula	Fraction	Lit.ref.
Akuammicine	$C_{20}H_{20}O_2N_2$	B	17,29
Ajmalicine	$C_{21}H_{24}O_3N_2$	A,B,B_1	17
Tetrahydroalstonine	$C_{21}H_{24}O_3N_2$	A,E	18
Serpentine	$C_{21}H_{22}O_3N_2$	F	17,18
Mitraphylline	$C_{21}H_{24}O_4N_2$	A	29
Lochnerine	$C_{20}H_{24}O_2N_2$	B	19
Ammocalline	$C_{19}H_{22}N_2$	B	29
Perividine	$C_{20}H_{22}O_4N_2$	A	29
Cavincine	$C_{20}H_{24}O_2N_2$	A	29
Lochneridine	$C_{20}H_{24}O_3N_2$	A	26
Perivine	$C_{20}H_{24}O_3N_2$	B	3,23
Catharanthine	$C_{21}H_{24}O_2N_2$	A	22,28
Lochnericine	$C_{21}H_{24}O_2N_2$	E	22,24
Vindolinine	$C_{21}H_{24}O_2N_2$	A	22
Sitsirikine	$C_{21}H_{26}O_3N_2$	B	26
Isositsirikine	$C_{21}H_{26}O_3N_2$	B	-
Virosine	$C_{22}H_{26}O_4N_2$	A	3,23
Vinosidine	$C_{22}H_{26}O_5N_2$	A	29
Lochnerivine	$C_{24}H_{28}O_5N_2$	A	29
Vindoline	$C_{25}H_{32}O_6N_2$	A,A_1,E	22,27
Vindolicine	$C_{25}H_{32}O_6N_2$	(A+B)	26
Leurosidine	$C_{41}H_{54}O_9N_4$	A	29
Vincarodine	$C_{44}H_{52}O_{10}N_4$	A	4
Catharine	$C_{46}H_{52}O_9N_4$	(A+B)	4,26
Catharicine	$C_{46}H_{52}O_{10}N_4$	A	4
Leurocristine	$C_{46}H_{54}O_{10}N_4$	A	4,29

Table 1 (Cont.)

Name	Formula	Fraction	Lit.ref.
Pleurosine	$C_{46}H_{56}O_{10}N_4$	A	4
Neoleurocristine	$C_{46}H_{56}O_{12}N_4$	A	4
Vincaleukoblastine	$C_{46}H_{58}O_9N_4$	A	1,25
Leurosine	$C_{46}H_{58}O_9N_4$	A	23,25
Isoleurosine	$C_{46}H_{60}O_9N_4$	A	26
Neoleurosidine	$C_{48}H_{62}O_{11}N_4$	A	4
Vindolidine	$C_{48}H_{64}O_{10}N_4$	A	4
Vincamicine		B	26
Leurosidine		A	4
Carosidine		A	4
Pericalline		B	29
Ammorosine		A	29
Perosine		B	29
Cavincidine		B	29
Maandrosine		B	29
Cathindine		B	29

The alkaloids isolated by the Lilly group from the various fractions are listed in Table 1, which includes all those whose characterisation has been published up to April 1964. However, Dr.M.Gorman very recently indicated in a private communication that over fifty alkaloids have now been isolated from Vinca rosea Linn., so that even this table is already out of date. Of the alkaloids tabulated, the structures were already known for ajmalicine (1), tetrahydroalstonine (1), serpentine (2), mitraphylline (3) and

akuammicine (4), and have since been determined for catharanthine[28](5), perivine[30](6), vindoline[27](7), vindolicine[31] (8), vindolinine[32](9), lochnerine[33](10), and lochneridine[34] (11). The structures of vincaleukoblastine (80) and leurocristine (vincristine)[68] are discussed below.



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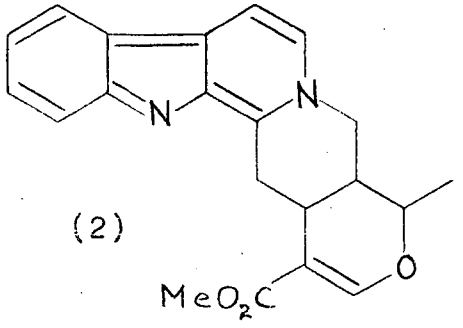

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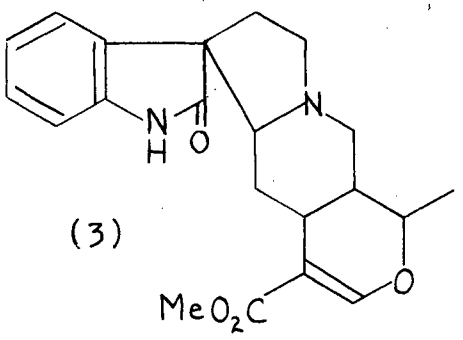

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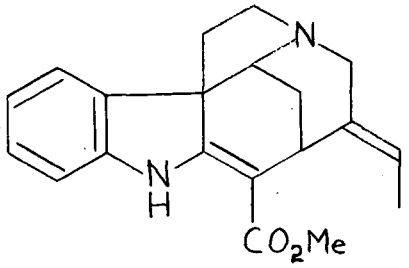

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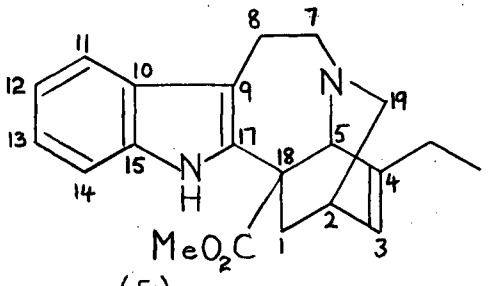

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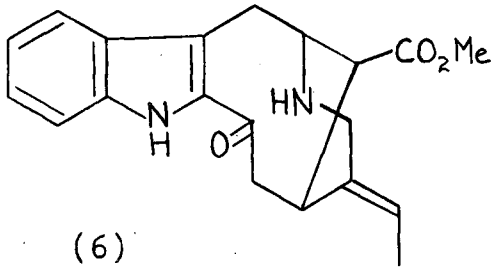

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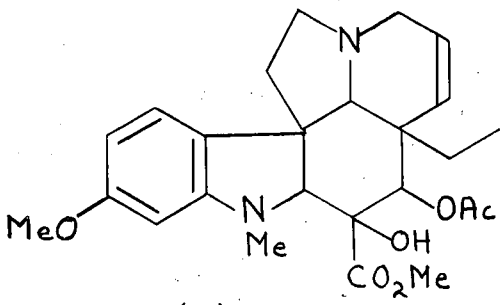

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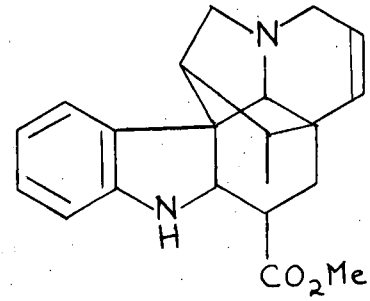

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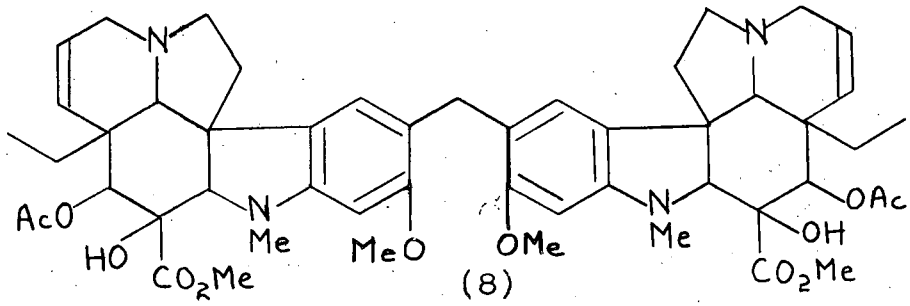

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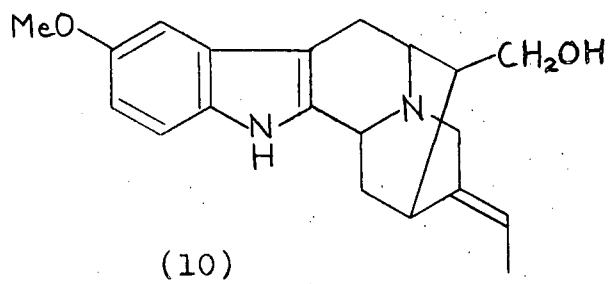

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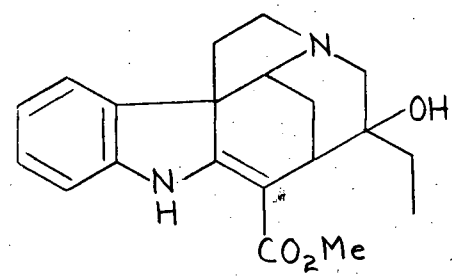


(11)

In Part I of this thesis is presented the evidence which led to the assignment of structures to sitsirikine, dihydrositsirikine and isositsirikine, three minor alkaloids isolated from Vinca rosea Linn. Part II discusses some aspects of the chemistry of cleavamine, an acid rearrangement product of the Vinca alkaloid catharanthine, and the

use of cleavamine and its derivatives in partial syntheses of Iboga and Aspidosperma-type alkaloids.

PART I

The Structural Elucidation of Sitsirikine, Dihydrositsirkine and Isositsirikine

A. Sitsirikine and Dihydrositsirikine

Sitsirikine was isolated as a minor alkaloid from Vinca rosea Linn. by the Lilly group[26]. Chromatography of fraction B (see Scheme 3) of the alkaloidal extract yielded several fractions from which perivine (6) was crystallised. The mother liquors and some of the following fractions were combined and converted to the sulphate salts. After perivine sulphate had been removed by recrystallisation from methanol, sitsirikine sulphate was obtained as blades from ethanol, and analysed for $C_{21}H_{26}O_3N_2 \cdot \frac{1}{2}H_2SO_4$. The free base was obtained only as an amorphous powder. On the basis of an infra-red comparison with β-yohimbine, it was suggested that sitsirikine might represent a new yohimbine isomer[26].

Through the kind co-operation of Dr.M.Gorman, Lilly Research Laboratories, Indianapolis, Indiana, U.S.A., we obtained a sample of sitsirikine for further structural studies. Our preliminary work revealed that the original alkaloid was a mixture of at least three compounds, since on thin-layer chromatography a separation into three distinct spots was observed.

Several different purification techniques were applied without success — chromatography on alumina and silica gel, sublimation, fractional recrystallisation of the salts. Finally, it was found that after several recrystallisations of the base from acetone-petroleum ether, a material melting

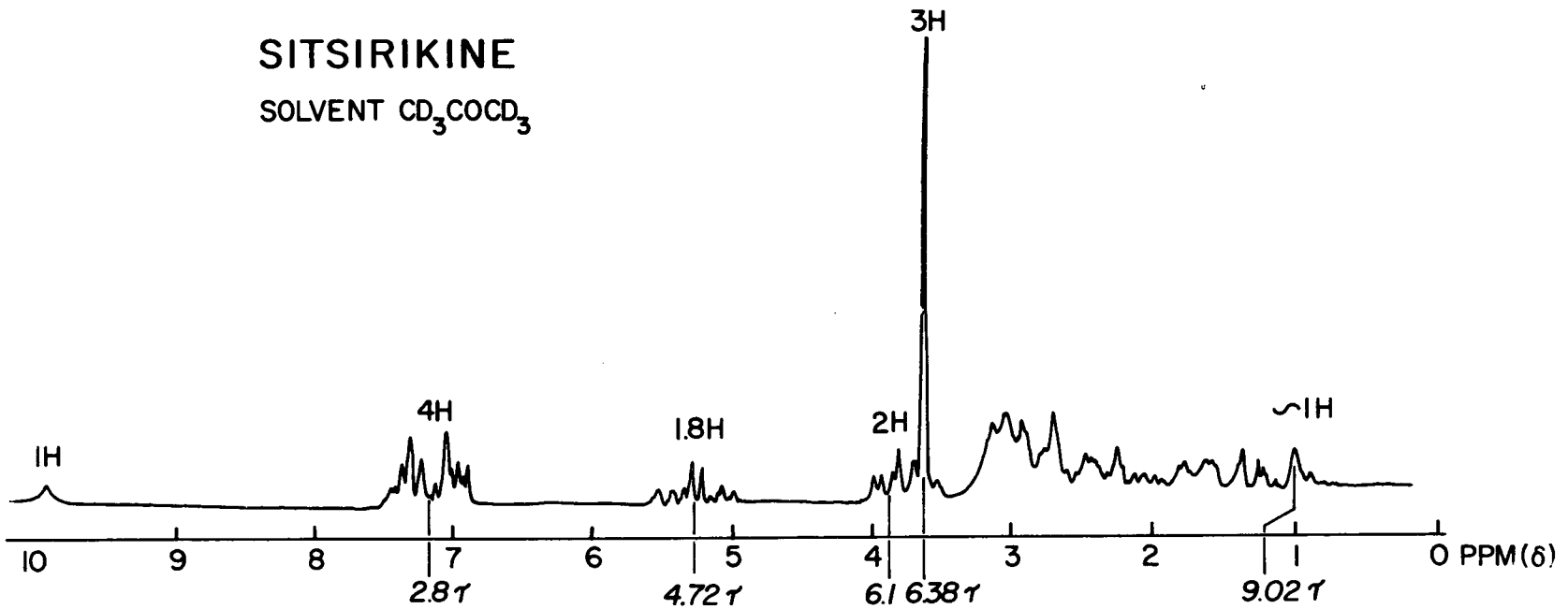

SITSIRIKINE
SOLVENT CD_3COCD_3
3H
4H
1.8H
2H
1H
~1H
10
9
8
7
6
5
4
3
2
1
0 PPM(δ)
2.8τ
4.72τ
6.1 6.38τ
9.02τ

Figure 1

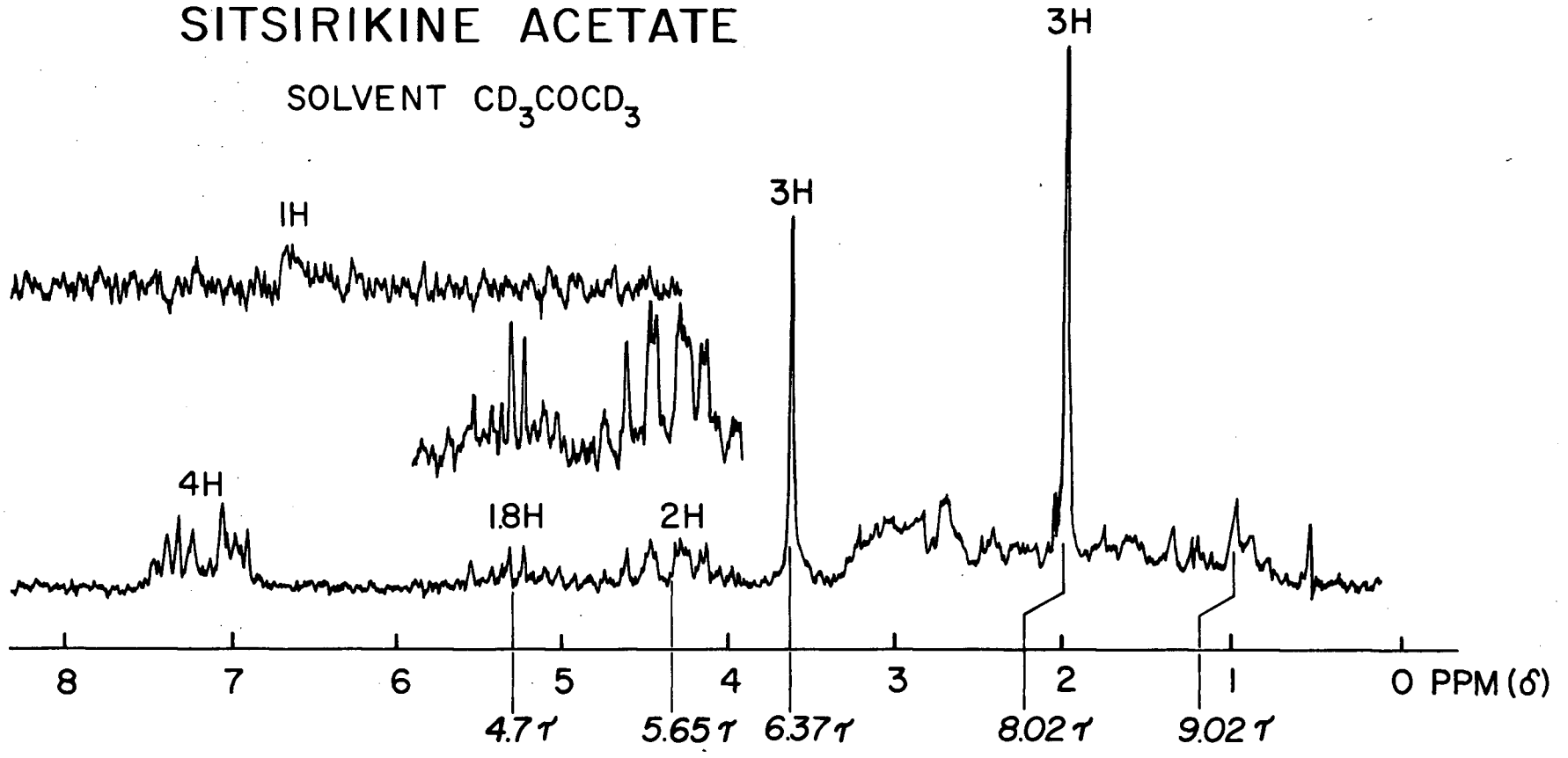

SITSIRIKINE ACETATE
SOLVENT CD_3COCD_3
3H
3H
1H
4H
1.8H
2H
8
7
6
5
4
3
2
1
0 PPM (δ)
4.7τ
5.65τ
6.37τ
8.02τ
9.02τ

Figure 2

sharply at 181° was obtained. This showed only two spots on thin-layer chromatography and analysed for the acetone solvate, $C_{21}H_{26}O_3N_2 \cdot CH_3COCH_3$. Attempts to separate the two components were of no avail, and indeed the mixture behaved as a homogeneous compound except on thin-layer chromatography. The unsolvated alkaloid was subsequently obtained from aqueous methanol as needles, m.p. 206-208°, $[\alpha]_D^{26}$ -58° (MeOH), and analysed well for $C_{21}H_{26}O_3N_2$. This molecular formula was supported by elemental analyses on the picrate, m.p. 226-228° (dec.) and finally substantiated by a mass spectral molecular weight determination (354).

The ultra-violet spectrum of sitsirikine, with maxima at 226, 282 and 290 mμ indicated an unsubstituted indole chromophore. This was confirmed by signals in the nuclear magnetic resonance (N.M.R.) spectrum (Figure 1) at 0.06 τ (indolic NH) and in the region 2.5-3.1 τ (four aromatic protons). A strong band in the infra-red spectrum at 1705 $cm.^{-1}$ was readily attributed to a carbonyl group and an absorption at 3360 $cm.^{-1}$ was compatible with the presence of NH and/or hydroxyl groups. In addition to a spike at 6.38 τ (CH_3O), the N.M.R. spectrum of sitsirikine displayed a two-proton multiplet centred at 6.1 τ that was possibly due to the methylene protons of a primary alcoholic function. The presence of a hydroxyl group was confirmed by the formation of a monoacetate, $C_{23}H_{28}O_4N_2$, m.p. 198°, $[\alpha]_D^{26}$ -26° (MeOH), whose N.M.R. spectrum (Figure 2) was particularly instructive. Apart from the expected signal at 8.02 τ due

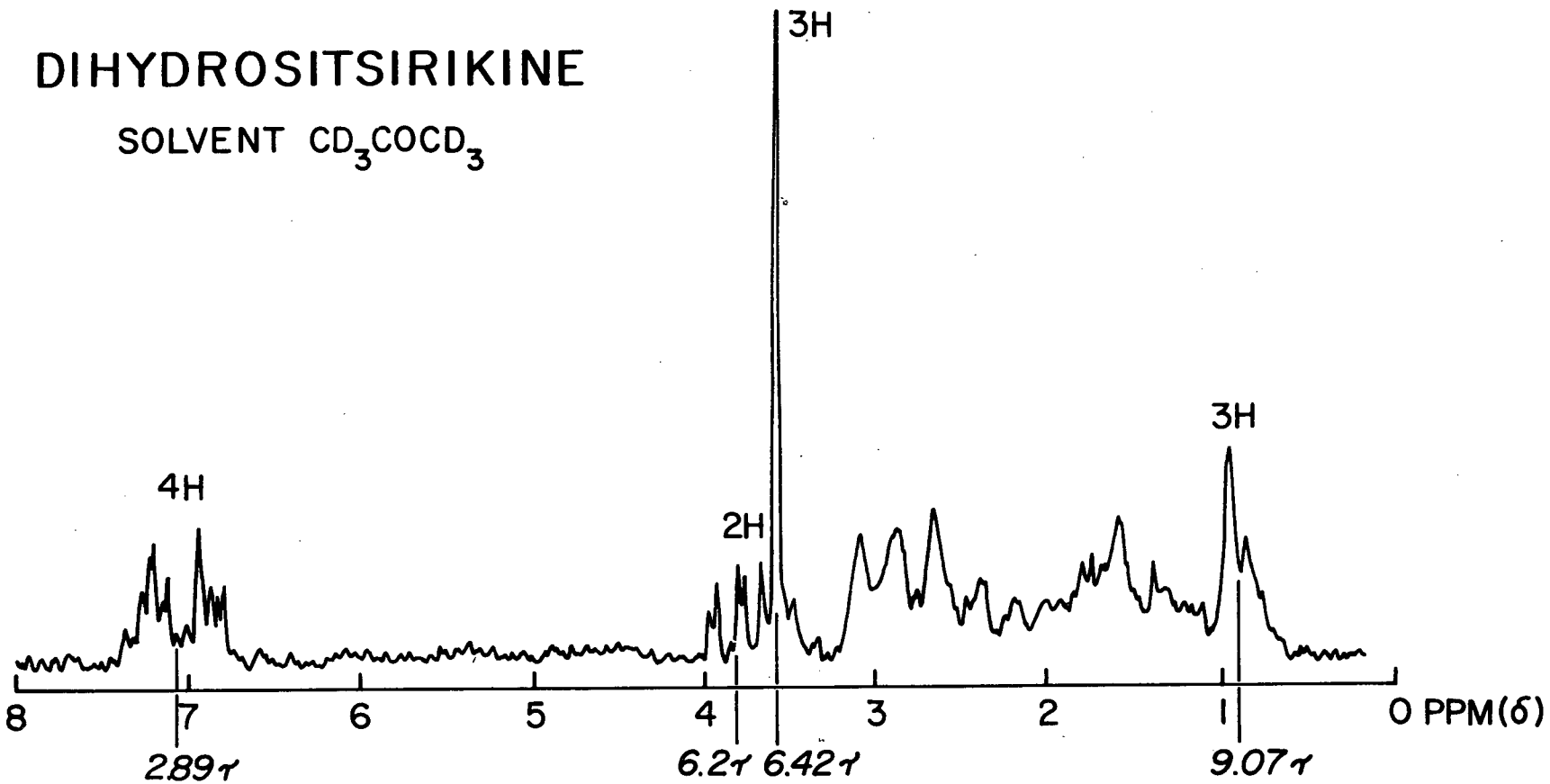

DIHYDROSITSIRIKINE
SOLVENT CD_3COCD_3
3H
4H
2H
3H
8
7
6
5
4
3
2
1
0 PPM(δ)
2.89τ
6.2τ
6.42τ
9.07τ

Figure 3

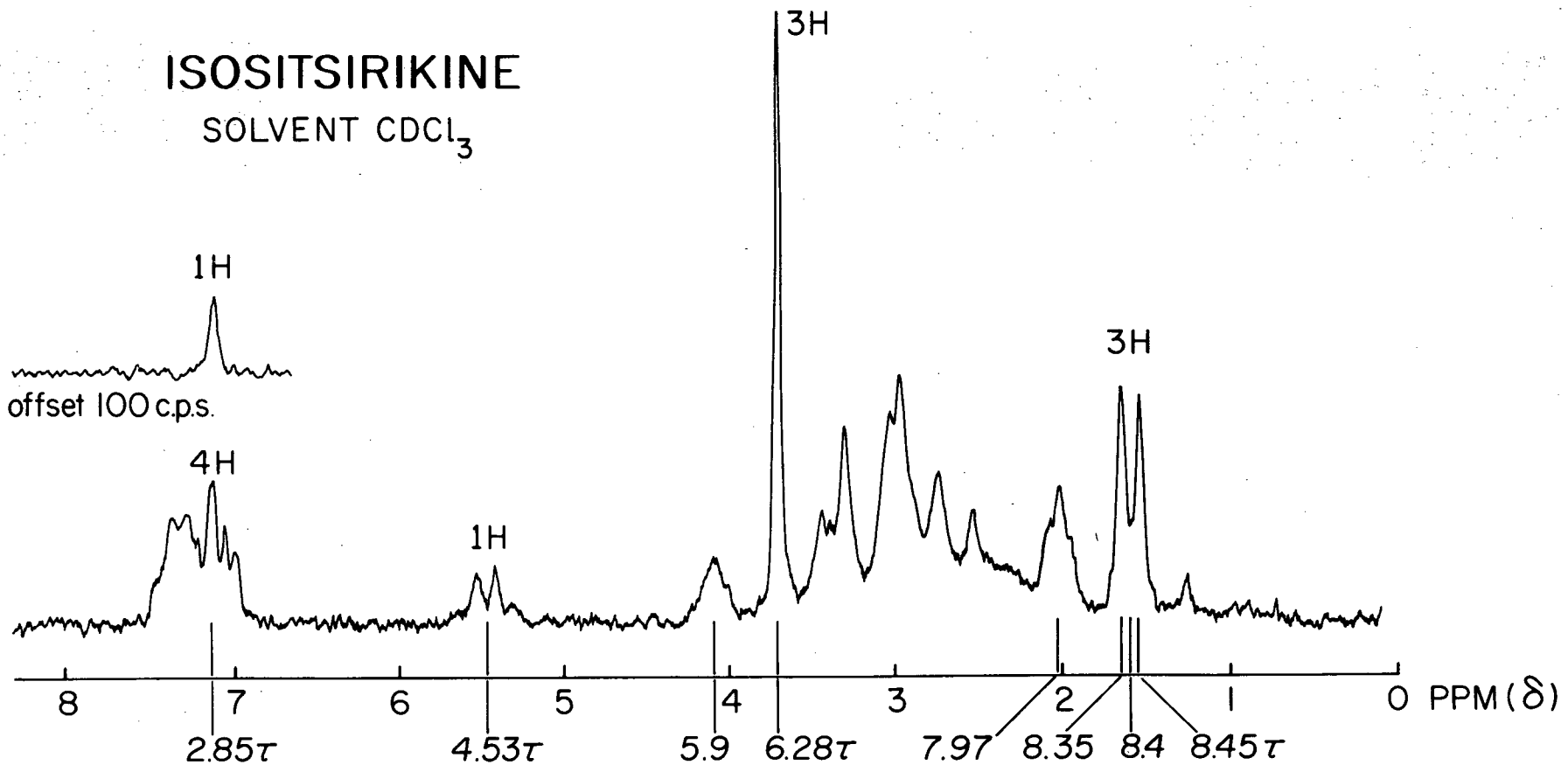

ISOSITSIRIKINE
SOLVENT CDCl3
1H
offset 100 c.p.s.
4H
1H
3H
3H
8
7
6
5
4
3
2
1
0 PPM (δ)
2.85τ
4.53τ
5.9
6.28τ
7.97
8.35
8.4
8.45τ

Figure 4

to the acetyl group, the multiplet present at 6.1τ in the spectrum of the alcohol had shifted downfield and now appeared at 5.6τ. This shift of 0.5τ upon acetylation is characteristic of primary alcohols, whereas the corresponding shift for secondary alcohols is about 1τ unit[35].

Besides the above-mentioned signals the N.M.R. spectra of sitsirikine and its acetate displayed a multiplet centred at 4.7τ due to olefinic protons, which integrated for rather less than two hydrogen atoms. A series of micro-hydrogenations was run on sitsirikine and it was found that only 0.6-0.7 mol. of hydrogen was taken up. Moreover, the product gave only one spot on thin-layer chromatography whose Rf value corresponded to the smaller of the two spots exhibited by the original alkaloid. This evidence suggested that the two components of the mixture differed from each other merely by the presence of an olefinic bond in one of the alkaloids. The unsaturated alkaloid was named sitsirikine, whereas the corresponding dihydro derivative will be referred to as dihydrositsirikine. This conclusion was fully borne out by subsequent work.

Catalytic hydrogenation on a larger scale and recrystallisation from acetone afforded solvated dihydrositsirikine, m.p. 180°, which analysed for $C_{21}H_{28}O_3N_2 \cdot CH_3COCH_3$. Further recrystallisations from aqueous methanol gave the unsolvated alkaloid, $C_{21}H_{28}O_3N_2$, m.p. 215°, $[\alpha]_D^{26}$ -55°(MeOH). The N.M.R. spectrum of dihydrositsirikine (Figure 3) showed a complete disappearance of the olefinic

proton absorption. A strong band at 1710 cm.$^{-1}$ in the infra-red spectrum of the reduced material excluded any conjugation between the carbonyl group and the double bond in sitsirikine, and the ultra-violet spectrum was unchanged with maxima at 226, 282 and 290 mμ. Elemental analyses on the crystalline picrate, m.p. 228-230°(dec.), acetate, m.p. 187°, $[\alpha]_D^{26}$-31°(MeOH), and p-bromobenzoate, m.p. 174°, supported the formula assigned to dihydrositsirikine, and final confirmation was obtained from a mass spectral molecular weight determination (356).

Evidence that the olefinic linkage in sitsirikine was in fact a terminal double bond was provided by the appearance of a new C-methyl absorption at 9.07τ in the N.M.R. spectrum of the reduction product. This was corroborated when ozonolysis of the original alkaloid gave formaldehyde, identified by paper chromatography of its 2,4-dinitrophenylhydrazone[36]. A conventional Kuhn-Roth determination on dihydrositsirikine indicated 0.93 mol. C-methyl, while a modified procedure[37] yielded propionic acid and thus showed that the new C-methyl function was in fact part of a C-ethyl group. These experiments established the presence of a vinyl group in sitsirikine.

However, it was still necessary to explain why the olefinic proton absorption in the N.M.R. spectrum of sitsirikine integrated for two rather than the three hydrogen atoms expected for a vinyl group. The thin-layer chromatography and microhydrogenation results had suggested that

the impurity present in the original alkaloid was dihydrositsirikine, and a close scrutiny of the N.M.R. spectrum of the original sitsirikine (Figure 1) revealed a slight absorption at 9.02 τ integrating for about one hydrogen, whereas the mass spectrum indicated a small peak at m/e 356 in addition to that at m/e 354. A Kuhn-Roth determination on the mixture showed 0.38 mol. C-methyl, and the modified method afforded propionic acid. From these results it was deduced that the original alkaloid was a mixture of sitsirikine and dihydrositsirikine in an approximate ratio of 2:1.

Since dihydrositsirikine was the only component which could be obtained pure, it was used as the starting material in all subsequent studies.

Besides providing the information discussed above, the N.M.R. spectrum of dihydrositsirikine (Figure 3) was very useful in establishing the nature of the two oxygen functions present in addition to the carbonyl group. In the region of 6.1 τ there was a two-proton absorption, attributable to hydrogen atoms attached to an oxygen-bearing carbon atom, which upon acetylation moved down to 5.6 τ. This paralleled the behaviour of the original sitsirikine and confirmed the presence of a primary alcohol. The nature of the third oxygen atom was indicated by a spike at 6.42 τ readily assigned as before to the three protons of a methoxyl function. A Zeisel determination on dihydrositsirikine showed the presence of one methoxyl group and gave

ample support to the N.M.R. designation.

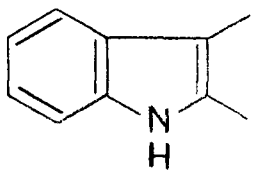
Since the ultra-violet and N.M.R. spectra excluded the possibility that the methoxyl was attached to the indole system, the presence of absorption bands at 1705 and 1165 $cm.^{-1}$ in the infra-red region suggested that it was part of a carbomethoxy group[38]. An attempted saponification under mild conditions was unsuccessful, but after more drastic treatment an unsaturated acid was isolated as the hydrochloride, m.p. 260-263°. The N.M.R. spectrum (in trifluoroacetic acid) of this substance showed loss of the methoxyl group.

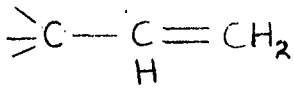
Reduction of dihydrositsirikine with lithium aluminium hydride yielded a crystalline diol, m.p. 203°, $[\alpha]_D^{26}$ -3° (MeOH), which analysed for $C_{20}H_{28}O_2N_2$. The infra-red spectrum of the diol showed no carbonyl absorption, and the N.M.R. spectrum indicated a complete absence of the methoxyl signal. The presence of a carbomethoxy group in dihydrositsirikine was thus confirmed.

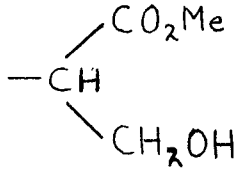
Treatment of the diol with acetone containing p-toluenesulphonic acid afforded an acetonide, m.p. 105-109°, as shown by a six-proton N.M.R. signal (gem-dimethyl) at 8.62τ. Since both alcoholic groups were primary the formation of this derivative meant that the hydroxyl groups were in a 1,3-relationship, and hence dihydrositsirikine itself must contain a β-hydroxy-ester grouping. The N.M.R. spectrum of the acetonide was even more instructive in that the signal at 6.25τ, due to the methylene protons

on the two oxygen-bearing carbon atoms, was split into a doublet, thereby indicating that there must be one proton on the carbon atom linking the hydroxymethyl and carbomethoxy groups in dihydrositsirikine.

At this point it had been established that sitsirikine possessed a tetracyclic skeleton and the following features:



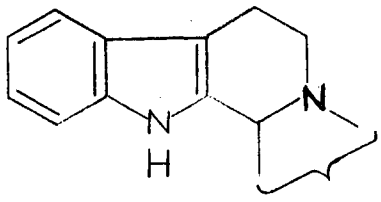




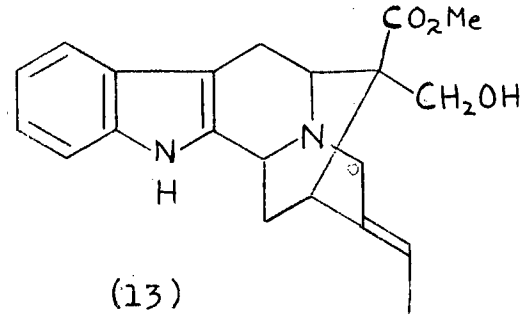
During the course of the chemical investigations, mass spectrometric analyses of sitsirikine and various derivatives were undertaken. The mass spectrum of dihydrositsirikine (Figure 8), run by the direct inlet procedure[39], was most helpful and is discussed in some detail.

The molecular ion peak at m/e 356 established the molecular formula assigned to dihydrositsirikine, and fragments at m/e 338 ($M-H_2O$) and 325 ($M-CH_2OH$) were consistent with the presence of a primary alcohol. A strong peak at m/e 253 was considered to arise from loss of the entire oxygen-containing portion of the molecule, i.e. $M-CH\langle^{CH_2OH}_{CO_2Me}$. More important, however, were the ions at m/e 184, 170, 169 and 156. It was immediately apparent from these four peaks that rings A,B, and C of the dihydrositsirikine skeleton were of the type (12) encountered in the yohimbine and related alkaloid classes[40],

where these ions are also attributed to the fragments shown in Figure 8. The assignments made in the case of yohimbine were quite rigorously established by deuterium labelling, as well as by studying the effect of functional substituents in various positions of the molecule. Moreover, the occurrence of significant peaks at m/e 169 and 170 and <u>not</u> at m/e 168



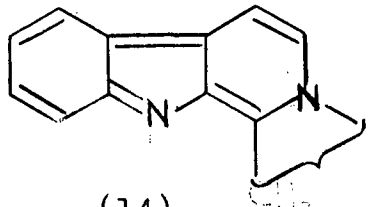
(12)



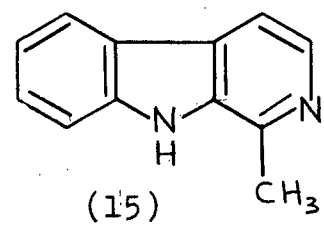
(13)

and 169 excluded the type of pentacyclic ring system found in polyneuridine (13)[40].

Further information regarding the ring skeleton was obtained from semimicro dehydrogenation experiments. Treatment of dihydrositsirikine with lead tetracetate afforded a product which exhibited ultra-violet spectra (λmax. 253, 308 and 365 mμ in neutral or acid solution; λmax. 284 and 328 mμ in alkaline solution) in good agreement with those of tetradehydroyohimbine and similar compounds (14)[41]. Dehydrogenation of dihydrositsirikine with 10% palladium-charcoal at 250° gave a mixture of



(14)



(15)

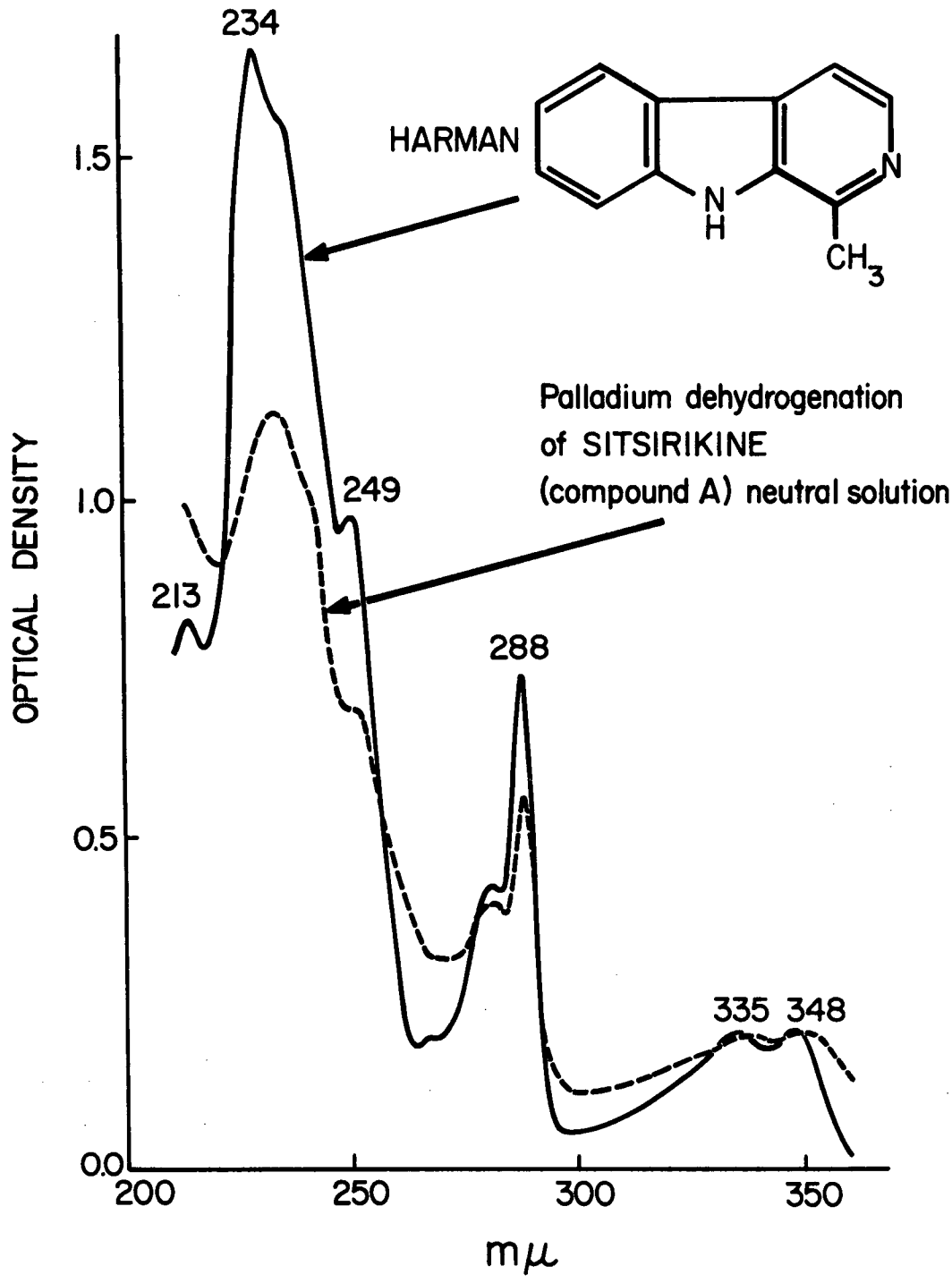

234
HARMAN
N
H
N
CH3
1.5
1.0
0.5
0.0
OPTICAL DENSITY
Palladium dehydrogenation
of SITSIRIKINE
(compound A) neutral solution
249
213
288
335 348
200
250
300
350
mμ

Figure 5

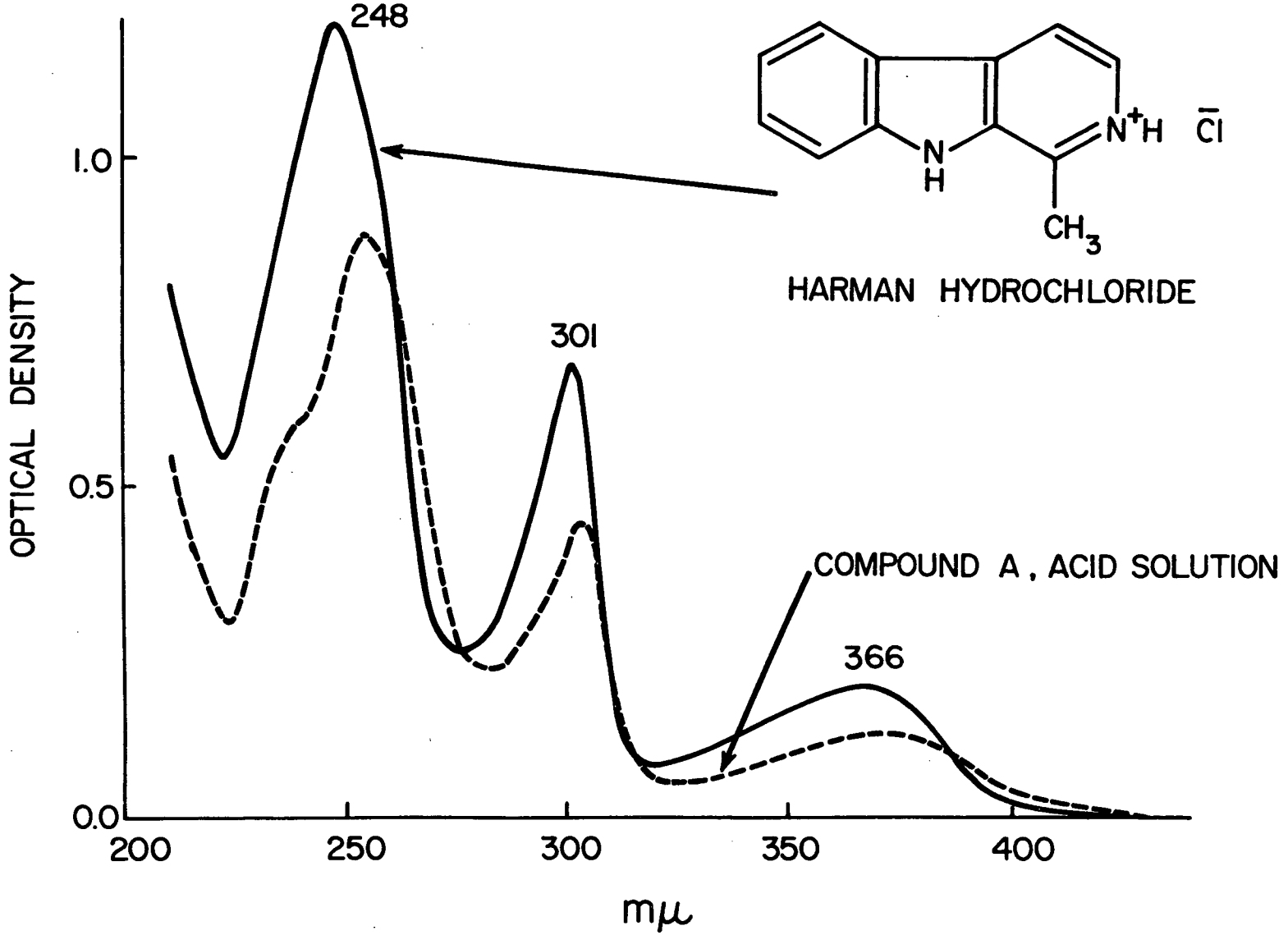
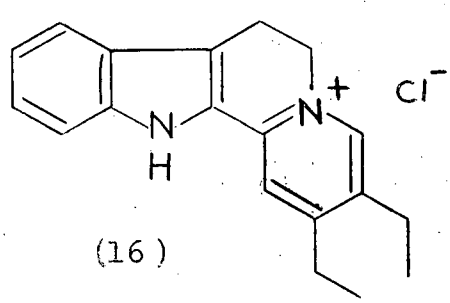

248
301
366
1.0
0.5
0.0
OPTICAL DENSITY
200
250
300
350
400
mμ
N
H
N+H
Cl
CH3
HARMAN HYDROCHLORIDE
COMPOUND A, ACID SOLUTION

Figure 6

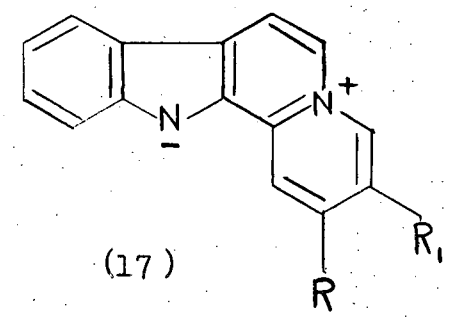
products which were separated by thin-layer chromatography. The main product (compound A) displayed ultra-violet spectra (Figures 5 and 6) similar to those of harman (15). In neutral and alkaline media the spectra were the same, with maxima at 234, 250, 282, 288, 337 and 349 mμ, whereas in acid solution there was a bathochromic shift to 254, 303 and 372 mμ.

These results were sufficient to confirm the tetrahydro-β-carboline structure (12) indicated by the mass spectrum.

Dihydrositsirikine hydrobromide was then subjected to palladium dehydrogenation at 280°, and the resulting mixture separated by thin-layer chromatography. The ultra-violet absorption of one major fraction (compound B) was in close correspondence with that of 5,6-dihydroflavocoryline hydrochloride (16)[43] with maxima at 221, 312, and 386 mμ. This provided the first piece of evidence for the entire ring system in sitsirikine.



(16)



(17)

Final confirmation was obtained when the dehydrogenation product was oxidised further with 2,3-dichloro-5,6-dicyano-p-benzoquinone to compound C, which possessed a completely aromatised ring system. The ultra-violet spectrum (Figure 7) with maxima at 237, 291, 345 and 385 mμ was in good agreement

QUINONE DEHYDROGENATION OF COMPOUND B (compound C, HCl salt)

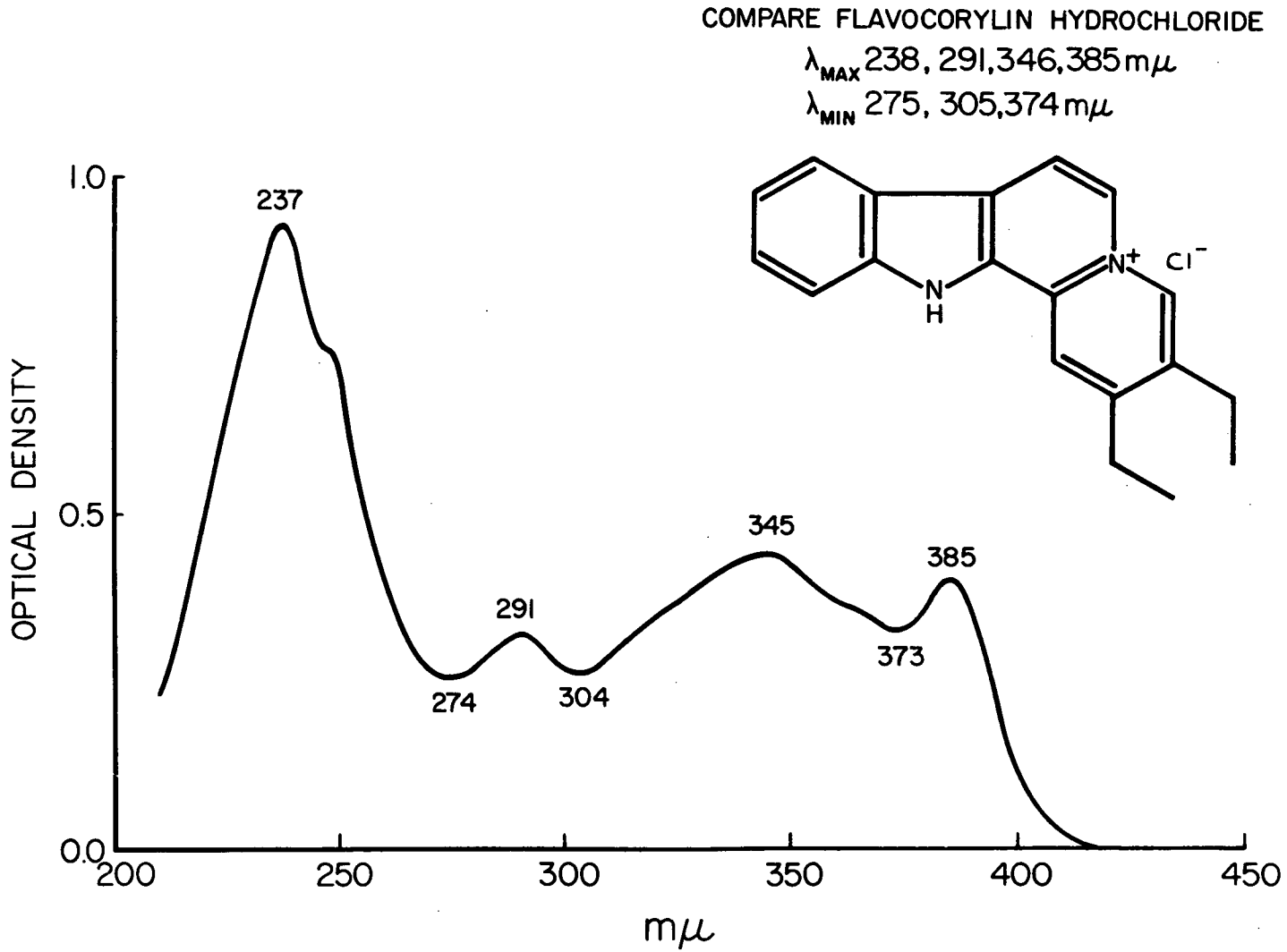


Figure 7

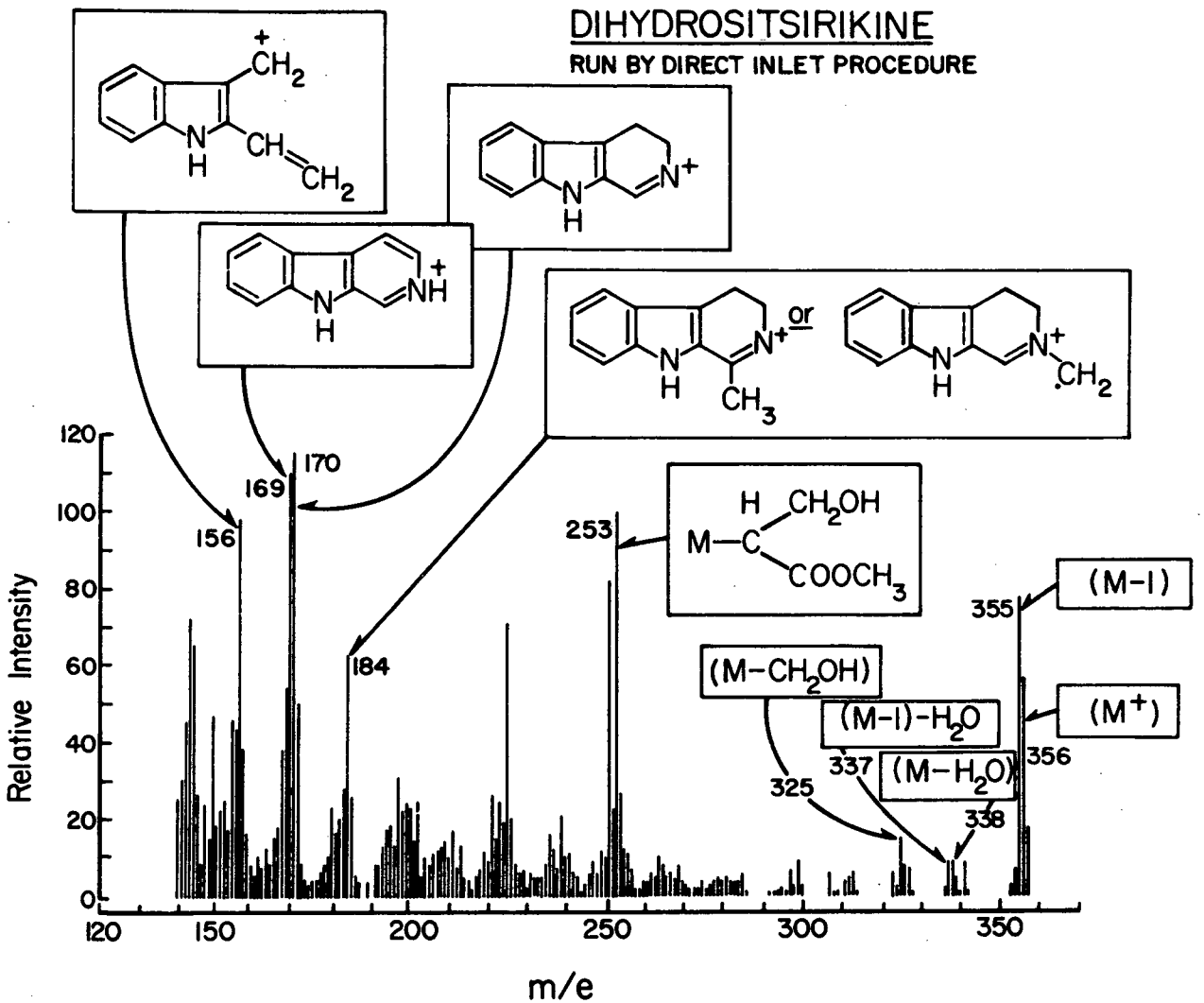

DIHYDROSITSIRIKINE
RUN BY DIRECT INLET PROCEDURE
Relative Intensity
m/e
120
100
80
60
40
20
0
120
150
200
250
300
350
156
169
170
184
253
325
337
338
355
356
(M-1)
(M-CH2OH)
(M-1)-H2O
(M-H2O)
(M+)
or

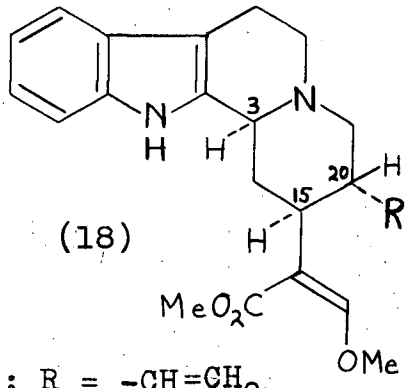
Figure 8

with that reported[43] for flavocoryline (17, $R = R_1 = Et$) but showed differences from other compounds (17)[43] with the same chromophore (see Table 2).

Table 2

R	R_1	$\lambda_{max.}$ (mμ)			
H	H	244	294	345	388
H	Et	235	295	350	390
Et	Et	238	291	346	385
$(CH_2)4$		242	295	342	388
i-Pr	Et	240	292	346	386

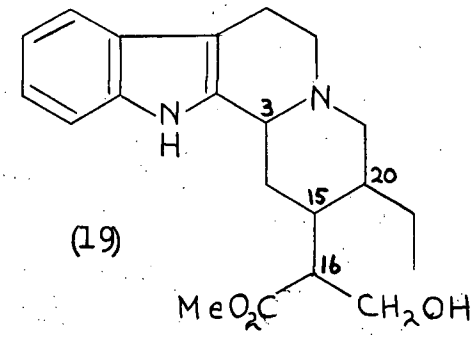
An authentic sample of flavocoryline hydrochloride was kindly provided by Dr.G.A. Swan, Chemistry Department, King's College, University of Durham, England, and the ultra-violet spectrum found to be superimposible on that of the compound derived from dihydrositsirikine. Furthermore, the two materials had the same Rf value on paper chromatograms run in several different solvent systems. Although the minute amounts of dehydrogenation products available prevented complete characterisation, the ultra-violet spectral data established the ring structure, and also suggested that sitsirikine was a relative of the corynantheine (18a) class of alkaloids. A provisional structure such as (19) could thus be considered for dihydrositsirikine.



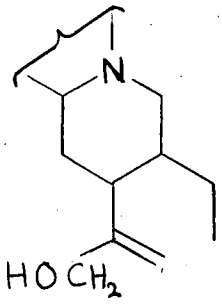
(18)

a: R = $-CH=CH_2$

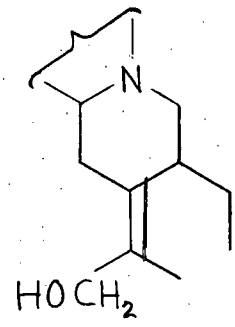
b: R = $-CH_2CH_3$



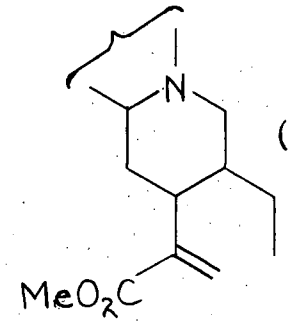
(19)



(20)



(21)



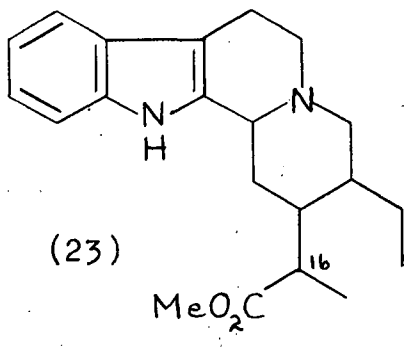
(22)

The orientation of the hydrogen atom at C-3 in (19) was indicated as α by the presence of Bohlmann bands[44] at 2810 and 2760 $cm.^{-1}$ in the infra-red spectrum of dihydrositsirikine. Furthermore, the hydrogen atom at C-15 could be assumed to have the α-configuration since this orientation has been found to be constant at the corresponding position in all related alkaloids[45].

During their investigations on corynantheine (18a) Karrer and co-workers[42] had reduced dihydrocorynantheine (18b) with lithium aluminium hydride and isolated two isomeric alcohols: desmethoxy-dihydrocorynantheine alcohol (20) and iso-desmethoxy-dihydrocorynantheine alcohol (21). Since the configurations at C-3 and C-15 in dihydrocorynantheine were the same as those projected for the corresponding positions in dihydrositsirikine, it seemed feasible to attempt a correlation between dihydrositsi-

rikine and dihydrocorynantheine. Dehydration of dihydrositsirikine to give the unsaturated ester (22), followed by hydride reduction should yield the known alkaloid (20), provided, of course, that the configuration at C-20 was also the same as in corynantheine.

Accordingly, dihydrositsirikine was treated with sodium methoxide in dry methanol to afford the $\alpha\beta$-unsaturated ester (22), which recrystallised from aqueous methanol as needles, m.p. 84-89°, $[\alpha]_D^{26}$ +2° (MeOH), and analysed for the methanol solvate, $C_{21}H_{26}O_2N_2 \cdot CH_3OH$. The presence of a terminal olefin was shown by a band in the infra-red spectrum at 1620 $cm.^{-1}$ and N.M.R. signals at 3.73 and 4.41τ, each of which integrated for one proton. On hydrogenation one mol. of hydrogen was taken up to give desoxy-dihydrositsirikine (23) as a mixture of the two C-16 epimers, m.p. 172-177°. The infra-red and N.M.R. spectra showed the disappearance of the double bond, and the analysis was in excellent agreement with the formula $C_{21}H_{28}O_2N_2$.

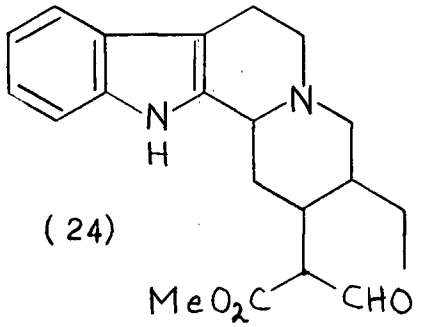


(23)

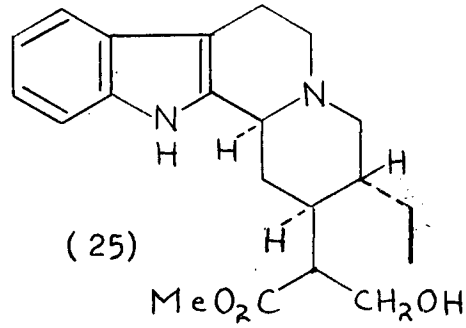
$\xleftarrow[Pd]{H_2}$ (22) $\xrightarrow{LiAlH_4}$ (20) + (21)

The olefinic ester was then reduced with lithium aluminium hydride, but the product contained relatively little terminal olefin, as determined by the N.M.R. spectrum. By a combination of chromatographic and recrystallisation techniques the major component was obtained as light brown needles, m.p. 204°, $[\alpha]_D^{26}$ -24.3° (MeOH), which showed one spot on thin-layer chromatography and analysed well for $C_{20}H_{26}ON_2$. The melting point and specific rotation were in excellent agreement with the corresponding constants — m.p. 204°, $[\alpha]_D^{17}$ - 24.0° — quoted[42] for iso-desmethoxy-dihydrocorynantheine alcohol (21). This was good evidence for the structure (19) for dihydrositsirikine but was not entirely conclusive, since unfortunately no sample of the iso-alcohol could be obtained for direct comparison.

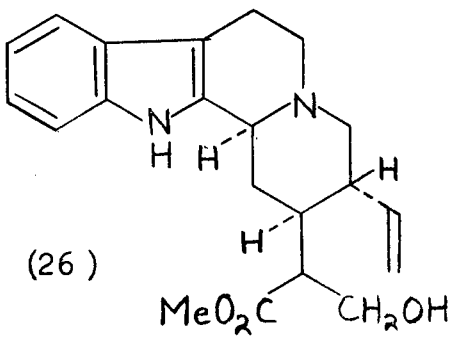
However, the correlation between dihydrocorynantheine and dihydrositsirikine was achieved by the following sequence of reactions. Mild acid hydrolysis converted dihydrocorynantheine (18b) to desmethyl-dihydrocorynantheine (24), which on reduction with sodium borohydride yielded a product (25) identical in every respect with dihydrositsirikine. Having thus established the structure of dihydrositsirikine, we could immediately assign structure (26) to sitsirikine.



(24)



(25)



(26)

B. Isositsirikine

From the amorphous post-perivine fractions of the chromatography of fraction B (Scheme 3), the Lilly group isolated another alkaloid which they also referred to as "sitsirikine" but to which we have given the name "isositsirikine" since we have now shown that it is a new alkaloid. Although the base, $[\alpha]_D^{26}$ -20° ($CHCl_3$), was an amorphous powder, it was homogeneous by thin-layer chromatography and gave a sharp-melting crystalline sulphate, m.p. 263.5°, and picrate, m.p. 216°. Analyses on isositsirikine and its salts indicated a formula of $C_{21}H_{26}O_3N_2$ for the base. This formula was established by a mass spectral molecular weight determination which showed a value of 354. Standard Kuhn-Roth and Zeisel determinations showed the presence of one C-methyl and one O-methyl group respectively. Maxima at 224, 283 and 291 mμ in the ultra-violet spectrum were characteristic of an unsubstituted indole chromophore, and an absorption band at 1720 $cm.^{-1}$ in the infra-red region gave evidence for a carbonyl group. The N.M.R. spectrum of isositsirikine (Figure 4) confirmed that the indole system was unsubstituted, with signals at 1.33τ (NH) and in the region 2.4-3.1τ (four

aromatic hydrogens), and a sharp three-proton singlet at 6.28τ was readily attributed to the methoxyl group found in the Zeisel determination.

The presence of one olefinic hydrogen atom was shown by a quartet centred at 4.53τ, whereas a doublet at 8.40τ indicated that the methyl group was attached to an olefinic carbon atom. Since the coupling constants were the same (7 c/s) in both cases, these signals almost certainly denoted an ethylidene group containing a trisubstituted double bond. Catalytic hydrogenation resulted in the uptake of one mol. of hydrogen to afford an amorphous mixture of two dihydro-isositsirikines, $C_{21}H_{28}O_3N_2$, as shown by thin-layer chromatography. The major component (which was <u>not</u> the same as dihydrositsirikine) was separated by chromatography and characterised as the crystalline picrate, m.p. 187°. In the N.M.R. spectrum of the dihydro compound the signals due to the ethylidene group had disappeared, and a new methyl absorption at 9.03τ became evident. Final confirmation of the ethylidene group was obtained when ozonolysis of isositsirikine yielded acetaldehyde, identified by paper chromatography of its 2,4-dinitrophenylhydrazone[36].

Carbonyl and methoxyl functions accounted for two of the oxygen atoms in isositsirikine. The nature of the third oxygen atom was revealed when acetylation gave an amorphous acetate, $C_{23}H_{28}O_4N_2$, which displayed the appropriate bands in the infra-red region at 1730 (C=O) and 1235 (OAc) $cm.^{-1}$. The

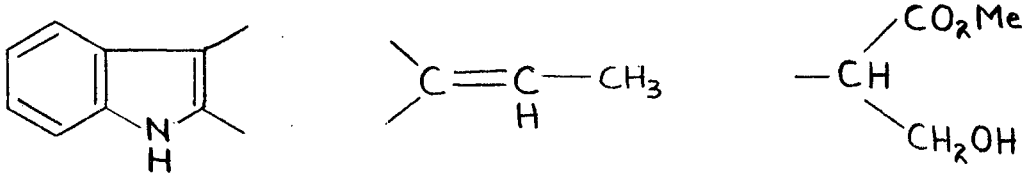
N.M.R. spectrum of this acetate was particularly instructive. Apart from the expected new sharp signal at 8.15τ due to the acetyl group, a doublet integrating for two hydrogens now appeared at 6.05τ, whereas a two-proton absorption present at 6.6τ in the spectrum of the alcohol had disappeared. The most obvious interpretation was that the doublet was due to the methylene protons of a primary hydroxyl group which had undergone a downfield shift of 0.5τ unit on acetylation[35]. Moreover, the splitting of the signal into a doublet suggested that the methylene protons were part of an A_2X system, and hence the hydroxymethyl group in isositsirikine was probably attached to a carbon bearing one hydrogen atom, i.e. H-C(-)-CH_2OH.

When isositsirikine was reduced with lithium aluminium hydride a diol was obtained, which showed neither a carbonyl absorption in the infra-red region nor a methoxyl signal in the N.M.R. spectrum. This evidence demonstrated the presence of a carbomethoxy group in isositsirikine.

Treatment of the reduction product with acetone containing p-toluenesulphonic acid gave an acetonide which crystallised from methanol as needles, m.p. 105-109°, $[\alpha]_D^{26}$-53° ($CHCl_3$). This derivative analysed well for the solvate, $C_{23}H_{30}O_2N_2 \cdot CH_3OH$, and the N.M.R. spectrum clearly indicated a gem-dimethyl group with a pair of sharp signals at 8.63 and 8.68τ. Since the diol had two primary alcohol functions, the formation of an isopropylidene derivative meant that the hydroxyl groups were in a 1,3 relationship. Hence isositsirikine itself must

possess a β-hydroxy-ester grouping similar to that found in sitsirikine.

At this point the following features of the alkaloid structure had been established:



The nature of the ring system was revealed by dehydrogenation of a small amount of isositsirikine sulphate with palladium black at 280° . The resulting mixture was separated by thin-layer chromatography, and two significant fractions were obtained. One of the dehydrogenation products gave ultra-violet spectra of the harman(15) type (Figures 5 and 6) with maxima at 232, 282, 289, 336 and 347 mμ in neutral solution, which shifted to 251, 302 and 374 mμ upon acidification. Even more instructive was the ultra-violet spectrum of the other fraction, since it was similar to that of flavocoryline hydrochloride (27) with maxima at 237, 291, 345 and 385 mμ (cf. Figure 7) and thus provided evidence for the entire tetracyclic ring system of isositsirikine.

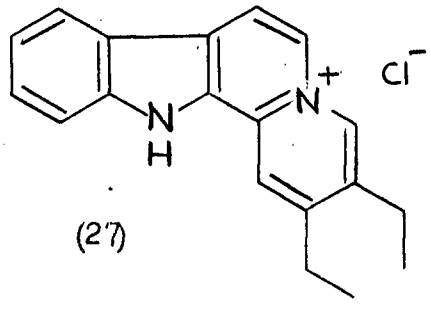
It was subsequently shown by paper chromatography, using an ethyl acetate-pyridine-water (8:2:1) system, that the latter dehydrogenation product was actually a mixture of two compounds. The major component (Rf 0.35) of this mixture was <u>not</u> flavocoryline (Rf 0.43) but the minor component

had the same Rf value as flavocoryline, indicating that some of this known alkaloid had presumably been obtained in the dehydrogenation.

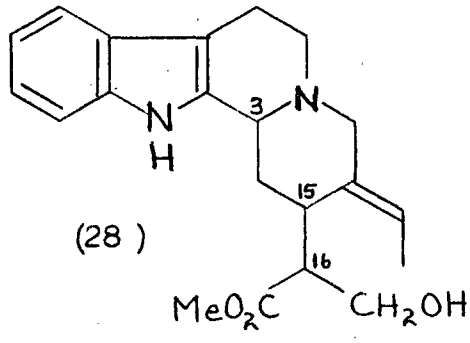
Since it had been possible to degrade dihydrositsirikine to flavocoryline by a combination of palladium and quinone dehydrogenation reactions, a similar procedure was followed with dihydro-isositsirikine.

The hydrogenation product of isositsirikine was converted to the amorphous hydrochloride. The salt (without purification) was then heated with palladium black at 280° , the residue taken up in acetic acid, and treated with 2,3-dichloro-5,6-dicyano-p-benzoquinone. From the reaction mixture was isolated a crystalline hydrochloride, m.p. 280-282° , which was identical in every respect with authentic flavocoryline hydrochloride (27): same melting point and undepressed mixed melting point; superimposible ultra-violet and infra-red spectra; identical Rf values on thin-layer and paper chromatography.

From these results the gross structure (28) could be assigned with some certainty to iso-sitsirikine, since the alternative structure with the positions of the ethylidene and β-hydroxy-ester functions interchanged was considered very unlikely on biogenetic grounds. However, the structure (28)



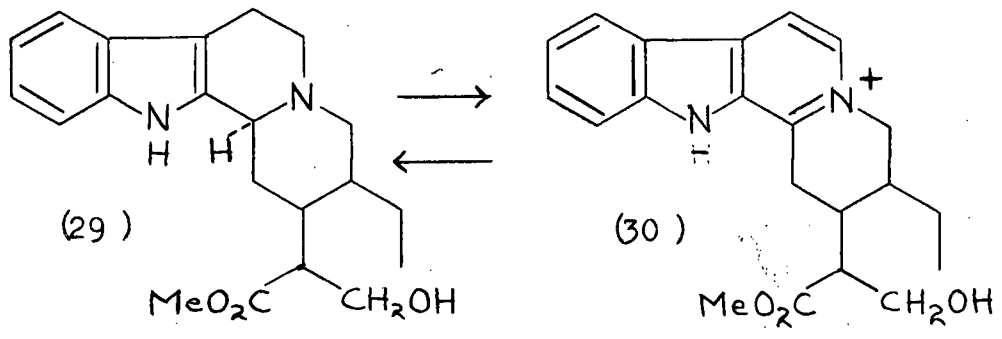
(27)



(28)

contained three asymmetric centres at C-3, C-15, and C-16 whose configurations still remained to be determined.

At first, the C-3 hydrogen atom was thought to have a β-orientation, since the infra-red spectrum of isositsirikine did not display Bohlmann bands[44] in the 2800 $cm.^{-1}$ region. However, dihydro-isositsirikine exhibited strong absorptions at 2810 and 2760 $cm.^{-1}$, and thus presumably had the α-configuration at C-3. This suggestion was substantiated when lead tetracetate oxidised dihydro-isositsirikine (29) to the tetradehydro compound (30), which on subsequent reduction with sodium borohydride regenerated the starting material. Since this sequence is known to give the isomer with the C-3 hydrogen atom in the α-orientation[46], it followed that dihydro-isositsirikine, and hence isositsirikine, must have this configuration at C-3.



The α-orientation of the hydrogen atom at C-15 could be assumed on the basis of Wenkert's empirical rule[45], but there was no way of readily finding the configuration at C-16. Therefore the structure postulated for isositsirikine was (31), and

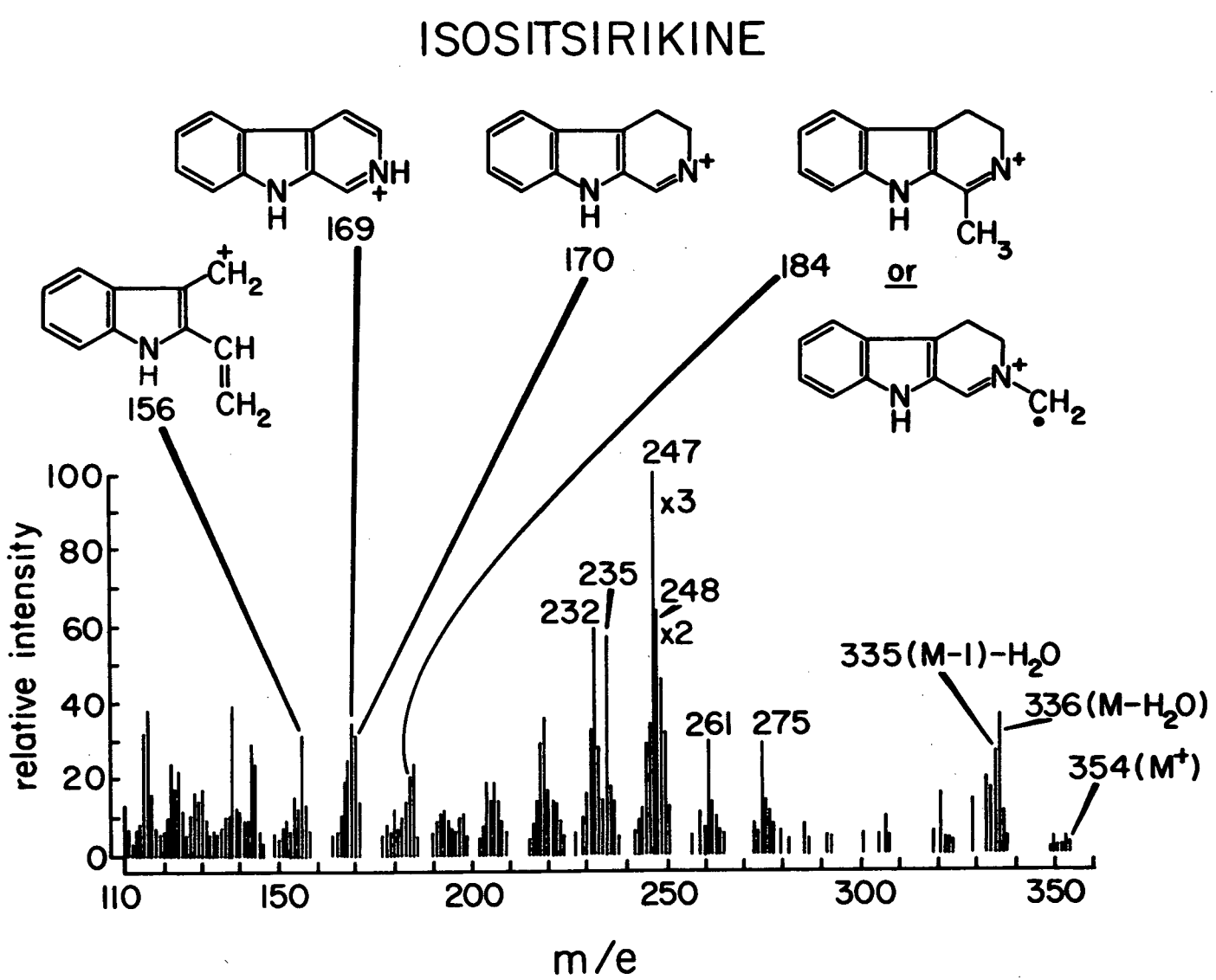
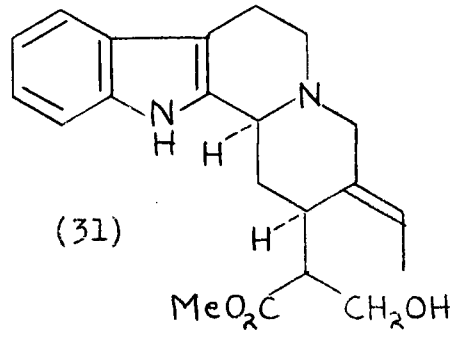

ISOSITSIRIKINE
169
170
184
or
156
247
x3
235
232
248
x2
335(M-1)-H2O
336(M-H2O)
261
275
354(M+)
relative intensity
100
80
60
40
20
0
110
150
200
250
300
350
m/e

Figure 9

it is noteworthy that this alkaloid bears a close relationship to sitsirikine (26).

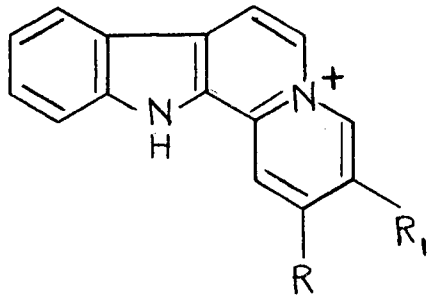


(31)

After the evidence indicated above for isositsirikine was already on hand, a mass spectrum (Figure 9) of this alkaloid was obtained. In addition to an accurate molecular weight, the mass spectrum provided valuable evidence about the structure, and thus supplemented the chemical investigations.

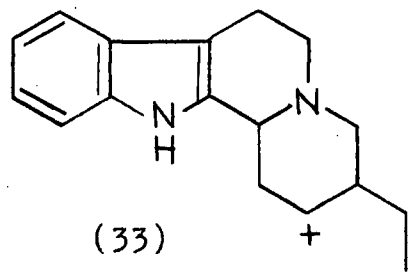
The peaks at m/e 336 ($M-H_2O$) and 335 ($M-1-H_2O$) were considerably stronger than the parent ions at m/e 354 (M^+) and 353 (M-1) — a facile dehydration compatible with the presence of a labile proton at C-16. A series of ions at m/e 184, 170, 169 and 156 corresponded to a similar sequence displayed by dihydrositsirikine, and were considered to be fragments derived from a tetrahydro-β-carboline system[40]. However, the mass spectrum of isositsirikine (Figure 9) differed markedly from the spectrum of dihydrositsirikine (Figure 8), inasmuch as a series of strong signals were obtained at m/e 275, 261, 247, 232 and 219 that were not found in the latter spectrum. These peaks could be plausibly attributed to various radical ions (32a,b,c,d, e) in which the entire tetracyclic ring structure of isositsiri-

kine had been aromatised. It must be emphasised that the

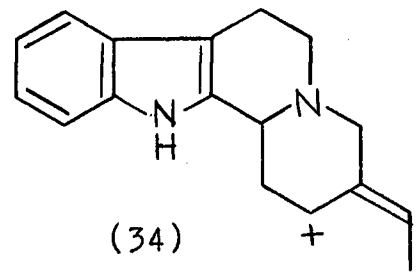


(32) a: R = R_1 = Et; m/e 275
b: R = Me, R_1 = Et; m/e 261
c: R = H, R_1 = Et; m/e 247
d: R = CH_2, R_1 = H; m/e 232
e: R = R_1 = H; m/e 219

structures shown are only tentative, since no evidence is available to decide which among several alternative structures are correct. Since these ions are not produced in the fragmentation of dihydrositsirikine (or sitsirikine) it must be assumed that the presence of a double bond exo to the D-ring leads to its ready aromatisation, and subsequently to that of the C-ring. The difference is most clearly demonstrated by the respective base peaks, both of which arise by loss of the β-hydroxy-ester group. With dihydrositsirikine this ion (33) is quite stable and is registered at m/e 251, but in the case of isositsirikine, dehydrogenation of the corresponding ion (34) occurs to give the m/e 247 fragment for which the structure (32c) is suggested.



(33)



(34)

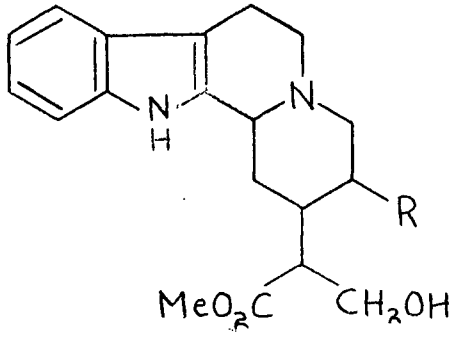
C. Other Work on Sitsirikine, Dihydrositsirikine and Isositsirikine

At about the same time as the publication of our work on sitsirikine and dihydrositsirikine[47], a report[48] appeared on the isolation of several related alkaloids from Aspidosperma oblongum A.DC. Spiteller and Spiteller-Friedmann separated trace amounts of alkaloids by means of thin-layer chromatography and postulated structures on the basis of mass spectral cracking patterns. From one fraction was obtained an olefin (M.W. 354), which on hydrogenation gave a dihydro compound (M.W. 356). A partial structure containing a tetrahydro-β-carboline system was deduced when both compounds displayed signals at m/e 184, 170, 169 and 156[40], whereas peaks at M-31 and M-59 suggested the presence of primary alcohol and methyl ester functions. Since both compounds showed a strong peak at M-103 it was tentatively assumed that the oxygen-containing groups were present as a β-hydroxy-ester unit, $-CH\begin{smallmatrix}CH_2OH\\CO_2Me\end{smallmatrix}$. Reduction of the olefin with lithium aluminium hydride gave a compound of molecular weight 326, which confirmed the methyl ester. This product also displayed a peak at m/e 251, corresponding to loss of $-CH\begin{smallmatrix}CH_2OH\\CH_2OH\end{smallmatrix}$, which substantiated the proposed β-hydroxy-ester group in the original alkaloid.

These deductions were supported by a parallel series of reactions and mass spectra on an accompanying alkaloid of molecular weight 384. This was considered to be merely a derivative of the above alkaloid (M.W. 354) with a methoxy substituent

in the aromatic ring, since the cracking pattern was similar to the above except that the peaks were shifted upwards by 30 mass units.

On the basis of these results the authors suggested the structures (35a) or (35b) for the alkaloid of molecular weight 354, and (35c) for the dihydro derivative (M.W. 356).



(35) a: R = $=CHCH_3$

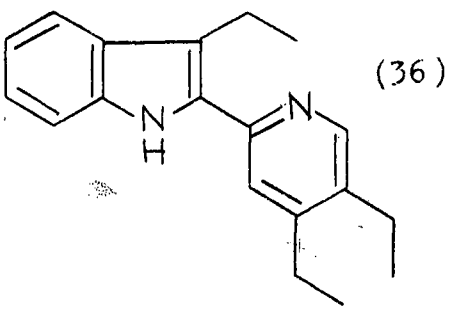
b: R = $-CH=CH_2$

c: R = $-CH_2CH_3$

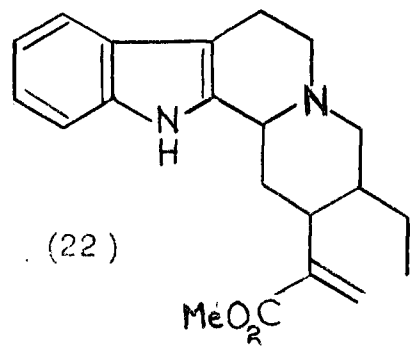
Subsequent comparison by Spiteller of the mass spectrum of the dihydro compound with the mass spectrum of our dihydro-sitsirikine indicated that they were practically identical. The small differences in the spectra were considered by Spiteller to be due to impurities in their compound, since the minute amounts available in their investigation prevented them from rigorously purifying their substance. Because the M-103 peak was larger in the olefin than in the dihydro compound, the isositsirikine structure (35a) was favoured for their parent alkaloid (M.W. 354), inasmuch as the cation (34) resulting from loss of the β-hydroxy-ester unit would be stabilised by the allylic double bond. However, our mass spectrum of isosit-sirikine was very different from that of Spiteller's alkaloid, and consequently the above rationalisation is open to question.

Some months after our publication, two Dutch workers[49] described the isolation of an alkaloid, $C_{21}H_{28}O_3N_2$, m.p. 216°, from Pausinystalia yohimbe Pierre. These authors independently derived a structure[50] which corresponded to dihydrositsirikine, and, indeed, the infra-red spectrum of their alkaloid was superimposible on that of our compound. The route by which their structure was determined was somewhat different from ours, and hence is summarised below.

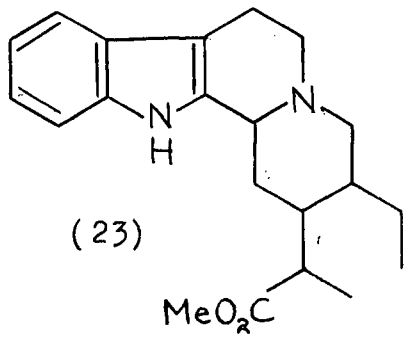
Dehydrogenation with selenium afforded alstyrine (36), which established the ring system and the position of substituents. Kuhn-Roth oxidation indicated one C-methyl group, which was considered as part of an ethyl group in view of the dehydrogenation results. The presence of a hydroxyl group was proven by formation of an acetate. From a Zeisel determination of one methoxyl group and a carbonyl band in the infra-red spectrum, a methyl ester was inferred, and this was confirmed by a saponification-reesterification sequence. The relation between the oxygen functions was elucidated by dehydration to an αβ-unsaturated ester (22) and hydrogenation to a mixture of the two desoxy-dihydrositsirikines (23). A Kuhn-Roth oxidation revealed the presence of an additional C-methyl group, and hence established a β-hydroxy-ester unit in the original alkaloid.



(36)



(22)



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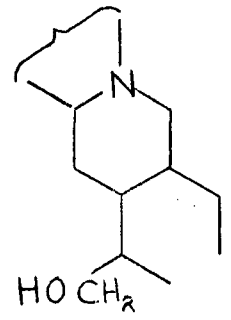


(37)

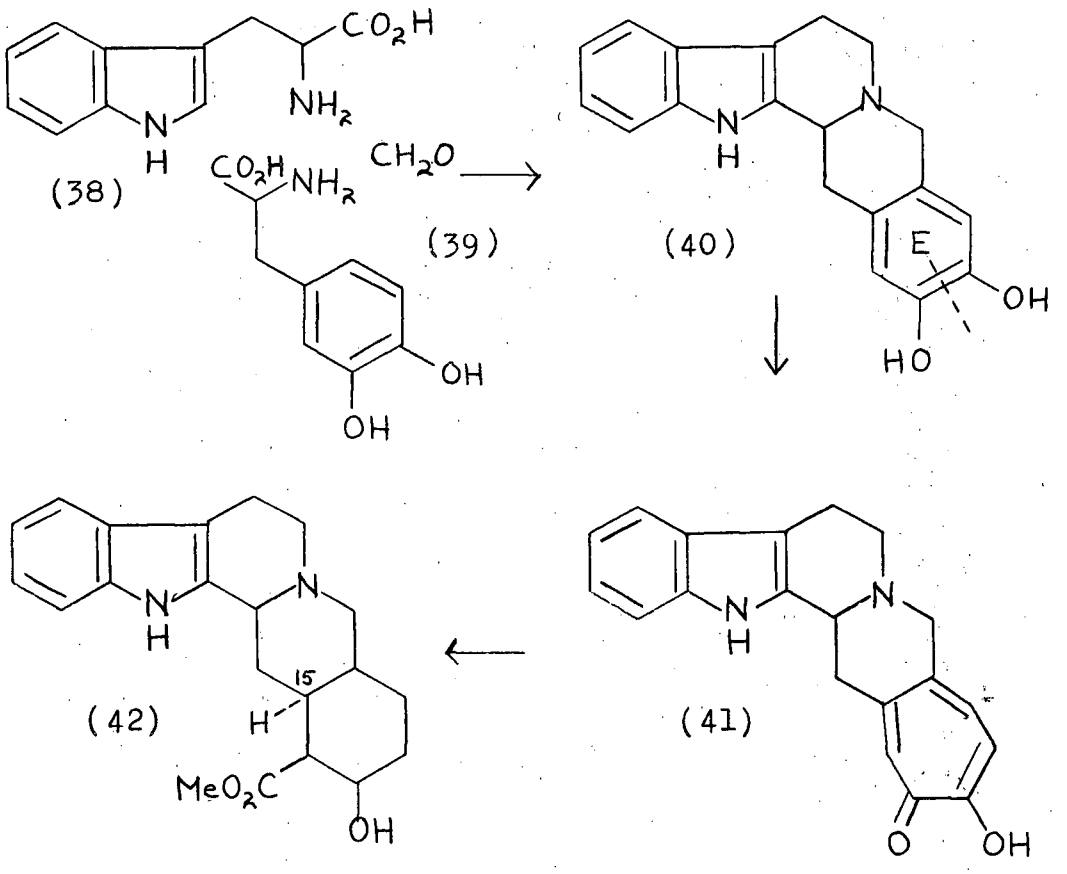
From these results a dihydrositsirikine structure (25) was deduced, and confirmed by a correlation with the known dihydrocorynantheine derivative (37). This correlation was achieved by hydride reduction of (23) to a mixture of two alcohols, one of which was isolated and found to be identical with (37).

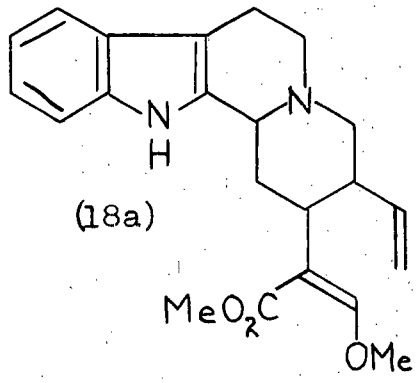
D. The Biogenesis of Yohimbine, Corynantheine and Ajmaline Type Alkaloids

It is of interest to discuss briefly some of the biosynthetic ideas pertaining to indole alkaloids, and more particularly to the corynantheine series, in order to consider the possible biogenetic relationship of sitsirikine and its relatives.For many years it has been considered that indole alkaloids related to yohimbine (42) are derived in part from tryptophan (38), a hypothesis that has been substantiated in every instance where tracer experiments have been performed; as, for example, with reserpine, ajmaline and serpentine[51]. Hence the main interest at the present time is in the non-tryptophan portion of the molecules.

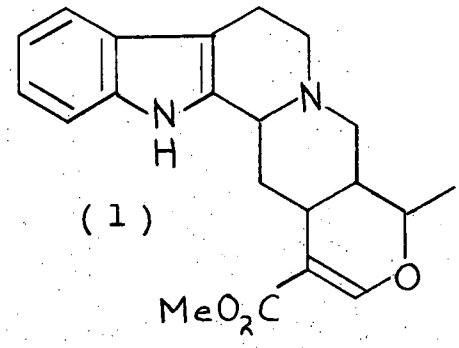
Thus in the Robinson-Woodward[52,53] theory, which is based on an earlier scheme due to Barger and Hahn[54], yohimbine (42)

is produced via the intermediate (40) formed by condensation of a dihydroxyphenylalanine unit (39) and formaldehyde with tryptophan (38). Introduction of a carbomethoxy group into (40) and appropriate reduction steps are then postulated to lead to yohimbine (42). Robinson's suggestion[52] to account for the carbomethoxy group is that the hydroxylated aromatic ring E is expanded to a tropolone (41), which then crumples to a keto-acid. A cleavage of ring E along the dotted line, known as a "Woodward fission"[55], is invoked to account for alkaloids such as corynantheine (18a), and ajmalicine (1). Subsequent ring closures are required to afford polyneuridine (13)[40] and ajmaline (43)[53].

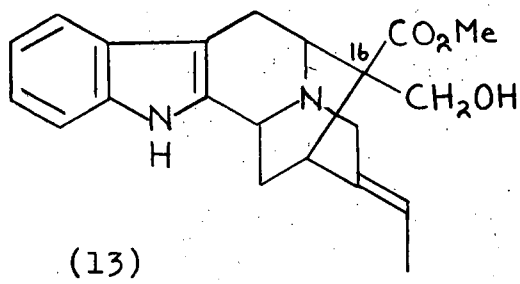




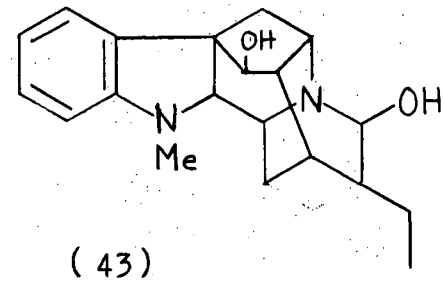
(18a)



(1)

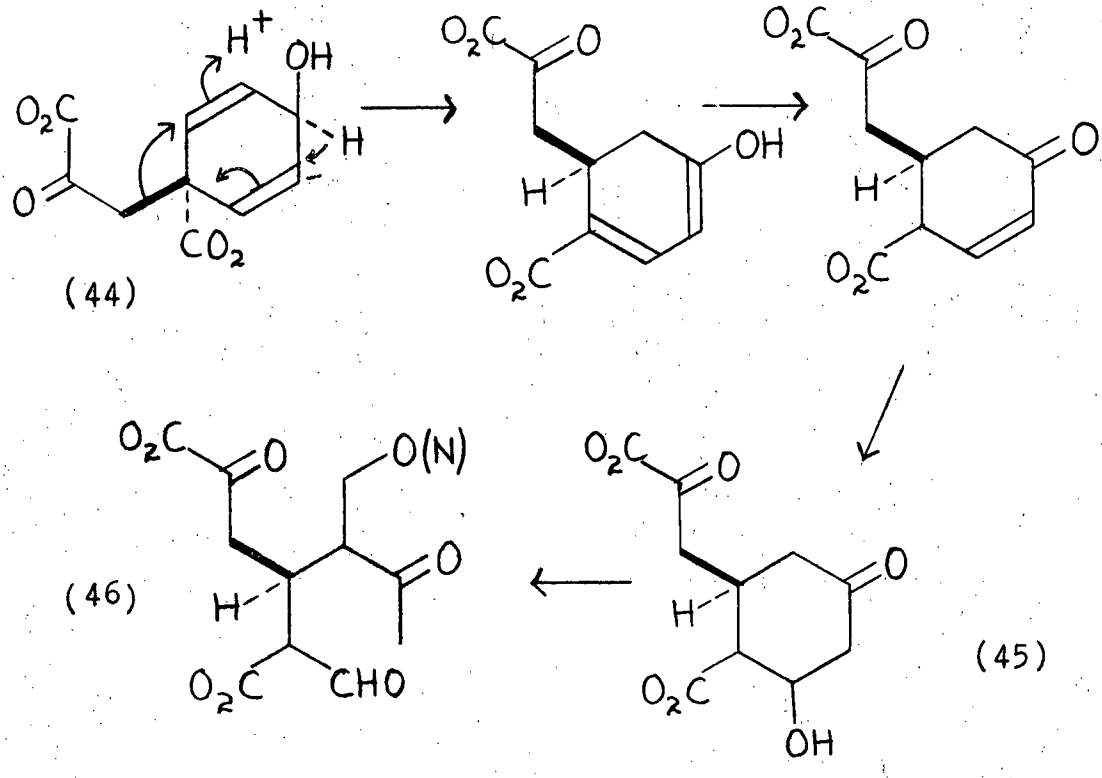


(13)



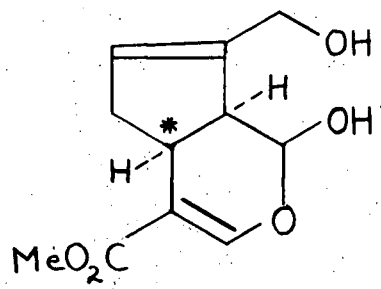
(43)

An elegant alternative scheme involving prephenic acid (44) has been elaborated by Wenkert[56]. Rearrangement of prephenic acid by a 1,2-shift of the pyruvate residue with retention of configuration, followed by hydration, affords a unit (45) readily discernible in yohimbine (42). Condensation with a formaldehyde equivalent and retro-aldolisation then yields a "seco-prephenate-formaldehyde" (SPF) group (46), that can condense with tryptamine to yield the ring-opened alkaloids typified by corynantheine (18a) and ajmalicine (1).

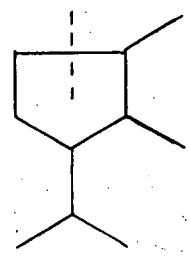


One of the attractive features of Wenkert's scheme is that it predicts the correct stereochemistry at the position corresponding to C-15 of yohimbine (42) in the various alkaloids. The hydrogen atom at this position is found to have the α-configuration in all related indole alkaloids of known stereochemistry[45], except for ψ-akuammicine[57] and the Aspidosperma alkaloids discussed later in this thesis.

A group of cyclopentane glucosides, one example of which is genipin (47)[58], has been found to have the same absolute configuration at the position [starred in (47)] corresponding to C-15 in (42). These compounds appear to have their structure based on the monoterpene unit (48), cleavage of which along the dotted line would give a skeleton analogous to the SPF unit.



(47)



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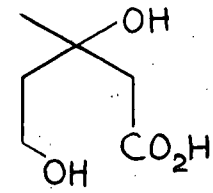
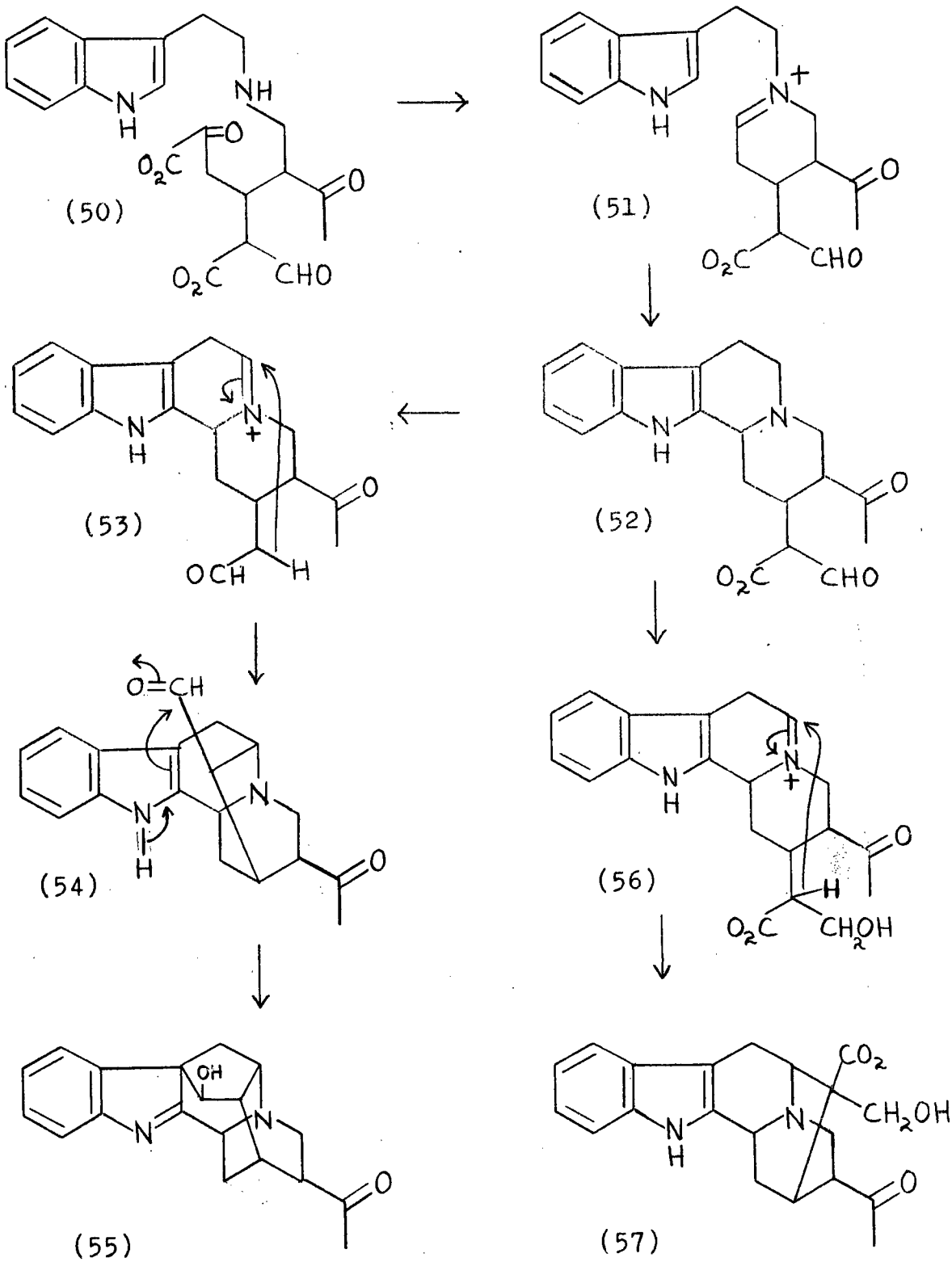


(49)

Wenkert thus considered the hypothesis (previously suggested by Thomas[59]) that the indole alkaloids may have a monoterpenoid precursor derived from mevalonic acid (49), or equally, that the cyclopentano-monoterpenes evolve from prephenic acid.

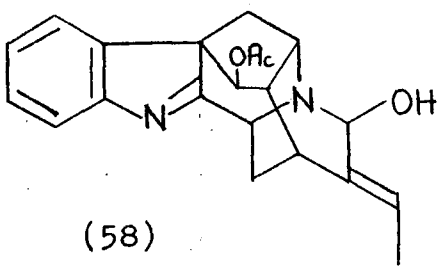
One can readily visualise the role of the SPF unit (46) in the biosynthesis of corynantheine (18a) and related alkaloids. Formation of an SPF-tryptamine complex (51) via (50) is followed by a Mannich-type condensation at the α-position of the indole system to give (52), which can then undergo appropriate modifications.

Sitsirikine (26) and its relatives constitute an interesting variation of the corynantheine series which may lie on one possible biogenetic pathway to pentacyclic alkaloids such as polyneuridine (13)[40]. The intermediate (52) proposed by Wenkert can be visualised as undergoing decarboxylation and oxidation to the immonium ion (53)[60], which then cyclises to (54). Addition of the aldehyde function to the β-position of the indole can afford a plausible precursor (55) of the ajmaline-type alkaloids such as vomeniline (58)[61]. If, however, reduction of the aldehyde group in (52) occurs before decarboxylation,


(50)
(51)
(52)
(53)
(54)
(55)
(56)
(57)

then a sitsirikine type is obtained. The corresponding immonium ion (56) can cyclise only to a pentacyclic precursor (57) of polyneuridine (13), or its C-16 epimer, akuammidine.

Recently Leete[62,63] has attempted to test the hypotheses described above by feeding labelled compounds to Rauwolfia serpentina and degrading the ajmaline (43) obtained. In both the Robinson-Woodward and the Wenkert schemes C-21 of ajmaline derived from a formaldehyde equivalent, a theory which is supported by incorporation of ^{14}C-formate at this position[62].



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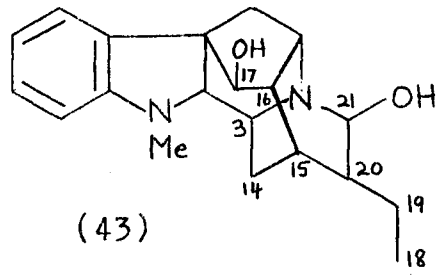


(43)

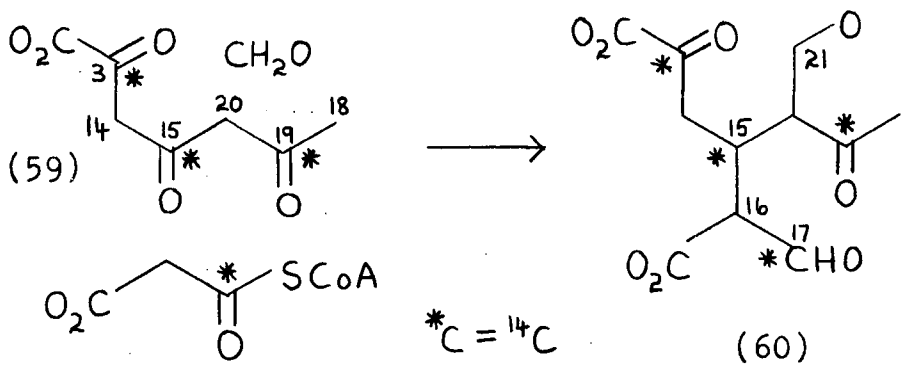
As phenylalanine is a known precursor of dihydroxyphenylalanine, it might be expected, on the basis of the Robinson-Woodward hypothesis, that administration of phenylalanine-2-^{14}C would provide ajmaline-3-^{14}C, but the ajmaline extracted was inactive, as were the reserpine and serpentine[63].

If Wenkert's prephenic acid hypothesis were correct, then alanine-2-^{14}C, which would label prephenic acid by way of pyruvate, should give activity at C-3 in ajmaline. However, only 2% of the radioactivity was attributable to this position[63].

Ajmaline isolated from a plant which had been fed mevalonate-2-^{14}C, an established precursor of terpenes, was completely inactive[63]. This result rendered unlikely another

biosynthetic route in which the non-tryptophan moiety was supposed to be formed from a monoterpene unit (48)[56,59].

A fourth hypothesis was then put forward by Leete[64], on the basis of a degradation of ajmaline, labelled by acetate-1-^{14}C incorporation, which showed that C-3 and C-19 each contained a quarter of the total activity, whereas C-21 was inactive. If it were assumed that the remaining half of the activity was shared between C-15 and C-17, then this would support a theory, previously advanced by Schlittler and Taylor[65], in which the carbon chain 18-19-20-15-14-3 originates by condensation of three molecules of acetyl-coenzyme A to a poly-β-keto fragment (59). Further condensations with a formaldehyde equivalent at C-20, and with the methylene group of malonyl-coenzyme A (derived from acetyl-coenzyme A[66]) at C-15, were postulated to afford an intermediate (60) very



similar to Wenkert's SPF unit (46). It should be emphasised that (60) would be expected to form a complex with tryptamine essentially identical to the tryptamine-SPF complex (51), and hence the latter part of Wenkert's scheme in which the various indole alkaloids are derived would still be valid.

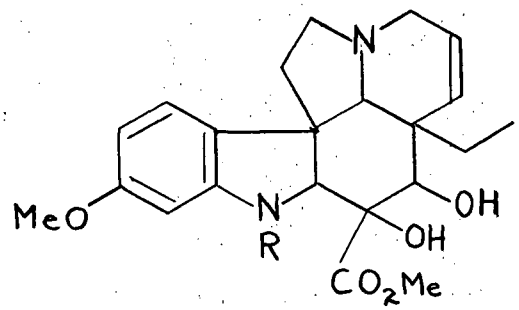
Unfortunately in a repetition of the work on R. serpentina, Battersby and co-workers[67] were unable to reproduce the above results, and it was found that the radioactive label in ajmaline (43) from both acetate and formate was scattered. Hence, at the present time no one hypothesis has been established to the exclusion of others, and the origin of the non-tryptophan portion of these indole alkaloids is still a subject of controversy.

PART II

Some Aspects of the Chemistry of Catharanthine and Cleavamine[112]

Introduction

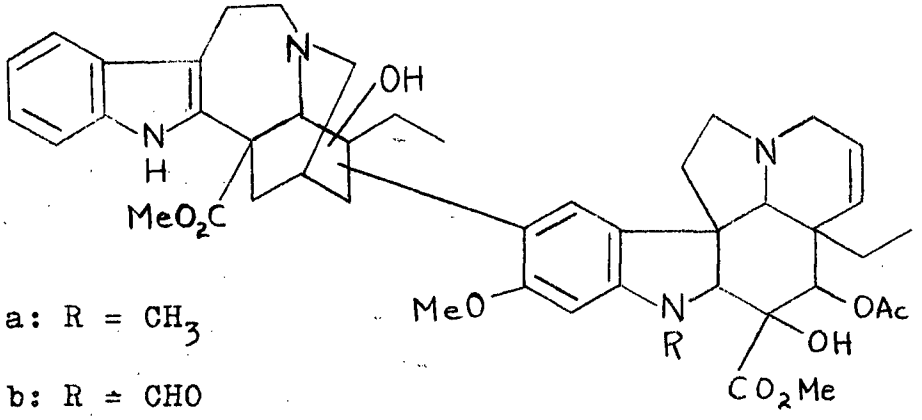
During investigations by the Lilly group[69] on the dimeric Vinca alkaloids, vincaleukoblastine (VLB), leurosine, and leurocristine, it was found that each was cleaved by concentrated hydrochloric acid to an indole compound and a vindoline derivative (61). In the instances of VLB and leurosine, the latter was desacetylvindoline (61a), whereas leurocristine gave des-$N_{(a)}$-methyl-desacetylvindoline (61b). Both VLB and leurocristine afforded the same indole derivative, velbanamine, $C_{19}H_{26}N_2O$, but the corresponding compound with leurosine was cleavamine, $C_{19}H_{24}N_2$. Velbanamine was considered to be a hydroxy-dihydrocleavamine, since it yielded some cleavamine on prolonged heating with acid[68].



(61) a: R = Me
b: R = H

When catharanthine (5)[69] was subjected to the same acid treatment, one of the products was found to be cleavamine, which suggested that the dimeric alkaloids were constituted of vindoline and catharanthine-like moieties. Moreover, the infra-red spectrum of VLB could be approximated by an equimolar mixture

of vindoline and catharanthine[70]. When the structure of these alkaloids had been established[27,28], the Lilly research group postulated the partial structure (62a) for VLB, the precise points of attachment of the vindoline unit and the position of the hydroxyl group still remaining in doubt. Leurosine was thought to be the anhydro-analogue, whereas leurocristine was probably des-$N_{(a)}$-methyl-$N_{(a)}$-formyl VLB (62b).

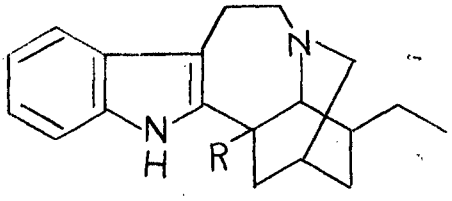


(62)a: R = CH_3

b: R = CHO

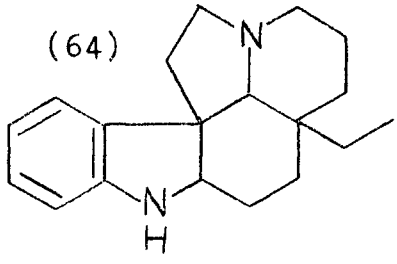
However, the identity of the indole portion of VLB could not be established directly, since it seemed that a rearrangement was taking place during the acid cleavage, and consequently the indole compound isolated did not necessarily possess the skeleton present in the original alkaloid. It therefore became imperative to establish the structure and mode of formation of cleavamine, and also to characterise the other products from the acid treatment of catharanthine. Establishment of the mechanism of the catharanthine-cleavamine transformation would furnish evidence for the structure of VLB, since catharanthine constituted an excellent model for the postulated indole moiety.

Although the study of this reaction was originally undertaken mainly in connection with the structure of VLB, the ultimate scope of the work went far beyond this aspect. Consideration of likely intermediates from a mechanistic standpoint led to the use of cleavamine analogues in the synthesis of immonium compounds, which in turn were found to undergo transannular cyclisations to Iboga (63) and Aspidosperma (64) - like skeleta.



(63) R = H or CO_2Me

(64)

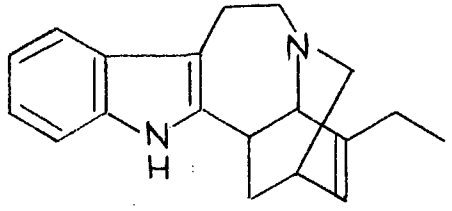


Hence the discussion of the work on cleavamine and its congeners can be divided into two sections: the catharanthine -cleavamine transformation and a possible mechanism are presented in section A, whereas the synthetic use of the cleavamines and their biogenetic implications will be discussed in section C. A review of current biosynthetic theories pertinent to the discussion on transannular cyclisations is given in section B.

A. The Catharanthine-Cleavamine Transformation

When catharanthine (5) was treated with concentrated hydrochloric acid in the presence of tin and stannous chloride, and the resulting mixture separated by chromatography, two of the products obtained were descarbomethoxycatharanthine (65) and cleavamine (66). The structure (66) of cleavamine had been

suggested mainly on the basis of a comparison of the mass spectral cracking patterns of cleavamine and dihydrocleavamine with that of quebrachamine[72] (67), and was finally established by an X-ray analysis of cleavamine methiodide[73].



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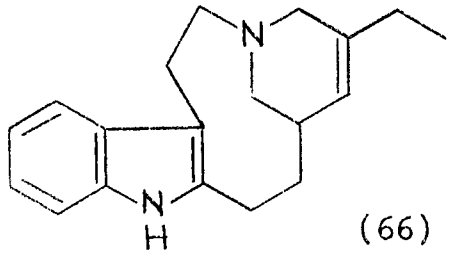

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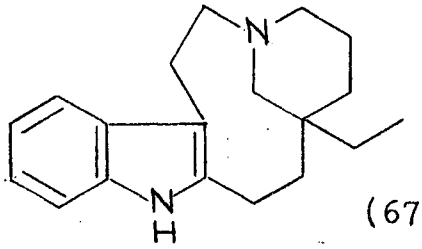

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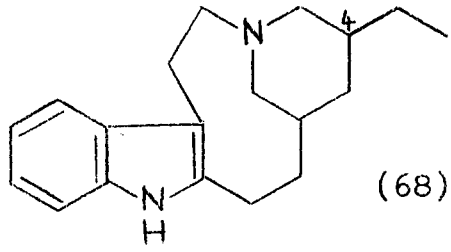
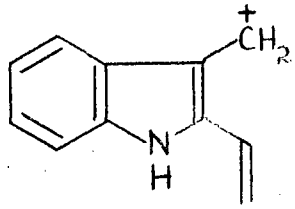


(68)

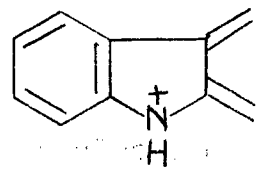
Since the combined yield of cleavamine and descarbomethoxycatharanthine was only about 20%, it was decided to examine some of the other components more closely. In particular, it was felt desirable to isolate other compounds which might provide information about the mechanism of this interesting rearrangement. The cleavamine mother liquors and several cleavamine-containing fractions were combined and subjected to a careful column chromatography. Apart from the many fractions containing intractable gums and resins, one fraction was obtained which could be well characterised. It is appropriate to discuss this in some detail, since additional evidence was furnished which was germane to any mechanistic interpretation.

This fraction, designated B9, was a mixture of two com-

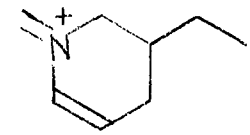
pounds, as shown by thin-layer chromatography. No olefinic protons were apparent in the N.M.R. spectrum of B9, and the methyl triplet normally present at 8.96τ in cleavamine had shifted to 9.13τ. The mass spectrum showed a molecular ion at m/e 282, and other significant peaks at m/e 156, 143, 138, 124 could be attributed to the following fragments, which are given by 4"α"-dihydrocleavamine (68)*:



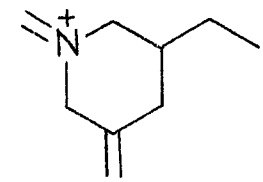
m/e 156



m/e 143



m/e 124



m/e 138

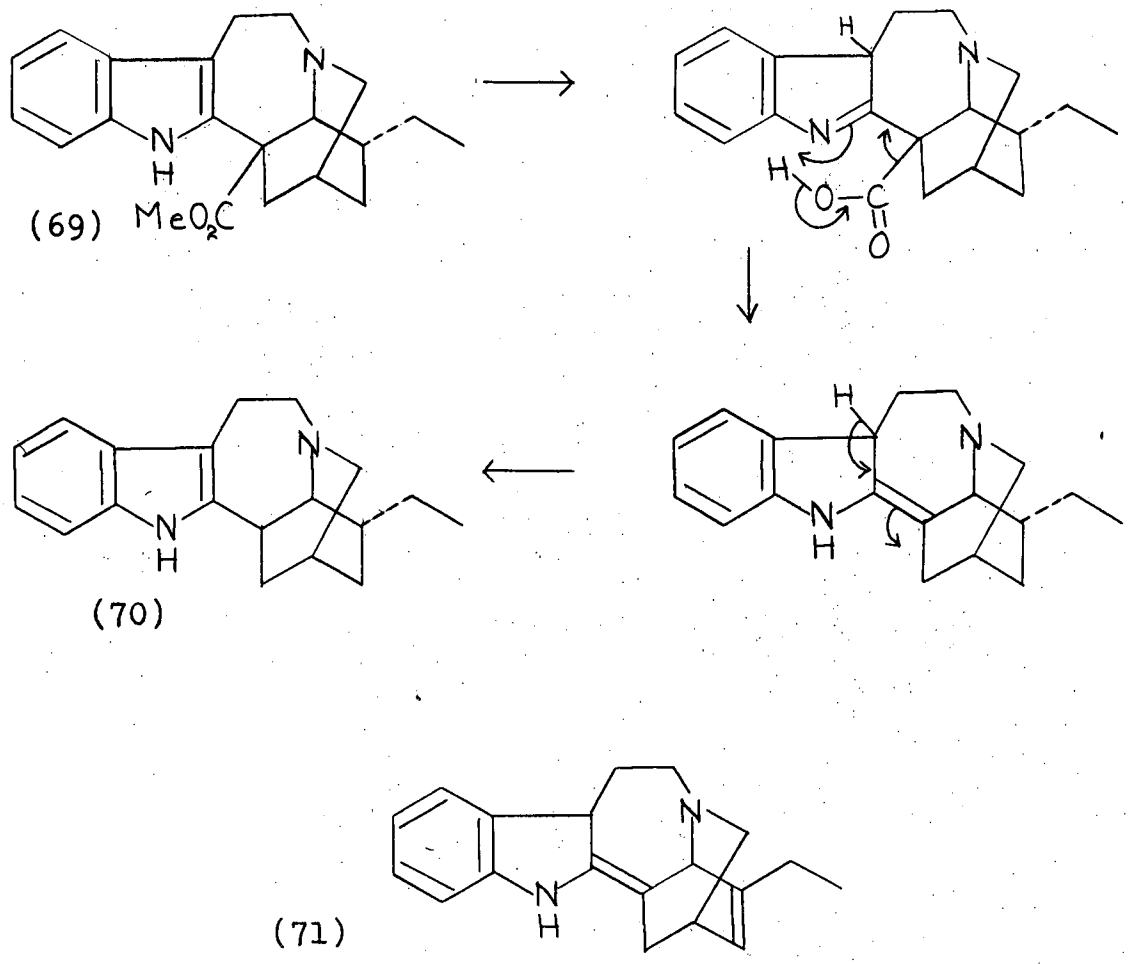
In general the mass spectrum of B9 was practically superimposible on that of 4"α"-dihydrocleavamine, and also the infra-red spectra were fairly similar. The leading spot of the mixture had the same Rf as 4"α"-dihydrocleavamine on thin-layer chromatography and the second spot was thought to be due to a dihydrocleavamine epimeric at C-4, which we designated as 4"β"-dihydrocleavamine. From other studies at the Lilly laboratories, a C-4 epimer of 4"α"-dihydrocleavamine was isolated, and a sample provided by Dr.M. Gorman, Lilly Research Laboratories for comparison purposes. It was possible to demonstrate

*For the sake of clarity, the dihydrocleavamine (68) obtained by catalytic hydrogenation of cleavamine[73] is referred to as 4"α"-dihydrocleavamine. This does not imply any definite stereochemistry, but is used merely to differentiate this compound from the corresponding C-4 epimer, 4"β"-dihydrocleavamine.

that our 4"β"-dihydrocleavamine was identical to the Lilly sample. A synthetic mixture of the dihydrocleavamines duplicated the behaviour of fraction B9 on thin-layer chromatography and gave an identical infra-red spectrum.

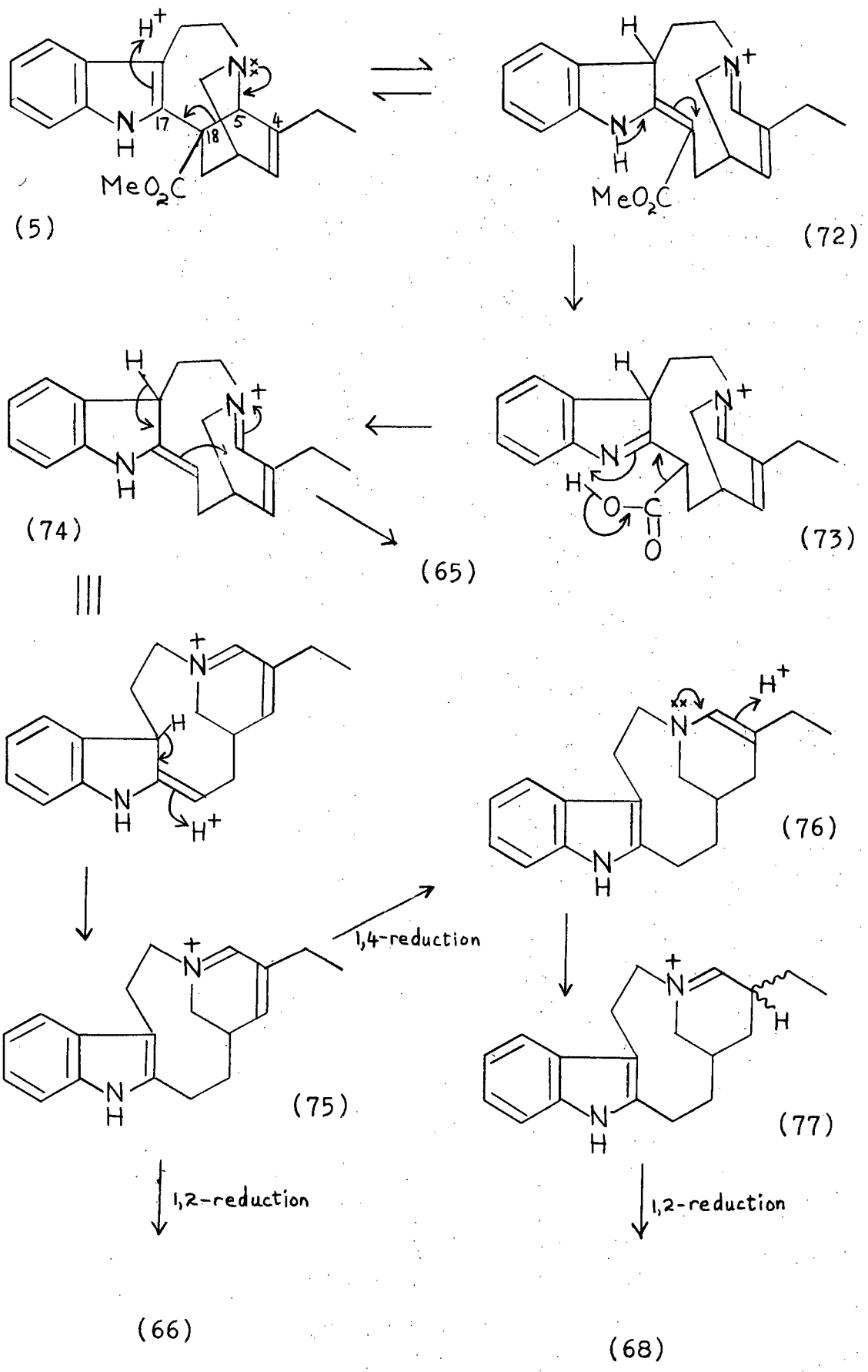
Two dihydrocleavamines epimeric at C-4 could be obtained in this reaction either from dihydrocatharanthine (present as an impurity or formed by reduction of catharanthine), or by reduction of cleavamine (or an equivalent reaction intermediate). Since the catharanthine was homogeneous, and in any case dihydrocatharanthine (69) had been shown to decarboxylate without formation of dihydrocleavamine[113], the former possiblity could be dismissed. We were thus left with the latter alternative, that had to be incorporated into a mechanism which would account for the formation of cleavamine (66), descarbomethoxycatharanthine (65) and the two dihydrocleavamines (68).

Some other results pertinent to any proposed mechanism had been obtained by the Lilly group[113]. Dihydrocatharanthine (69) was decarboxylated readily to epi-ibogamine(70) by heating with hydrazine in ethanol[74], or by hydrolysis with either aqueous potassium hydroxide or lithium iodide in pyridine followed by heating with dilute mineral acid[75]. These results fitted the proposed mechanism[75] for the decarboxylation of Iboga alkaloids:



However, none of these procedures was successful in decarboxylating catharanthine (5), presumably because the corresponding intermediate (71) would be too strained to form[113].

Therefore, the formation of descarbomethoxy-catharanthine (65), albeit in poor yield, upon treatment of catharanthine (5) with concentrated hydrochloric acid must involve some other mechanism. In order to explain the occurrence of cleavamine (66) and the epimeric dihydrocleavamines (68) a ring-opened intermediate must be present at some stage, and moreover, a route has to be provided whereby the olefinic linkage present in catharanthine can be reduced. With these considerations in mind we postulate the speculative mechanism on p. 51


(5)
(72)
(74)
(65)
(73)
(76)
(75)
(77)
(66)
(68)
1,4-reduction
1,2-reduction
1,2-reduction

for the reaction.

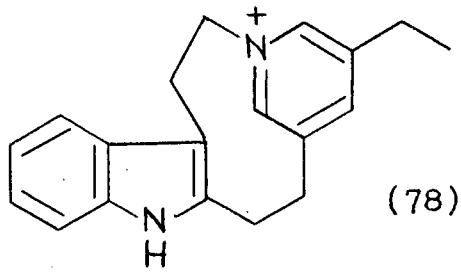
The lone pair of electrons on the $N_{(b)}$-atom in (5) can participate in a rearrangement to form an immonium ion, with concurrent ring cleavage and protonation at the β-position of the indole. The resulting ring-opened intermediate (72) is stabilised by two factors: (i) the allylic nature of the immonium ion, and (ii) the conjugation of the newly generated double bond between C-17 and C-18 with both the ester and anilino functions. After acid hydrolysis of the ester, decarboxylation may then occur via (73) in an analogous manner to the Iboga alkaloids. Absence of a C-5/C18 bond renders the molecule more flexible, and the decarboxylated product (74) is obtained, whereas the corresponding intermediate (71) required in the usual mechanism (see above, p. 50) cannot be formed.

The crucial intermediate (74) may follow either of two reaction paths. If the original electron flow is reversed, then the C-5/C-18 bond is regenerated and the product will be descarbomethoxycatharanthine(65). But if there is merely an allylic shift of a proton (perhaps because the immonium system has already been reduced) then the ring-opened tetracyclic compounds must ultimately be formed. Assuming that (75) is the actual intermediate, 1,2-reduction of the immonium ion will give cleavamine directly. On the other hand, 1,4-reduction can also occur to afford an eneamine (76), which rearranges in the well-known manner[76] to the immonium compound (77) with subsequent reduction to (68). A mixture of 4"α"- and 4"β"- di-

hydrocleavamines (68) is obtained because the approach of the proton to C-4 in (76) can occur from above or below the plane of the ring with essentially equal facility.

The reduction of catharanthine with zinc in glacial acetic acid to carbomethoxy-4"β"-dihydrocleavamine (119) showed that the reduction and decarbomethoxylation were separate processes. Much more substantial support for the mechanism, however, was the transannular cyclisation of an immonium ion derived from carbomethoxy-4"β"-dihydrocleavamine to an Iboga skeleton (see section C, p.79). This demonstrated that a similar cyclisation proposed for the formation of descarbomethoxy-catharanthine (65) from the intermediate (74) was actually feasible.

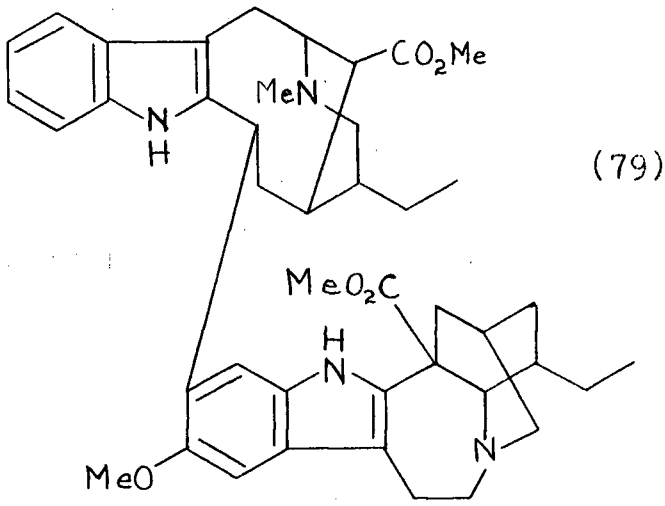
In the absence of an inorganic reducing agent, the reduction step possibly takes place via an intramolecular redox reaction of two molecules of the intermediate (75) to yield one molecule of cleavamine (66) and one of a pyridinium compound (78). The increase in yield of cleavamine in a reducing medium is thus explicable on the grounds that (75) is reduced directly to cleavamine and no pyridinium compound is formed.



(78)

Further interest in the chemistry of cleavamine was

stimulated by the recent work of Büchi and co-workers[77] on voacamine (79) which indicated that the original structure (62a) proposed by the Lilly group for VLB was probably wrong, and suggested that the indole moiety was a cleavamine (66) rather than a catharanthine (5) type.

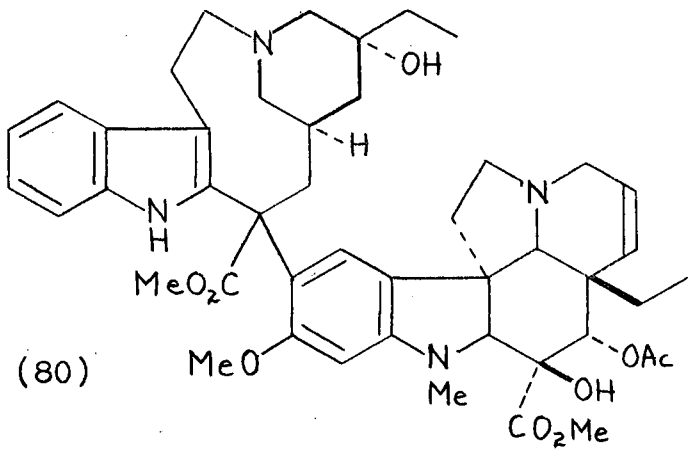


(79)

It was noted that voacamine also represented a "dimeric" alkaloid constituted from two indole moieties. Even more important was the observation that voacamine could be cleaved into the respective monomeric units by means of acidic reagents under conditions similar to those used for VLB. The carbon-carbon bond linking the two halves is labile and is ruptured during acid treatment.

One of the weak features in the Lilly structure (62) for VLB had been the nature of the linkage between the indole and dihydro-indole portions. As mentioned before, VLB is cleaved by acid into desacetylvindoline and hydroxy-dihydro-cleavamine[69], but it was difficult to rationalise such a fracture on the basis of structure (62). One would not expect

a bond comprised of an aliphatic carbon on one hand and an aromatic carbon on the other to react in this manner. Consideration of the chemistry of voacamine led to a review of the structure for VLB and further studies were undertaken. A high-resolution mass spectrum gave a molecular weight for VLB of 810.4219, which showed that the correct formula was $C_{46}H_{58}O_9N_4$ and not $C_{46}H_{56}O_9N_4$ as previously thought[78]. Thus VLB must contain a carbomethoxy-cleavamine rather than a catharanthine unit as the indole portion of the molecule. On the basis of this and other evidence, a revised structure (80) was very recently proposed for VLB[68].



(80)

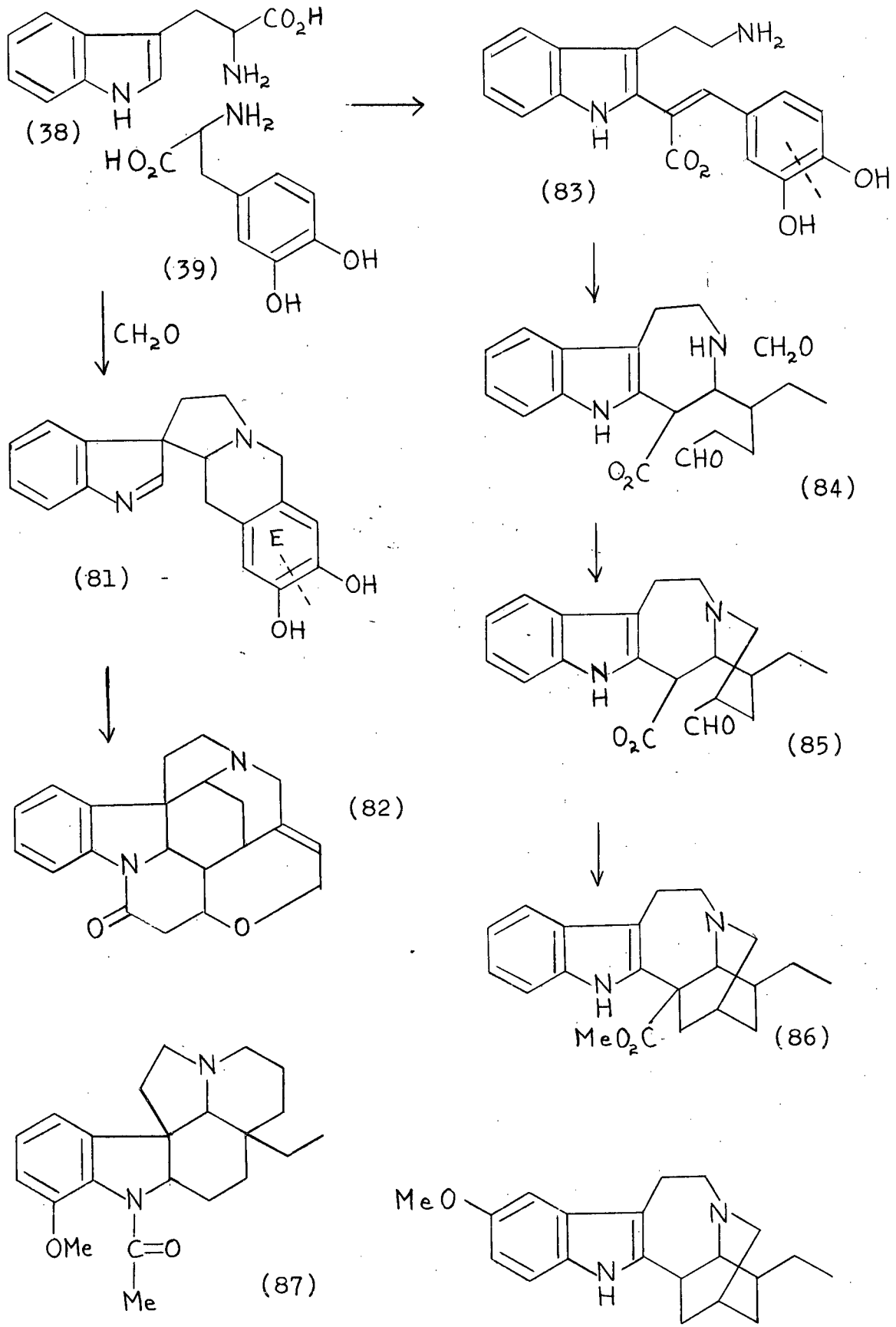
Although the study of the catharanthine-cleavamine transformation was initiated to throw light upon a corresponding reaction thought to occur with VLB and its congeners, it was also realized at an early stage of the investigation that the results were of wider potential interest in the areas of synthesis and biogenesis of indole alkaloids. These aspects were subsequently considered in some detail, and are discussed in the following sections.

B. The Biogenesis of Strychnos, Iboga and Aspidosperma Alkaloids

In order to be in a position to discuss fully the results of the transannular cyclisations (section C), it is pertinent to review briefly the various theories advanced for the biosynthesis of alkaloids of the Strychnos, Iboga and Aspidosperma species, as typified by strychnine (82), coronaridine (86) and aspidospermine (87).

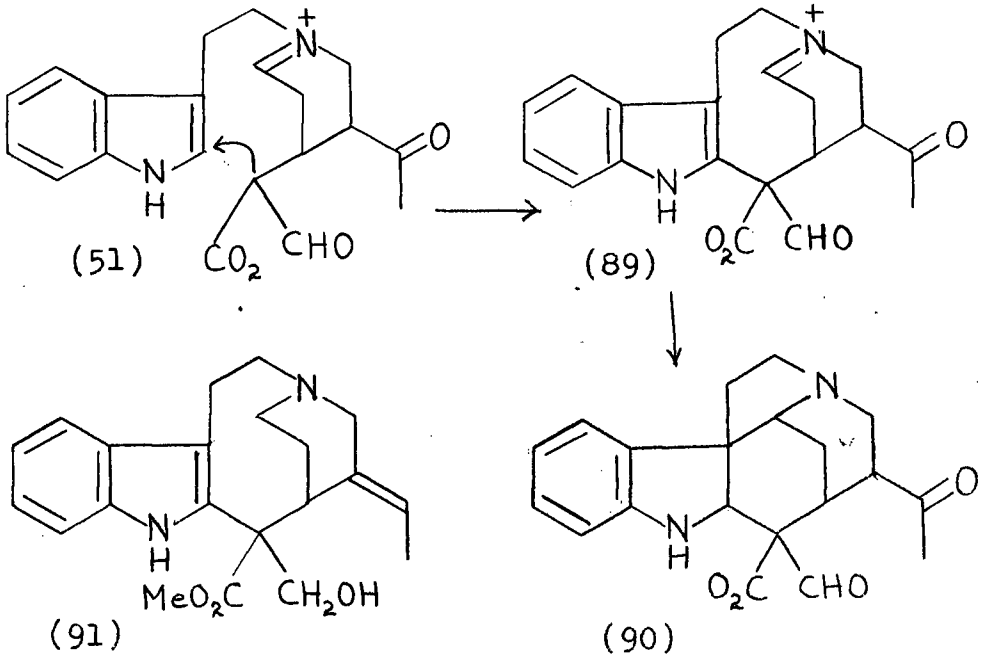
These compounds are considered to be related biogenetically (see below), and indeed have been found to occur together in the same plants, as for example Vinca rosea Linn. (see introduction to this thesis) and Stemmadenia donell-smithii (Rose) Woodson[71,86]. It has recently been demonstrated that labelled tryptophan (38) is incorporated into vindoline (7)[27] and ibogaine (88)[79], and hence one may assume that the related alkaloids are constructed in part from tryptophan.

Strychnine (82) may thus be derived, according to the Robinson-Woodward theory[52,53] mentioned earlier (p.35), from a condensation of a dihydroxyphenylalanine unit (39) and formaldehyde with tryptophan (38) to afford the intermediate (81), which undergoes subsequent "Woodward fission"[55] of ring E (along the dotted line) and appropriate cyclisations to provide the Strychnos skeleton. In order to accommodate the Iboga alkaloids, a variation of this above scheme has been proposed by Taylor[80], whereby condensation of tryptophan (38) and 3,4-dihydroxyphenylalanine (39) affords the $\alpha\beta$-unsaturated acid (83). The latter intermediate by a Michael addition, and

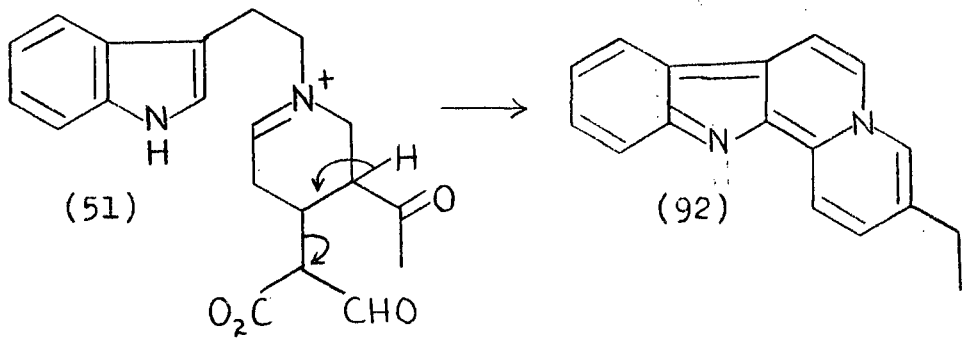

(38)
(39)
(81)
(82)
(83)
(84)
(85)
(86)
(87)
(88)
CO_2H
NH_2
HO_2C
OH
CH_2O
E
OMe
C=O
Me
CO_2
HN
CHO
MeO_2C
MeO

Woodward fission of the aromatic ring, provides the intermediate (84), which then participates with formaldehyde in a Mannich reaction to yield the tetracyclic compound (85). Subsequent aldol condensation, dehydration, and reduction lead to the Iboga skeleton (86).

A more comprehensive scheme for the Strychnos and Iboga alkaloids, that has the additional merit of encompassing the Aspidosperma series, is furnished by Wenkert's prephenic acid hypothesis[56], which was discussed earlier (p.37) in relation to the corynantheine-type bases. According to this theory, the strychnine (82) group evolves from the tryptamine-SPF complex (51) by attack of the formyl acetate residue at the α-position of the indole to give the immonium ion (89), which bears on obvious resemblance to the known alkaloid stemmadenine (91)[89]. A transannular cyclisation then provides the strychnine precursor (90).

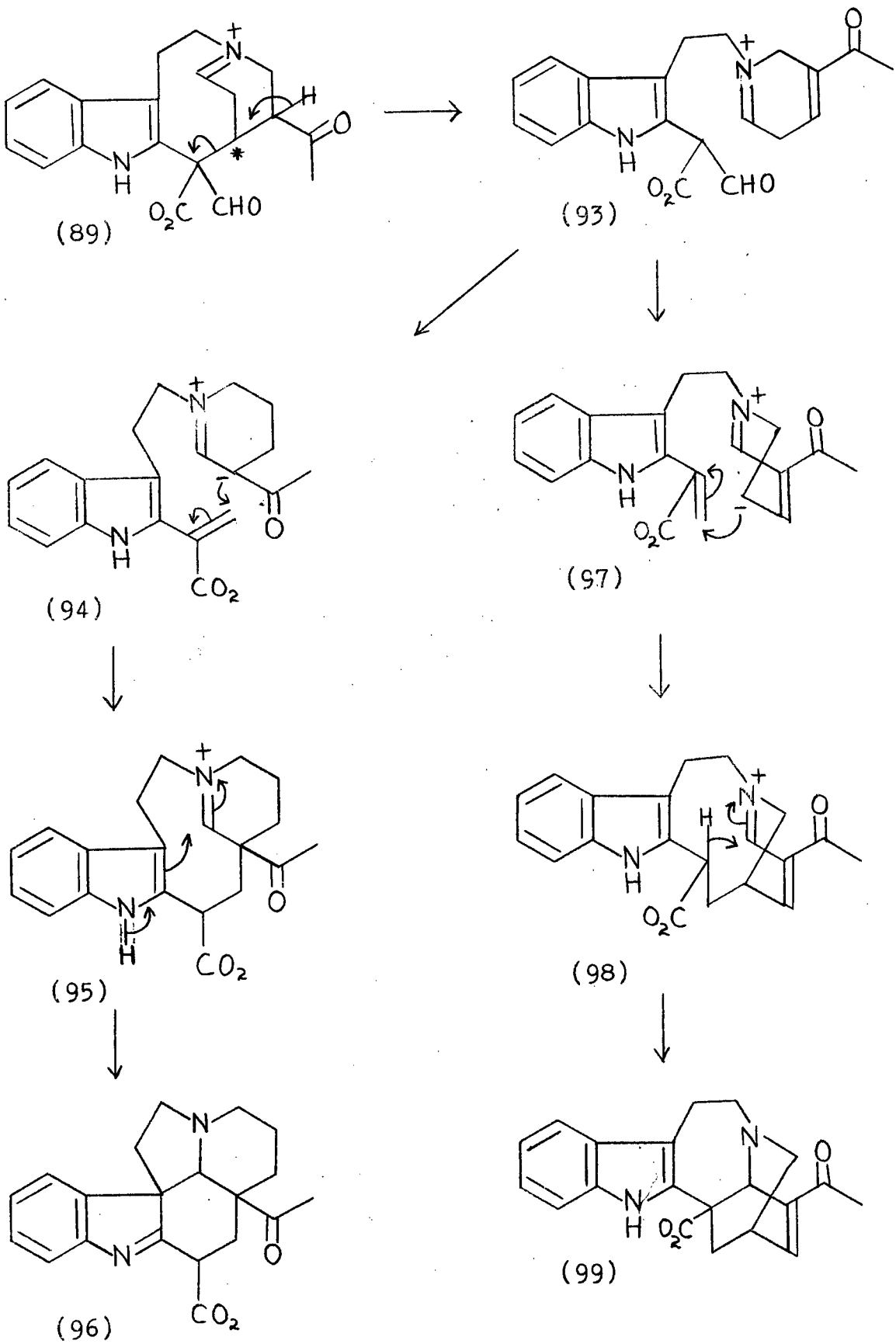


Derivation of the Iboga and Aspidosperma systems is less straightforward, since it is evident that they arise from rearranged SPF units. The crucial rearrangement can be seen as proceeding via a retro-Michael reaction of the interme-

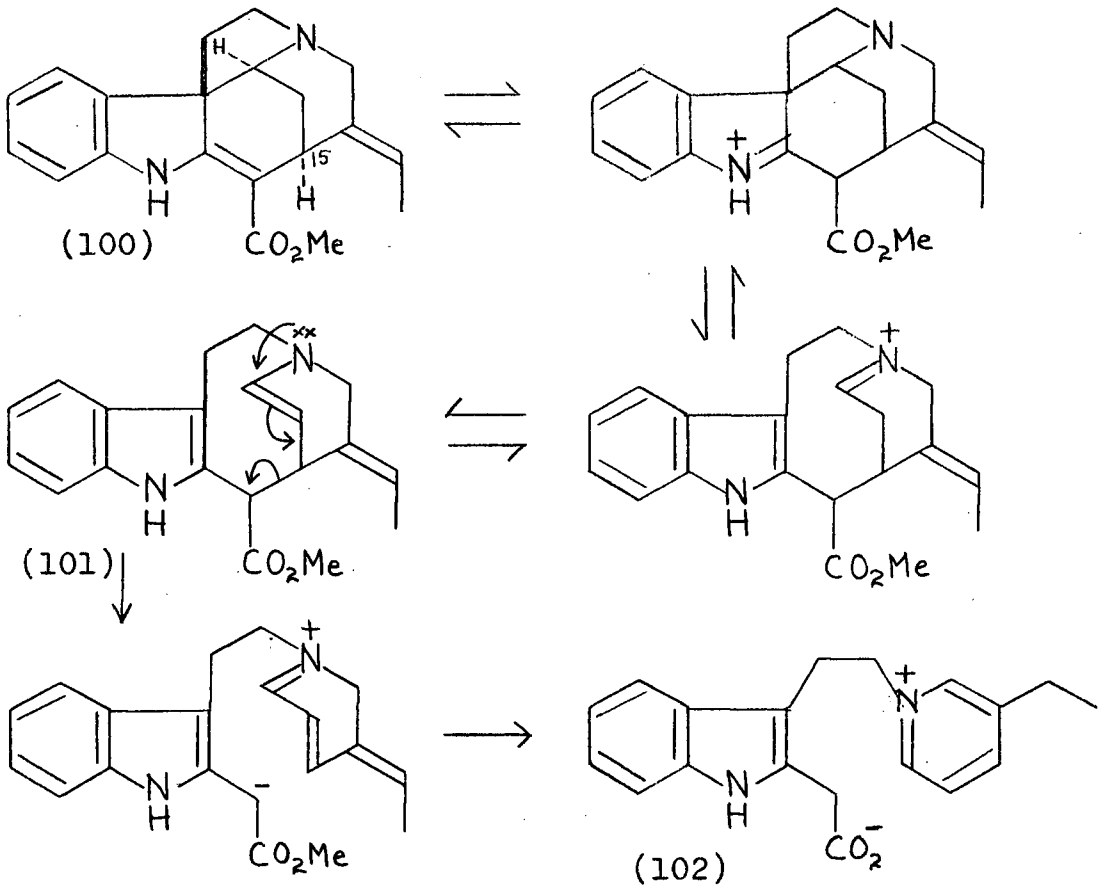


diate (89), involving an activated hydrogen atom on a carbon atom α to either the immonium system or to the acetyl group, with resultant cleavage of the SPF unit. If the original tryptamine-SPF complex (51) underwent this reaction, the formyl acetate residue would be lost and a pathway to the flavopereirine (92) structure revealed. However, were the retro-Michael process to occur at a later stage, when the formyl acetate moiety could not be lost because of its attachment to the indole system, as in (89), then the cleavage product (93) could be modified by unexceptional reactions to give either an Aspidosperma (94) or an Iboga (97) precursor. These compounds could then undergo a parallel series of reactions: Michael additions to the αβ-unsaturated acid systems would afford the nine-membered ring compounds (95) and (98), which could then, by transannular cyclisations, yield the Aspidosperma and Iboga skelata, (96) and (99) respectively.

No direct proof of Wenkert's hypothesis has been pub-

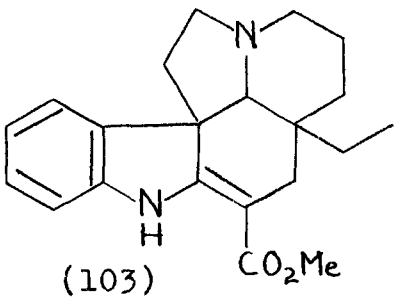

(89)
(93)
(94)
(97)
(95)
(98)
(96)
(99)

lished to date, but the latter portion of his proposal is supported by a considerable weight of circumstantial evidence. For instance, the retro-Michael reaction of a strychnine-type alkaloid is exemplified by a cleavage that Smith and Edwards[81] have found to occur with akuammicine (100). In order to explain the formation of the betaine (102) when akuammicine was heated in methanol at 100° for three hours, the authors proposed a mechanism involving a retro-Michael cleavage of an intermediate such as (101).

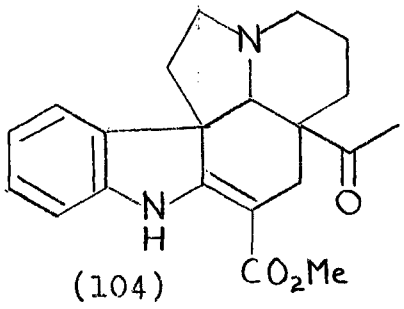


It is worthy of note that Wenkert predicted the occurrence in Nature of Aspidosperma alkaloids carboxylated as in (96) and this has since been verified by the isolation of

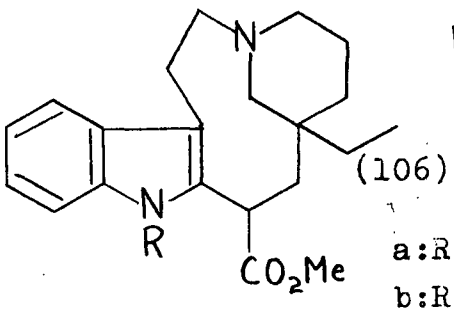
several alkaloids related to vincadifformine (103). Perhaps the best example is minovincine (104)[82], which possesses not only a carbomethoxy group in the predicted position but also the acetyl function. Counterparts of the conjugated enone system in the postulated Iboga-type precursor (99) have also been found: the double bond in catharanthine (5), and the carbonyl group in voacryptine[83] (105). Furthermore, the ring-opened precursor (95) is represented in Nature by vincadine (106a)[84], and vincaminorine (106b)[90], whereas the carbometh-



(103)

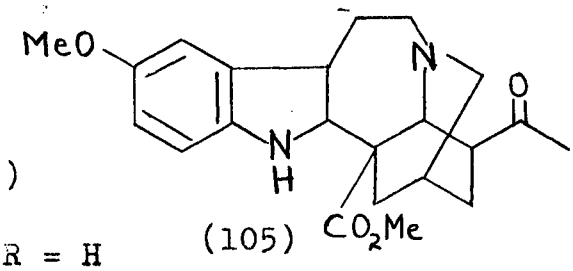


(104)



(106)

a:R = H
b:R = Me



(105)

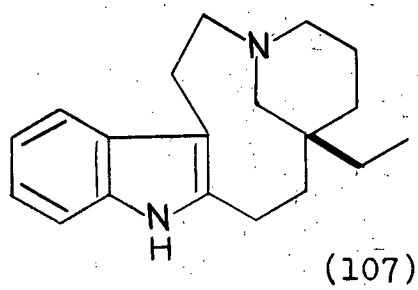
oxy-cleavamine portion of the VLB molecule (80) is readily derived from (98).

A significant point in favour of the above scheme is that it predicts the correct absolute configuration at C-15 in akuammicine (100), which has been found to be constant in this and related Strychnos alkaloids[89]. The sole exception

is the d,l mixture ψ-akuammicine, the formation of which can be attributed to a reversible transformation of (89) to (93) prior to the complete evolution of the former to the Strychnos system. The occurrence in Nature of racemic vincadifformine (103)[91,92], the optical antipodes of quebrachamine (107)[85], (108)[86], and of vincadifformine (103)[82,92], and the enantiomeric alkaloids (—)-O-methylaspidocarpine (113)[87] and (+)-pyrifolidine (114)[88] can be interpreted on the basis of the above biosynthetic scheme. Since intervention of the non-asymmetric intermediate (93) in Aspidosperma biosynthesis destroys the fixed configuration of the starred position in (89), no optical relationship can exist between the alkaloids of this family and other indole bases. Randomisation of the absolute configuration of Aspidosperma alkaloids is therefore considered by Wenkert to be due to a lack of optical consistency in the Michael reaction (94) to (95).

Evidence which tends to support the postulated transannular cyclisation of (95) to (96) has been accumulated in studies of the relative configurations of various Aspidosperma alkaloids. Some of these have been correlated with (-)-aspidospermine (115), whose absolute configuration is known from the X-ray structure determination by Mills and Nyburg[93]. (—)-Quebrachamine has been shown to have the same configuration as aspidospermine at the asymmetric centre involving the ethyl group[94], and hence has the structure (107); the epimeric (+)-quebrachamine must then be (108).

It is attractive to speculate that the Aspidosperma alkaloids may be divided into two stereochemical series, one of which is theoretically derived by a transannular cyclisation of (−)-quebrachamine (107), the other by cyclisation of the (+)-enantiomer (108). The reverse processes can



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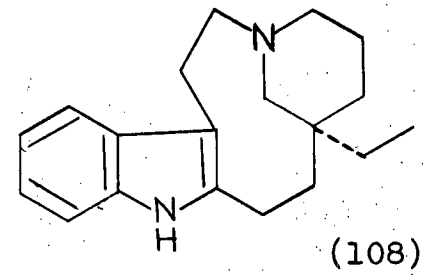
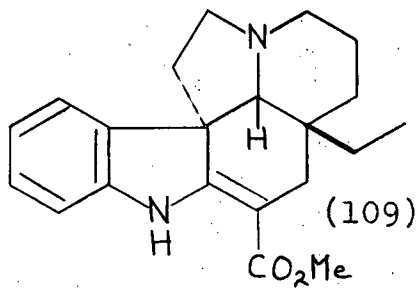


(108)



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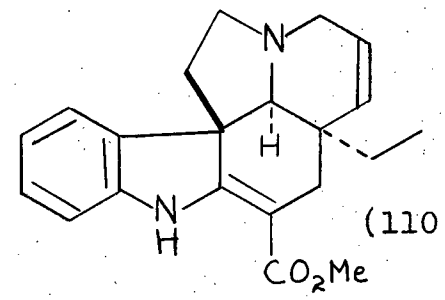
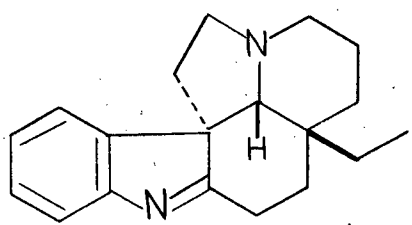
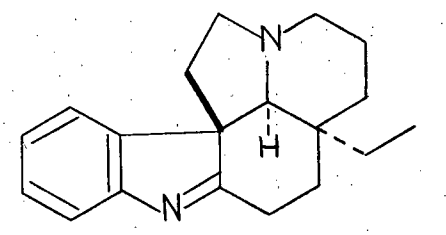


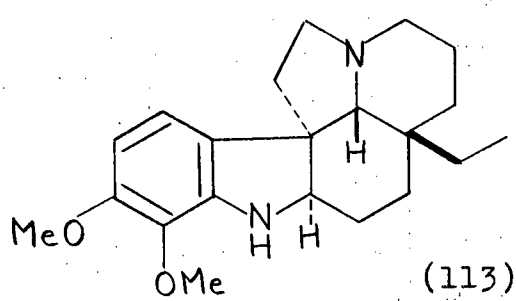
(110)



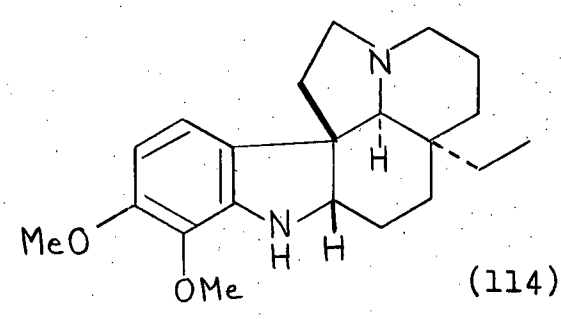
(111)



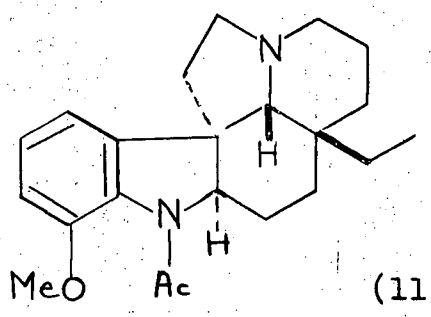
(112)



(113)



(114)



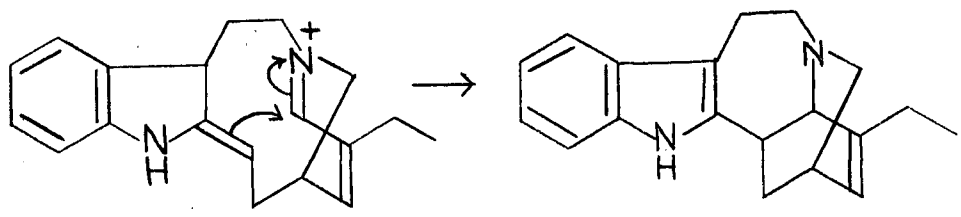
(115)

often be achieved in the laboratory[92,94,95]. In several cases the orientation of the ethyl equivalent of the acetyl group in (95) and (96) seems to be characteristic of the alkaloids of a particular plant species. Thus (—)-quebrachamine (107) has been found in conjunction with (—)-aspidospermine (115) and (—)-pyrifolidine (113) [(—)-O-Methyl-aspidocarpine] in Aspidosperma quebracho blanco[85], and with (+)-vincadifformine (109) and (+)-1,2-dehydroaspidospermidine (111) in Rhazya stricta[92]. On the other hand, (+)-quebrachamine occurs together with (—)-tabersonine (110) [(—)-6,7-dehydrovincadifformine] in Stemmadenia species[95], and with (-)-1,2-dehydroaspidospermidine (112) in Pleiocarpa tubicina[96]. The stereochemistry seems to be determined by the orientation of the acetyl group in the quebrachamine-like precursor (95) since the Mannich-type closure of the nine-membered ring requires formation of a cis-perhydroquinoline system in (96). Furthermore, Wenkert[56] suggested that the cis-anti-cis backbone exhibited by the Aspidosperma alkaloids may be the consequence of the Mannich condensation (and any subsequent reduction) following the path of least steric resistance. The isolation of stereochemically related alkaloids in the same

plant certainly supports this suggestion, and furnishes circumstantial evidence for the occurrence in Nature of a transannular cyclisation such as postulated in Wenkert's hypothesis. Experimental evidence which demonstrates the feasibility of such cyclisations will be presented in section C.

C. Transannular Cyclisations of Cleavamine Derivatives

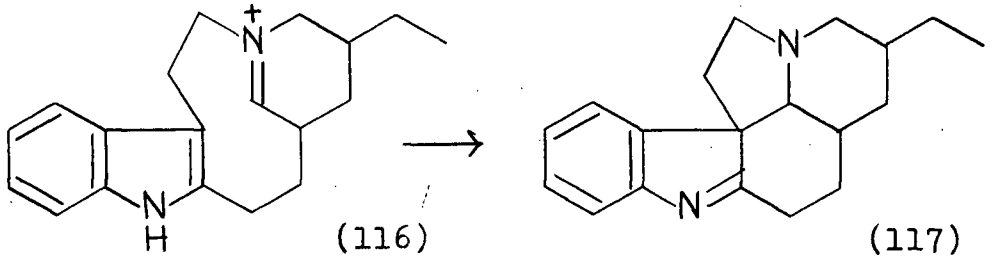
A consideration of the mechanistic aspects of the catharanthine (5)—cleavamine (66) transformation led us in turn to examine the various biosynthetic hypotheses that have been outlined above. In particular, our interest was drawn to the part of Wenkert's scheme dealing with the Iboga and Aspidosperma alkaloids, which was found to have direct relevance to the work on cleavamine. First of all, when one considered the conversion of intermediate (98) to the pentacyclic structure (99) of the Iboga alkaloids, it was apparent that the transannular cyclisation of a cleavamine-like skeleton was involved. This was immediately reminiscent of an identical transannular cyclisation of (74) that had been proposed in the mechanism (p.51) to explain the formation of descarbomethoxycatharanthine (65). Secondly, the intermediate (95)



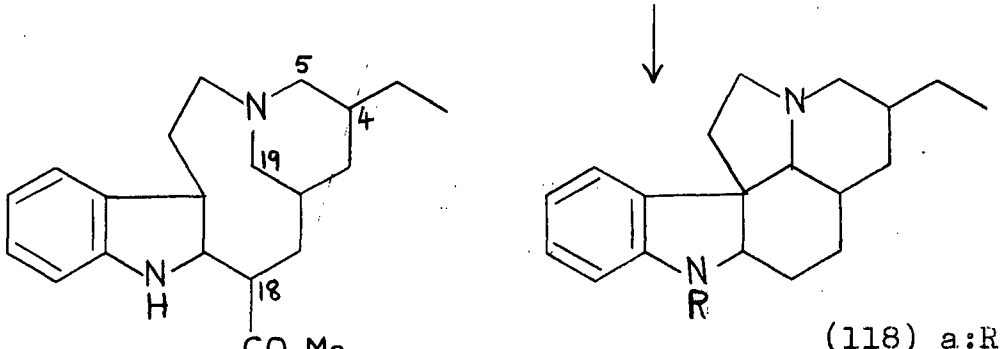
(74) (65)

advanced by Wenkert as the direct precursor of the Aspidosperma system (96) was again similar to cleavamine (66), essentially differing only in the position of the ethyl group. It was clear that cleavamine or one of its derivatives might be converted into an immonium intermediate of the type proposed by Wenkert, and thereby afford an excellent opportunity for evaluating the feasibility of such transannular cyclisation processes.

Accordingly, oxidation of 4"α"-dihydrocleavamine (68) with mercuric acetate gave an immonium ion (116), which underwent transannular cyclisation to an Aspidosperma-like skeleton (117). This could not be isolated as such, but the corresponding dihydro-indole (118a) was obtained after reduction with lithium aluminium hydride[97].



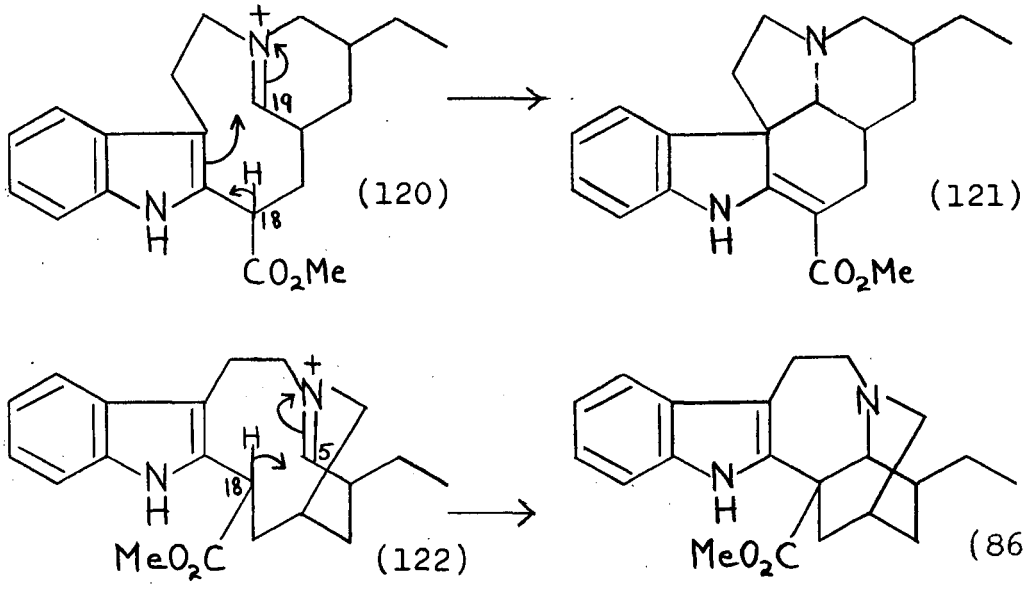
(116) (117)



(119) (118) a:R = H

b:R = Ac

This result suggested that entry into the vincadifformine (103) type of system could be realised by use of the appropriate carbomethoxy-dihydrocleavamine (119)[112]. Moreover, with this cleavamine derivative there was also a possibility of obtaining an Iboga alkaloid system, since the C-18 hydrogen atom was rendered labile by the carbomethoxy group. Reaction with mercuric acetate would be expected to generate an intermediate with the immonium system ($\geq\overset{+}{N}=C<$) involving either C-19 or C-5. The intermediate (120) with the C-19 immonium group could, by the appropriate transannular cyclisation, afford a vincadifformine-like system (121), whereas that (122) with the $\geq\overset{+}{N}=C$-5 grouping could yield an Iboga alkaloid (86). We were able to demonstrate that in fact both processes operate.



Carbomethoxy-4"β"-dihydrocleavamine (119)[68] was prepared by reduction of catharanthine (5) with zinc and acetic acid[98]. Acid hydrolysis and decarboxylation of this product afforded 4"β"-dihydrocleavamine which was <u>not</u> identical to

that obtained by hydrogenation of cleavamine, and hence must have the ethyl group in a different orientation. Oxidation of carbomethoxy-4"β"-dihydrocleavamine with mercuric acetate in acetic acid afforded a complex mixture which was subjected to chromatography on alumina. This procedure resulted in the isolation of one major component and two other alkaloids in smaller amounts. The latter substances, which were found to be the known alkaloids coronaridine[71] and dihydrocatharanthine[28], will be presented later, while the former is discussed immediately below.

(i) Pseudo-vincadifformine and its Derivatives

The major product, which we have termed pseudo-vincadifformine, was obtained from the initial benzene fractions of the chromatography in about 25% yield[99]. It was a white amorphous powder, $[\alpha]_D^{26}$ -503° (EtOH), which analysed well for $C_{21}H_{26}O_2N_2$. Final confirmation of the molecular formula was obtained when a mass spectrometric molecular weight determination showed a value of 338. Maxima in the ultra-violet spectrum at 226, 298 and 326 mμ, and absorption bands in the infra-red region at 1675 and 1610 cm.$^{-1}$ clearly indicated an αβ-unsaturated ester function conjugated with the dihydro-indole system in the same manner as in vincadifformine (103). The N.M.R. spectrum exhibited a singlet at 1.05τ (NH), a complex pattern in the region 2.4-3.3τ corresponding to four aromatic protons, and a spike at 6.23τ due to the methoxyl group. A very strong signal at m/e 124 in the mass spectrum (Figure 10) was indicative of an Aspidosperma

PSEUDO—VINCADIFFORMINE

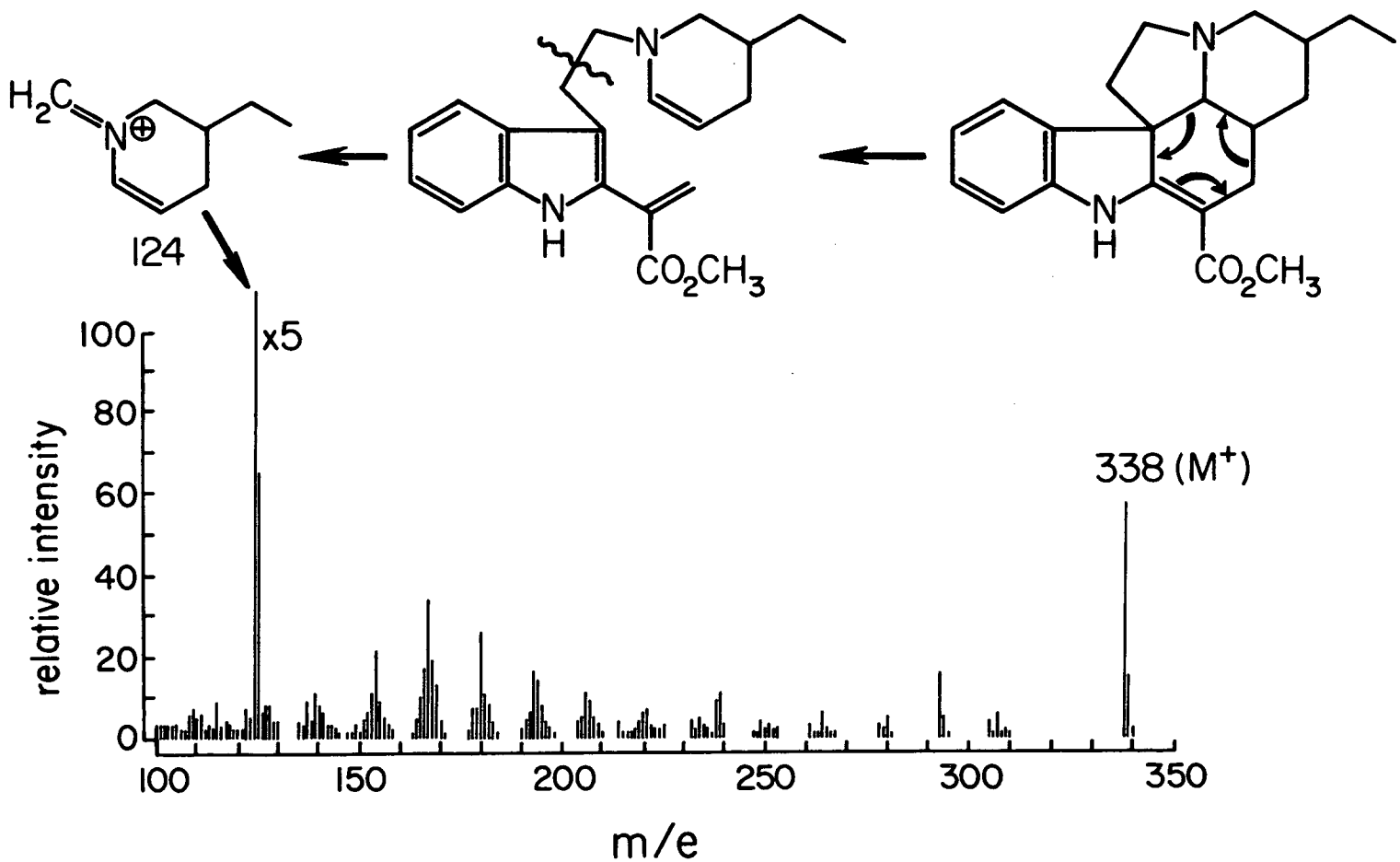


Figure 10

DIHYDRO—PSEUDO—VINCADIFFORMINE

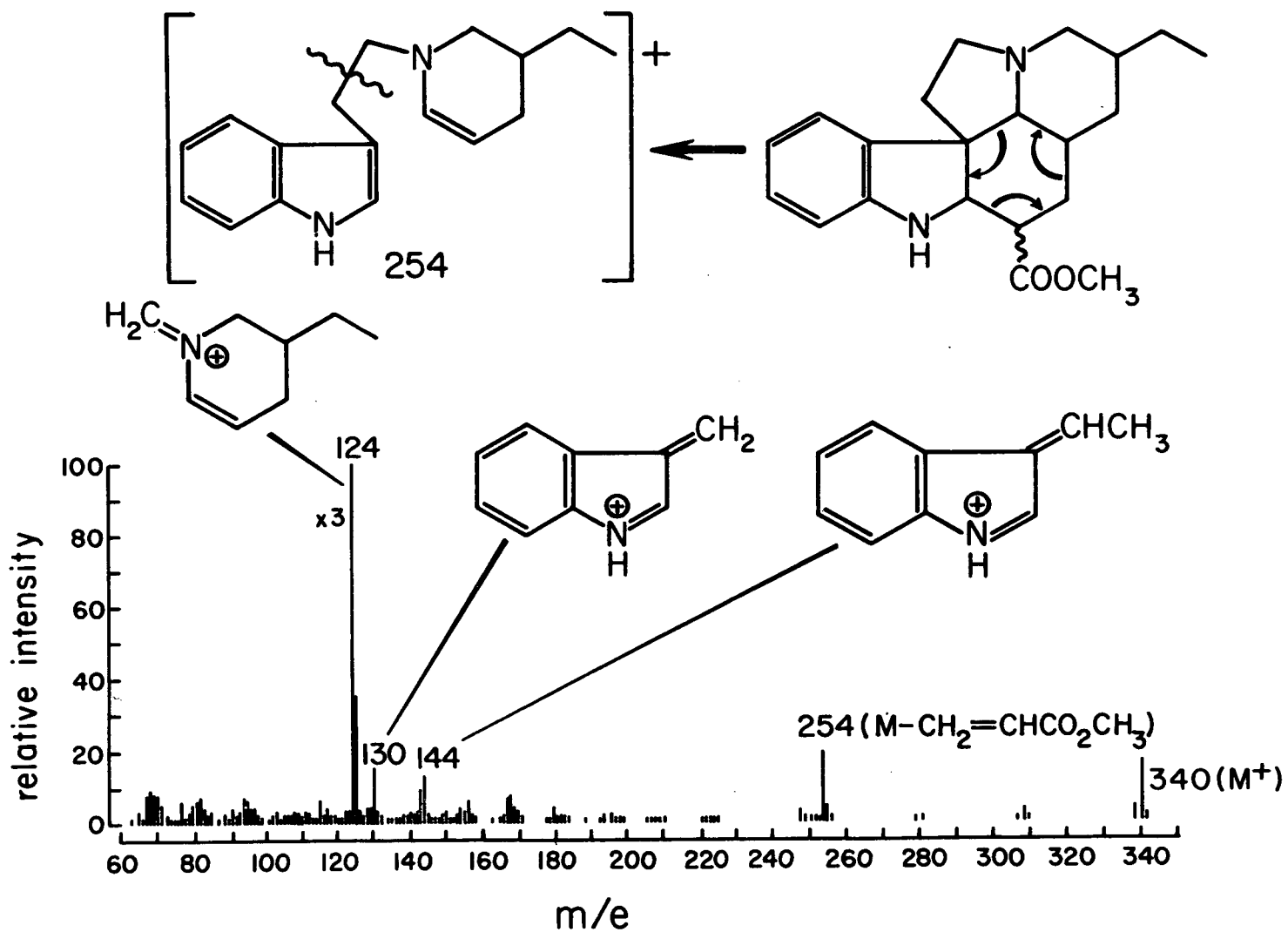
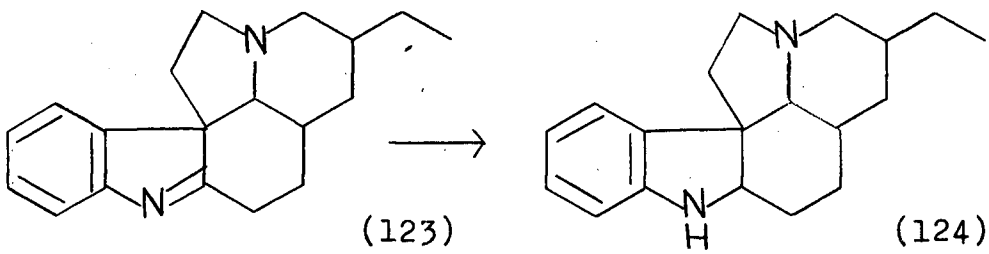


Figure 11

-type skeleton[100] (see later), and, indeed, the spectrum was very similar to that of vincadifformine.

Chemical evidence in support of the conjugated ester system was provided by acid-catalysed hydrolysis and decarboxylation of pseudo-vincadifformine (121) to yield a gummy product (123) which showed the expected spectral properties of an indolenine system[100,91], [$\lambda_{max.}$ 221, 227 (inflection) and 250 (broad) mμ; no carbonyl or NH absorption in the infra-red region]. Subsequent reduction of the latter substance with lithium aluminium hydride afforded a crystalline product, m.p. 89-90°, $[\alpha]_D^{26}$ -60° ($CHCl_3$), for which the structure (124) was deduced from the following evidence. Elemental ana-



(123) (124)

lyses suggested a formula $C_{19}H_{26}N_2$, which was confirmed by a mass spectrometric molecular weight (282). The reduction of the indolenine system was clearly indicated by a typical dihydro-indole ultra-violet spectrum ($\lambda_{max.}$ 243 and 295 mμ) and the appearance of a new absorption at 3230 $cm.^{-1}$ in the infra-red spectrum (NH). Moreover, a complex pattern of lines in the N.M.R. spectrum in the region 2.7-3.6 τ, due to four aromatic protons, was in complete

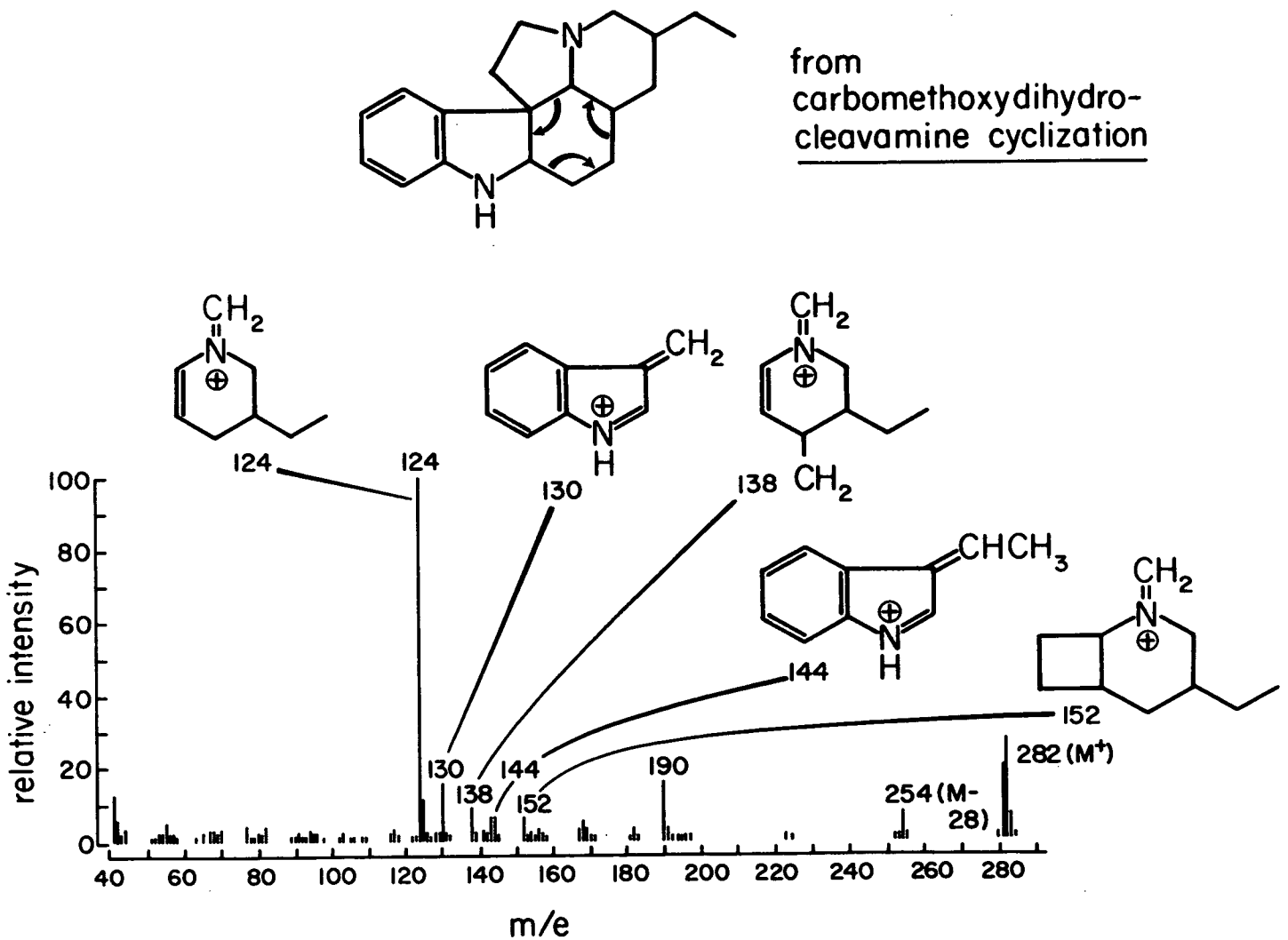
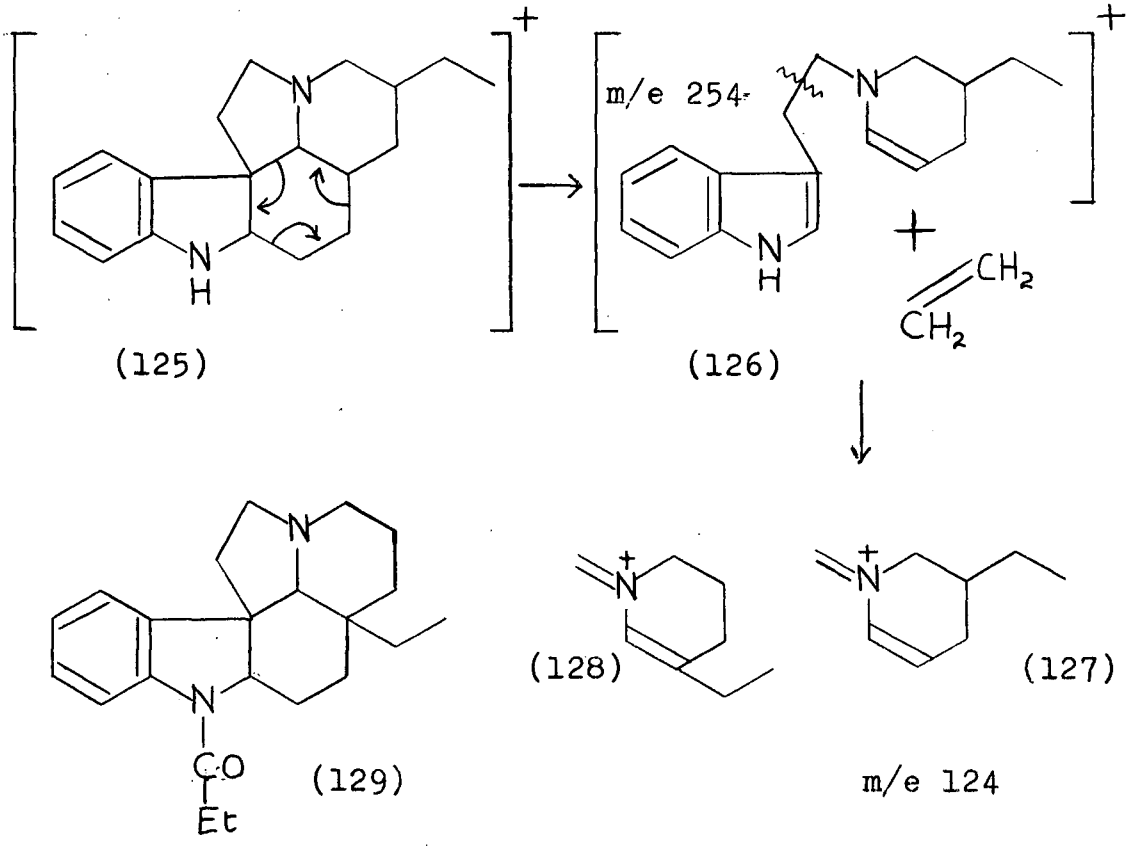

from
carbomethoxydihydro-
cleavamine cyclization
relative intensity
m/e
124
124
130
138
144
152
190
254 (M-28)
282 (M+)

Figure 12

agreement with known Aspidosperma alkaloid systems[102].

Invaluable information was provided by the mass spectrum (Figure 12) of the dihydro-indole (124), which showed significant peaks at m/e 282 (M^+) and 254 (M-28), and a very strong signal at m/e 124. It was recently observed by Biemann et al.[100] that the appearance of M-28 and m/e 124 peaks may be considered diagnostic of an Aspidosperma-type skeleton. In this instance, a similar fragmentation process leading to a m/e 124 ion may be postulated, whereby the molecular ion (125) expels ethylene to yield the m/e 254 fragment (126), which is subsequently cleaved to the m/e 124 ion (127). This ion differs only in the position of the ethyl group from that (128) proposed[100] for the corresponding m/e 124 peak displayed by the

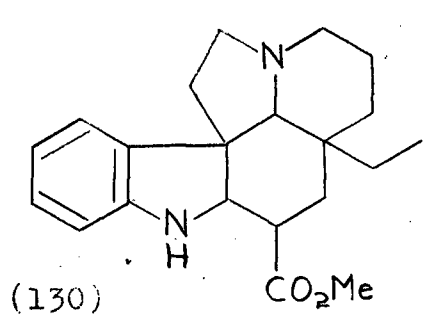


Aspidosperma alkaloids. Furthermore, the mass spectrum of (124) was superimposible on that of the similar compound (118a)previously synthesised from 4"α"-dihydrocleavamine[97].

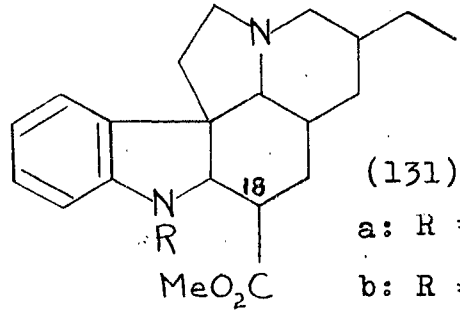
Further evidence for the structure of (124) was obtained from the N-acetyl derivative, m.p. 107.5-109°, $C_{21}H_{28}O_2N_2$. The ultra-violet spectrum of the latter displayed maxima at 253, 279 and 289 mμ , and the complex multiplet in the aromatic region of the N.M.R. spectrum of (124) had now collapsed into a broad three-proton peak centred at 2.85τ and a signal at 1.87τ due to one proton. These spectral data were in excellent agreement with the acetate (118b) previously derived from 4"α"-dihydrocleavamine[97] and with the known Aspidosperma alkaloid demethoxypalosine (129)[103].

Additional chemical proof of the conjugated ester system was provided by reduction of pseudo-vincadifformine with zinc and sulphuric acid to yield two isomeric dihydro derivatives. The major product, dihydro-pseudovincadifformine, $[\alpha]_D^{24}$-16° (EtOH), analysed for $C_{21}H_{28}O_2N_2$, a formula which was established by a mass spectrometric molecular weight of 340. The ultra-violet spectrum ($\lambda_{max.}$ 244 and 299 mμ) was characteristic of a dihydro-indole chromophore, and the ester carbonyl absorption in the infra-red had now moved to 1725 $cm.^{-1}$. Acetylation afforded a N-acetate, $C_{23}H_{30}O_3N_2$, $[\alpha]_D^{24}$+28°(EtOH), which showed ultra-violet maxima at 253, 282 and 291 mμ , and the appropriate appearance of an amide band at 1660 $cm.^{-1}$ with concurrent loss of the NH peak in the infra-red spectrum. Besides the expected signal at

7.75τ ($CH_3C=O$) in the N.M.R. spectrum, there was an unexpected upfield shift of the methoxyl signal from 6.33 to 6.83τ, which will be discussed later. In general, the spectral properties of dihydro-pseudo-vincadifformine and its acetate were in agreement with the structure (131), for which confirmation was found in the mass spectral cracking pattern (Figure 11).



(130)

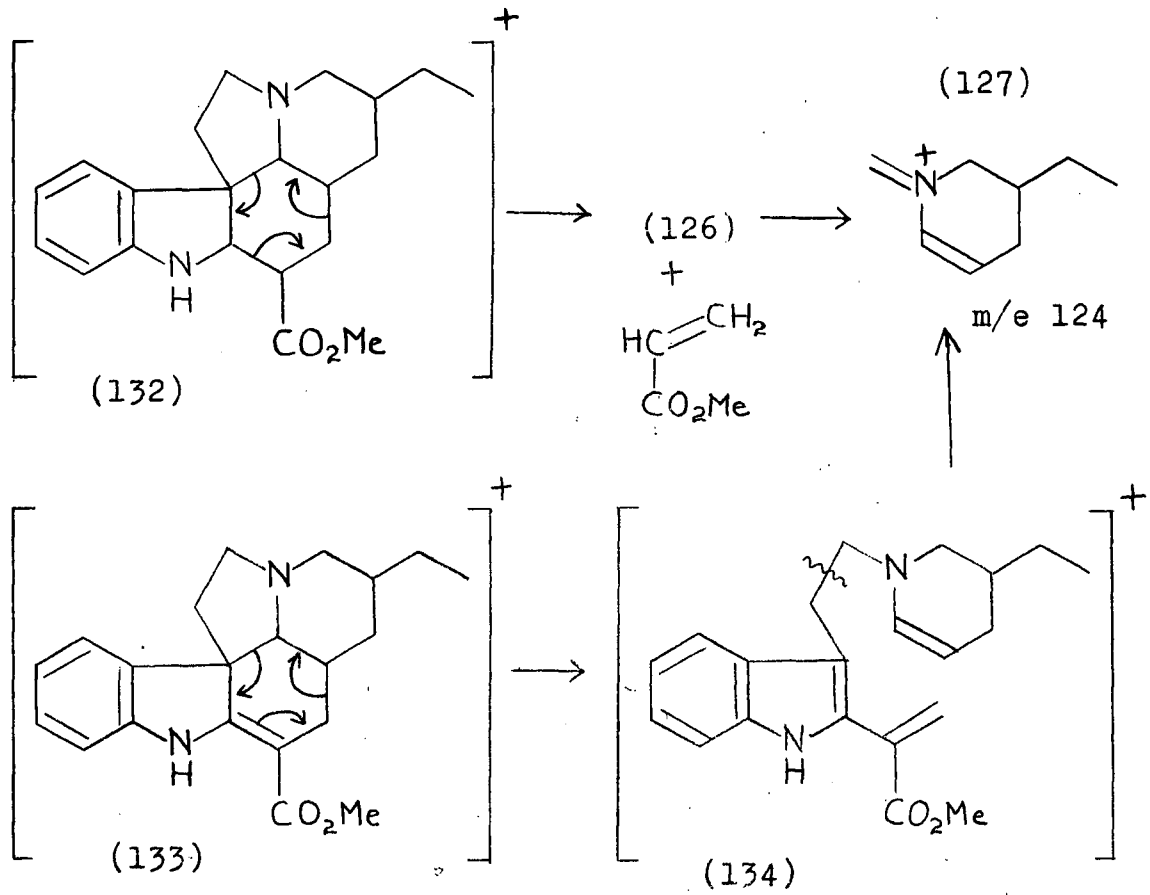


(131)

a: R = H

b: R = Ac

The base peak of the mass spectrum was m/e 124, whereas the second most intense peak was at m/e 254. A fragmentation process entirely analogous to that discussed above (125) to (127) was obviously occurring, in which the molecular ion (132) eliminated a molecule of methyl acrylate instead of ethylene to give an identical m/e 254 fragment (126), which then cleaved as before to afford the m/e 124 ion (127). The absence of a significant m/e 254 peak in the mass spectrum (Figure 10) of pseudo-vincadifformine (121) itself was due to the presence of a double bond, which prevented any elimination of methyl acrylate (or its equivalent) from the molecular ion (133).



However, the rearrangement product (134) could still cleave to yield the m/e 124 ion. Finally, a comparison of the mass spectra of pseudo-vincadifformine (121) and dihydro-pseudo-vincadifformine (131a) with those of authentic vincadifformine (103) and dihydrovincadifformine (130) revealed that both pairs of spectra were identical.

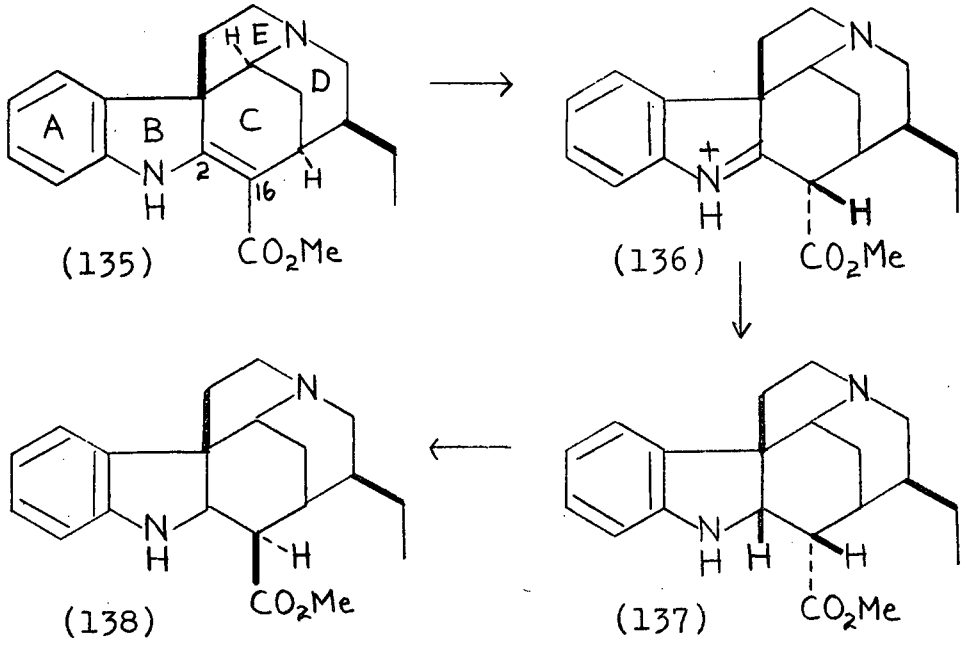
The minor product, $[\alpha]_D^{24}$ -132° (EtOH), from the zinc /sulphuric acid reduction of pseudo-vincadifformine also analysed for $C_{21}H_{28}O_3N_2$, and spectral data indicated the presence of dihydro-indole and saturated ester functions. Acetylation afforded a N-acetate, $C_{23}H_{30}O_3N_2$, $[\alpha]_D^{24}$ +3° (EtOH), whose ultra-violet spectrum ($\lambda_{max.}$ 250, 278 and 296 mμ) showed small but distinct differences from the ace-

tate of the major dihydro compound. Moreover, the methoxyl absorption in the N.M.R. spectrum of the acetate was in a more usual position (6.42 τ).

Vigorous treatment with sodium methoxide converted the major component into a substance which proved to be identical with the minor product. It was thus established that the minor component, which we refer to as iso-dihydro-pseudo-vincadifformine, also had the gross structure (131a) and that the compounds were in fact stereoisomers, which differed only in the configuration at the carbon atom (C-18) bearing the carbomethoxy group.

The isolation and interconversion of the two dihydro compounds in the reduction of pseudo-vincadifformine (121) could be rationalized by analogy with a similar series of reactions performed by Smith and Edwards[104] in the akuammicine series. Reduction of dihydroakuammicine (135) with zinc and sulphuric acid afforded tetrahydroakuammicine (137), which was epimerised with sodium methoxide to iso-tetrahydroakuammicine (138). The authors suggested that the first step in the reduction was protonation of C-16 to give the immonium ion (136). The proton added to the β-face in order to allow the carbomethoxy group to take up the more stable equatorial orientation, with ring C in the boat conformation. Reduction of the immonium system then proceeded with addition of hydrogen at C-2, again from the β-face, to give a compound (137) in which the B/C ring junction was the more stable cis-form and rings C and D had chair conformations. This forced the

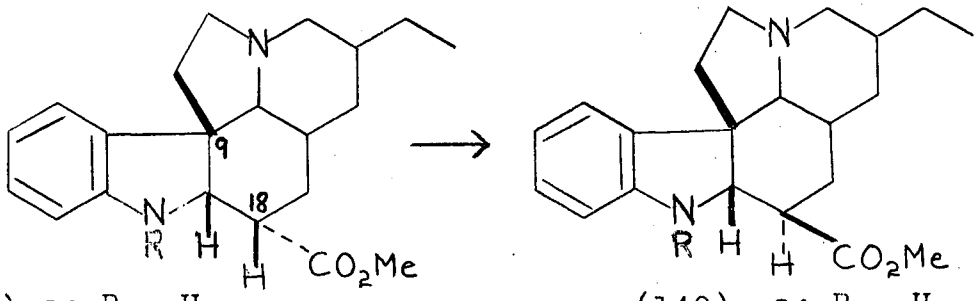
carbomethoxy group into an unfavourable axial orientation.



Epimerisation of tetrahydroakuammicine (137) to the isomeric base (138) was then readily understood as involving a change of orientation of the carbomethoxy group from axial to equatorial. This mechanism enabled the authors to explain the hydrogen bonding of the carbonyl group indicated by the infra-red spectrum of(137), which did not occur in the case of (138).

Although the akuammicine derivatives (137) and (138) are not strictly comparable to dihydro-pseudo-vincadifformine (131a) and its epimer, it is nevertheless likely that the reduction of pseudo-vincadifformine (121) follows a similar steric course. If, for the sake of argument, the β-configuration is assumed at C-9, then the predominant isomer would also be the kinetically favoured one (139a),

which has the carbomethoxy group at C-18 in the axial α-orientation. Treatment with base would then afford the thermodynamically more stable iso-dihydro compound (140a) with an equatorial carbomethoxy substituent in the β-orientation.



(139) a: R = H
b: R = Ac

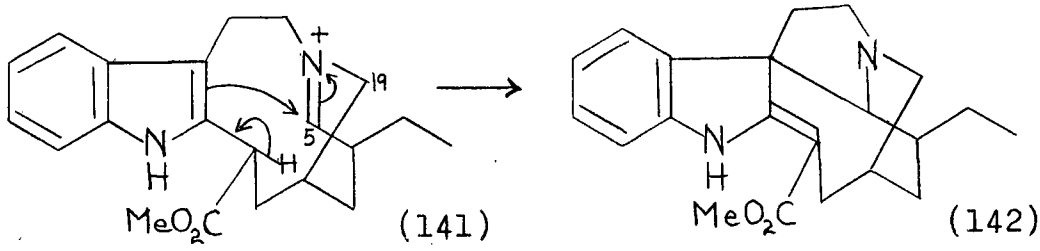
(140) a: R = H
b: R = Ac

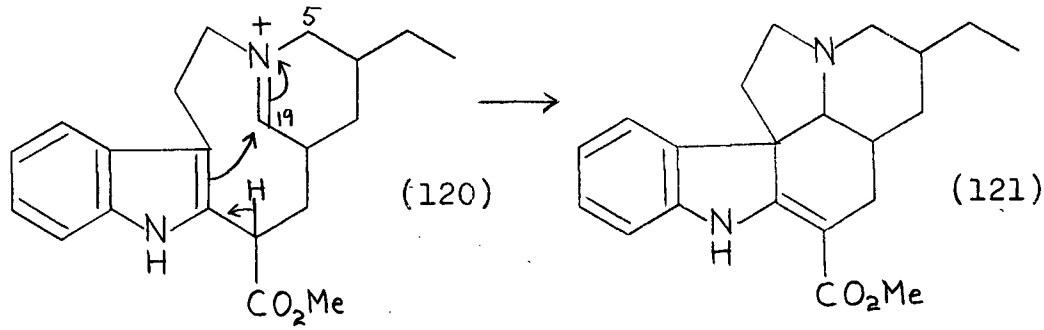
It must be emphasised that, since the stereochemistry of pseudo-vincadifformine still remains to be established, the above arguments are not conclusive.

If one constructs models of the corresponding acetates (139b) and (140 b), the methoxyl group of the axial carbomethoxy function of (139b) is found to come in close proximity to the benzene ring, whereas the methoxyl group in (140b) cannot do so. Thus the high position (6.83 τ) of the methoxyl proton signals in the N.M.R. spectrum of dihydro-pseudo-vincadifformine acetate (139b) may be due to diamagnetic shielding by the benzene ring.

The chemical and spectral evidence cited above established a vincadifformine-type structure (121) for pseudo-vincadifformine. It should be mentioned at this time that, in fact, the mercuric acetate oxidation of carbomethoxy-4"β"-dihydrocleavamine (119) can, and does, proceed in two

directions to provide immonium derivatives involving either C-19 or C-5. At the outset it was therefore necessary to consider the alternative cyclisation of (141) to (142).





However, studies of models showed that steric repulsions made this cyclisation extremely unfavourable, and the structure (142) was excluded even before experiments were run. Hence pseudo-vincadifformine (121) must be derived from the C-19 immonium ion (120) as shown.

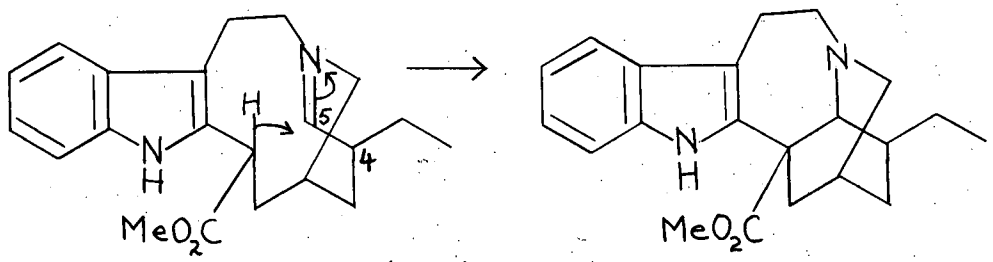
(ii) Coronaridine and Dihydrocatharanthine

The chromatography of the mixture resulting from mercuric acetate oxidation of carbomethoxy-4"β"-dihydrocleavamine (119) yielded, in addition to pseudo-vincadifformine, small amounts of two other alkaloids[105]. From the later benzene fractions of the chromatography was isolated an amorphous alkaloid , which afforded a crystalline hydro-

chloride, m.p. 221-223° . The ultra-violet spectrum of the base indicated an indole chromophore with maxima at 226, 285, and 293 mμ . The presence of an ester carbonyl absorption at 1705 $cm.^{-1}$, and the absence of the strong Bohlmann bands in the region between 2700 and 2800 $cm.^{-1}$ displayed by the starting material, suggested that this alkaloid was a member of the Iboga series (86). Indeed, comparison of our alkaloid (infra-red spectra and thin-layer chromatographic mobility) with an authentic sample of coronaridine (145)[71] showed that they were the same[106]. Further comparison (mixed melting-point and infra-red spectra) of the crystalline hydrochlorides completely established the identity.

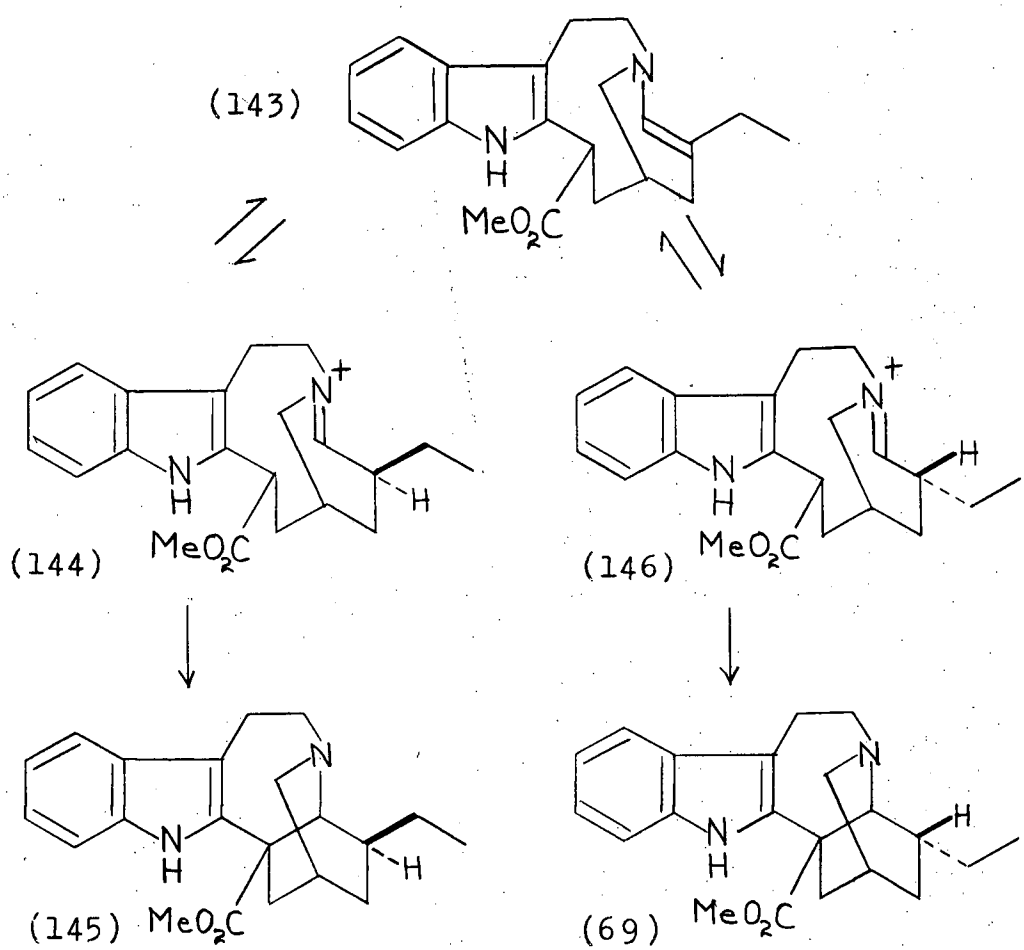
The other alkaloid, eluted with benzene-ether (1:1), was crystalline, m.p. 143-145.5° , $[\alpha]_D^{26}$+49° ($CHCl_3$), and the spectral data showed the presence of an indole system and a saturated ester function. An authentic sample of dihydrocatharanthine was prepared by hydrogenation of catharanthine (5), and a direct comparison (mixed melting-point, infra-red spectra, thin-layer chromatographic mobility) confirmed that our product was actually dihydrocatharanthine (69)[28,107].

Thus it was established that the transannular cyclisation of the other possible mercuric acetate oxidation product (122) with the $>\overset{+}{N}=C-5$ immonium system, led to the Iboga skeleton (86).



(122) (86)

The isolation of both coronaridine (145) and dihydrocatharanthine (69) from this reaction indicated that an isomerisation of the ethyl group at C-4 was taking place.



This was not unexpected, since the immonium ion (144) or (146) formed by oxidation of carbomethoxy-4"β"-dihydro-

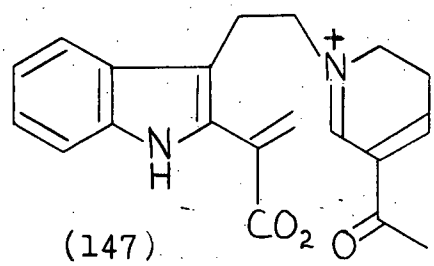
cleavamine could readily isomerise to the other epimer via the eneamine (143) before cyclisation. The mobility of the immonium-eneamine system is well known[76].

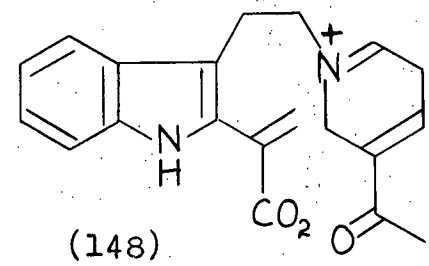
D. Discussion

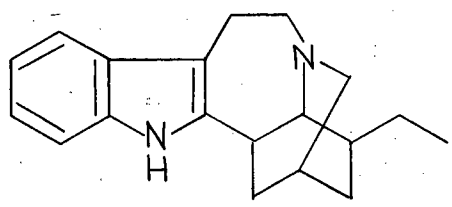
The cyclisation to Iboga alkaloids provides some support for the mechanism postulated (p.51) for the catharanthine -cleavamine transformation. An essential part of the mechanism is the transannular cyclisation of a cleavamine-like intermediate (74) to descarbomethoxycatharanthine (65), and the above results demonstrate the feasibility of this process. Moreover, the immonium-eneamine tautomerism required to accommodate coronaridine and dihydrocatharanthine is also involved to explain the formation of 4"α"- and 4"β"-dihydrocleavamines (68) in the cleavage of catharanthine (5) with acidic reagents.

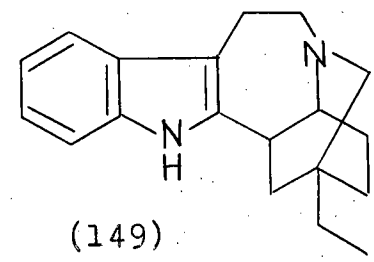
It is apparent that the three products obtained in our reaction prove that the kind of transannular cyclisation of immonium ions proposed in Wenkert's biosynthetic scheme (p. 60) does take place quite readily. Although these results do not prove that this is the actual biogenetic pathway, they certainly lend support to the likelihood of such reactions. It appears that the Aspidosperma (64) and Iboga (63) alkaloids may very well evolve from a common biogenetic precursor [such as (147)], that can afford either a quebrachamine (67), or cleavamine (66) skeleton, which subsequently undergoes

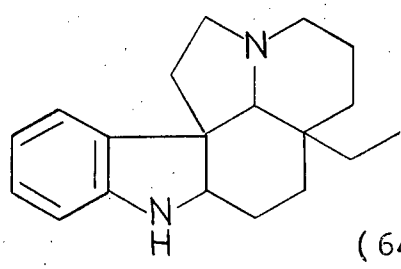
cyclisation in the manner we have now realised in the laboratory. The alkaloids of these types are theoretically derivable from the same immonium ion (147), which involves the carbon atom between the nitrogen and ethyl-bearing carbon atoms. It will be of interest to see whether alkaloid systems

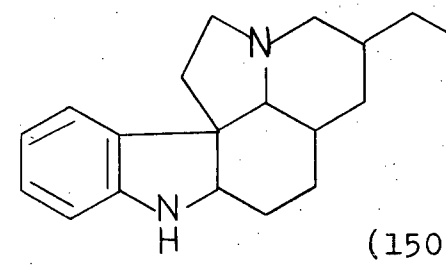

(147)


(148)


(63)

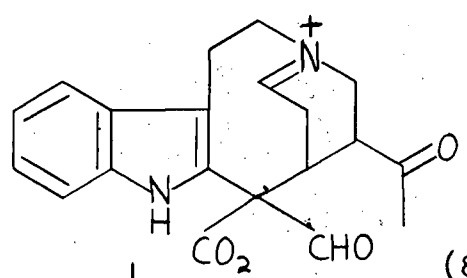

(149)


(64)

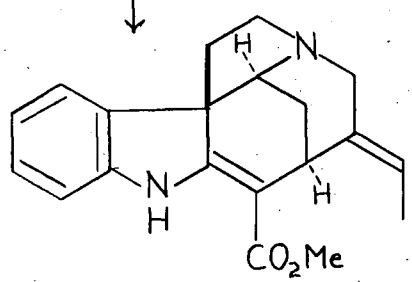

(150)

such as (149) and (150), which could be formed from the alternative ion (148), will be found in Nature. This would then parallel the relationship between akuammicine (100)[104] and condylocarpine (152)[108], which can be considered as arising biogenetically from a related pair of immonium ion precursors, (89) and (151) respectively. Indeed, the Swiss workers[108] who recently interrelated akuammicine and condy-

locarpine proposed the transannular cyclisation of ionic intermediates similar to (89) and (151).



(89)



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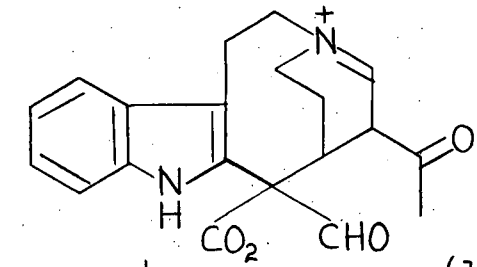
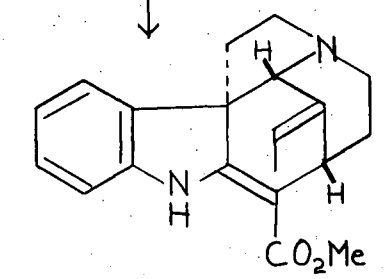
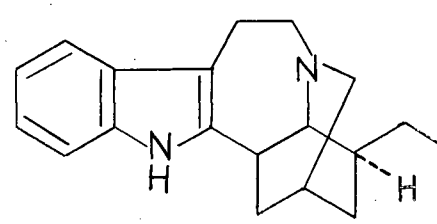


(151)



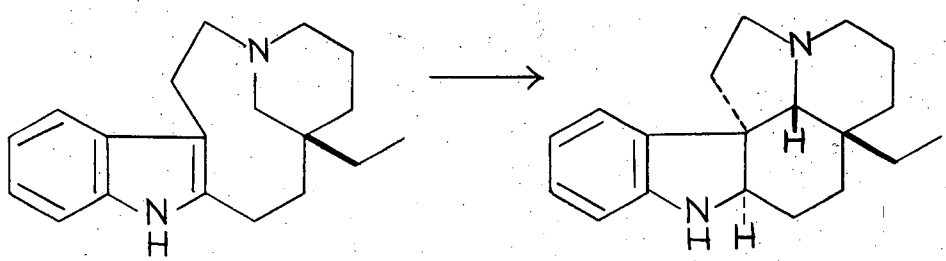
(152)

The formation of coronaridine (145) and dihydrocatharanthine (69) in the mercuric acetate reaction constitutes an attractive entry into the Iboga alkaloid series. Since the removal of the carbomethoxy group is readily accomplished[74] this sequence obviously also provides partial syntheses of ibogamine (153)[71] and epi-ibogamine (70)[28]. It is apparent that if a synthesis of carbomethoxy-dihydrocleavamine (119) can be developed, this would effect the total synthesis of the Iboga skeleton, which has not yet been accomplished.



(153)

An interesting potential route to the Aspidosperma system, particularly of the vincadifformine (103) type, is revealed by the cyclisation of the immonium ion (120) to pseudo-vincadifformine (121). The obvious extension of this reaction to an alkaloid such as vincadine (106a) should provide a synthesis of vincadifformine and its relatives. Since it has recently proved possible to cyclise (−)-quebrachamine (107) to (+)-aspidospermidine (154) by means of mercuric acetate oxidation and subsequent hydride reduction[109], the synthesis of vincadifforimine from vincadine is now highly probable.



(107) (154)

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Ultra-violet (U.V.) absorption curves were measured in methanol solution on a Cary 14 spectrometer, and infra-red (I.R.) spectra were taken on a Perkin-Elmer Model 21 spectrophotometer. Nuclear magnetic resonance (N.M.R.) spectra were recorded at 60 megacycles/sec. on a Varian A60 instrument; the line positions or centres of multiplets are given in the Tiers τ scale with reference to tetramethylsilane as the internal standard; the multiplicity, and integrated area and type of protons are indicated in parentheses. Silica gel G plates were used for thin-layer chromatography (T.L.C.) and were developed by ethyl acetate, ethyl acetate-chloroform or ethyl acetate-ethanol mixtures as given below. The alumina used for column chromatography was Shawinigan reagent grade, deactivated with 3% of 10% aqueous acetic acid, unless otherwise stated. Analyses were performed by Dr.A. Bernhardt and his associates, Mulheim (Ruhr), Germany and by the Microanalytical Laboratory, University of British Columbia. Every molecular weight (M.W.) quoted was determined mass spectrometrically.

Part I Experimental Section

Isolation of Sitsirikine

The crude sulphate (1 g.) provided by Dr.M. Gorman,

Eli Lilly Research Laboratories, was dissolved in methanol (20 ml.) and water (250 ml.), the solution cooled in ice and made basic with aqueous ammonia. The precipitate was taken up in ether (200 ml.) the layers separated, and the aqueous portion further extracted with ether (3 x 100 ml.). After drying over magnesium sulphate, the combined ethereal extracts were evaporated to give a powder, which showed three spots on T.L.C. (EtOAc).

Several recrystallisations from acetone-petroleum ether (b.p. 60-80°) afforded needles (320 mg.) m.p. 178-179°, which now displayed only two spots on T.L.C. Repeated recrystallisations failed to resolve the mixture, which behaved as a pure compound by all criteria except T.L.C., and hence this was called sitsirikine. The purest samples of the alkaloid and its derivatives are described below.

Sitsirikine crystallised from acetone with one molecule of solvent as needles, m.p. 181°, $[\alpha]_D^{26}$ -52° (MeOH). Found: C, 69.52; H, 7.84. Calc. for $C_{21}H_{26}O_3N_2 \cdot Me_2CO$: C, 69.88; H, 7.82.

The unsolvated material was obtained from aqueous methanol as stout needles, m.p. 206-208°; $[\alpha]_D^{26}$ -58° (MeOH); $\lambda_{max.}$ (log ϵ): 226 (4.56), 282 (3.90), 290 (3.84) mμ; $\bar{\nu}_{max.}$ (Nujol): 3360 (NH and/or OH), 1705 (C=O), 740 (o-disubstituted benzene) cm.$^{-1}$; N.M.R. signals (CD_3COCD_3): 2.8 (multiplet, 4H, aromatic), 4.7 (multiplet, 1.8H, olefinic), 6.1 (multiplet, 2H, CH_2O), 6.38 (singlet, 3H, CH_3O), 9.02 (1H) τ. Found: C, 71.15, 71.43; H, 7.51, 7.66; O, 14.00;

N, 7.89, 7.77; C-Me, 1.61; M.W. 354. Calc. for $C_{21}H_{26}O_3N_2$: C, 71.16; H, 7.39; O, 13.54; N, 7.90; (1) C-Me, 4.24; M.W. 354.

Sitsirikine Picrate

A saturated alcoholic solution of picric acid (2 ml.) was added to sitsirikine (50 mg.) in ethanol (1 ml.) and the mixture heated to boiling. The precipitate was recrystallised from methanol to afford yellow hexagonal prisms (45 mg.), m.p. 226-228° (dec.). Found: C, 55.55, 55.70; H, 5.46, 5.26; N, 12.10. Calc. for $C_{27}H_{29}O_{10}N_5$: C, 55.57; H, 5.01; N, 12.00.

Sitsirikine Acetate

Sitsirikine (110 mg.) was dissolved in pyridine-acetic anhydride (1:1, 2 ml.) and left overnight. The solution was poured into ice-water (10 ml.), basified with ammonia, and the precipitate taken up in ether. After washing several times with water, the ethereal solution was dried over magnesium sulphate and evaporated. Recrystallisation from aqueous methanol afforded the acetate as needles (100 mg.), m.p. 198°; $[\alpha]_D^{26}$ -26° (MeOH); two spots on T.L.C. (EtOAc-$CHCl_3$, 1:1); $\bar{\nu}_{max.}$ (Nujol): 3340 (NH), 1735 (C=O), 1700 (C=O), 1240 (OAc) $cm.^{-1}$; N.M.R. signals (CD_3COCD_3): 2.7 (multiplet, 4H, aromatic), 4.7 (multiplet, 1.8H, olefinic), 5.6 (multiplet, 2H, CH_2OAc), 6.37 (singlet, 3H, CH_3O), 8.02 (singlet, 3H, $CH_3C=O$), 9.02 (1H) τ. Found: C, 69.65; H, 7.32; N, 7.01. Calc. for $C_{23}H_{28}O_4N_2$: C, 69.67; H, 7.12; N, 7.07.

Dihydrositsirikine (25)

Sitsirikine (450 mg.), in methanol (10 ml.), was hydrogenated over palladium black (24 mg.). The hydrogen uptake ceased after 30 minutes when 0.65 mol. had been absorbed. After removal of the catalyst and solvent, the product was recrystallised twice from acetone-petroleum ether (b.p. 60-80°) to give dihydrositsirikine (405 mg.), m.p. 177-179°. This compound displayed only one spot on T.L.C. (EtOAc) which corresponded to one of the two spots shown by sitsirikine. Therefore the impurity which could not be removed from sitsirikine was in fact the dihydro compound. Dihydrositsirikine crystallised from acetone, with one molecule of solvent, as needles, m.p. 180° . Found: C, 69.42; H, 7.91; N, 6.97. Calc. for $C_{21}H_{28}O_3N_2 \cdot Me_2CO$: C, 69.53; H, 8.27; N, 6.76.

Recrystallisation from aqueous methanol afforded the unsolvated alkaloid as prisms, m.p. 215°; $[\alpha]_D^{26}$ -55° (MeOH); $\lambda_{max.}$ (log ϵ): 226 (4.61), 282 (3.95), 290 (3.87) mμ; $\lambda_{min.}$ (log ϵ): 247 (3.40), 287.5 (3.85) mμ ; $\bar{\nu}_{max.}$ ($CHCl_3$): 3480 (NH and OH), 2810 and 2760 (Bohlmann bands)[44], 1710 (C=O) cm.$^{-1}$; $\bar{\nu}_{max.}$ (Nujol): 3400 (NH), 3200 (OH), 1710 (C=O)cm.$^{-1}$; N.M.R. signals (CD_3COCD_3): 2.8 (multiplet, 4H, aromatic) 6.1 (multiplet, 2H, CH_2O), 6.42 (singlet, 3H, CH_3O), 9.07 (broad singlet, 3H, CH_3C) τ . Found: C, 70.80; H, 7.78; O, 13.49; N, 7.77; O-Me, 9.01; C-Me, 3.95; M.W. 356. Calc. for $C_{21}H_{28}O_3N_2$: C, 70.76; H, 7.92; O, 13.47, N, 7.86; (1) O-Me, 8.72; (1) C-Me, 4.22; M.W. 356.

Dihydrositsirikine Picrate

Dihydrositsirikine (60 mg.) and a solution of picric acid were reacted in the manner described above and the derivative recrystallised from methanol to yield amber prisms (55 mg.), m.p. 228-230° (dec.). Found: C, 55.30, 55.46; H, 5.55,5.71; N, 11.95. Calc. for $C_{27}H_{31}O_{10}N_5$: C, 55.38; H, 5.34; N, 11.96.

Dihydrositsirikine Acetate

The acetate was prepared by treatment of dihydrositsirikine (200 mg.) with acetic anhydride in pyridine as above. The product was recrystallised twice from acetone-petroleum ether (b.p. 60-80°) to afford needles (155 mg.), m.p. 187° ; one spot on T.L.C. (EtOAc-$CHCl_3$, 1:1); $[\alpha]_D^{26}$ -31° (MeOH); $\bar{\nu}_{max.}$ (Nujol): 3390 (NH), 1740 (C=O), 1705 (C=O), 1250 (OAc) $cm.^{-1}$; N.M.R. signals ($CDCl_3$): 1.34 (singlet, 1H, NH), 2.8 (multiplet, 4H, aromatic), 5.6 (multiplet, 2H, CH_2OAc), 6.38 (singlet, 3H, CH_3O), 7.96 (singlet, 3H, $CH_3C=O$), 9.02 (broad singlet, 3H, CH_3C)τ. Found: C, 69.39, 68.98; H, 7.75, 7.59; O, 16.46; N, 6.85. Calc. for $C_{23}H_{30}O_4N_2$: C, 69.32; H, 7.69; O, 16.06; N, 7.03.

Dihydrositsirikine p-Bromobenzoate

p-Bromobenzoyl chloride (130 mg.) was added to dihydrositsirikine (65 mg.) in dry pyridine (3 ml.). After standing overnight the solution was poured into ice-water, made basic with aqueous ammonia and stirred for 10 minutes.

The precipitate was taken up in ether, the ethereal solution washed twice with water and dried over magnesium sulphate. Removal of the solvent gave a gum which was dissolved in benzene and filtered through alumina (5 g.). The benzene eluate was concentrated and petroleum-ether (b.p. 60-80°) added dropwise to the boiling solution until a permanent turbidity was obtained. The product was recrystallised from acetone-petroleum ether (b.p. 60-80°) to yield the p-bromobenzoate as slender needles (60 mg.), m.p. 174°; one spot on T.L.C.; $\bar{\nu}_{max.}$ (Nujol): 3370 (NH), 1725 (C=O), 1705 (C=O) cm.$^{-1}$; N.M.R. signals ($CDCl_3$): 2.5 (multiplet, 8H, aromatic), 5.4 (multiplet, 3H, CH_2O), 6.37 (singlet, 3H, CH_3O), 9.10 (broad singlet, 3H, CH_3C) τ. Found: C, 62.34; H, 5.70; N, 5.06. Calc. for $C_{28}H_{31}O_4N_2Br$: C, 62.33; H, 5.79; N, 5.19.

Saponification of Dihydrositsirikine (25)

Dihydrositsirikine (200 mg.) was heated under reflux with 2 N methanolic sodium hydroxide (20 ml.) for 2 hours. After removal of the solvent, the residue was taken up in water and extracted with methylene chloride to remove unsaponified material. The aqueous solution was acidified with hydrochloric acid and evaporated to dryness. The residue was leached with absolute ethanol and the solution filtered from sodium chloride. Evaporation and recrystallisation from aqueous alcohol gave an $\alpha\beta$-unsaturated acid hydrochloride,

m.p. 260-263° ; $\bar{\nu}_{max.}$ (Nujol): 1695 (C=O), 1620 (C=C) cm.$^{-1}$; N.M.R. signals (CF_3CO_2H): 3.2 (multiplet, 4H, aromatic), 3.77 (singlet, 1H, olefinic) and 4.20 (singlet, 1H, olefinic)τ.

Dihydrositsirikine Diol

A solution of dihydrositsirikine (250 mg.) in tetrahydrofuran (10 ml.) was run slowly into a stirred suspension of lithium aluminium hydride (200 mg.) in tetrahydrofuran (10 ml.) and heated under reflux for 3 hours. After the mixture had stood overnight, the excess hydride was decomposed with saturated aqueous sodium sulphate solution (10 ml.), followed by water (20 ml.). The aqueous suspension was then extracted with methylene chloride (4 x 25 ml.), and the combined extracts dried over sodium sulphate. Removal of the solvent and recrystallisation from aqueous acetone gave the diol as needles (180 mg.), m.p. 203° ; one spot on T.L.C. (EtOAc-EtOH, 1:1); $[\alpha]_D^{26}$-3° (MeOH); $\lambda_{max.}$ (log ε): 226 (4.53), 282 (3.85), 290 (3.78)mμ ; $\bar{\nu}_{max.}$ (Nujol): 3210 (NH and OH) cm.$^{-1}$; N.M.R. signals (CD_3COCD_3): 2.8 (multiplet, 4H, aromatic), 6.4 (multiplet, 4H, 2 CH_2O), 9.02 (broad singlet, 3H, CH_3C)τ. Found: C, 73.30; H, 8.46; N, 8.41. Calc. for $C_{20}H_{28}O_2N_2$: C, 73.13; H, 8.59; N, 8.53.

Acetonide of Dihydrositsirikine Diol

The above diol (150 mg.) was dissolved in dry acetone (20 ml.) and p-toluenesulphonic acid (110 mg.) added. After

standing at room temperature for 48 hours, the solution was neutralised with aqueous ammonia and the acetone removed under vacuum. The product was isolated with ether, the ethereal solution dried over magnesium sulphate, and evaporated to leave a gum. On trituration with a little anhydrous ether crystals formed, which were filtered off and found to be unreacted diol (95 mg.).

The filtrate was evaporated, the residue taken up in benzene and passed throught a column of alumina (3 g.). Removal of the solvent gave the acetonide as an amorphous powder (30 mg.) which crystallised from methanol with one molecule of solvent, m.p. 105-109° ; one spot on T.L.C. (EtOAc-$CHCl_3$, 1:1); $\bar{\nu}_{max.}$ (Nujol): 3200 (NH) $cm.^{-1}$; N.M.R. signals ($CDCl_3$): 1.61 (singlet, 1H, NH), 2.8 (multiplet, 4H, aromatic), 6.25 (doublet, 4H, 2 CH_2O), 8.62 (singlet, 6H, $(CH_3)_2CO_2$), 9.06 (broad singlet, 3H, CH_3C)τ. Found (powder): C, 74.56; H, 9.03; N, 7.31. Calc. for $C_{23}H_{32}O_2N_2$: C, 74.96; H, 8.75; N, 7.60. Found (solvate): C, 72.21; H, 8.71. Calc. for $C_{23}H_{32}O_2N_2 \cdot MeOH$: C, 71.96; H, 9.06.

Modified Kuhn-Roth Oxidation[37] of Dihydrositsirikine (25)

Dihydrositsirikine (5 mg.) and 10% chromic acid (2 ml.) were put in a distillation apparatus, double-distilled water (2 ml.) added, and distillation begun immediately. It was continued with periodic addition of water until 30 ml. of distillate had been collected. This was neutralised with

2 N aqueous potassium hydroxide (pH meter), and evaporated to dryness. The residue was taken up in pure water (0.3 ml.) and put on a small column of Dowex 50 acid resin (2 x 0.5 cm.); the flask was washed with water (2 x 0.5 ml.) and this also added to the column. To the filtrate was added a few drops of 70% aqueous ethylamine, and it was then concentrated under vacuum at 30-40° down to 1-2 drops. This was spotted on Whatman No. 1 paper together with standard solutions of the ethylamine salts of acetic, propionic and butyric acids.

The paper was developed by descending chromatography, using a 0.025 M ethylamine solution in water-saturated n-butanol as the stationary phase, and water-saturated n-butanol as the mobile phase[37]. After 24 hours the paper was sprayed with alcoholic bromocresol green solution, when the acids became visible as blue spots on a yellow background.

Dihydrositsirikine gave acetic and propionic acids, and a blank oxidation showed only the barest trace of acetic acid.

Sitsirikine under these conditions also gave acetic and propionic acids, while isositsirikine gave acetic acid only.

Ozonisation of Sitsirikine (26) and Isositsirikine (31)

Sitsirikine (5 mg.) in glacial acetic acid (1 ml.) was ozonised for 5 minutes, then transferred to a distillation apparatus containing 5% aqueous ferrous sulphate (15 ml.).

After 30 minutes the mixture was steam distilled into an aqueous solution of 2,4-dinitrophenylhydrazine sulphate until about 10 ml. of water had passed over. The solution was extracted several times with benzene, the combined extracts dried with magnesium sulphate and flushed through a column of Fisher acid-washed alumina (10 g.). After concentration of the benzene solution to about 0.5 ml., a few drops were spotted on Whatman No. 1 paper, together with standard solutions (5 mg./ml.) of the 2,4-dinitrophenylhydrazones of formaldehyde, acetaldehyde and acetone.

The paper was developed by descending chromatography[36], using methanol-heptane as the stationary phase and heptane as the mobile phase. After 6 hours the paper was sprayed with 10% aqueous sodium hydroxide solution. The 2,4-dinitrophenylhydrazone of formaldehyde (red-brown spot, Rf 0.10) was clearly indicated, and a trace of acetone (dark-brown spot, Rf 0.30) was also present. Blank experiments gave no spots corresponding to formaldehyde or acetaldehyde, but always showed a trace of acetone.

Repetition of the same procedure for isositsirikine (5 mg.) showed formation of acetaldehyde 2,4-dinitrophenylhydrazone (Rf 0.19).

Lead Tetracetate Dehydrogenation of Dihydrositsirikine

Lead tetracetate (400 mg.) was added in small portions over a period of 10 minutes to a solution of dihydrositsi-

rikine (100 mg.) in glacial acetic acid (10 ml.). The mixture was kept at 50-60° for a further 20 minutes, then poured into ice-cold 50% aqueous sodium hydroxide solution and extracted with chloroform. The chloroform extract was washed with a little water, dried over sodium sulphate, and acidified to Congo red with 8 N ethanolic hydrogen chloride. Evaporation of the solvent afforded tetradehydro-dihydrositsirikine hydrochloride as a gum (70 mg.), which could not be induced to crystallise; $\lambda_{max.}$ (acid and neutral solution): 253, 308, 365 mμ; $\lambda_{max.}$ (alkaline solution): 284, 328 mμ; $\bar{\nu}_{max.}$ ($CHCl_3$): 1700 (C=O), 1630 (aromatic) $cm.^{-1}$.

Attempted Palladium Dehydrogenation of Tetradehydro -dihydrositsirikine Hydrochloride

The hydrochloride (55 mg.) from the previous reaction was mixed with palladium black (50 mg.) and heated at 250-270° under nitrogen for 7 minutes. The residue was leached with hot methanol, the solution filtered, and the U.V. spectrum run directly on this solution. It was unchanged from that of the starting material.

The reaction was repeated twice, heating at 280° for 15 and 30 minutes; however, no change was observed in the U.V. spectrum.

Palladium-charcoal Dehydrogenation of Dihydrositsirikine (25)

Dihydrositsirikine (50 mg.) was well mixed with 10% palladium-charcoal (250 mg.) and heated under nitrogen at 250°

for 15 minutes. The residue was extracted with hot methanol and the U.V. spectrum run; $\lambda_{max.}$: 230, 290, 310, 385 mμ.

After removal of the methanol, the product was taken up in ether-water, the ether layer separated and dried over sodium sulphate. Removal of the solvent gave a gum (30 mg.); $\lambda_{max.}$: 230, 288, 317 mμ.

The aqueous portion was made strongly alkaline (pH>10) and extracted with chloroform. After drying, the chloroform solution was evaporated to afford a gum (5 mg.); $\lambda_{max.}$: 295, 313, 348, 389 mμ.

The neutral extract was dissolved in a few drops of methanol and spotted on a preparative T.L.C. plate (silica gel, 0.5 mm. thick). The plate was developed in chloroform-ethyl acetate (3:1) for 45 minutes, dried, and then developed two more times. Under U.V. light four bands could be seen, and each was cut out and extracted with methanol in a Soxhlet apparatus for several hours. The extract from the main band (compound A) displayed U.V. spectra similar to those of harman[43] (Figures 5 and 6); $\lambda_{max.}$ (neutral and alkaline solution): 234, 250, 282, 288, 337, 349 mμ; $\lambda_{max.}$ (acid solution): 254, 303, 372 mμ. Evaporation of the methanol yielded a gum (10 mg.); $\bar{\nu}_{max.}$ ($CHCl_3$): 1710 (C=O), 1625 (C=C) $cm.^{-1}$.

Palladium-charcoal Dehydrogenation of Dihydrositsirikine Hydrobromide

The hydrobromide (50 mg.) was well mixed with 10% palladium-charcoal (200 mg.) and heated under nitrogen at 280° for 15 minutes. The residue was extracted with hot methanol, filtered, and the U.V. spectra run; $\lambda_{max.}$ (neutral and acidic solution): 221, 250, 308, 366, 380 mμ; $\lambda_{max.}$ (alkaline solution): 282, 310, 382 mμ.

The solvent was removed and the residue dissolved in water, the solution made basic (pH 8) with ammonia and shaken with ether. After separation and drying the ether was removed to leave a gum (5 mg.); $\lambda_{max.}$: 290, 355 mμ.

50% Aqueous sodium hydroxide was added to the aqueous portion until it was strongly alkaline (pH>10), and the solution was then extracted with chloroform. The red chloroform extract was washed with water, dried, and acidified with 8 N ethanolic hydrogen chloride (yellow solution). Evaporation of the solvent afforded a gum (22 mg.); $\lambda_{max.}$ (acid and neutral solution): 222, 308, 383 mμ; $\lambda_{max.}$ (alkaline solution): 283, 315, 380 mμ.

This material was dissolved in a little methanol and spotted on a preparative T.L.C. plate (silica gel, 0.5 mm. thick). This plate was run for 20 minutes in ethyl acetate, and then twice in ethanol-ethyl acetate (1:1) for 40 minutes.

Under U.V. light a separation into two main bands was observed. These were cut out and extracted with methanol in

a Soxhlet apparatus for several hours.

One fraction gave a U.V. spectrum analogous to that of tetradehydro-dihydrositsirikine hydrochloride; $\lambda_{max.}$: 251, 307, 365 mμ. The other fraction (compound B) gave a U.V. spectrum similar to that of 5,6-dihydroflavocoryline hydrochloride[43]; $\lambda_{max.}$: 221, 312, 386 mμ; $\lambda_{min.}$: 215, 277, 338 mμ.

Quinone Dehydrogenation of Compound B

The methanolic solution of compound B was evaporated to give a gum (2 mg.), which was dissolved in glacial acetic acid (0.5 ml.). 2,3-Dichloro-5,6-dicyano-p-benzoquinone (10 mg.) was added, and the mixture heated at 80-90° for 6 hours. The solution was then diluted with water and extracted several times with ether. After making strongly alkaline with 50% aqueous sodium hydroxide, the aqueous solution was shaken with chloroform, the organic layer separated, washed with a little water and dried over sodium sulphate. Acidification with 8 N ethanolic hydrogen chloride and removal of the solvent afforded a yellow gum (0.8 mg.). This (compound C) displayed a U.V. spectrum (Figure 7) very similar to that of flavocoryline hydrochloride[43]; $\lambda_{max.}$: 237, 291, 345, 385mμ $\lambda_{min.}$: 274, 304, 373 mμ.

The compound displayed the same Rf value (0.43) as an authentic sample of flavocoryline on paper chromatography using an ethyl acetate-pyridine-water (8:2:1) system.

Dihydrositsirikine Olefinic Ester (22)

Dihydrositsirikine acetate (1.3 g.) was heated under reflux with 0.1 N sodium methoxide in dry methanol (60 ml.) for 45 minutes. Solid carbon dioxide was then added, the methanol removed under vacuum, and the residue taken up in ether-water. The ethereal solution was dried and the solvent removed. Chromatography of the product on alumina (50 g.) afforded the desired material (410 mg.) on elution with benzene-ether (19:1). Ether eluted dihydrositsirikine (605 mg.).

Recrystallisation from methanol afforded the solvated olefinic ester as needles (290 mg.), m.p. 84-89° ; one spot on T.L.C.(EtOAc-$CHCl_3$, 1:1); $[\alpha]_D^{26}$ +2° (MeOH); $\bar{\nu}_{max.}$ (Nujol): 3160 (NH), 1708 (C=O), 1622 (C=C); N.M.R. signals ($CDCl_3$): 1.60 (singlet, 1H, NH), 2.8 (multiplet, 4H, aromatic), 3.73 (singlet, 1H, olefinic), 4.41 (singlet, 1H, olefinic), 6.23 (singlet, 3H, CH_3O), 9.06 (broad singlet, 3H, CH_3C)τ. Found: C, 71.41; H, 8.08; O, 12.71; N, 7.12. Calc. for $C_{21}H_{26}O_2N_2 \cdot MeOH$: C, 71.32; H, 8.16; O, 12.96; N, 7.56.

Desoxy-Dihydrositsirikine (23)

The above olefinic ester (95 mg.) in methanol was hydrogenated over palladium black (15 mg.). Uptake of hydrogen ceased after 3 hours when 1.04 mol. had been absorbed. The solution was filtered, heated to boiling, and water added dropwise until a permanent turbidity was observed. On cooling the saturated ester crystallised out as prisms (80 mg.), m.p.

172-177° ; two spots on T.L.C. (EtOAc-$CHCl_3$, 1:1); $\bar{\nu}_{max.}$ (Nujol): 3370 (NH), 1710 (C=O) $cm.^{-1}$. Found: C, 74.04; H, 8.43; N, 8.36. Calc. for $C_{21}H_{28}O_2N_2$: C, 74.08, H, 8.29; N, 8.23.

Lithium Aluminium Hydride Reduction of Dihydrositsirikine Olefinic Ester (22)

The olefinic ester (150 mg.) and lithium aluminium hydride (100 mg.) in ether-tetrahydrofuran (1:1, 30 ml.) were heated under reflux for 2 hours. Excess hydride was decomposed with saturated aqueous sodium sulphate solution (20 ml.), the organic layer separated, and the aqueous suspension extracted with methylene chloride (3 x 10 ml.). The combined organic extracts were dried over magnesium sulphate and evaporated to leave a crystalline solid (140 mg.). This material had no carbonyl absorption in the I.R. region, but showed three spots on T.L.C. The N.M.R. spectrum indicated that the mixture contained only 25% of the expected terminal olefinic alcohol.

The product was taken up in benzene and chromatographed on alumina (5 g., deactivated with 0.3% of glacial acetic acid). Ethyl acetate eluted the major fraction (70 mg.), which was recrystallised twice from acetone-petroleum ether (b.p. 60-80°) and once from aqueous methanol to afford light brown needles (23 mg.), m.p. 204° ; one spot on T.L.C. (EtOAc); $[\alpha]_D^{26}$ -24.3° (MeOH); $\bar{\nu}_{max.}$ (Nujol): 3200 $cm.^{-1}$ (NH and OH); N.M.R. signals ($CDCl_3$): 1.25 (singlet, 1H, NH), 2.8 (multiplet, 4H, aromatic)τ, no olefinic protons. Found: C, 77.39; H, 8.49; N, 8.79.

Calc. for $C_{20}H_{26}ON_2$: C, 77.38; H, 8.44; N, 9.03.

The constants quoted in the literature[42] for iso-desmethoxy-dihydrocorynantheine alcohol (21) are m.p. 204°, $[\alpha]_D^{17}$ -24.0° (MeOH).

Dihydrocorynantheine (18b)

Crude corynantheine[110] (700 mg., two spots on T.L.C.) was hydrogenated in ethanol (5 ml.) over palladium black. Uptake of hydrogen ceased after 20 minutes when 0.6 mol. had been absorbed. After filtration the solution was warmed to 65° and water added dropwise until the solution remained cloudy. On cooling dihydrocorynantheine was obtained as needles (610 mg.), m.p. 174-176°; one spot on T.L.C. (EtOAc); $\bar{\nu}_{max.}$ ($CHCl_3$): 3420 (NH), 1690 (C=O), 1628 (C=C) $cm.^{-1}$. N.M.R. signals (CD_3COCD_3): 0.43 (singlet, 1H, NH), 2.8 (multiplet, 5H, aromatic and olefinic), 6.27 (singlet, 3H, CH_3O), 6.40 (singlet, 3H, CH_3O), 9.1 (broad singlet, 3H, CH_3C)τ. Found: C, 71.78; H, 7.91; N, 7.56. Calc. for $C_{22}H_{28}O_3N_2$: C, 71.71; H, 7.66; N, 7.60.

The m.p. quoted[111] for dihydrocorynantheine is 173-174°.

Desmethyl-dihydrocorynantheine (24)

A solution of dihydrocorynantheine (500 mg.) in acetone (50 ml.) was cooled in ice and dry hydrogen chloride passed in for 15 minutes. After standing at 5° for 15 hours, the solution was evaporated under vacuum to small bulk, diluted with water (100 ml.) and extracted with chloroform (10 x 50 ml.).

The water layer was then made basic with aqueous ammonia, extracted with ether (3 x 50 ml.) and the combined ether extracts dried over magnesium sulphate. Removal of the ether afforded desmethyl-dihydrocorynantheine as an amorphous powder (240 mg.), which gave a positive ferric chloride test (purple); $\bar{\nu}_{max.}$ ($CHCl_3$): 3480 (NH) 1715 (C=O), 1658 (HO-C=C-C=O), 1607 (C=C) $cm.^{-1}$. N.M.R. signals (CD_3COCD_3): 1.01 (singlet, 1H, NH), 2.8 (multiplet, 4H, aromatic), 6.28 and 6.52 (2 singlets, 3H, CH_3O), 9.1 (broad singlet, 3H, CH_3C)τ. Found: C, 71.22; H, 7.91; N, 8.21. Calc. for $C_{21}H_{26}O_3N_2$: C, 71.16, H, 7.39; N, 7.90.

Synthesis of Dihydrositsirikine (25)

Desmethyl-dihydrocorynantheine (200 mg.), in methanol (10 ml.), was reduced with sodium borohydride (500 mg.). After 1 hour the methanol was removed under vacuum, the residue treated with water and then extracted with ether. The ethereal layer was separated, dried over magnesium sulphate and the solvent evaporated. The resulting material was dissolved in benzene and chromatographed on alumina (10 g.). Elution with ether yielded a solid which was recrystallised twice from petroleum ether (b.p. 60-80°) to afford stout needles (65 mg.), m.p. 215° ; one spot on T.L.C. (EtOAc); $[\alpha]_D^{26}$ -56° (MeOH); $\bar{\nu}_{max.}$ (Nujol): 3400 (NH), 3200 (OH) 1710 (C=O) $cm.^{-1}$. Found: C, 70.81; H, 7.94; N, 7.96. Calc. for $C_{21}H_{28}O_3N_2$: C, 70.76; H, 7.92; N, 7.86.

This compound was identical with dihydrositsirikine by all criteria: m.p. and mixed m.p.; optical rotation; Rf value on T.L.C.; superimposible I.R. spectra.

Isositsirikine (31)

Isositsirikine was obtained from Dr.M. Gorman, Eli Lilly Laboratories as the crystalline sulphate, m.p. 263.5°. Found: C, 62.70, H, 6.69; O, 20.14; N, 6.93; S, 3.97; C-Me, 2.95; O-Me, 7.65. Calc. for $C_{21}H_{26}O_3N_2 \cdot \frac{1}{2}H_2SO_4$: C, 62.52; H, 6.74; O, 19.84; N, 7.00; S, 3.97; (1) C-Me, 3.72; (1) O-Me, 7.69.

The free base was an amorphous powder, $[\alpha]_D^{25}$ -20° ($CHCl_3$); one spot on T.L.C. (EtOAc); $\lambda_{max.}$ (log ε): 224 (4.55), 283 (3.92), 291 (3.84) mμ; $\bar{\nu}_{max.}$ ($CHCl_3$): 3400 (NH and OH), 1725 (C=O) cm.$^{-1}$, no Bohlmann bands[44]; $\bar{\nu}_{max.}$ (Nujol): 3300 (NH and OH), 1720 (C=O), 740 (o-disubstituted benzene) cm.$^{-1}$; N.M.R. signals ($CDCl_3$): 1.33 (singlet, 1H, NH), 2.7 (multiplet, 4H, aromatic), 4.53 (quartet, J = 7 c.p.s., 1H, C=<u>CH</u>-CH_3), 6.28 (singlet, 3H, CH_3O), 8.40 (doublet, J = 7 c.p.s., 3H, <u>CH_3</u>-CH=C) τ. Found: C, 70.65; H, 7.75; O, 14.15; N, 7.53; C-Me, 3.28; O-Me, 9.17; M.W. 354. Calc. for $C_{21}H_{26}O_3N_2$: C, 71.16; H, 7.39; O, 13.54; N, 7.90; (1) C-Me, 4.24; (1) O-Me, 8.76; M.W. 354.

Isositsirikine picrate was prepared in the manner described for sitsirikine and recrystallised from methanol as yellow needles, m.p. 216°. Found: C, 55.60, 55.69; H, 5.20, 5.38; O, 27.29; N, 12.17, 11.92. Calc. for $C_{27}H_{29}O_{10}N_5$:

C, 55.57; H, 5.01; O, 27.42; N, 12.00.

Acetylation of isositsirikine with acetic anhydride in pyridine gave an amorphous monoacetate which was homogeneous by T.L.C. (EtOAc-$CHCl_3$, 1:1); $\bar{\nu}_{max.}$ (CCl_4): 3380 (NH), 1730 (C=O), 1235 (OAc) $cm.^{-1}$; N.M.R. signals ($CDCl_3$): 1.40 (singlet, 1H, NH), 2.7 (multiplet, 4H, aromatic), 4.39 (quartet, 1H, C=$\underline{CH}$-CH_3), 6.05 (doublet, 2H, CH-$\underline{CH_2}$OAc) 6.27 (singlet, 3H, CH_3O), 8.15 (singlet, 3H, CH_3C=O), 8.36 (doublet, 3H, $\underline{CH_3}$-CH=C)τ. Found: C, 69.84; H, 7.78; N, 7.44. Calc. for $C_{23}H_{28}O_4N_2$: C, 69.67, H, 7.12; N, 7.07.

Acetonide of Isositsirikine Diol

A solution of isositsirikine (300 mg.) in tetrahydrofuran (10 ml.) was run into a suspension of lithium aluminium hydride (300 mg.) in ether (5 ml.), and the mixture heated under reflux for 3 hours. Saturated aqueous sodium sulphate (10 ml.) was added with stirring and the organic layer separated. After dilution with water (20 ml.) the aqueous layer was extracted with methylene chloride (3 x 20 ml.), and the combined organic extracts were dried over sodium sulphate. Evaporation of the solution afforded a gum (240 mg.); $\bar{\nu}_{max.}$: 3200 (OH and NH) $cm.^{-1}$, no carbonyl absorption; N.M.R. signals (CD_3COCD_3): 2.8 (multiplet, 4H, aromatic), 4.53 (quartet, 1H, C=$\underline{CH}$-CH_3), 8.37 (doublet, 3H, $\underline{CH_3}$-CH=C)τ , no methoxyl absorption.

Since it could not be induced to crystallise, the gum was taken up in dry acetone (10 ml.), p-toluenesulphonic acid

(150 mg.) added, and the mixture heated under reflux for 30 minutes. After standing overnight the solution was made basic with aqueous ammonia, and the acetone removed under vacuum. The residue was extracted with ether, the ethereal solution dried over sodium sulphate and evaporated to leave a gum. This was taken up in benzene and filtered through alumina (5 g.). Removal of the benzene and recrystallisation from methanol afforded isositsirikine acetonide as needles (42 mg.), m.p. 105-109° ; one spot on T.L.C. (EtOAc); $[\alpha]_D^{26}$ -53° ($CHCl_3$); $\bar{\nu}_{max.}$ (Nujol): 3200 (NH) $cm.^{-1}$; N.M.R. signals ($CDCl_3$): 1.87 (singlet, 1H, NH), 2.8 (multiplet, 4H, aromatic), 4.40 (quartet, 1H, C=<u>CH</u>-CH_3), 8.30 (doublet, 3H, CH_3-CH=C), 8.63 (singlet, 3H, $(CH_3)_2CO_2$), 8.68 (singlet, 3H, $(CH_3)_2CO_2$)τ. Found: C, 72.10, 72.19; H, 8.39; 8.46; N, 7.20. Calc. for $C_{23}H_{30}O_2N_2 \cdot$ MeOH: C, 72.33, H, 8.60; N, 7.03.

<u>Dihydro-isositsirikine (29)</u>

Isositsirikine base (200 mg.), in methanol (5 ml.), was hydrogenated over palladium black (20 mg.). Uptake of hydrogen ceased after 5 hours, when 1.02 mol. had been absorbed. Removal of the catalyst and the solvent yielded an amorphous product, which showed two spots on T.L.C. (EtOAc). The major component (120 mg.) was isolated from the ether-benzene (1:3) eluate during chromatography on alumina (10 g.).

Dihydro-isositsirikine was an amorphous powder which could not be induced to crystallise, but was homogeneous on T.L.C.; $\lambda_{max.}$ ($\log \epsilon$): 226 (4.57), 284 (3.92), 291 (3.84) $m\mu$;

$\bar{\nu}_{max.}$ ($CHCl_3$): 3480 (NH and OH), 2810 and 2760 (Bohlmann bands)[44] 1720 (C=O) $cm.^{-1}$; N.M.R. signals ($CDCl_3$): 2.01 (singlet, 1H, NH), 2.8 (multiplet, 4H, aromatic), 6.20 (singlet, 3H, CH_3O), 9.03 (broad singlet, 3H, CH_3C)τ . Found: C, 70.30; H, 7.53; N, 8.13. Calc. for $C_{21}H_{28}O_3N_2$: C, 70.76; H, 7.92; N, 7.86.

Dihydro-isositsirikine picrate was formed in the usual manner and recrystallised from aqueous methanol as yellow platelets, m.p. 187° . Found: C, 54.65, 54.30; H, 5.68, 4.84; O, 27.57; N, 11.89. Calc. for $C_{27}H_{31}O_{10}N_5 \cdot \frac{1}{2}H_2O$: C, 54.56; H, 5.43; O, 28.23; N, 11.79.

Lead Tetracetate Oxidation of Dihydro-isositsirikine (29)

The dihydro compound (100 mg.) was dissolved in acetic acid (10 ml.), lead tetracetate (400 mg.) added in small portions, and the mixture heated at ca. 60° for 2 hours. After removal of the solvent the residue was taken up in water (20 ml.), made strongly alkaline with 50% aqueous potassium hydroxide and extracted with chloroform (3 x 20 ml.).

The combined extracts were dried over sodium sulphate, acidified with 8 N ethanolic hydrogen chloride and evaporated to dryness. Tetradehydro-dihydro-isositsirikine (30) hydrochloride was thus obtained as a red glass (70 mg.); $\lambda_{max.}$: 253, 308, 360 mμ; $\bar{\nu}_{max.}$ ($CHCl_3$, free base): 1730 (C=O), 1615 $cm.^{-1}$; T.L.C. (EtOAc) showed no starting material.

Sodium Borohydride Reduction of Tetradehydro-dihydro-isositsirikine (30)

The above hydrochloride (60 mg.), in methanol (5 ml.), was treated with sodium borohydride (200 mg.), and heated under reflux for 1 hour. The solvent was removed under vacuum, the residue taken up in water (10 ml.), and extracted with ether (4 x 10 ml.) After drying, the ethereal solution was evaporated, the product taken up in benzene, and chromatographed on alumina(3 g.). Benzene-ether (3:1) eluted a compound (21 mg.) which was found to be identical to dihydro-isositsirikine (U.V. and I.R. spectra, T.L.C.).

Palladium Dehydrogenation of Isositsirikine Sulphate

Isositsirikine sulphate (60 mg.) was intimately mixed with palladium black (60 mg.) and heated at ca. 270° under a nitrogen atmosphere for 10 minutes. The product was taken up in hot methanol, the solution filtered, evaporated down to a few drops and spotted on a preparative T.L.C. plate (silica gel, 0.5 mm. thick). The plate was developed twice in ethanol-ethyl acetate (1:1), then viewed under U.V. light and the fluorescent bands cut out. Each section was extracted with methanol in a Soxhlet apparatus for several hours, and the U.V. spectrum run on the solution. Significant spectra were shown by two fractions:
(i) The fraction with Rf 0.9 had a U.V. absorption similar to that of harman[43] (cf. Figures 5 and 6); $\lambda_{max.}$ (neutral solution): 232, 282, 289, 336, 347 mμ ; $\lambda_{min.}$: 273, 305, 340 mμ ;

$\lambda_{max.}$ (acid solution): 251, 302, 374 mμ ; $\lambda_{min.}$: 233, 279, 330 mμ .

(ii) The fraction with Rf 0.1 had a spectrum reminiscent of flavocoryline[43]; $\lambda_{max.}$ (neutral and acid solution): 237, 247, 291, 345, 385 mμ ; $\lambda_{min.}$: 245, 274, 304, 373 mμ .

The methanolic solution of (ii) was evaporated and the residue taken up in a little water. 50% Aqueous potassium hydroxide was then added until the solution was strongly alkaline, and the mixture extracted with chloroform (3 x 10 ml.) After drying over potassium carbonate the chloroform extract was acidified with 8 N ethanolic hydrogen chloride and evaporated to leave a yellow gum (9 mg.). The I.R. spectrum was similar but not identical to that of flavocoryline hydrochloride.

Paper chromatography showed that most of the material obtained from isositsirikine was <u>not</u> flavocoryline. Using an ethyl acetate-pyridine-water (8:2:1) system, the major component of fraction (ii) had an Rf of 0.35, whereas flavocoryline had a corresponding value of 0.43.

Palladium Dehydrogenation of Dihydro-isositsirikine Hydrochloride

Dihydro-isositsirikine (200 mg.) was converted to the amorphous hydrochloride salt, which was then intimately mixed with palladium black (200 mg.) and heated under a nitrogen atmosphere at ca. 280° for 10 minutes. The residue was taken up in hot glacial acetic acid (5 ml.), the solution filtered

and 2,3-dichloro-5,6-dicyano-p-benzoquinone (200 mg.) added. The mixture was then heated at 90-95° for 5 hours. After removal of the solvent under vacuum, dilute aqueous ammonia (20 ml.) was added (pH 8), and the solution extracted several times with ether to remove weak bases. The aqueous solution was then made strongly alkaline (pH>10) with 50% aqueous potassium hydroxide and extracted with chloroform (3 x 20 ml.). When the chloroform extract had been dried over potassium carbonate, it was acidified with 8 N ethanolic hydrogen chloride and evaporated to yield a yellow gum (40 mg.), which displayed a flavocoryline-type U.V. spectrum. Paper chromatography (as above) indicated that it was a mixture of two compounds — the major constituent having the same Rf value as flavocoryline, whereas the other corresponded to the compound obtained from isositsirikine. Three recrystallisations from chloroform afforded the major component as yellow needles (6 mg.), m.p. 280-282° (block preheated to 150°); $\lambda_{max.}$ (logϵ): 238 (4.54), 247 (4.50), 290 (4.13), 345 (4.27), 384 (4.23) mμ ; $\lambda_{min.}$ (log ϵ): 210 (4.26), 244 (4.49), 273 (4.04), 303 (4.01), 373 (4.15) mμ; $\bar{\nu}_{max.}$ (Nujol): 3350 (NH), 1650, 1630, 1515 cm.$^{-1}$; one spot on paper chromatography.

This compound was found to be identical with authentic flavocoryline hydrochloride in all respects: m.p. and mixed m.p.; superimposible I.R. and U.V. spectra; same Rf values on paper chromatography.

Part II Experimental Section

Isolation of Cleavamine (66) and Descarbomethoxy-catharanthine (65)

A mixture of catharanthine hydrochloride (40 g.)[101], stannous chloride (44 g.) and mossy tin (4 g.) in concentrated hydrochloric acid (520 ml.), was heated under reflux in a nitrogen atmosphere for 75 minutes. By the end of this time a red gum had formed. The acidic solution was decanted from the gum and washed with methylene chloride (3 x 100 ml.). The washings were combined with the red gum, then methanol (50 ml.) and methylene chloride (100 ml.) were added so that a clear solution was obtained. This solution was shaken with 1 N aqueous sodium hydroxide (600 ml.), separated and washed with water (200 ml.); the sodium hydroxide solution was washed with ether (2 x 100 ml.) and the ethereal extract added to the methylene chloride solution. After drying over magnesium sulphate, the organic solution was evaporated to leave a reddish oil (32 g.), which was taken up in benzene and chromatographed on alumina (1200 g.).

Cleavamine[101] was eluted in the initial benzene-petroleum ether (b.p. 30-60°) (1:1) fractions and recrystallised from methanol to give needles (2.4 g.), m.p. 117-119° ; one spot on T.L.C. (EtOAc); $[\alpha]_D^{26}$ +73° ($CHCl_3$); $\lambda_{max.}$ (log ϵ): 225 (4.60), 285 (3.87), 292 (3.86) mμ ; $\bar{\nu}_{max.}$ (Nujol): 3420 (NH), 2800, 2740 and 2700 (Bohlmann bands)[44] $cm.^{-1}$, no carbonyl absorption; N.M.R. signals (CD_3COCD_3): 0.87 (singlet, 1H, NH) 2.8 (multiplet, 4H, aromatic), 4.72 (doublet, 1H, olefinic),

8.96 (triplet, 3H, $\underline{CH_3}CH_2$)τ. Found: C, 81.30; H, 8.54; N, 10.18; M.W. 280. Calc. for $C_{19}H_{24}N_2$: C, 81.38; H, 8.63; N, 9.99; M.W. 280.

Benzene-chloroform (1:1) eluted starting material (~ 7 g.) in the initial fractions and descarbomethoxy-catharanthine (1.0 g.) in the later fractions. The latter material was recrystallised twice from ether to yield needles, m.p. 103-104°, which were identical with authentic descarbomethoxy-catharanthine[101] (I.R., T.L.C.); $\bar{\nu}_{max.}$ (Nujol): 3140 (NH) cm.$^{-1}$. Found: C, 81.70; H, 8.12; N, 10.10. Calc.for $C_{19}H_{22}N_2$: C, 81.97; H, 7.97; N, 10.06.

4"α"-Dihydrocleavamine (68)

Cleavamine (1.5 g.), in ethyl acetate (20 ml.), was hydrogenated over Adam's catalyst (150 mg.). Uptake of hydrogen ceased after 50 minutes when 1 mol. of hydrogen had been absorbed. Filtration and evaporation gave 4"α"-dihydrocleavamine, which recrystallised from methanol as prisms (1.2 g.), m.p. 136-138°; one spot on T.L.C. (EtOAc); $\bar{\nu}_{max.}$ (Nujol); 3410 (NH), 2790 and 2750 (Bohlmann bands) cm.$^{-1}$; N.M.R. signals (CD_3COCD_3): 0.88 (singlet, 1H, NH), 2.8 (multiplet,4H, aromatic), 9.17 (triplet, 3H, $\underline{CH_3}CH_2$)τ. Found: C, 81.02; H, 9.59; N, 9.88; M.W. 282. Calc. for $C_{19}H_{26}N_2$: C, 80.80; H, 9.28; N, 9.92; M.W. 282.

Isolation of B9

The later benzene-petroleum ether (1:1) fractions from the above chromatography (p.110) displayed three spots on T.L.C.

(EtOAc) with Rf values of 0.77, 0.53, and 0.27. The compound with a Rf value of 0.77 was found to correspond to cleavamine. These fractions were combined (5.4 g.) and placed on alumina (450 g.). Elution was begun with benzene-petroleum ether (1:2), and the first four fractions afforded cleavamine(1.4 g.) after recrystallisation from methanol. Later fractions contained progressively less cleavamine and more of the other two constituents. The last (B9, 55 mg.) of the nine fractions obtained with this eluent contained no cleavamine, displaying only two spots on T.L.C. (Rf 0.53 and 0.27). Recrystallisation from aqueous ethanol gave light brown prisms, m.p. 127-132° ; $\bar{\nu}_{max.}$ (Nujol): 3410 (NH),2790 and 2740 (Bohlmann bands) $cm.^{-1}$; N.M.R. signals ($CDCl_3$): 2.05 (singlet, 1H, NH), 2.8 (multiplet, 4H, aromatic), 9.13 (broad singlet, 3H, CH_3C)τ, no olefinic proton absorption. Found: C, 80.56; H, 9.46; N, 10.04; M.W. 282. Calc.for $C_{19}H_{26}N_2$: C, 80.80; H, 9.28; N, 9.92; M.W. 282.

Fraction B9 was shown to be a mixture of 4"α"- and 4"β"-dihydrocleavamine by comparison (T.L.C., I.R. spectra) with authentic samples. The 4"α"-dihydrocleavamine was prepared by catalytic hydrogenation of cleavamine (see above), and a sample of 4"β"-dihydrocleavamine was kindly provided by Dr.M. Gorman, Lilly Research Laboratories.

Carbomethoxy-4"β"-dihydrocleavamine (119)[98]

A mixture of catharanthine (5) (30 g.) and zinc dust (300 g.), in glacial acetic acid (750 ml.), was heated under

reflux in a nitrogen atmosphere for 4 hours. The hot solution was decanted, most of the solvent removed under vacuum, and the residue taken up in water (100 ml.). The solution was made basic with aqueous ammonia, extracted with ether (4 x 150 ml.), and the combined ether extracts dried over magnesium sulphate. Removal of the ether afforded an oil which was taken up in hot methanol (100 ml.). A crystalline solid (6.3 g.) was deposited on cooling. This material showed three spots on T.L.C. (EtOAc-$CHCl_3$, 1:1) and consequently was chromatographed on alumina (200 g.). Elution with benzene-petroleum ether (b.p. 30-60°) (1:1) provided carbomethoxy-4"β"-dihydrocleavamine (4.8 g.), which was recrystallised from methanol to afford stout needles, m.p. 172° ; one spot on T.L.C. (EtOAc-$CHCl_3$ 1:1); $[\alpha]_D^{26}$ +100° ($CHCl_3$); $\lambda_{max.}$ (log ε): 227 (4.47), 286 (3.87), 293 (3.84) mμ ; $\bar{\nu}_{max.}$ (Nujol): 3430 (NH), 2790 (Bohlmann band)[44], 1722 (C=O) $cm.^{-1}$; N.M.R. signals ($CDCl_3$): 1.00 (singlet, 1H, NH), 2.7 (multiplet, 4H, aromatic), 6.28 (singlet, 3H, CH_3O), 9.31 (triplet, 3H, $\underline{CH_3}CH_2$)τ, no olefinic proton absorption. Found: C, 74.26; H, 8.35; N, 8.26. Calc. for $C_{21}H_{28}O_2N_2$: C, 74.07; H, 8.29; N, 8.23.

Neuss et al.[68] quote m.p. 64-66°, $[\alpha]_D^{26}$ +96° ($CHCl_3$) for carbomethoxy-dihydrocleavamine.

4"β"-Dihydrocleavamine (68)

Carbomethoxy-4"β"-dihydrocleavamine (500 mg.), in 5N hydrochloric acid (30 ml.), was heated on the water-bath under

a nitrogen atmosphere for 8 hours. The solution was cooled in ice, made basic with aqueous ammonia, and extracted with methylene chloride (3 x 50 ml.). The organic extract was dried, concentrated to a small volume and filtered through alumina (10 g.). Evaporation of the solvent gave an amorphous powder (340 mg.) which could not be induced to crystallise; one spot on T.L.C. (EtOAc); $\bar{\nu}_{max.}$ (Nujol): 3350(NH), 2750 (Bohlmann band) $cm.^{-1}$, no carbonyl absorption.

This material was identical (T.L.C., I.R. spectra) with an authentic sample of 4"β"-dihydrocleavamine kindly supplied by Dr.M. Gorman, Lilly Research Laboratories.

Mercuric Acetate Oxidation of Carbomethoxy-4"β"-dihydrocleavamine (119)

Carbomethoxy-4"β"-dihydrocleavamine (4.5 g.) and mercuric acetate (10.5 g.), in glacial acetic acid (150 ml.), were stirred under a nitrogen atmosphere for 40 hours. The solution was then filtered from the precipitated mercurous acetate (8.2 g, 90%) and heated under reflux for 5 hours. The solvent was removed as far as possible under vacuum, the residue made basic with dilute ammonia (50 ml.) and extracted with methylene chloride (3 x 50 ml.).

After drying over sodium sulphate the methylene chloride was removed, and the dark brown product chromatographed on alumina (200 g., deactivated with 0.6 ml. of glacial acetic acid). The initial benzene fractions afforded pseudo-vincadifformine

(121) as a white powder (1.15 g.); $[\alpha]_D^{26}$ -503° (EtOH); one spot on T.L.C. (EtOAc-$CHCl_3$, 1:9); $\lambda_{max.}$ (log ε): 226 (4.07), 298 (4.12), 326 (4.24) mμ; $\bar{\nu}_{max.}$ (CCl_4): 3380 (NH), 2780 (Bohlmann band), 1675 (C=O), 1610 (C=C) $cm.^{-1}$; N.M.R signals ($CDCl_3$): 1.05 (singlet, 1H, NH), 2.8 (multiplet, 4H, aromatic), 6.23 (singlet, 3H, CH_3O), 9.07 (triplet, 3H, $\underline{CH_3}CH_2$)τ. Found: C, 74.48, 74.69; H, 7.80, 7.52; O, 9.62; N, 8.27; M.W. 338. Calc. for $C_{21}H_{26}O_2N_2$: C, 74.52; H, 7.74; O, 9.46; N, 8.28; M.W. 338.

The later benzene fractions yielded an amorphous powder (105 mg.); one spot on T.L.C. (EtOAc-$CHCl_3$, 1:9); $\lambda_{max.}$: 226, 285, 293 mμ; $\bar{\nu}_{max.}$ (CCl_4): 3400 (NH), 1705 (C=O) $cm.^{-1}$; N.M.R. signals ($CDCl_3$): 2.01 (singlet, 1H, NH), 2.8 (multiplet, 4H, aromatic), 6.30 (singlet, 3H, CH_3O), 9.10 (triplet, 3H, $\underline{CH_3}CH_2$)τ. The Rf value on T.L.C. and the I.R. spectrum were identical to those of coronaridine (145)[71].

The amorphous material was taken up in anhydrous ether and the hydrochloride salt formed by passing in dry hydrogen chloride. Two recrystallisations from acetone-ether afforded the hydrochloride as needles, m.p. 221-223° (dec.); $\bar{\nu}_{max.}$ (Nujol): 3160 (NH), 2530 ($\overset{+}{N}H$), 1715 (C=O) $cm.^{-1}$. An authentic sample of coronaridine hydrochloride, m.p. 221-223°, prepared from a sample of coronaridine kindly provided by Dr.M. Gorman, Lilly Research Laboratories, did not depress the melting point, and the I.R. spectra were identical.

Benzene-ether (1:1) eluted a compound (85 mg.), which was recrystallised from petroleum ether (b.p. 60-80°) to afford

prisms, m.p. 143-145.5°; $[\alpha]_D^{23}$+49° ($CHCl_3$); $\lambda_{max.}$: 225, 286, 293 mμ; $\bar{\nu}_{max.}$(KBr): 3350 (NH) 1700 (C=O) cm.$^{-1}$; N.M.R. signals ($CDCl_3$): 2.00 (singlet, 1H, NH), 2.8 (multiplet, 4H, aromatic), 6.38 (singlet, 3H, CH_3O), 9.04 (triplet, 3H, $\underline{CH_3}CH_2$)τ. This material was identified as dihydrocatharanthine (69)[28] by comparison (m.p., mixed m.p., T.L.C., I.R. spectra) with an authentic sample prepared by hydrogenation of catharanthine (5).[107]

Acid Hydrolysis of Pseudo-vincadifformine (121)

Pseudo-vincadifformine (100 mg.) was heated with 2 N hydrochloric acid (3 ml.) in a sealed tube at 110° for 6 hous. The solution was made basic with aqueous ammonia and the precipitate taken up in ether. Evaporation of the ethereal solution yielded a gummy product (123) which exhibited the spectral properties of an indolenine; $\lambda_{max.}$: 221, 250 (broad) mμ; $\bar{\nu}_{max.}$ (CCl_4): 1605, 1575 cm.$^{-1}$, no NH absorption.

The gum was dissolved in tetrahydrofuran (5 ml.) and heated under reflux with lithium aluminium hydride (100 mg.) for 3 hours. The excess of hydride was destroyed with saturated aqueous sodium sulphate (10 ml.) and the product isolated with ether. Removal of the solvent afforded (124) as an oil which crystallised from acetone as needles, m.p. 89-90°; $[\alpha]_D^{26}$-60° ($CHCl_3$); one spot on T.L.C. (EtOAc-$CHCl_3$, 1:9); $\lambda_{max.}$ (log ε): 243 (3.81), 295 (3.45) mμ; $\bar{\nu}_{max.}$ (Nujol): 3230 (NH), 1600 (aromatic C=C) cm.$^{-1}$; N.M.R. signals ($CDCl_3$): 3.1 (multiplet, 4H, aromatic) 9.10 (triplet, 3H, $\underline{CH_3}CH_2$)τ. Found: C, 81.00;

H, 9.38; N, 9.86; M.W. 282. Calc. for $C_{19}H_{26}N_2$: C, 80.80, H, 9.28; N, 9.92; M.W. 282.

Acetylation with acetic anhydride in pyridine afforded the N-acetate, which was recrystallised from petroleum ether (b.p. 60-80°), m.p. 107.5-109° ; one spot on T.L.C. (EtOAc-$CHCl_3$, 1:9); $\lambda_{max.}$ (log ϵ): 212 (4.35), 253 (4.13), 279 (3.58), 289 (3.51)mμ ; $\bar{\nu}_{max.}$ (KBr): 1655 (N-C=O), 1595 (aromatic C=C) cm.$^{-1}$; N.M.R. signals ($CDCl_3$): 1.87 (broad singlet, 1H, aromatic), 2.85 (multiplet, 3H, aromatic), 7.78 (singlet, 3H, $CH_3C=O$), 9.10 (triplet, 3H, $\underline{CH_3}CH_2$) τ. Found: C, 77.14; H, 8.64; O, 5.07; N, 8.97. Calc. for $C_{24}H_{28}ON_2$: C, 77.73; H, 8.70; O, 4.93; N, 8.63.

Reduction of Pseudo-vincadifformine (121) with Zinc and Sulphuric Acid

Pseudo-vincadifformine (1.0 g.) and zinc dust (150 g.), in 10% methanolic sulphuric acid (500 ml.), were heated under reflux for 30 minutes. The methanol was removed under vacuum, the acid neutralised with aqueous sodium carbonate, and the solution extracted with ether (3x 200 ml.). After drying, evaporation of the solvent afforded a gum (0.8 g.) which showed two spots on T.L.C. (EtOAc-$CHCl_3$, 1:9). This material was then chromatographed on alumina (40 g.). The major product (640 mg.), dihydro-pseudo-vincadifformine (139a), eluted with benzene-petroleum ether (b.p. 30-60°) (1:1) was an amorphous powder (from ether); $[\alpha]_D^{24}$ -16° (EtOH); one spot on T.L.C. (EtOAc-$CHCl_3$, 1:9); $\lambda_{max.}$ (log ϵ): 244 (3.82), 299 (3.45)mμ ; $\bar{\nu}_{max.}$

(CCl_4): 3380 (NH), 1720 (C=O), 1605 (aromatic C=C) $cm.^{-1}$; N.M.R. signals ($CDCl_3$): 3.1 (multiplet, 4H, aromatic), 6.33 (singlet, 3H, CH_3O), 9.12 (triplet, 3H, $\underline{CH_3}CH_2$)τ. Found: C, 73.97; H, 8.13; N, 8.32; M.W. 340. Calc. for $C_{21}H_{28}O_2N_2$: C, 74.08; H, 8.29; N, 8.23; M.W. 340.

Acetylation with acetic anhydride in pyridine gave an amorphous N-acetate, $[\alpha]_D^{23}$+28° (EtOH); one spot on T.L.C. (EtOAc-$CHCl_3$, 1:9); $\lambda_{max.}$ (log ϵ): 253 (4.01), 282 (3.49), 291 (3.45)mμ; $\bar{\nu}_{max.}$ (CCl_4): 1725 (C=O), 1660 (N-C=O), 1595 (aromatic C=C) $cm.^{-1}$; N.M.R. signals (CCl_4): 2.3 (broad singlet, 1H, aromatic), 3.0 (multiplet, 3H, aromatic), 6.83 (singlet, 3H, CH_3O), 7.75 (singlet, 3H, CH_3C=O), 9.08 (triplet, 3H, $\underline{CH_3}CH_2$)τ. Found: C, 72.26; H, 8.24; N, 7.51. Calc. for $C_{23}H_{30}O_3N_2$: C, 72.22; H, 7.91; N, 7.32.

The minor product (80 mg.), iso-dihydro-pseudo-vincadifformine (140a), was eluted with benzene-ether (1:1) and was also an amorphous powder, $[\alpha]_D^{24}$-132° (EtOH); one spot on T.L.C. (EtOAc-$CHCl_3$, 1:9); $\lambda_{max.}$ (log ϵ): 244 (3.83), 297 (3.45) mμ; $\bar{\nu}_{max.}$ (CCl_4); 3380 (NH), 1720 (C=O), 1605 (aromatic C=C) $cm.^{-1}$; N.M.R. signals ($CDCl_3$): 3.2 (multiplet, 4H, aromatic), 6.32 (singlet, 3H, CH_3O), 9.10 (triplet, 3H, $\underline{CH_3}CH_2$)τ. Found: C, 74.38; H, 8.32; N, 8.29. Calc. for $C_{21}H_{28}O_2N_2$: C, 74.08; H, 8.29; N, 8.23.

Acetylation with acetate anhydride in pyridine afforded an amorphous N-acetate, $[\alpha]_D^{24}$+3° (EtOH); one spot on T.L.C. (EtOAc-$CHCl_3$, 1:9); $\lambda_{max.}$ (log ϵ): 250 (4.09), 278 (3.47), 296

(3.39)mμ ; $\bar{\nu}_{max.}$ (CCl_4): 1725 (C=O), 1660 (N-C=O), 1595 (aromatic C=C) $cm.^{-1}$; N.M.R. signals (CCl_4): 2.9 (multiplet, 4H, aromatic), 6.42 (singlet, 3H, CH_3O), 7.86 (singlet, 3H, $CH_3C=O$), 9.10 (triplet, 3H, $\underline{CH_3}CH_2$)τ. Found: C, 72.57; H, 8.10. Calc. for $C_{23}H_{30}O_3N_2$: C, 72.22; H, 7.91.

Epimerisation of Dihydro-pseudo-vincadifformine (130)

A solution of dihydro-pseudo-vincadifformine (200 mg.) in methanol (2 ml.) was sealed in a tube together with sodium methoxide (60 mg.) and saturated methanolic magnesium methoxide solution (1 ml.), and heated at 100° for 5 hours. The solution was then poured into water (20 ml.) and extracted immediately with ether (4 x 20 ml.). After drying over sodium sulphate the ethereal extract was evaporated to give an amorphous powder (165 mg.), which was identical with iso-dihydro-pseudovincadifformine (140) (T.L.C., U.V. and I.R. spectra).

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