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THE UNIVERSITY OF ALBERTA

SOME CONSTITUENTS OF LYCOPODIUM LUCIDULUM

by

Ⓒ LESLIE FRANK BALL
B.Sc. (Hons),
Leeds, England

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTY
EDMONTON, ALBERTA
SPRING, 1971

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled SOME CONSTITUENTS OF LYCOPODIUM LUCIDULUM submitted by LESLIE FRANK BALL, B.Sc. (Hons.) in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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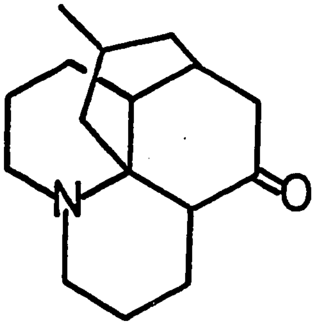
Date

ABSTRACT

An investigation of some of the constituents of *Lycopodium lucidulum* has been carried out. Particular interest has been directed towards the isolation and characterization of the compounds comprising the weakly basic constituents of this plant. A total of fourteen alkaloids have been isolated and characterized, which include lycopodine (2), lycodine (12), luciduline (33), anhydrolycodoline (50) and dihydroluciduline (73) of known structure.



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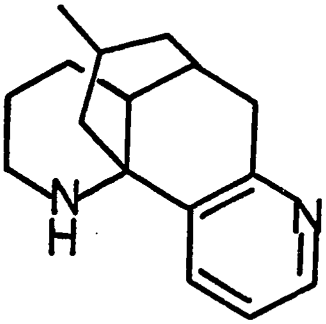

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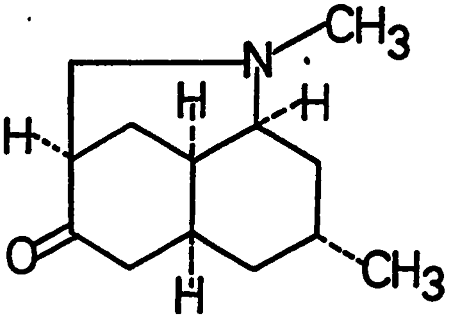

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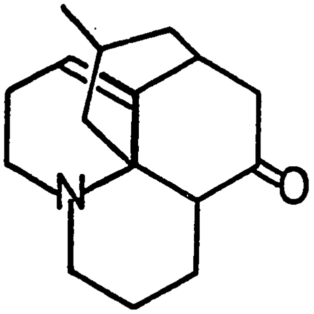

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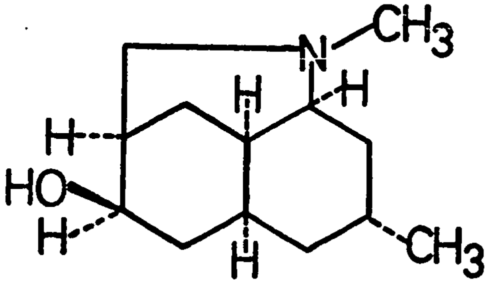
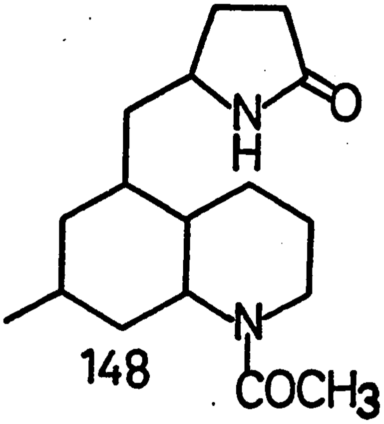


73

Structures 148 and 153 have been tentatively assigned to two hitherto undetected alkaloids.



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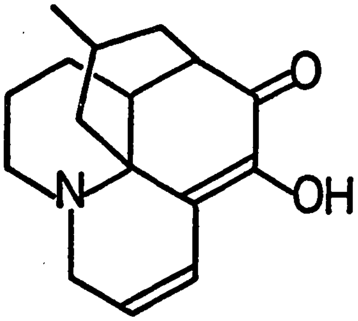
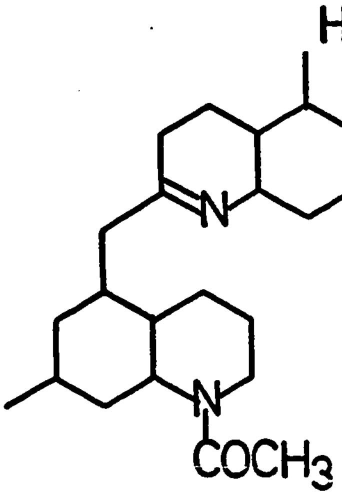


153

The major part of this thesis has been devoted to the characterization of the remaining seven alkaloids. Evidence is presented which indicates that these alkaloids contain a similar $C_{30}N_3$ carbon-nitrogen skeleton and suggests that the alkaloids consist of two distinct portions linked via one methylene group. Furthermore, the evidence suggests that the major portion is a $C_{17}N_2$ unit incorporating a 2,3,6-trisubstituted piperidine system, while the other is a $C_{12}N$ unit possessing a 7-methyldecahydroquinoline skeleton.

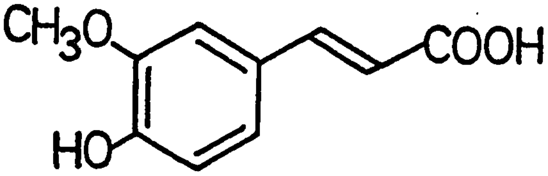
A consideration of the chemical and physical properties of these seven alkaloids, together with a consideration of the currently accepted mode of biosynthesis of the Lycopodium alkaloids, has led to the tentative

assignment of structure 121 to represent the carbon-nitrogen skeleton of these alkaloids.



121

A brief investigation of the acidic components of L. lucidulum has led to the isolation of ferulic acid (156).



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INTRODUCTION

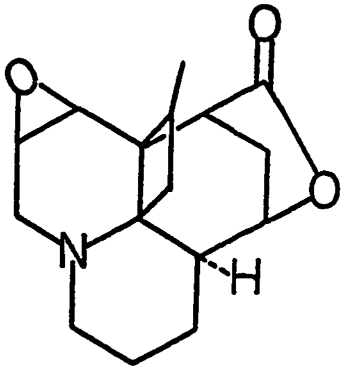
The Lycopodium alkaloids find their origin in the small evergreen perennial plants known as the Clubmosses, the best known of the fern allies.

The plants belong to the order LYCOPODIALES and, together with the SELAGINELLALES, or Spikemosses, they constitute the only two living orders in the class LYCOPODIINAE. The order LYCOPODIALES has one family, LYCOPODIACEAE, which in turn has two genera. One of these, the genus Phylloglossum, has only one species which is restricted to Australasia while the other, the genus Lycopodium, has more than 200 species throughout the world.

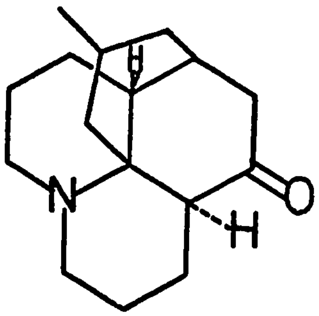
Chemical interest in the Lycopodium genus was first stimulated in 1881 when Bödeker[1] reported the isolation of an alkaloid from the species Lycopodium complanatum L. There is little doubt that this alkaloid is the one now known as lycopodine which, despite its early isolation, was not assigned the correct molecular formula $C_{16}H_{25}NO$ until 1938 following its isolation[2] from Lycopodium clavatum L.

In 1942 Marion and Manske initiated a systematic investigation of the Lycopodium genus and published a series of papers[3-13] over the next decade describing the isolation and characterization of some 35 alkaloids from a dozen Lycopodium species. At the present time, close to 100 alkaloids have been characterized[14].

The first structural elucidation of a *Lycopodium* alkaloid was in 1956 when Wiesner and Valenta proposed the structure 1 for annotinine[15], the major alkaloid of *Lycopodium annotinum* L. This structure was later confirmed by x-ray crystallographic studies[16] carried out on annotinine bromohydrin. In 1960, MacLean and Harrison[17] assigned structure 2 to lycopodine, the most widely distributed alkaloid in the LYCOPODIACEAE.



Annotinine
1



Lycopodine
2

The last decade has seen rapid progress in the characterization[18] and structural elucidation of the *Lycopodium* alkaloids, mainly because of improved methods of isolation and availability of a wider variety of spectroscopic techniques. Thin layer chromatography (t.l.c.), elution chromatography, counter current distribution and gas liquid partition chromatography (g.l.p.c.) all make large contributions to the isolation and characterization of these alkaloids. The rapid advances made in structural elucidation are due in great part to the development of improved physical methods. Mass spectrometry, ^1H nuclear magnetic resonance, infrared and ultraviolet spectroscopy have all played a large part in this field, while optical rotatory dispersion and circular dichroism have made an equally important but less extensive contribution. X-ray crystallography has been utilized both to confirm (e.g. annotinine[16] and luciduline[19]) and to establish (e.g. annopodine[20] and alopecurine[21]) structures in the *Lycopodium* genus. Now the application of ^{13}C nuclear magnetic resonance promises to be a very useful addition to the methods already available.

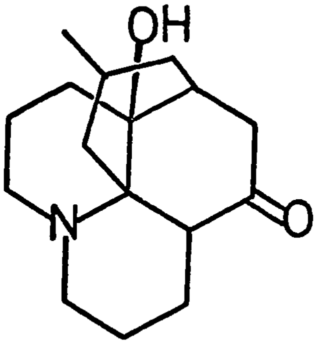
The contribution made by these techniques is typified by the fact that since 1960 the structures of some forty alkaloids have been established. Many of these alkaloids possess a carbon skeleton similar to that of lycopodine while others have appreciably different carbon skeletons.

These known alkaloids can be accommodated into ten structural groups, of which the lycopodine group is by far the largest. These groups are now introduced on a chronological basis according to the structural elucidation of their parent alkaloid.

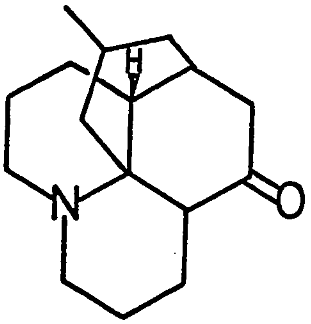
Annotinine (1) possesses a carbon skeleton now known to be unique among the *Lycopodium* alkaloids[15]. Considering lycopodine (2) had been known for some seventy years[1], it was perhaps surprising that the first *Lycopodium* alkaloid to yield to structural studies was instead annotinine. This is probably related to the fact that the region within easy access to the University of New Brunswick was found to be a rich source of *Lycopodium annotinum* L.

The isolation of 7-methylquinoline and 5,7-dimethylquinoline from the dehydrogenation[22] of lycopodine, together with an analysis of the products of its reaction with cyanogen bromide[23], proved to be the key to the structural elucidation of the alkaloid. The ready availability of lycopodine (2) has aided the structural solution of many alkaloids in various groups[14], usually by conversion of the structurally unknown alkaloid and lycopodine into a common derivative. Some typical members of the lycopodine group are presented in Chart I.

Ayer and Iverach[24], by dehydration followed by hydrogenation, were able to convert lycodoline (3) into a mixture of lycopodine (2) and 12-epilycopodine (11).



Lycodoline
3



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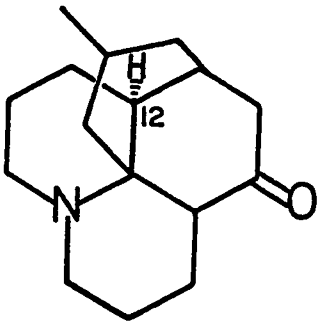

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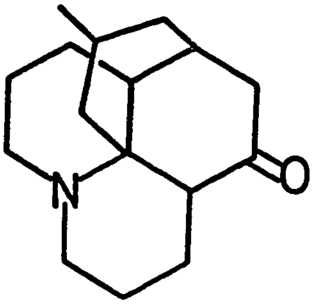
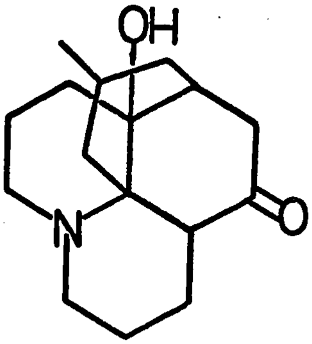
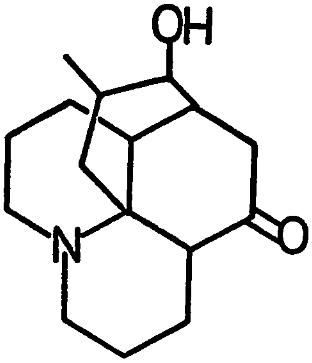
Chart I



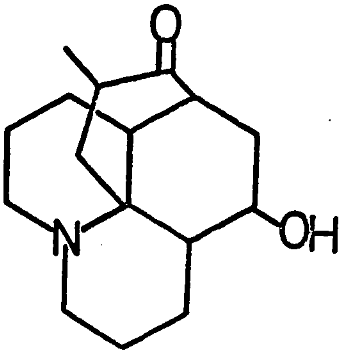
Lycopodine
2



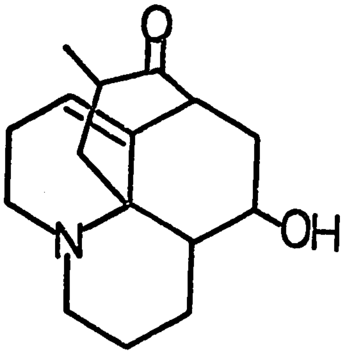
Lycodoline[24]
3



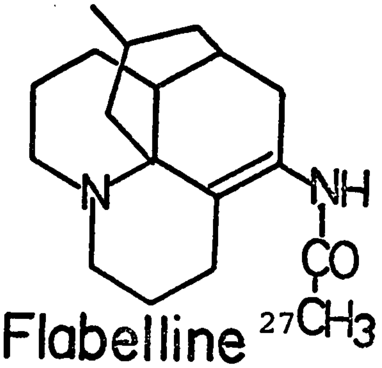
Clavolonine[29]
4



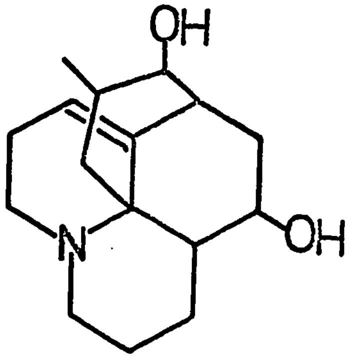
Annofoline[25]
5



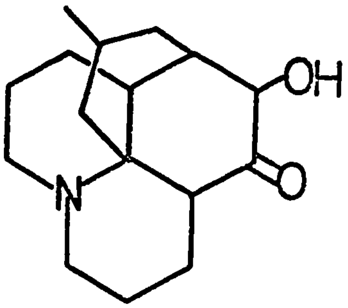
Acrifoline[30]
6



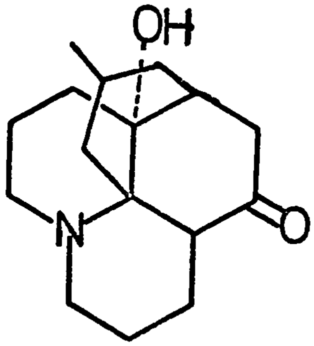
Flabelline[27]
7



Lycofoline[31,32]
8



L.20[26]
9

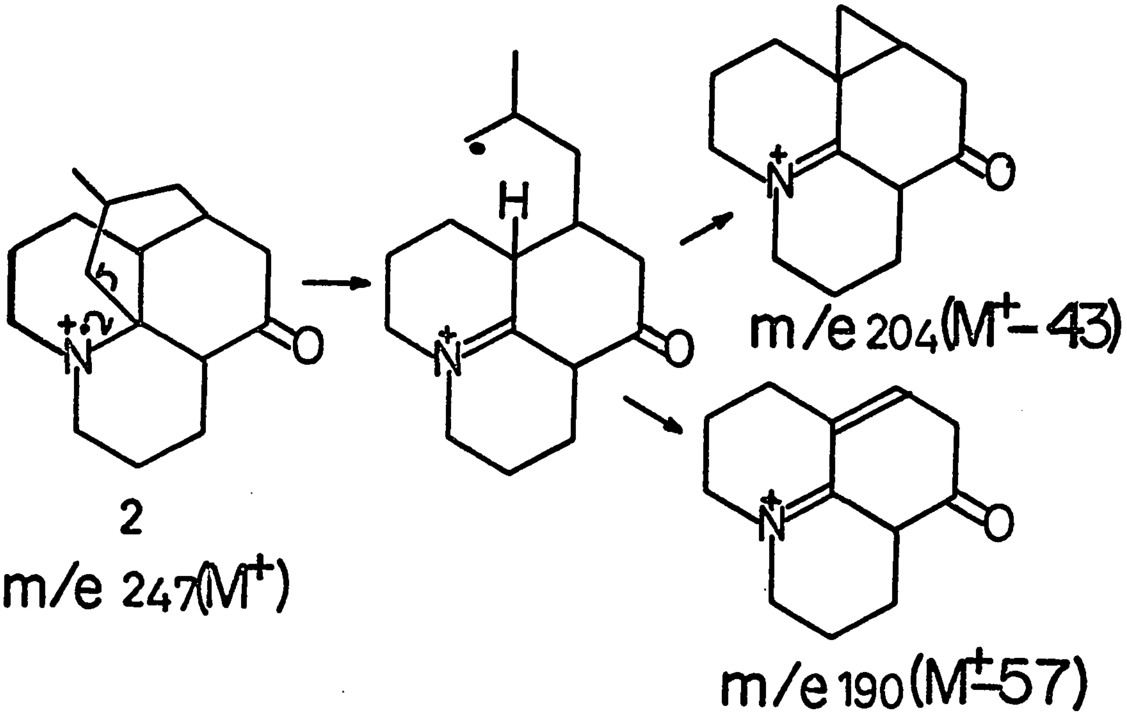


L.23[33]
10

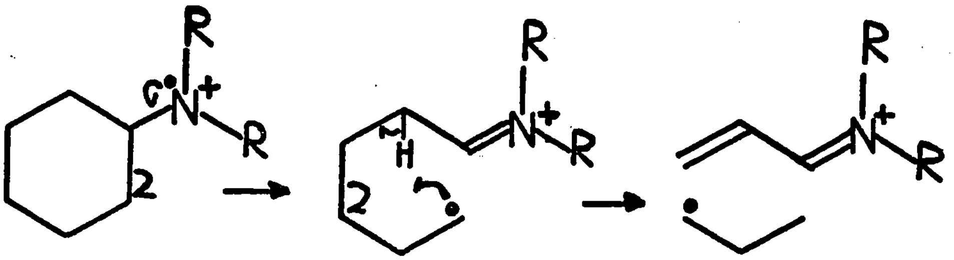
Lycopodine has also been converted into annofoline[25] (5), alkaloid L.20[26] (9) and flabelline[27] (7).

It is perhaps in its application to alkaloids of this lycopodine group that mass spectrometry has especially contributed to structural studies. Since the initial investigation by MacLean[28] the behavior of the Lycopodium alkaloids in the mass spectrometer has become better understood so that it is often possible, by studying the spectrum, to predict the positions of substituents on a lycopodine type carbon skeleton.

Upon electron impact lycopodine (2) shows strong peaks at m/e 247 (M^+), 204 (M^+ -43), 190 (M^+ -57, base peak), 162 (M^+ -85) and 134 (M^+ -113)[34].

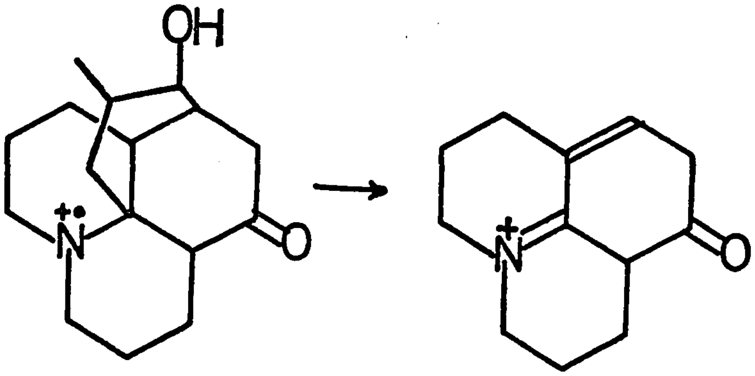


The latter represents a typical cyclohexylamine fragmentation pathway[35], e.g.,



Deuterium labeling experiments have established these fragmentation patterns. The subsequent formation of the ions at m/e 162 and m/e 134 is believed to be brought about by successive losses of ethylene and carbon monoxide from the ion at m/e 190. The actual order of these losses has not been established.

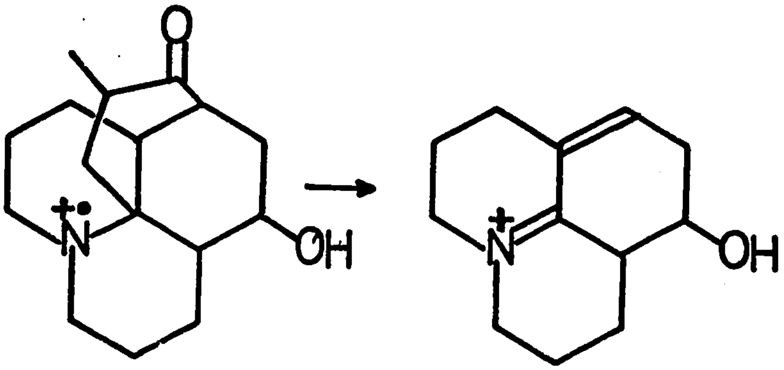
The presence of substituents on the bridge as in clavolonine[29] (4) and annofoline[25] (5) appears to have no effect on the fragmentation pathway.



4

m/e 263(M$^+$)

m/e 190(**base peak**)

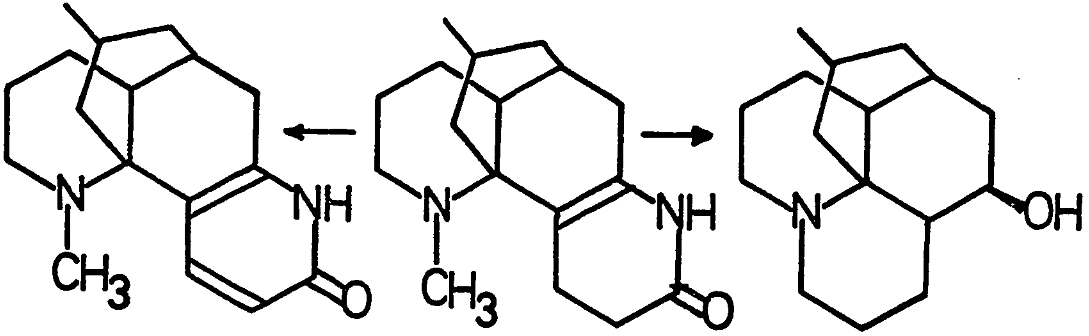


5

m/e 277(M$^+$)

m/e 192 (base peak)

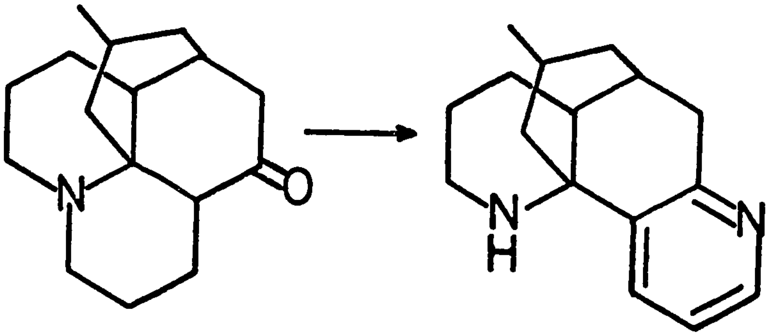
Lycodine (12) typifies the third group of alkaloids and its structure together with those of α-obscurine (13) and β-obscurine (14) were proposed by Ayer and Iverach[36,37] shortly after the solution to the lycopodine structure.



β-Obscurine
14

α-Obscurine
13

15

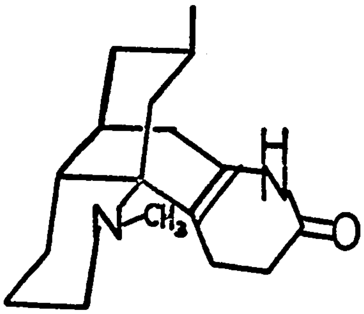


2

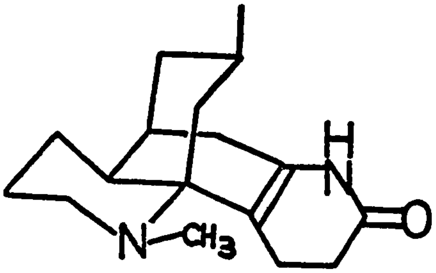
Lycodine
12

The confirmation of these structures soon followed. The transformation of α-obscurine into β-obscurine was easily accomplished and showed the former to be a dihydro derivative of the latter. Ayer and co-workers[38] were able to convert α-obscurine into dihydrolycopodine (15). Meanwhile, Anet and Rao[39] converted lycopodine (2) into lycodine (12).

Sauroxine, a major alkaloid of *Lycopodium saururus*[40] also belongs to the lycodine group. It is isomeric with α-obscurine (13) and the two exhibit strikingly similar infrared, ultraviolet and mass spectra. This similarity suggested they were stereoisomers and the supposition was proved correct in 1965 when Ayer and co-workers[41] were able to assign structure 16 to sauroxine.

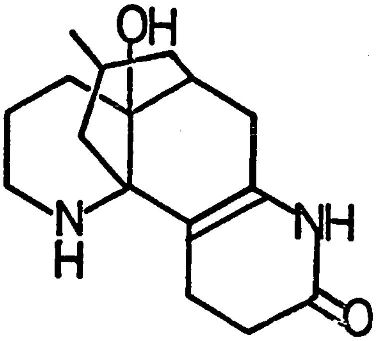


α-Obscurine
13



Sauroxine
16

Among the other alkaloids which have been assigned to this group are N-methyllycodine[38], des-N-methyl-α-obscurine[42], hydroxy-des-N-methyl-α-obscurine[42] (17) and selagine[43] (18).



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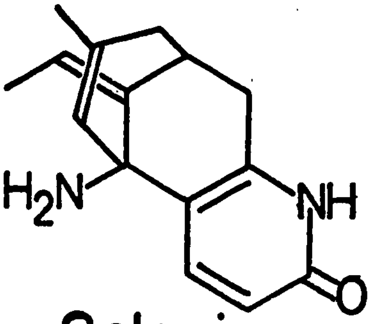
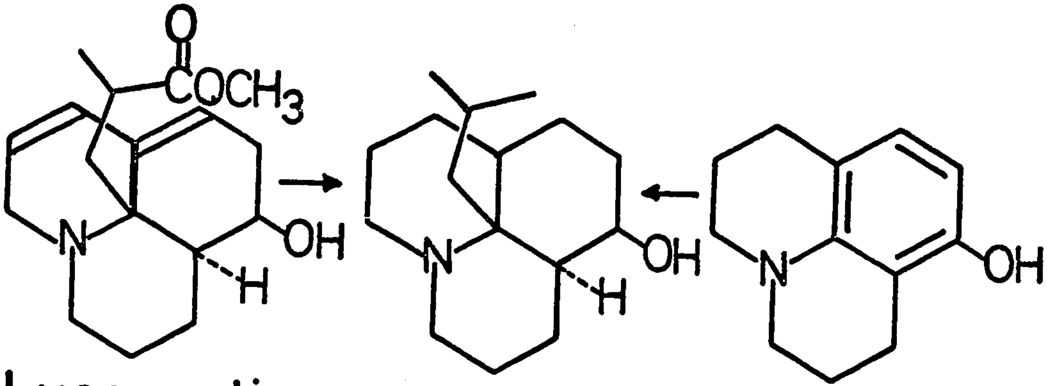


Selagine
18

In 1964 the structures of lyconnotine[44] (19) and annotine[45] (22) were established. The alkaloids possess unique carbon skeletons and at the present time remain the only members of their respective groups. Lyconnotine is a minor alkaloid of L. annotinum and its structural elucidation was followed by the synthesis[46] of one of its degradation products (20) from 8-hydroxyjulolidine[47] (21).

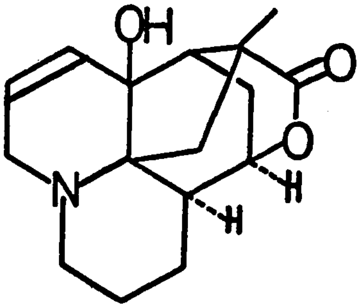


Lyconnotine
19

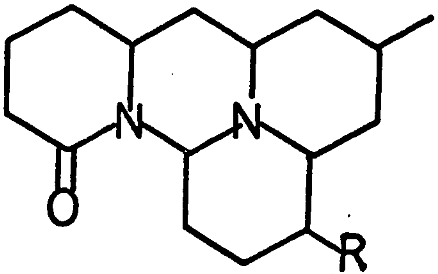
20

21

Annotine, as the name suggests, is isolated from L. annotinum. MacLean and co-workers[45] proposed structure 22 for the alkaloid, for which supporting evidence came from its mass spectrum[48]. Confirmation of this structure followed shortly afterwards as a result of chemical degradation.



Annotine
22

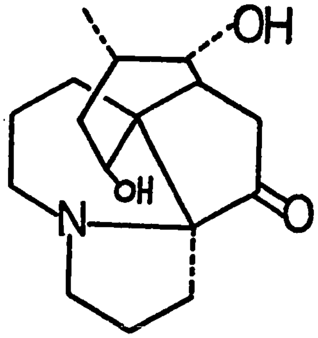


23.Cernuine(R=H)
24.Lycocernuine(R=OH)

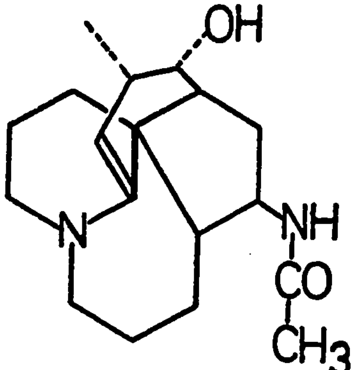
The structures of cernuine[49] (23) and lycocernuine (24) were also proposed in 1964, and their relative and absolute stereochemistry was established shortly afterwards by the same group[50,51,52]. These two alkaloids represent the bases so far that have been isolated from Lycopodium cernuum. This species is the only one to date which has been found to be void of lycopodine and lycodine type alkaloids. The structures of cernuine and lycocernuine were proposed largely on the basis of the isolation of 2-n-butyl-4-methyl-6-pentylpyridine and an unusually successful application of the biogenetic scheme proposed by Conroy[53], which will be discussed later in more detail.

The seventh group of alkaloids is exemplified by serratinine, the most abundant of seven new alkaloids isolated by Inubushi and co-workers from Lycopodium serratum var. thunbergii.

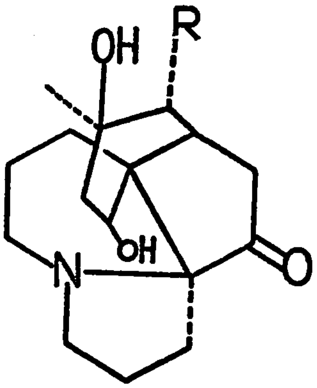
Following some extensive degradation work they proposed structure 25 for serratinine[54,55]. Shortly afterwards the structures of serratinidine[56] (26), serratine[57] (27) and serratanidine[57] (28) were established. Nuclear magnetic resonance spectroscopy played a predominant role in the solution of these structures.



Serratinine
25

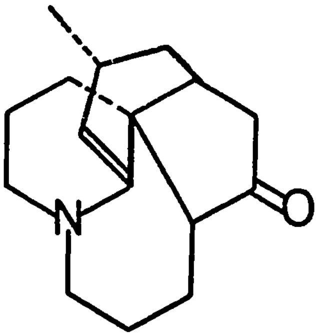


Serratinidine
26

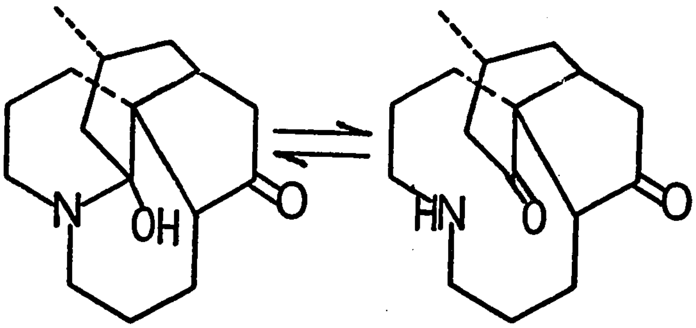


27.Serratine(R=H).
28.Serratanidine(R=OH).

Serratinine has been converted into fawcettidine[58](29) and as a result of the combined efforts of three research groups it has been shown that fawcettimine[59] (30) is related to the serratinine group[60].

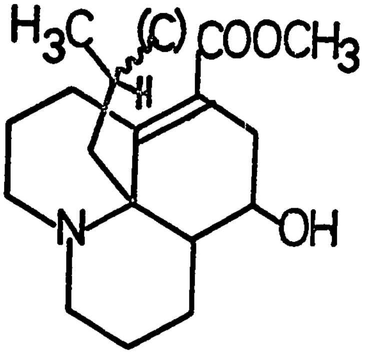


Fawcettidine
29

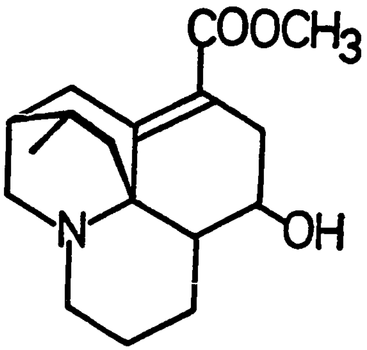


Fawcettimine
30

Annopodine, an alkaloid isolated from Lycopodium annotinum is another base possessing a unique carbon skeleton. Ayer and co-workers were able to propose the following partial structure 31 on the basis of chemical and biogenetic reasoning but a lack of material brought this approach to a halt. A little later the same group was able to assign structure 32 to annopodine as a result of an x-ray crystallographic analysis of the hydrobromide salt[20].



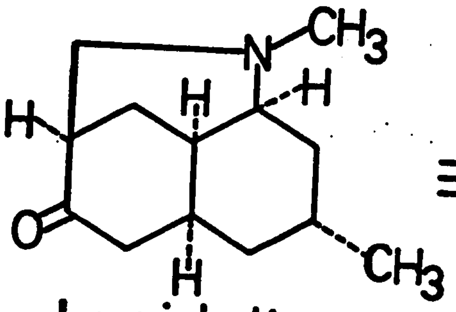
31



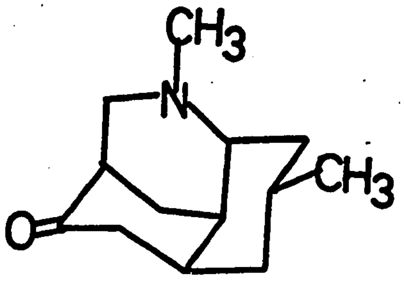
Annopodine

32

Yet another unique carbon skeleton was discovered in 1968 when the structure of luciduline (33), an alkaloid obtained from Lycopodium lucidulum, was established[19]. Since the derivation of its structure will be discussed in more detail later in this dissertation, it is sufficient here to state that again x-ray analysis was used to confirm a structure that had been proposed on chemical grounds.

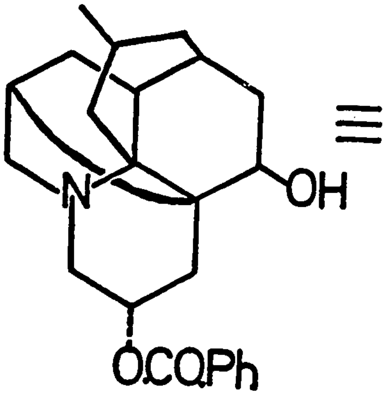


Luciduline
33

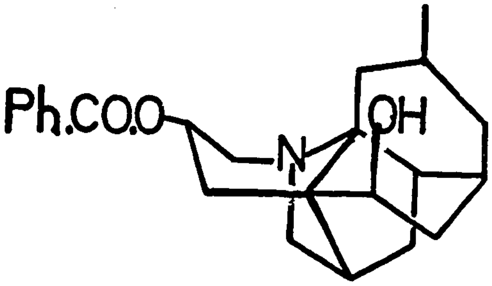


33a

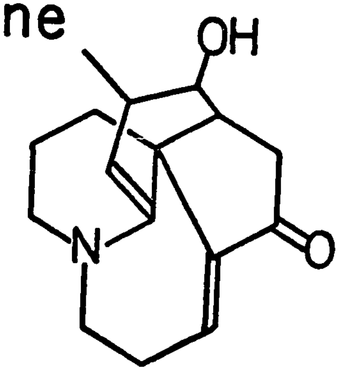
Quite recently Ayer and co-workers were able to isolate five new alkaloids from Lycopodium alopecuroides L. The first of these to yield to structural studies was alopecurine (34) whose structure was established by an x-ray analysis of debenzoylalopecurine hydrobromide[21]. The other alkaloids are



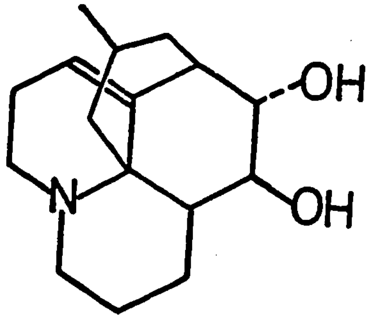
Alopecurine
34



34a

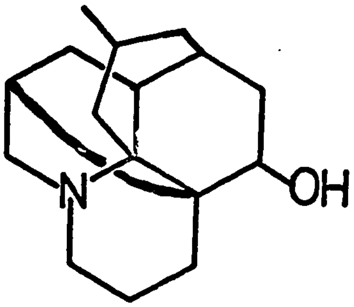


Alolycopine
35

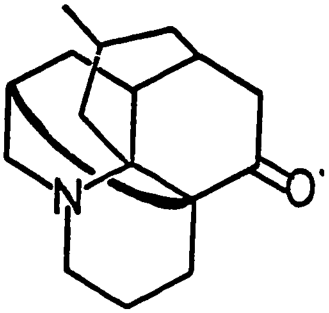


Lucidioline
36

acetyldebenzoylalopecurine, alolycopine[61] (35), alopecuridine and lycopecurine. The structural solutions of the first two of these alkaloids were reported in 1969 by this group, shortly after they had published the structural solution of lucidioline[62] (36), an alkaloid from L.lucidulum. Alopecuridine is thought to be a hydroxylated fawcettimine[63]. Lycopecurine, which Ayer and Masaki[64] have shown by x-ray analysis to possess structure 37, is readily transformed by Jones' reagent[65] to dehydrolycopecurine (38) which has recently been found[66] to be a natural constituent of Lycopodium inundatum L.



Lycopecurine
37



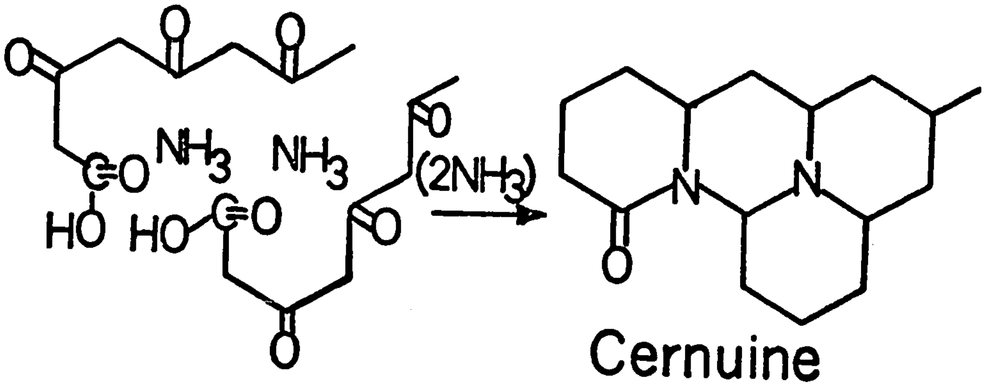
Dehydrolycopecurine
38

In the last decade an increasing interest in the biosynthesis of the Lycopodium alkaloids has developed. Applications of the earliest biosynthetic scheme proposed by Conroy[53] have played a particularly important part in the structural elucidation of cernuine[49,50] (23) and lycocernuine (24), and contributed, albeit to a lesser extent, to the structural solution of lycodoline[24] (3), annotine[45] (22), fawcettidine[58] (29) and fawcettimine[59] (30). Conroy suggested that the Lycopodium alkaloids could be biosynthesized through the condensation of two 3,5,7-triketooctanoic acid equivalents in combination with one or more equivalents of ammonia. The application of this hypothesis to the formation of lycopodine (2), lycodine (12) and selagine (18) is shown in Scheme I. The numbering system[67] employed in the Lycopodium alkaloids originates from this polyacetate hypothesis.

The two polyketooctanoic acid (39) units combine, via appropriate aldol condensations, to give 40 then 41, which in turn combines with one equivalent of ammonia and leads to the lycopodine skeleton. As shown, a ring closure involving two equivalents of ammonia can give rise to lycodine and selagine.

The route to annotinine (1) requires a slight modification to the general pattern. If in intermediate 40 carbon 8 is oxidized to carboxyl prior to the formation of 41, then ring closure via aldolization at C-8 is impossible. However, such a condensation can occur between C-12 and C-15 yielding a cyclobutane system (42) and subsequently leading to annotinine (1), as shown in Scheme II.

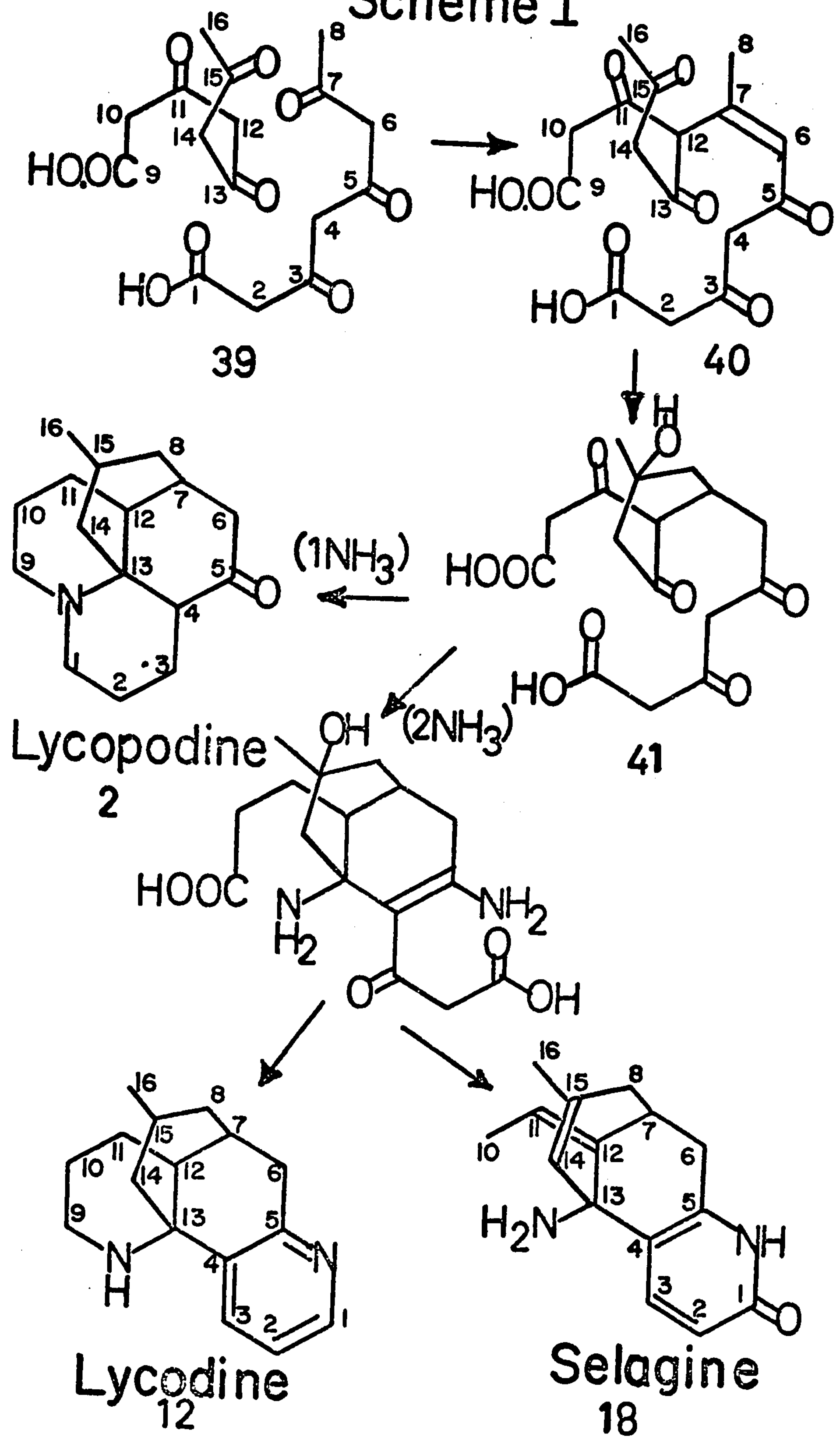
The biosynthesis of cernuine (23) can readily be accounted for by this polyacetate hypothesis.



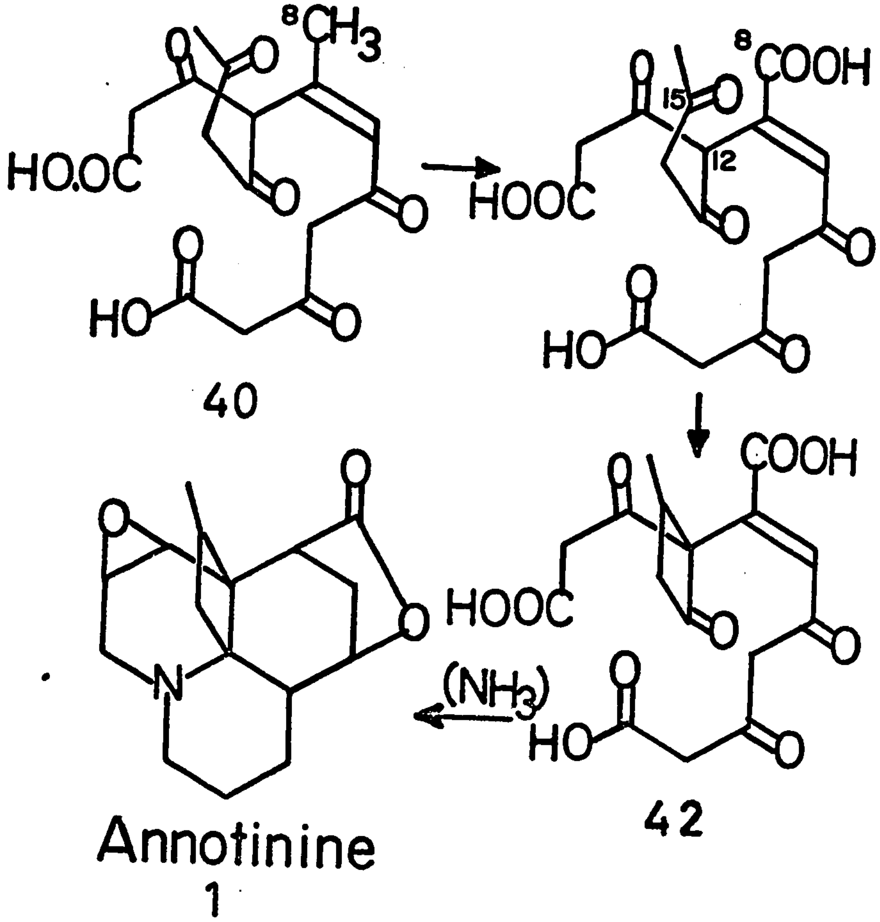
Cernuine

23

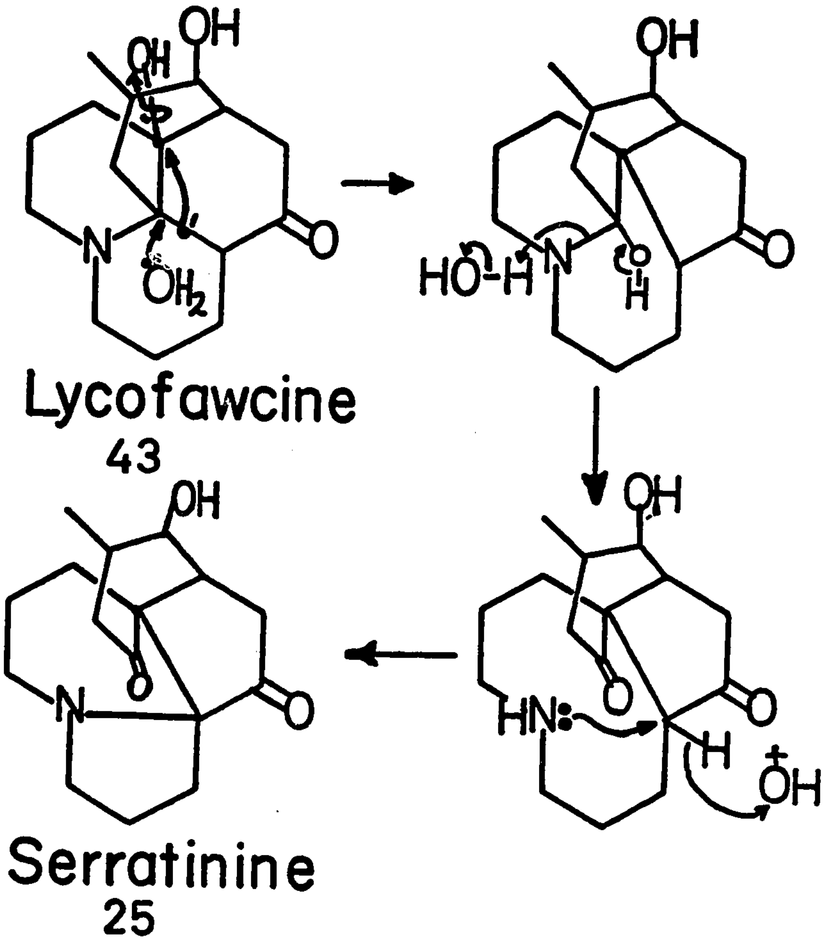
Scheme I


39
40
41
(1NH3)
(2NH3)
Lycopodine
2
Lycodine
12
Selagine
18
HO.OC
HOOC
HO
OH
NH2
H2N

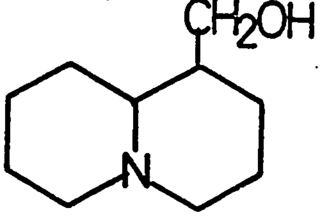
Scheme II



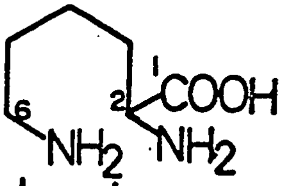
Inubushi[68] has proposed that the serratinine ring system can be formed in nature by rearrangement of a suitable lycopodine-type alkaloid. The transformation of lycofawcine (43) to serratinine (25), as depicted overleaf, serves to illustrate this idea.



Although the biosynthesis of the Lycopodium alkaloids could be explained in terms of the polyketooctanoic acid idea, other approaches could not be excluded. Wiesner[67] noted that the lupinine (44) skeleton, which is known to be derived from lysine[69] (45), was included in the framework of lycopodine (2).

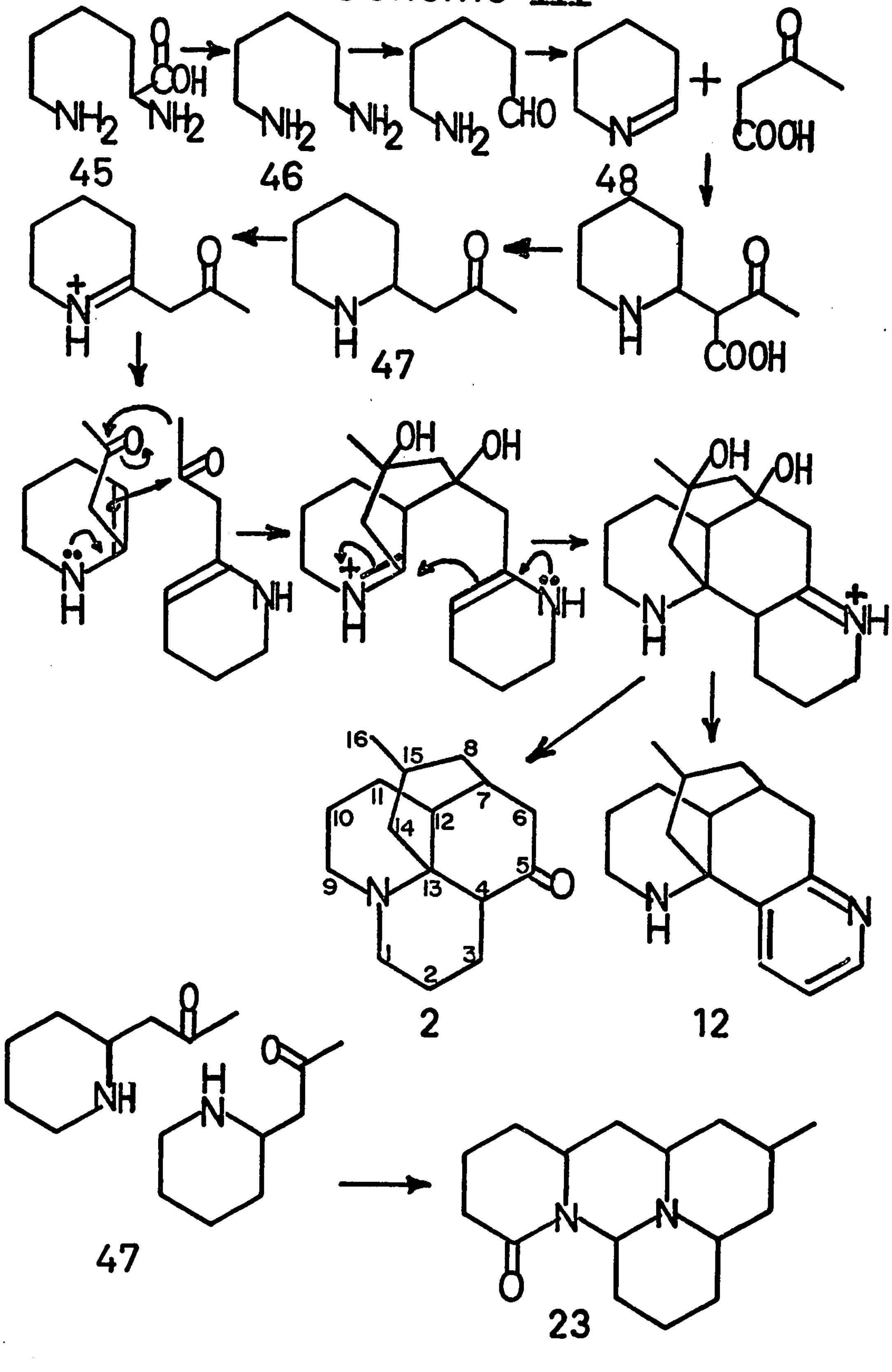


Lupinine
44

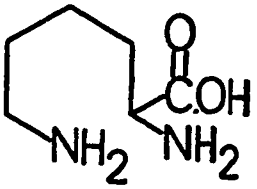


Lysine
45

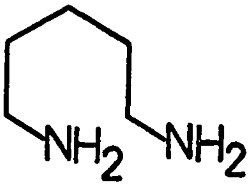
Scheme III


COOH
NH2
45
46
CHO
48
COOH
47
OH
NH
16
15
8
11
7
10
14
12
6
5
9
13
4
1
2
3
2
12
47
23

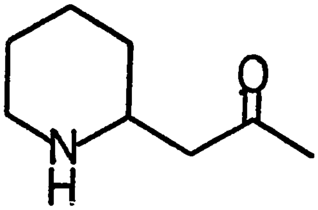
Several years later MacLean, Spenser and co-workers[70] carried out experiments with labeled lysine. They separately introduced 2-^{14}C-lysine and 6-^{14}C-lysine to Lycopodium flabelliforme. In each case, they isolated radioactive lycopodine containing one fourth of the total molecular activity at the carbonyl carbon. Lysine was therefore shown to be a specific precursor to lycopodine, as depicted in Scheme III on the previous page. Further investigations[71] led them to propose that the alkaloid represents a modified dimer of pelletierine[72] (47) which in turn is generated from lysine via a symmetrical intermediate (46).



Lysine



46

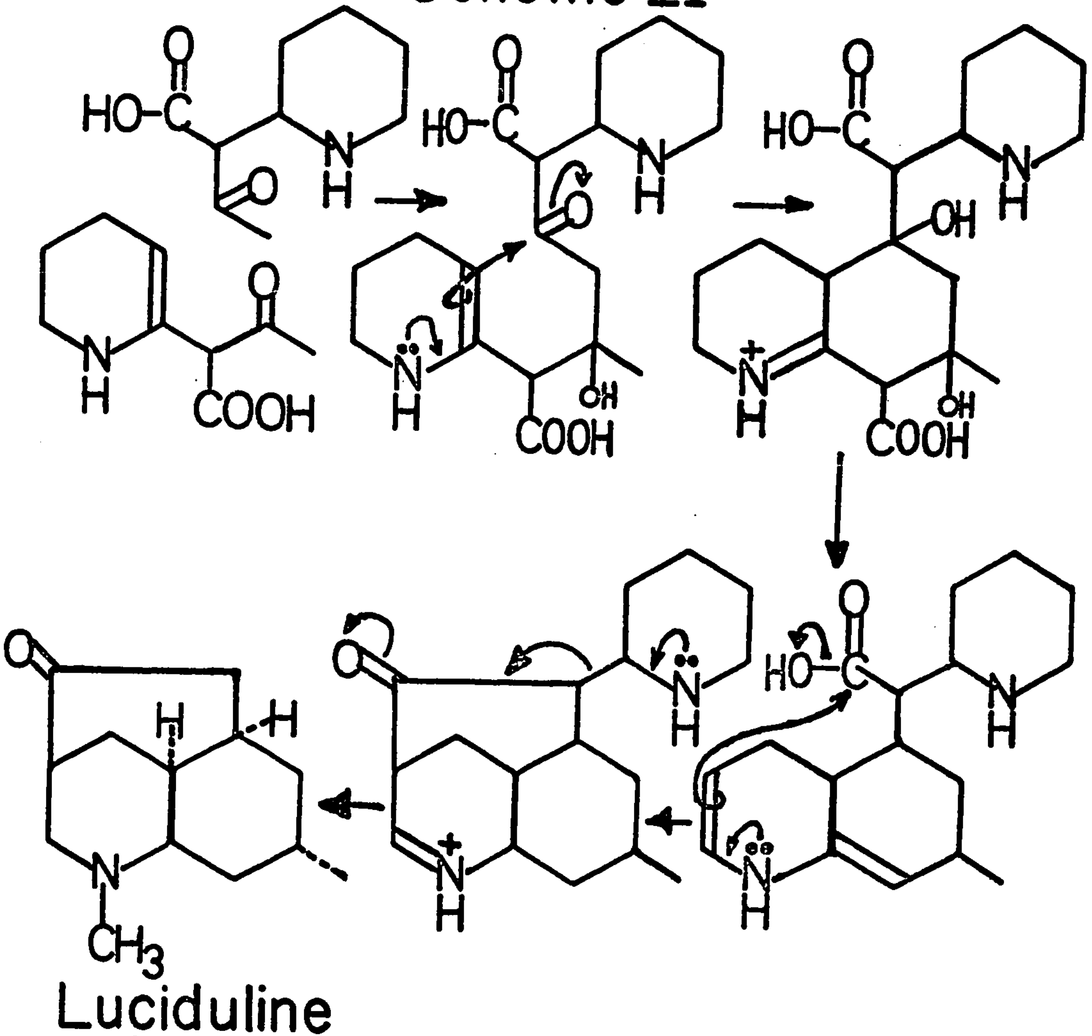


47

Scheme III also illustrates the application of the pelletierine hypothesis to the biosynthesis of lycodine (12) and cernuine (23), while the formation of luciduline (33) is depicted in Scheme IV overleaf.

This approach has been tested by the feeding of labeled pelletierine to Lycopodium tristachyum. The results indicate[71] that only one pelletierine unit is incorporated into lycopodine and that this is the origin of the C9-C16 unit in the alkaloid.

Scheme IV

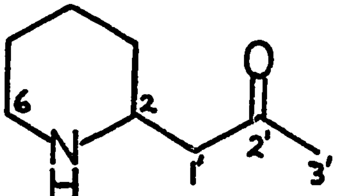


Luciduline

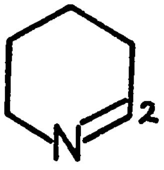
33

The origin of the C1-C8 unit is uncertain but like pelletierine, is known to be derived from lysine (45) and acetate. The C6-C8 and C14-C16 carbons of lycopodine are acetate derived, the latter arising from the side chain unit in the pelletierine.

More recently[73,74] studies have been made using 2-^{14}C-and 6-^{14}C-piperideine as well as 6-^{14}C-; 2,3'-$^{14}C_2$ and 4,5-$^{3}H_2$, 2-^{14}C-pelletierine. The results of the five labeling experiments show that both Δ^1-piperideine (48) and pelletierine (47) are precursors and again reveal the incorporation of only one unit of pelletierine into lycopodine.



Pelletierine
47



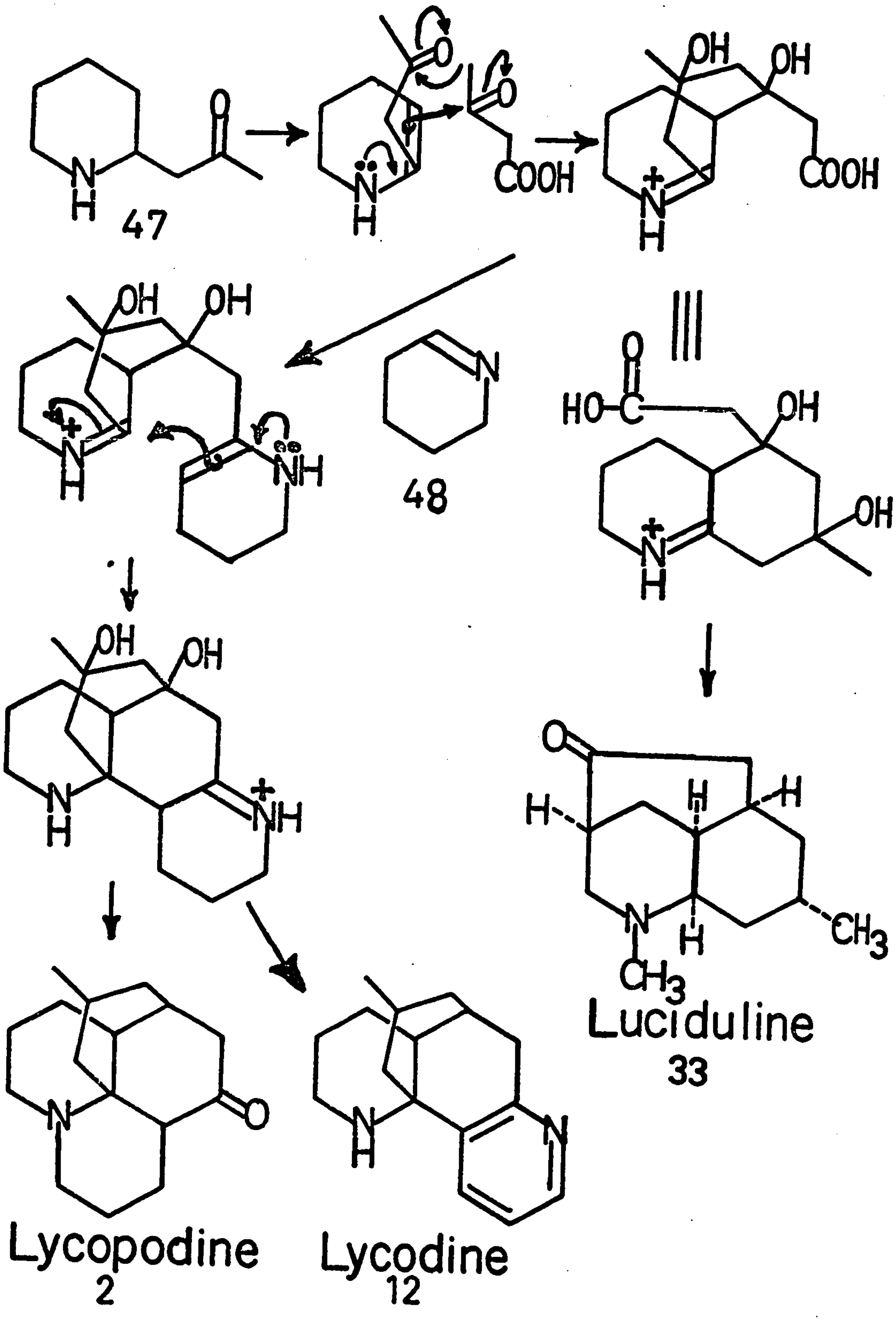
Δ^1-Piperideine
48

MacLean and co-workers[74] have suggested that lycopodine could be formed via dimerization of two C_8N units, possibly allyl piperidine with loss of one nitrogen at a late stage in the process and that pelletierine can replace one but not the other of these units in the biosynthesis.

An alternate mechanism to account for the biosynthesis of the <u>Lycopodium</u> alkaloids is outlined in Scheme V overleaf[75] and involves the incorporation of pelletierine (45), acetate and Δ^1-piperideine (46) into these alkaloids.

MacLean, Spenser and co-workers have recently shown[76] that cernuine (23) incorporates only one pelletierine molecule and it therefore seems likely that a similar process could be involved in the biosynthesis of luciduline contrary to the mechanism shown previously which involved two pelletierine units.

Scheme V



It appears that although the Conroy polyacetate hypothesis has contributed towards a better understanding of the biosynthesis and, in many cases, to the structural elucidation of the Lycopodium alkaloids, it nevertheless does not represent the actual biogenetic pathway. The combined efforts of MacLean, Spenser and their respective co-workers have established beyond doubt that the true biogenetic pathway requires the involvement of both acetate and lysine.

The Lycopodium alkaloids represent a very formidable synthetic challenge. The first of many attempted syntheses[77,78,79] of lycopodine (2) was reported in 1964 by Bohlmann and co-workers[80]. Success was achieved simultaneously in 1968 by Stork and co-workers[81] and by Ayer and co-workers[82] and represented, in a formal sense, a synthesis of many other alkaloids which had already been synthesized from lycopodine.

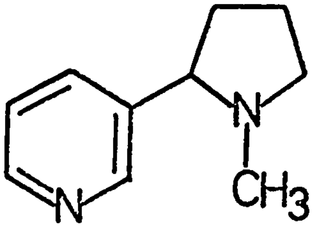
In the meantime, however, Wiesner and Poon[83] accomplished the successful synthesis of annotinine (1), which ten years earlier had been the first Lycopodium alkaloid to be structurally elucidated.

This brief survey of the chemistry of the Lycopodium alkaloids is intended to serve as background material for the work described in this thesis, a study of the weakly basic alkaloids of Lycopodium lucidulum, which is described in detail in the following sections.

DISCUSSION AND RESULTS

In 1946, as part of their systematic examination of various species of the Lycopodium genus, Marion and Manske reported[8] the results of an investigation of Lycopodium lucidulum Michx. With the exception of Lycopodium annotinum L. the aforementioned species is regarded as the most primitive of the genus.

From L. lucidulum, Marion and Manske isolated two alkaloidal extracts. The first, Extract A, consisted of the ether-soluble alkaloids while Extract B contained those which were insoluble in ether but soluble in chloroform. Marion and Manske were able to isolate nine alkaloids from Extract A, namely, nicotine (49), lycopodine (2), L.13, L.20 (9), L.21 (33), L.22, L.23 (10), L.24 and L.25. The melting points of the compounds and their respective hydroperchlorate salts, together with the results of their chemical analyses, formed the basis of the original character-



Nicotine

49

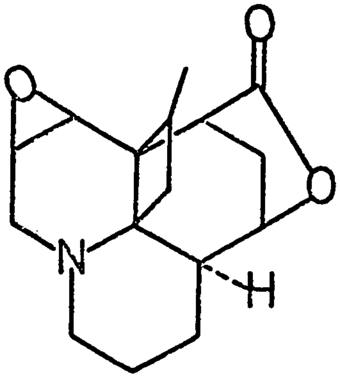
ization of these alkaloids, as depicted in Table I. At that time only nicotine (49) was of known structure[84].

TABLE I

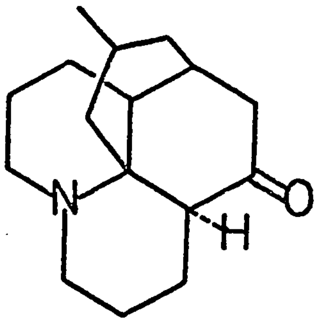
ALKALOID	M.p. (°C)	M.p. (°C) of HYDRO-PERCHLORATE SALT	MOLECULAR FORMULA
Nicotine			$C_{10}H_{14}N_2$
Lycopodine	116	283	$C_{16}H_{25}NO$
L.13	130	274	$C_{16}H_{25}NO$
L.20	258	271	$C_{17}H_{27}NO_2$
L.21		201	$C_{13}H_{21}NO$
L.22	108	254	$C_{16}H_{27}NO$
L.23	161	300	$C_{16}H_{25}NO_2$
L.24		278	$C_{16}H_{25}NO$
L.25		297	$C_{16}H_{25}NO_2$

Marion and Manske were unable to characterize any alkaloids from the brown resin-like contents of Extract B.

The structure elucidation of annotinine[15] (1) in 1956, and that of lycopodine (2) four years later[17], marked



1

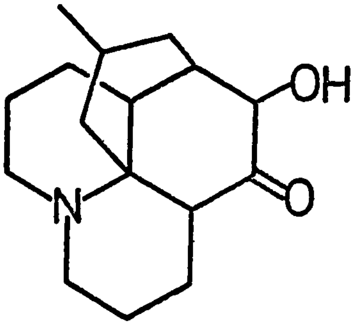


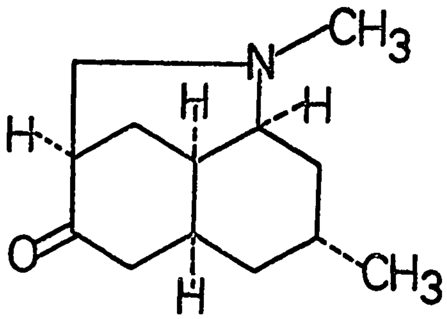
2

the introduction of a decade in which the structures of some 50 Lycopodium alkaloids, from various species, were elucidated.

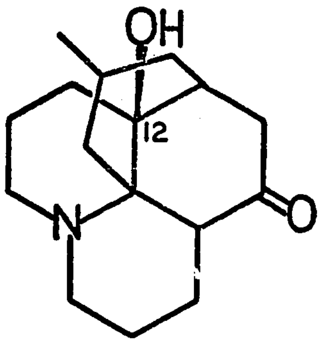
The more strongly basic alkaloidal fraction of L. lucidulum, which appears to correspond to Extract A above, has been the object of much detailed research. Of the nine alkaloids originally isolated by Marion and Manske, only the nature of L.22 and L.24 remains unresolved.

Ayer and co-workers[33] showed that the hydroperchlorate salt of L.13 is identical with lycopodine hydroperchlorate, and have suggested[19] that luciduline (33), which is isolated from the weakly basic alkaloidal fraction of L.lucidulum, is identical with L.21. In 1963, following the revision of its molecular formula to $C_{16}H_{25}NO_2$, the same group reported[26] the structure of alkaloid L.20 (9).


9


33

The structure of luciduline (33) was reported in 1968 by Ayer and co-workers[19] who a year later established[33] that alkaloid L.23 (10) is the C-12 epimer of the alkaloid lycodoline (3) whose structure they had proposed[24] in 1964. The presence of lycodoline, originally termed alkaloid L.8, in *L. lucidulum* was first reported in 1962[85].



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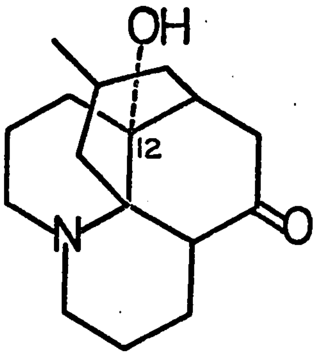

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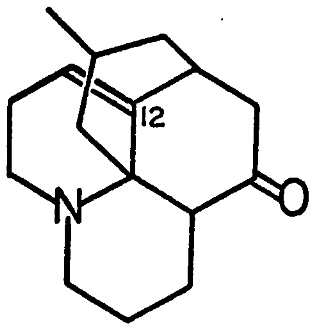
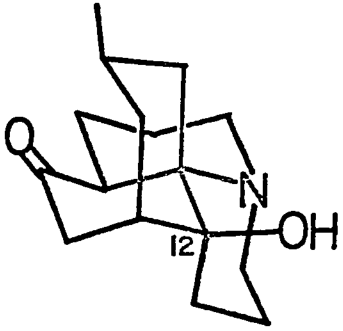
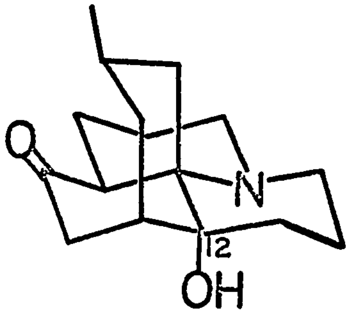


Anhydrolycodoline

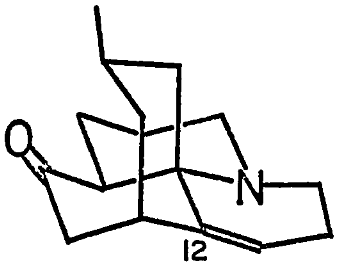
50



3a



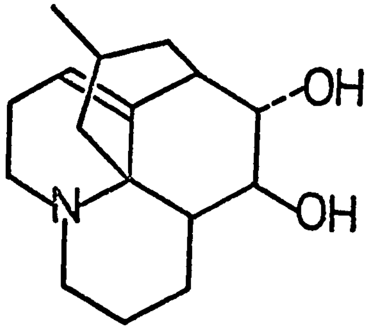
10a



50a

The above relationship between lycodoline (3) and alkaloid L.23 (10) followed from the discovery that they both[33] readily yielded anhydrolycodoline (50) on dehydration, and from the observation that they showed similar mass spectra. These facts, together with the appearance of Bohlmann bands[86,87] in the infrared spectrum of alkaloid L.23, which suggested the presence of a *trans*-quinolizidine system (10a), led to the assignment of structure 10 to alkaloid L.23. Meanwhile, the same group had discovered[88] that alkaloid L.23 is identical with the base originally named alkaloid L.25 by Marion and Manske[8].

Since the initial investigation by Manske and Marion, many other alkaloids have been isolated in these laboratories from L. lucidulum. These include lycodine[37] (12), anhydrolycodoline[89] (50), flabelliformine[90] (51), lucidioline[62] (36), the diosphenol[91] (52) and dihydroluciduline. Dihydroluciduline was found to be identical with the product formed by sodium borohydride reduction of luciduline (33). The diosphenol (52) was found to be the same as the diosphenol previously prepared[92] from alkaloid L.20 (9) and lycopodine (2). Lucidioline (36) is the only alkaloid of the above group whose structure was unknown at the time of its isolation from L. lucidulum. Ayer and co-workers[62] reported the structure elucidation of this alkaloid in 1969.



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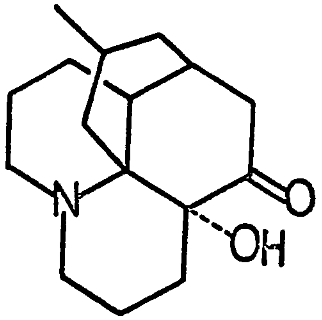
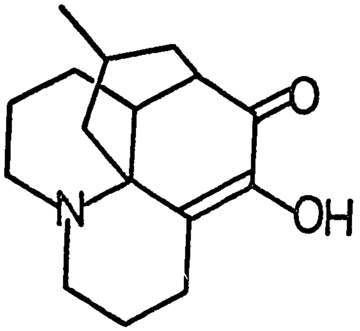
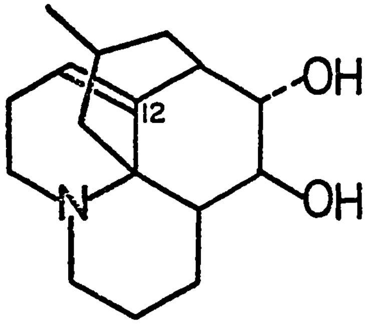


51

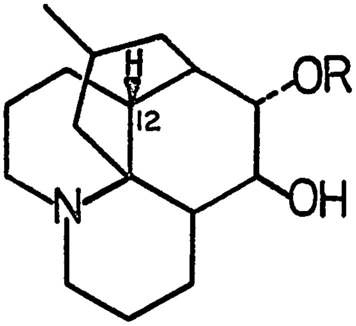


52

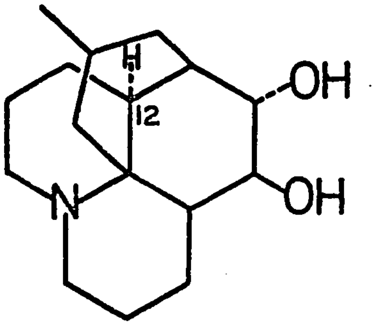
Lucidioline, $C_{16}H_{25}NO_2$, was converted into two isomeric dihydro derivatives by catalytic hydrogenation. One of these was found to be identical with desacetyllycoclavine (53), which is readily available from lycoclavine[92] (54), while the other product was readily converted into an O,O-diacetyl derivative which showed prominent Bohlmann bands in its i.r. spectrum. This evidence suggested that the dihydro compounds were epimeric at C-12 and consequently also at nitrogen. As a result of this and other evidence, the authors assigned structure 55 to the second hydrogenation product, making it 12-epi-desacetyllycoclavine, and therefore proposed structure 36 for lucidioline.



36



53 (R=H)
54 (R=COCH$_3$)



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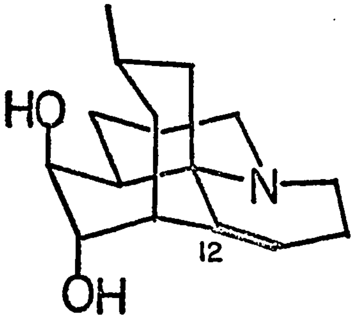
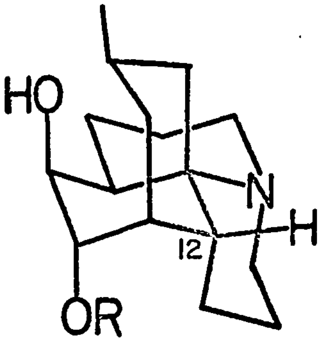
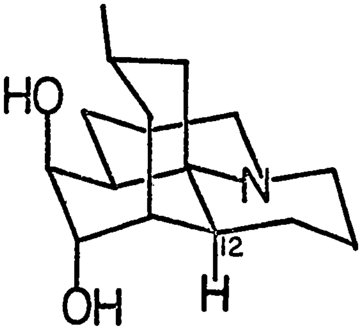


36a



53a (R=H)
54a (R=COCH$_3$)



55a

The investigation of the weakly basic alkaloids of *Lycopodium lucidulum*, which is the topic of this thesis, was begun in these laboratories in 1961, when Berezowsky[93] carried out a partial separation and preliminary examination of the alkaloids of this species. The total crude basic extract was dissolved in dilute hydrochloric acid, the pH adjusted to 8.0 with sodium bicarbonate and the solution extracted with chloroform. This yielded the so-called weakly basic alkaloid fraction. Further basification to pH$\geqslant$10 and extraction with chloroform yielded the strongly basic fraction.

The strongly basic fraction was found to be very similar in content to the Extract A described by Marion and Manske[8]. In addition to the alkaloids which they characterized, an examination of this fraction revealed the presence of lycodoline (3).

The only alkaloid that was isolated at that time from the weakly basic fraction was lycopodine (2), the most of which, however, was present in the other fraction. The rest of the weakly basic material was a brown resin-like gum, which had a very characteristic i.r. spectrum. The appearance of a band at 1640 cm.$^{-1}$ or 1620 cm.$^{-1}$ in the spectra determined in carbon tetrachloride or chloroform, respectively, was a discovery which has become the principal means of detection of these weakly basic alkaloids. It is reasonable to consider that this material corresponds to the "intractable" Extract B obtained by Marion and Manske[8].

A few years later the investigation of the alkaloids of L. lucidulum was continued in these laboratories by

Altenkirk and Nkunika. A different preliminary separation procedure was developed which was a marked improvement on the scheme just mentioned. This new procedure involved dissolving the total alkaloids in dilute hydrochloric acid, adjusting the pH to 5.5 with disodium hydrogen phosphate, and extraction of the solution with methylene chloride. Subsequently, the aqueous solution remaining was adjusted to pH$\geqslant$10 with ammonium hydroxide and extracted with chloroform.

The so-called strong bases were isolated from the chloroform extract and were investigated by Altenkirk[94]. The contents of the methylene chloride fraction were termed the weakly basic alkaloids and were investigated initially by Nkunika[95] and subsequently by the author of this thesis. This preliminary separation served in particular to advance the knowledge of the strongly basic alkaloidal components as their isolation was not now complicated by the presence of the weak bases. Altenkirk was able to isolate, characterize and elucidate the structures of most of the strong bases, while Nkunika carried out the initial characterization of many of the weak bases and elucidated the structure of one of them, luciduline[19] (33). The present work constitutes a continuation of the studies initiated by Nkunika.

Unlike the strong bases which are readily obtained in crystalline form following column chromatography, the weak bases are very difficult to separate in pure form and to date only one of them, which has been named lycolucine, has been isolated in crystalline form. Preliminary thin layer chromatographic studies utilizing basic alumina as adsorbent, indicate that the weakly basic alkaloidal fraction contains luciduline, lycopodine, lycodine and at least six other components.

Luciduline, an oil, is easily separated from the other weakly basic alkaloids by column chromatography or dry column chromatography. Its isolation is facilitated by the fact that it is the least polar (i.e. least strongly adsorbed on basic alumina) of all the alkaloids of L. lucidulum. Small quantities of lycopodine (2) and lycodine (12) have also been isolated from the weakly basic fraction by chromatography. These alkaloids are regarded as strong bases and only minor quantities are isolated from this fraction compared with the amounts isolated from the strongly basic fraction. Even by the most careful column chromatography it has been found impossible to isolate any of the other weakly basic alkaloids, characterized by bands in the i.r. at 1620 cm.$^{-1}$ in chloroform and at 1640 cm.$^{-1}$ in carbon tetrachloride, in a pure state. For their isolation more laborious techniques are involved and will be discussed shortly.

Although it is not the intention here to present a complete description of the studies related to the elucidation of the structure of luciduline[19] (33), it is pertinent to summarize the fundamental chemical and spectral characteristics of the alkaloid.

Luciduline, $C_{13}H_{21}NO$, which is an oil at room temperature, represents the first weakly basic alkaloid of *L. lucidulum* to yield to structural studies. It possesses properties which closely parallel those reported for the alkaloid L.21 isolated by Marion and Manske[8]. The infrared spectrum of luciduline, determined in carbon tetrachloride, shows absorption bands (figure 1) at 2780, 1690 and 1410 cm.$^{-1}$ attributed to N-methyl[96], carbonyl and methylene attached to carbonyl[97], respectively.

The results of a detailed examination of the n.m.r. spectra of luciduline and its simple derivatives in both neutral and acidic media, formed a major portion of the evidence upon which the structure elucidation of the alkaloid was based. When determined in deuterochloroform the n.m.r. of luciduline (figure 2) exhibits a three-proton doublet at τ 9.10 ($J = 6$ cps) assigned to a secondary C-methyl group, and a three-proton singlet at τ 7.90. When the n.m.r. spectrum is determined in perdeuteroacetic acid the latter singlet is observed at τ 7.00. The singlet therefore was attributed to the presence of a methyl group attached to a basic nitrogen[98].

The mass spectrum of luciduline (figure 3) shows prominent peaks at *m/e* 207 (parent and base peak), 206 (M^+ -1), 192 (M^+ -CH_3), 164, 150, 96, 70 and 44. The molecular composition of several of these ions was determined by high resolution mass spectrometry.

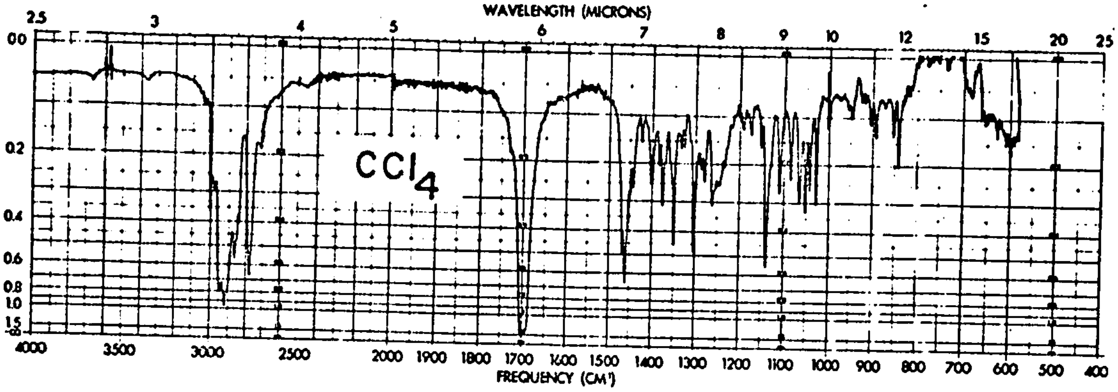


Figure 1

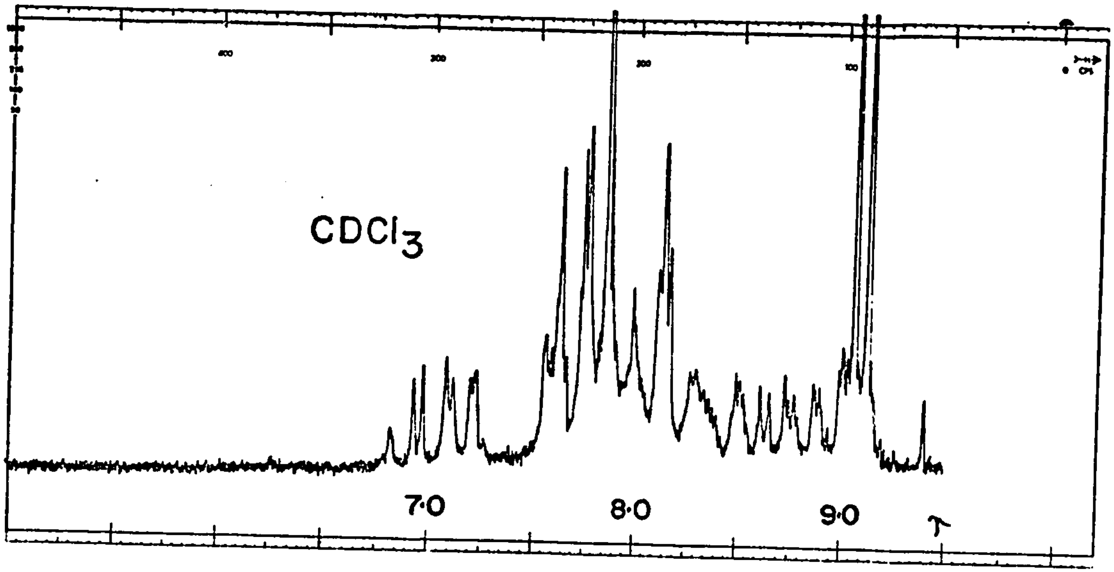


Figure 2

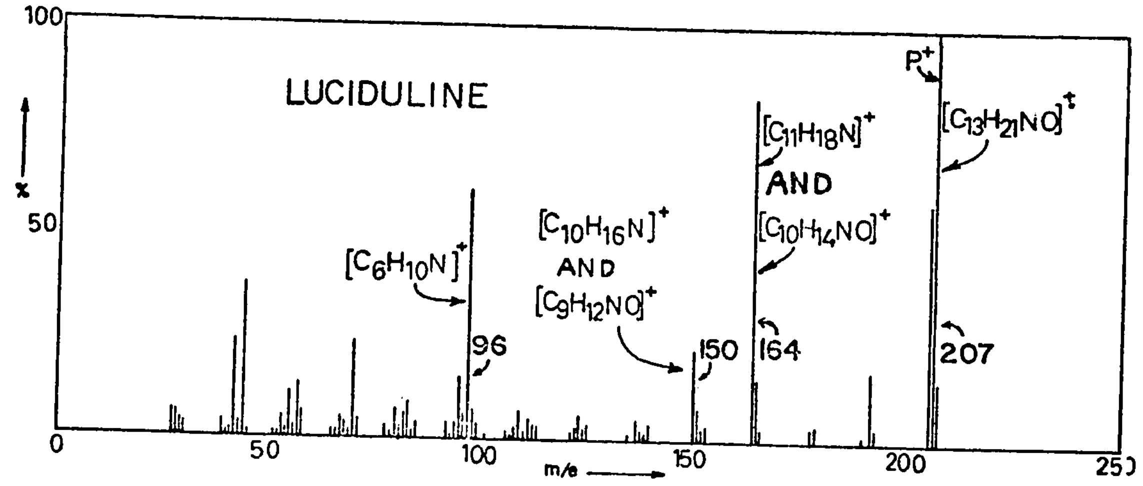
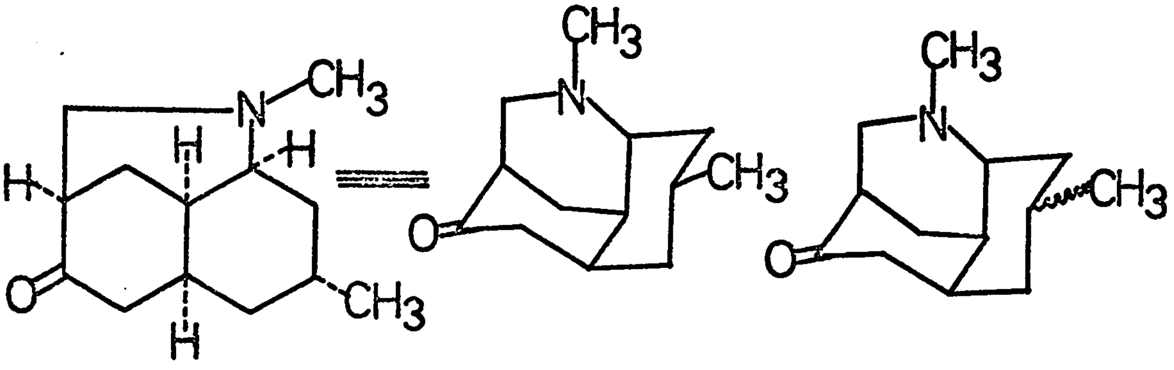


Figure 3

The reduction of luciduline by sodium borohydride afforded dihydroluciduline, which in turn readily formed an O-acetyl derivative. The selenium dehydrogenation of luciduline gave a good yield of 2,6-dimethylnaphthalene.

As a result of these and further investigations, Ayer and Nkunika were able to propose that the gross structure of luciduline could be represented by structure 56. This proposal was confirmed following the x-ray analysis[19] of p-bromobenzoyldihydroluciduline which also established the absolute configuration of the alkaloid, as depicted in structures 33 and 33a. Recent experiments have shown that luciduline is physiologically inactive.



33 33a 56

Although the weak base luciduline and the residual strong bases, lycopodine and lycodine, are readily separable from the bulk of the weak bases by elution or dry-column chromatography[99], these techniques alone are unable to effect a separation of the other six weak bases. However, early efforts had shown that two of them could be separated in a reasonably pure state by repeated dry-column chromatography. These two alkaloids were the first of the so-called 1620 compounds to be isolated. Mass spectrometry revealed that the two compounds were isomers of composition $C_{30}H_{49}N_3O$, while their infrared spectra in chloroform were characterized by intense absorption at 2780 and 1620 cm.$^{-1}$. With respect to one of their absorption bands the alkaloids became known initially as the 1620-A (less polar) and 1620-B compounds. However, what was required was a way of isolating all of the weak bases.

Of the many different separation techniques that were tested at this stage, countercurrent distribution[100] over 100 tubes, utilizing the Craig-Post automatic apparatus, involving chloroform as the stationary lower phase and a saturated aqueous solution of potassium hydrogen tartrate (pH 3.0) as the moving upper phase[101], was by far the best. When used in conjunction with the various chromatographic procedures this provided an efficient and reproducible method of separating the components of the weakly basic alkaloid mixture. The success of the method relies upon the fact that the slightly stronger of the bases, being more easily protonated by, and therefore soluble in, the weakly acidic moving upper phase, undergo more transfers and are consequently located nearer the tube 100 than the more weakly basic components of the original weak base mixture. The monitoring of the 100 samples by thin layer chromatography typically reveals the presence of five distinct sample types, some containing only one component, while others contain up to four components. Accordingly, we have named these samples Fractions A to E and a total of eight weakly basic alkaloids (i.e. one more than was detectable by the preliminary t.l.c. studies), have been characterized. One of these, luciduline, has been discussed earlier. The remaining seven are all characterized by absorption bands at 2780 and 1620 cm.$^{-1}$ in their i.r. spectra determined in chloroform, and two of these correspond to the 1620-A and 1620-B compounds described above.

The best results are obtained if the weak base sample is first subjected to elution chromatography to remove the easily separable luciduline, lycopodine and lycodine. Although gas liquid phase chromatography (g.l.p.c.) has aided the detection and isolation of luciduline and numerous lycopodine and lycodine type alkaloids, its contribution to the characterization of the seven weakly basic alkaloids has been negligible. However, it has played a prominent role in the detection and characterization of the dehydrogenation products of these alkaloids, particularly when used in conjunction with the mass spectrometer.

A variation to the separation procedure described above involves a preliminary ten-funnel countercurrent distribution which can be employed either instead of or in addition to the preliminary chromatography. The appropriate fractions are then subjected to the automatic 100 tube countercurrent experiment.

The general techniques involved in the isolation and separation of the alkaloids of Lycopodium lucidulum are schematically outlined in figure 4. The tube numbers associated with the ultimate isolation of the five weakly basic Fractions A to E represent a typical case. As stated earlier the analysis of each of these five fractions reveals that some contain just one component while others contain up to four. The summary of these findings is shown below, together with the quantities of each fraction usually isolated from a countercurrent experiment on 8 grams of weakly basic alkaloid material.

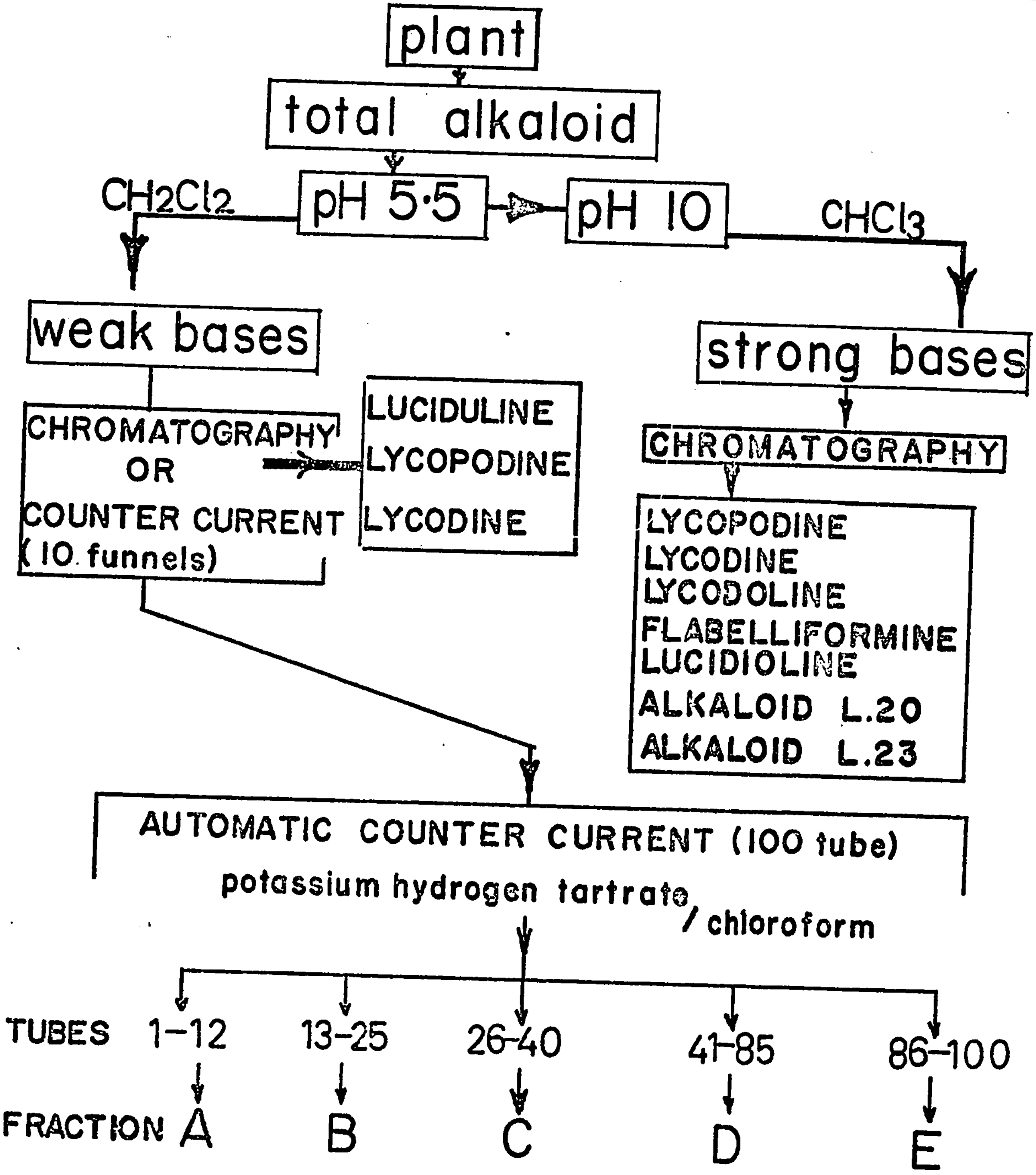
Tube Nos.	Fraction	Components	Quantity in grams
1-12	A	2	0.7 (9%)
13-25	B	3	0.4 (5%)
26-40	C	1	0.3 (4%)
41-85	D	4	5.5 (68%)
86-100	E	2	1.1 (14%)

The behavior of these Fractions A to E on thin layer chromatography (t.l.c.) is depicted in figure 5.

The following pages of this discussion will be devoted to a description of the further characterization of the alkaloid components found in these five fractions. Each fraction will be considered individually though they will not be treated alphabetically. Rather, it is the intention here to introduce and discuss the properties of these fractions

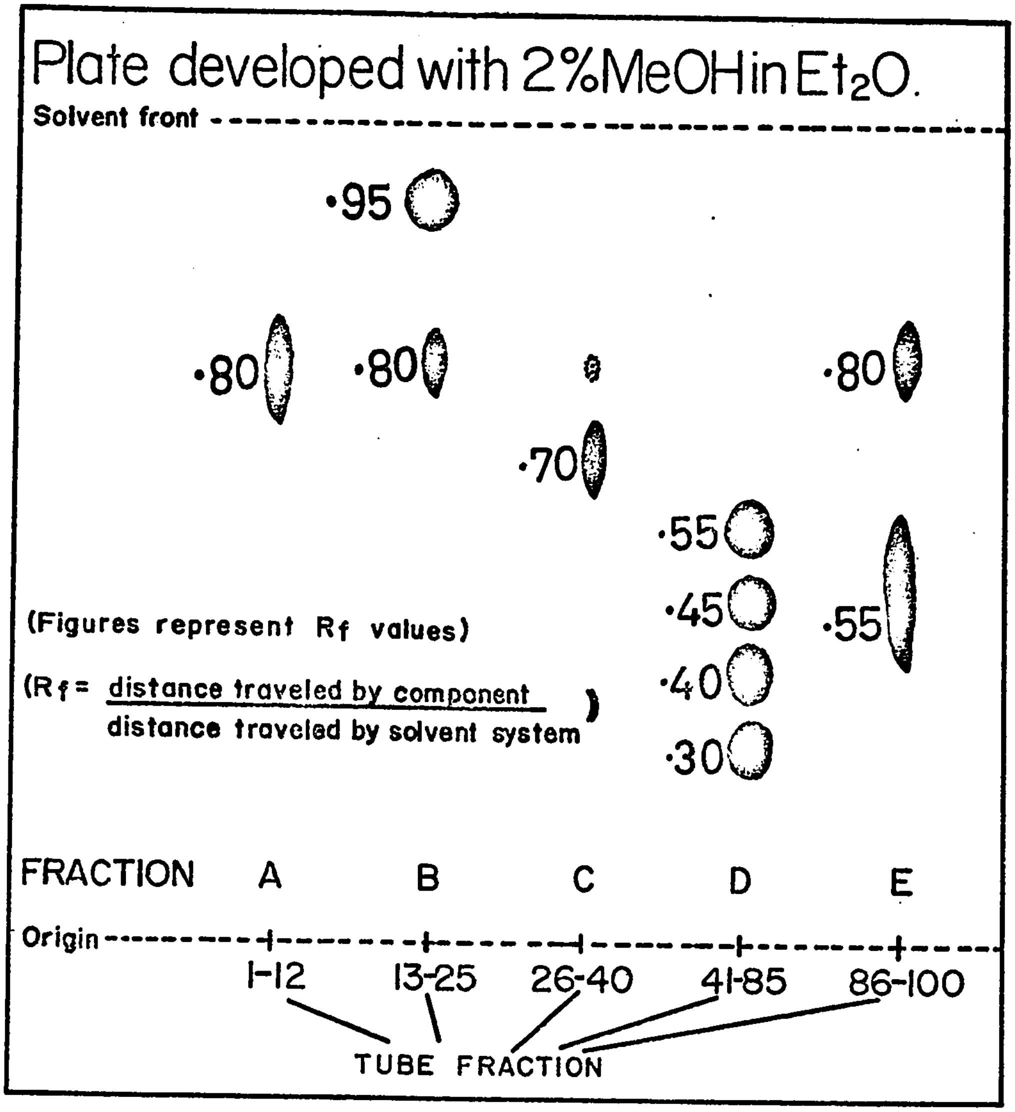
Figure 4

Isolation of the alkaloids of L.lucidulum



each Fraction further purified by various chromatographic techniques.

Figure 5



chronologically with respect to the order in which they were first systematically characterized.

In accordance with the foregoing concept the most weakly basic alkaloids of the weak base mixture are found in Fraction A while the most strongly basic alkaloids of the mixture are contained in Fraction E. Fraction E consists of two major components, lycopodine and lycodine which, for the purposes of the ensuing discussion, will be regarded as strong bases of L. lucidulum. The use of the term weak bases will be applied only to luciduline and those alkaloids characterized by an absorption at 1620 cm.$^{-1}$ in the infrared determined in chloroform.

Preliminary investigations of the five fractions revealed that the two least polar components of the 4-component Fraction D, the most abundant fraction, correspond to the alkaloids which were named 1620-A and 1620-B, following their isolation via repeated dry-column chromatography. Also the least polar component of Fraction B proved to be luciduline.

Since Fraction D was the most abundant fraction isolated from the countercurrent experiment, it was logical at the time to carry out most of the initial structural studies on its components. Since these studies led to the basis of our present knowledge of the 1620 compounds, it is appropriate that the characterization of the components of Fraction D be used to initiate the discussion.

The Alkaloids of Fraction D (part 1)

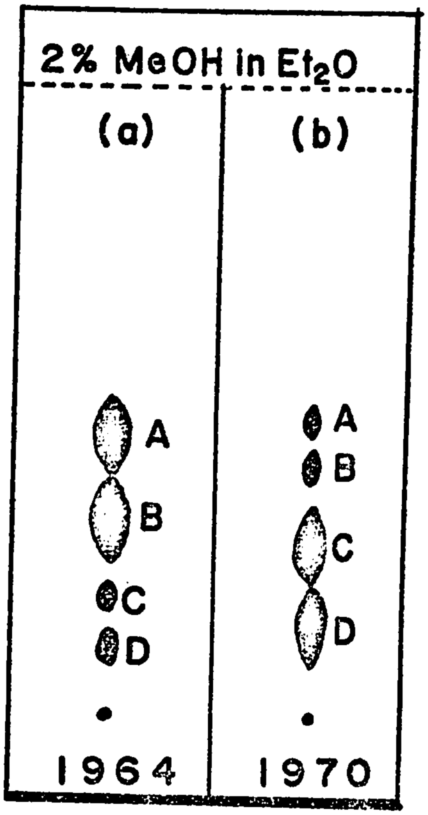


Figure 6

When the constitution of Fraction D was first investigated in 1964, t.l.c. indicated the presence of two less polar but major components, together with two minor, more polar components. It was natural then that the isolation, purification and characterization of the two less polar components became the immediate goal of the research studies at that time. However, more recent extractions of the same original batch of plant material lead subsequently to a Fraction D which contains the same four components but which is now dominated by the two more polar alkaloids. Accordingly, considerably more research has been carried out on these polar alkaloids in recent months. In keeping with the chronological presentation of this discussion, the more polar components will be discussed later under Fraction D (part 2).

The present discussion (part 1) will be limited to the less polar components which correspond to the alkaloids 1620-A and 1620-B mentioned previously.

By repeated dry-column chromatography it was possible to isolate the major two less polar components from the two minor more polar components and then to effect a reasonable separation of the former into the individual components, depicted as A and B in figure 6(a).

The less polar and more polar of these two components, originally known as the 1620-A and 1620-B compounds, have been named lucidine-A and lucidine-B respectively. Mass spectrometry shows that they are isomeric and of molecular composition $C_{30}H_{49}N_3O$. They exhibit similar absorption in the infrared and show strong peaks at 2780 and 1620 cm.$^{-1}$ in chloroform solution (figure 7). The former absorption inferred the presence of an N-methyl group while the latter was suspected of being due to an amide moiety[102].

The n.m.r. spectra of the two alkaloids are also very similar and the only noticeable difference lies in the region around τ 9.0. The spectrum of lucidine-A determined in deuterochloroform (figure 8) shows a complex six-proton multiplet at τ 9.15 while the spectrum of lucidine-B in this region contains two C-methyl doublets at τ 9.16 (J = 6 cps) and 8.93 (J = 7 cps) respectively. Both lucidine-A and -B show what was originally thought to be a three-proton doublet at τ 7.93 (J = 4 cps), together with a sharp singlet at τ 7.83 (3H). The latter signal exhibits a pronounced shift when the spectrum is determined in acidic media and is accordingly assigned to the N-methyl group. The chemical shift of the doublet at τ 7.93 is typical of an acetyl group and in view of the i.r. absorption at 1620 cm.$^{-1}$ it was

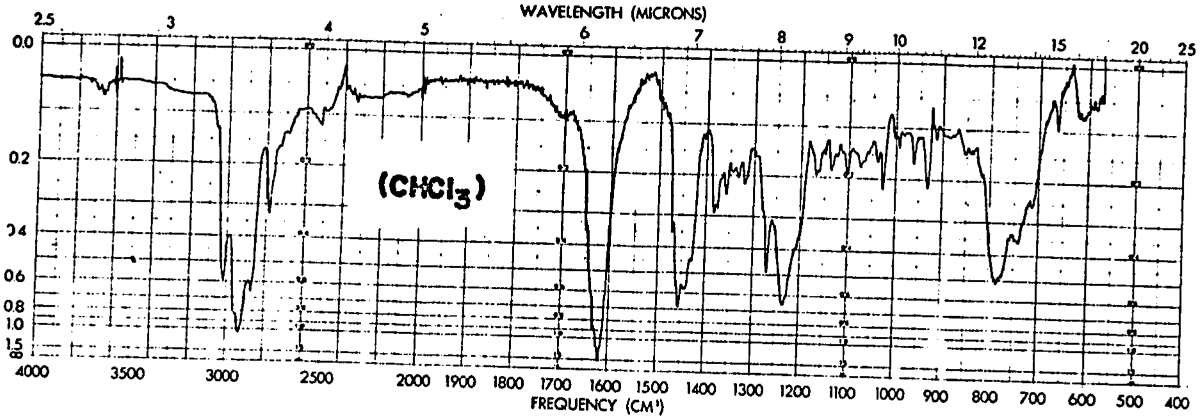


Figure 7

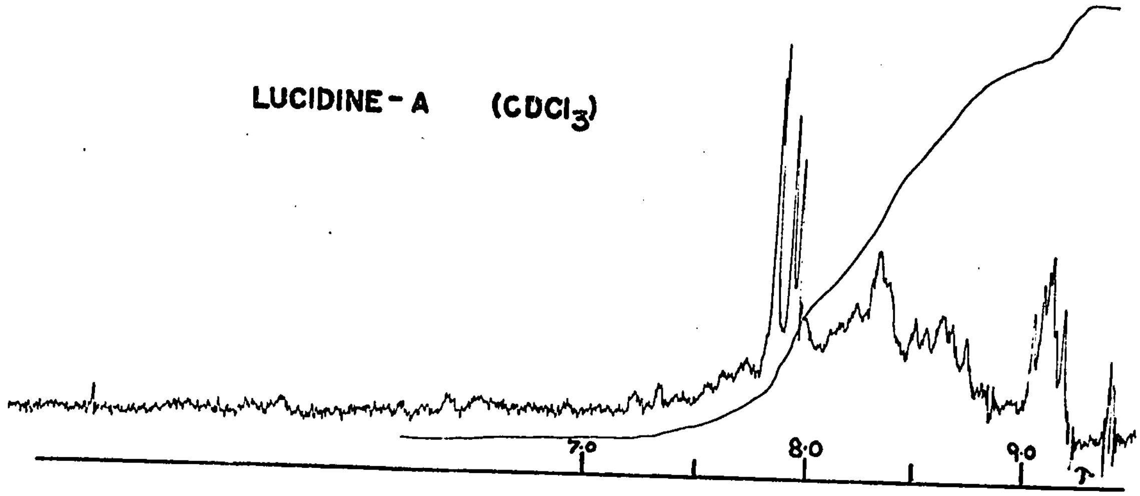


Figure 8

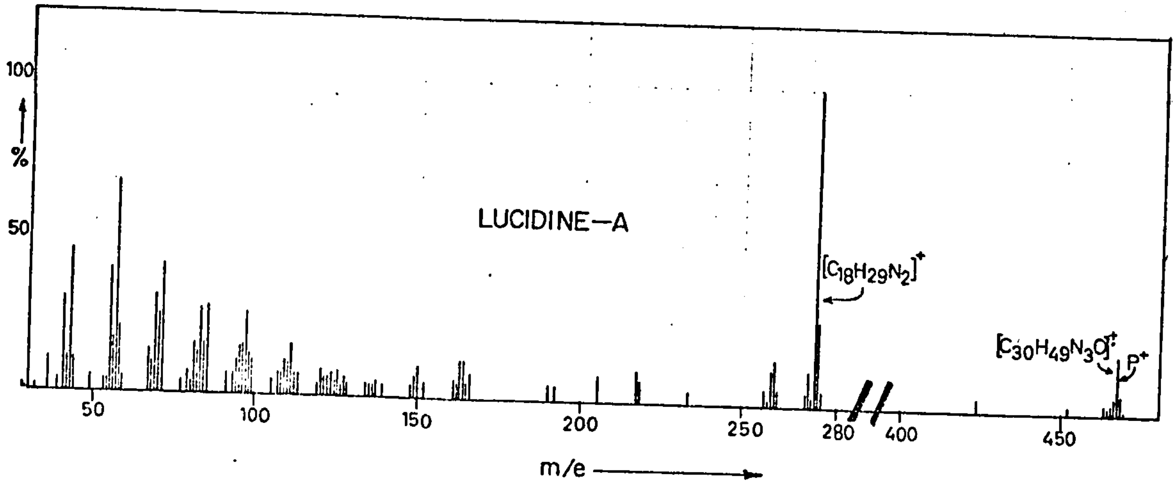


Figure 9

considered that an N-acetyl function might be present in the molecule.

Treatment of lucidine-A and lucidine-B with boiling aqueous sulphuric acid leads in each case to compounds of composition $C_{28}H_{47}N_3$ which correspond to desacetyllucidine-A and desacetyllucidine-B. Each compound lacks the absorption at 1620 cm.$^{-1}$ in the infrared but retains the peak at 2780 cm.$^{-1}$ associated with the N-methyl group. In addition, each hydrolysis product exhibits a peak at 1643 cm.$^{-1}$ of moderate intensity perhaps indicative of an imino ($>C{=}N-$) double bond[103]. This peak is not detectable in the original alkaloids due to the adjacent intense peak at 1620 cm.$^{-1}$. The n.m.r. of the desacetyl compounds is void of the doublet at τ 7.93 and this fact, together with the i.r. and mass spectral data, is reasonable proof that lucidine-A and lucidine-B do contain an N-acetyl group. The doublet nature of the signal pertaining to the acetyl group was suspected of being due to the presence of rotational isomers within each alkaloid.

The characterization of the three nitrogen atoms became the objective at this stage. It was already known that both lucidine-A and lucidine-B possessed N-methyl and N-acetyl and possibly an imino group, but whether these functionalities involved all three nitrogens instead of two was not established.

The i.r. spectra of the alkaloids lacked the absorption usually associated with an NH group and did not show an amide-II band[102]. When lucidine-A and lucidine-B are treated with sodium borohydride they form the respective dihydro compounds. The desacetyl compounds, obtained via acid hydrolysis of the alkaloids, are also reduced by sodium borohydride to yield products which lack both the 1620 and 1643 cm.$^{-1}$ absorption bands of their precursors.

A comparison of the chemical shift of the N-methyl signal in the n.m.r. spectra of either lucidine-A or -B carried out in deuterochloroform (τ 7.83) with that observed when the spectra are determined in trifluoroacetic acid[98] (τ 7.30), indicates that the nitrogen of the N-methyl group is reasonably basic. It seems unlikely then that this nitrogen is also bonded to an acetyl group since such a system would reveal the N-methyl absorption in the parent alkaloids at a considerably lower field position than τ 7.83, possibly in the region τ 7.2 to 7.0. This suggests then that the N-methyl and the N-acetyl groups involve different nitrogen atoms, but as yet, does not rule out the possibility that the methyl or acetyl groups could be attached to the imino nitrogen atom.

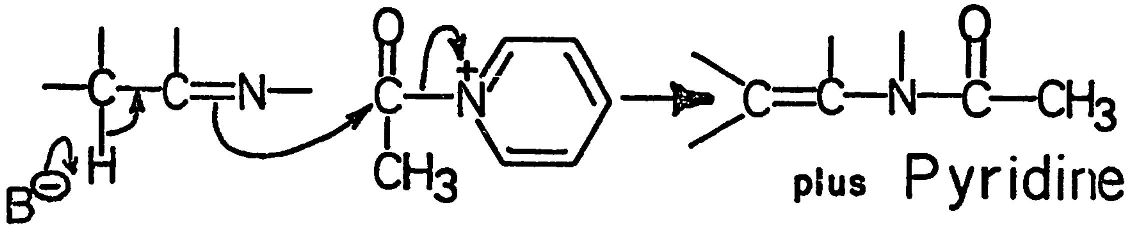
Treatment of lucidine-B with sodium borohydride led to the isolation of dihydrolucidine-B which readily acetylates to yield N-acetyldihydrolucidine-B, $C_{32}H_{53}N_3O_2$, which contains two N-acetyl groups. The n.m.r. of this compound in deuterochloroform reveals the N-methyl signal at τ 7.84, indicating the N-methylated nitrogen is still basic and infers, therefore, that this methyl group is attached to neither of the acetylated nitrogens now present. This evidence suggests that the N-methyl group is not attached to the imino nitrogen atom in lucidine-A or -B.

The alternative possibility that the acetyl group in lucidine-A or -B might be bonded to the imino nitrogen atom was ruled out since reduction of such a system would yield a secondary amido nitrogen which, being non-basic, would not be expected to further acetylate.

Treatment of the hydrolysis products of lucidine-A or -B (i.e., desacetyllucidine-A or -B) with acetic anhydride in pyridine, does cause acetylation but the products are not the original alkaloids. This suggests that either the hydrolysis or the re-acetylation reaction involves a skeletal change.

Lucidine-B itself is acetylated by acetic anhydride at room temperature to yield N-acetyllucidine-B, $C_{32}H_{51}N_3O_2$, mw 509. However, dihydrolucidine-B, which also contains a secondary amino function, still only yields a monoacetylation product, N-acetyldihydrolucidine-B, $C_{32}H_{53}N_3O_2$.

Since lucidine-B is believed to be devoid of $>$NH groups, the acetylation of this alkaloid may involve a rearrangement of the imino group as depicted overleaf104 in diagram 57.

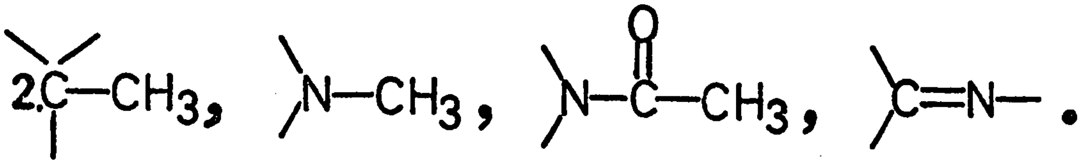


57

The absence of lucidine-A and -B from recently isolated alkaloidal material has left this problem unsolved. However, the presence of such a skeletally rearranged product should be detectable by its behavior towards sodium borohydride. Whereas lucidine-A and -B readily yield the respective dihydro compounds, the skeletally rearranged product should be inert to this reagent.

The N-acetyl groups of both the alkaloids and their dihydro derivatives are readily reduced to N-ethyl groups[105] by lithium aluminum hydride. The reduction products, which contain at least one NH group from the original imine function, yield only monoacetylation products on prolonged treatment with acetic anhydride. This supports the idea that the acetylated nitrogen and methylated nitrogen are tertiary and also the concept that the imine function is involved in the acetylation of the parent alkaloids, otherwise diacetylation products would be anticipated.

So far the evidence is consistent for the presence of the following groups (58) in lucidine-A and -B.



58

All attempts to crystallize lucidine-A or -B, or any of their derivatives, have been unsuccessful.

The molecular formula $C_{30}H_{49}N_3O$ requires a total of eight sites of unsaturation. The N-acetyl and imino functions account for two of these and the fact that the dihydro derivatives are recovered unchanged after being subjected to catalytic hydrogenation conditions at 50 atmospheres, indicates the absence of any other double bonds. Thus lucidine-A and lucidine-B appear to be hexacyclic.

Most of the foregoing reaction products were characterized via the mass spectrometric determination of their molecular composition. In cases where sufficient material was available chemical analyses were carried out. Although the latter were not sufficiently accurate to allow the determination of molecular formulae, the results were consistently close to the more accurate figures obtained by mass spectrometry. The high resolution analyses of the parent mass spectral peaks of some of the aforementioned compounds are shown as follows.

Compound	m/e Found	m/e Calculated	Molecular Formula
Lucidine A/B	467.3870	467.3875	$C_{30}H_{49}N_3O$
Dihydrolucidine A/B	469.4019	469.4032	$C_{30}H_{51}N_3O$
N-acetyllucidine B	509.3967	509.3981	$C_{32}H_{51}N_3O_2$
Tetrahydrodeoxy-lucidine A/B	455.4237	455.4250	$C_{30}H_{53}N_3$

In their mass spectra lucidine-A (figure 9, page 46), lucidine-B and desacetyllucidine-B all show a base peak at m/e 273. All the lucidine derivatives which possess the reduced imino system, such as dihydrolucidine-A or -B and tetrahydrodeoxyludicine-A or -B, possess a base peak at m/e 261 and, at the same time, usually still reveal a fairly intense peak at m/e 273. A characteristic feature in the spectrum of tetrahydrodeoxylucidine-A or -B, which contain an N-ethyl group in place of the original N-acetyl function, is the appearance of intense peaks at m/e 180 and 178. Other peaks which are common to all the lucidine-A or -B alkaloids and their respective derivatives are found at m/e 260, 218, 217, 166, 164, 150 and 96.

High resolution mass spectrometry has been utilized to determine the molecular composition of several of the above peaks.

m/e Found	m/e Calculated	Molecular Formula
273.2326	273.2330	$C_{18}H_{29}N_2$
261.2329	261.2331	$C_{17}H_{29}N_2$
260.2252	260.2257	$C_{17}H_{28}N_2$
180.1755	180.1752	$C_{12}H_{22}N$
164.1444	164.1439	$C_{11}H_{18}N$
150.1284	150.1283	$C_{10}H_{16}N$

The alkaloids are all further characterized by loss of 43 mass units (i.e., from the parent ion). This has been attributed to loss of either $C_3H_7\cdot$ or $CH_3CO\cdot$. The loss of $CH_3CO\cdot$ from the lucidine-A or -B compounds themselves would generate a fragment at m/e 424, $C_{28}H_{46}N_3$. An inspection of the total spectrum reveals that it is devoid of peaks between m/e 273 and m/e 424, which we interpret to mean that the alkaloid gives rise to two major fragments upon electron impact. It is also feasible, taking into account the molecular composition values above, to consider that the peaks at m/e 164 and 150 come from the smaller portion of the molecule instead of being derived via further fragmentation of the m/e 273 peak. For example, addition of the molecular composition values of the ions at m/e 273 and 150, 261 and 164, yield hypothetical ions at 423, $C_{28}H_{45}N_3$ and 425, $C_{28}H_{47}N_3$ respectively, which very closely correspond to the fragment at m/e 424, $C_{28}H_{46}N_3$ or the actual parent peak of desacetyllucidine-A or -B, mw 425, $C_{28}H_{47}N_3$. These correlations will be examined later in this discussion, together with those found in the mass spectra of the other components.

The Alkaloid of Fraction C

Dry-column chromatographic purification of Fraction C obtained from tubes 26-40 of a typical countercurrent experiment led to the isolation of lycolucine, an alkaloid which we originally termed the 1620-G compound. Lycolucine is dextrorotatory and exists as a colorless crystalline solid, m.p. 198-200°. It represents the only weakly basic alkaloid of L. lucidulum that has been isolated in the crystalline state. Its molecular formula $C_{30}H_{43}N_3O$ immediately led us to consider that it might be simply related to the lucidine alkaloids just discussed. Its spectral characteristics reaffirmed this initial concept. The i.r. spectrum in chloroform shows absorption at 2780, 1620, 1580 and 1565 cm.$^{-1}$. The first two peaks mentioned resemble the spectra of lucidine-A and -B, in which they were assigned to N-methyl and N-acetyl groups, while the latter peaks suggest the presence of an aromatic system. The infrared spectrum of lycolucine determined in nujol is shown in figure 10.

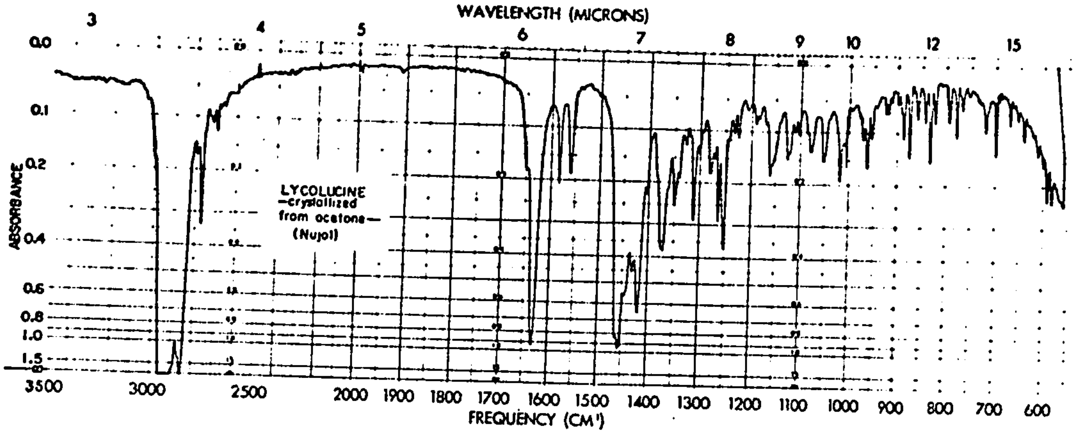
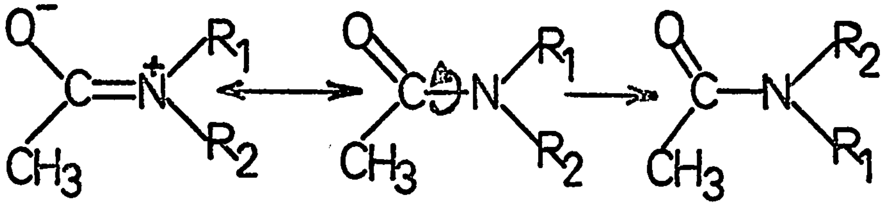


Figure 10

The behavior of lycolucine in the ultraviolet is very informative. When determined in methanol the u.v. spectrum of lycolucine shows absorption maxima at 217mμ, 261mμ (ε= 8,800), 272mμ (ε = 7,800) and 300mμ (ε = 10,200). The addition of one drop of dilute hydrochloric acid to the sample causes a distinct change and the new maxima at 212mμ, 258mμ, 268mμ and 319mμ (ε = 15,000). The first three maxima all appear as less intense bands than is the case in neutral methanol, while the absorption originally at 300mμ appears as a more intense peak at 319mμ. These facts indicate the presence of at least one basic nitrogen in the chromophore and suggest[106] that lycolucine contains a vinylpyridine skeleton.

N.m.r. spectroscopy yields more information about the structure of the alkaloid but the spectra are often complicated by what we believe is a rotomeric effect in the molecule attributable to the N-acetyl group. The interpretation of signals then is complicated since signals which appear as doublets are not necessarily coupled to another proton but may be a singlet which is influenced by the rotomeric effect.

Rotation about single bonds is usually so rapid that the detection of rotational isomers[107] by n.m.r. is only possible at very low temperatures. Resonance theory predicts that the C—N bond in amides has considerable double bond character due to delocalization of the lone pair electrons on the nitrogen. Such systems therefore may exhibit such a slow rate of rotation about this bond that n.m.r. can detect the individual isomers.



59 60 61

The double bond character of 60 shown in 59 slows down the rate of rotation so much that n.m.r. would show the superimposed spectra of the rotational isomers 60 and 61. We believe that this accounts for the added complexity of the lycolucine spectrum and for the "apparent" doublet nature of the N-acetyl moiety in the lucidine alkaloids.

When determined in deuterochloroform the n.m.r. spectrum of lycolucine shows (figure 11) a complex multiplet (6H) at τ 9.06 rather similar to that observed in the spectrum of lucidine-A, attributed to two C-methyl groups. In the region τ9-8 there are many signals all showing an apparent doublet nature (splitting about 2 cps).

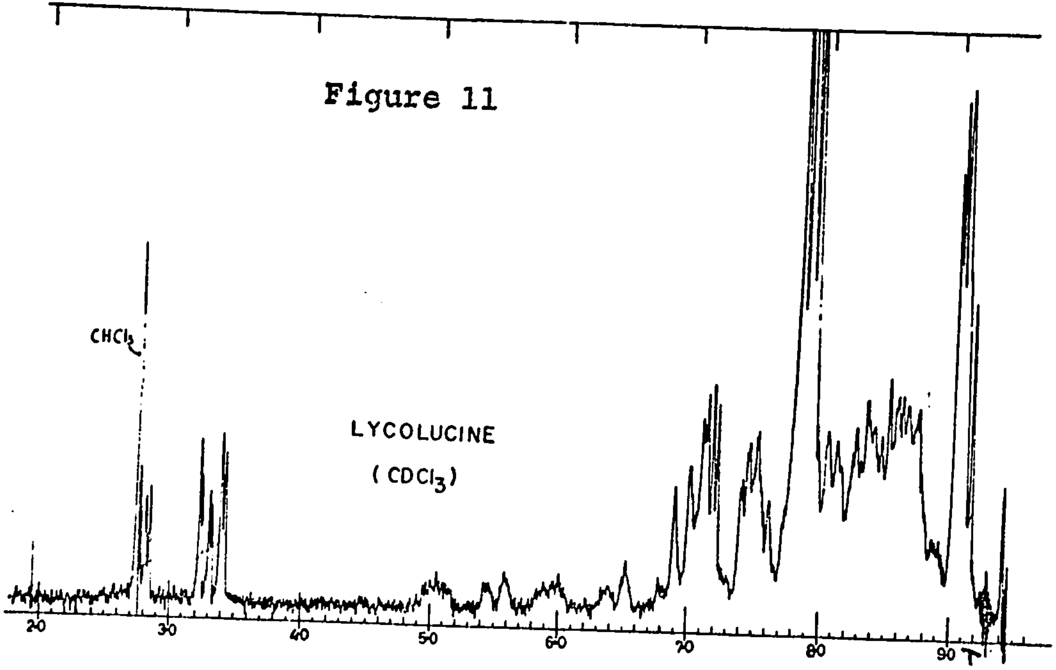
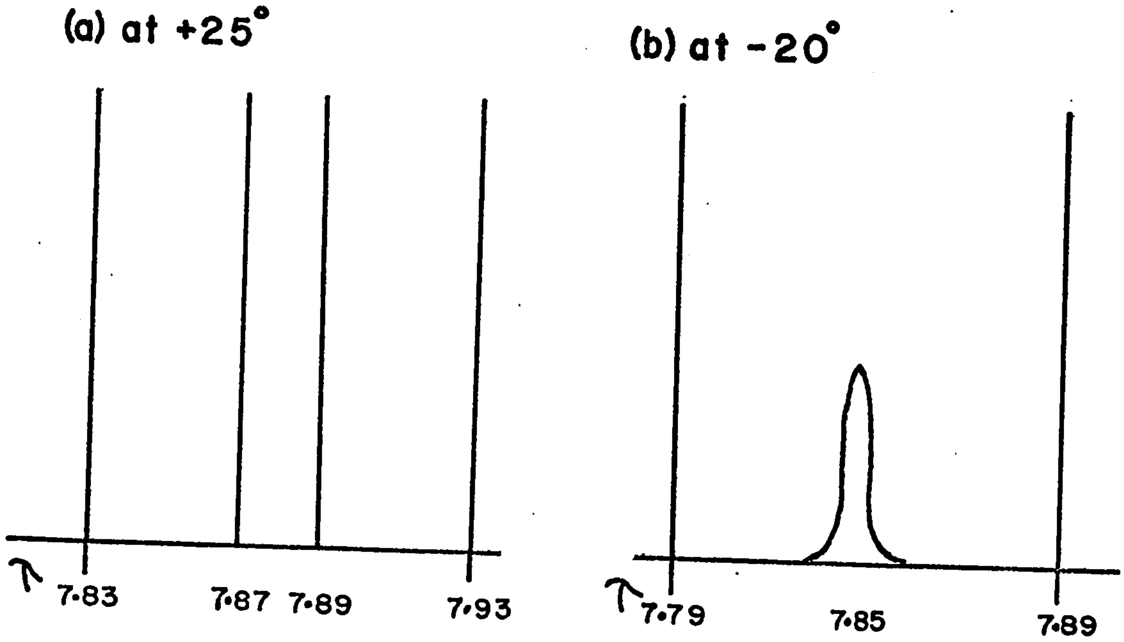


Figure 11

At τ7.93, 7.88 and 7.83 are three intense signals, the central one being most intense, which are regarded as being due to the N-acetyl and N-methyl protons. That these peaks are not in fact a triplet is revealed by examination of the 100 Mc and 220 Mc spectra in this region. In the former spectrum these signals are separated by 5 cps while in the latter they are separated by 11 cps. The 220 Mc spectrum also reveals that the central signal is in fact two separate signals with a separation of 2 cps. This region therefore consists of four signals, two of which are attributed to rotational isomeric N-COCH$_3$ protons while the other two are attributed to the N-CH$_3$ protons.

Recent 100 Mc n.m.r. studies show, as expected, that the intensity of these four signals is temperature dependent. The overall effect of lowering the temperature is to cause a net displacement downfield of all four signals, but at the same time, the two outer signals become more intense at the expense of the inner two. These observations are illustrated in the diagram below (62).



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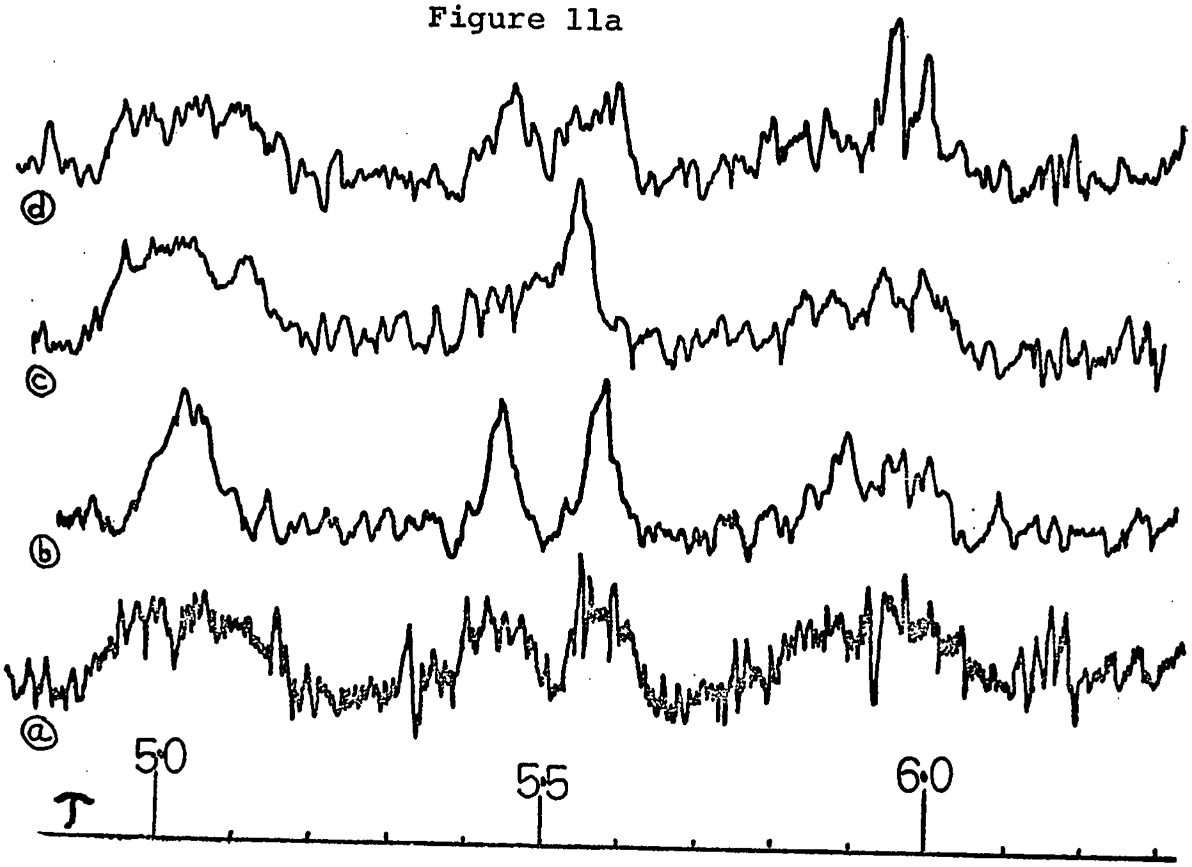
It is appropriate to mention here that desacetyllycolucine, formed by acid hydrolysis of lycolucine, shows only a singlet, attributed to the N-CH_3 group, in the τ 8.0 to 7.8 region. Its n.m.r. spectrum lacks any complications due to rotomerism.

The n.m.r. spectrum of lycolucine shows an AB quartet (J_{AB} = 8 cps) with centers at τ 3.26 and 2.80 and a singlet at τ 3.40. The further splitting observed with the quartet and singlet in lycolucine is absent in the spectrum of desacetyllycolucine and is attributed to the presence of a rotomeric effect in the former. The chemical shift of the quartet and the magnitude of its coupling constant infer that it represents the β and γ protons of a pyridine ring[108], while the singlet at τ 3.40 has a chemical shift typically associated with the presence of a vinyl proton alpha to a pyridine ring. Furthermore, a comparison of the n.m.r. spectra of 2-vinyl- and 4-vinylpyridine infers that the vinyl group in lycolucine is attached to one of the alpha positions of the pyridine ring[109].

The region τ 7.50 to 6.80 of the spectrum shows several well defined peaks including a doublet at τ 7.50 (J = 6 cps), a quartet at τ 7.16 (J = 4 cps) and singlets at τ 7.01 and 6.89. The region τ 6.80 to 4.90 shows many multiplets. A summary of the double irradiation experiments that were carried out is illustrated in figure 11a.

Double irradiation at τ 7.50 (doublet) causes the multiplets originally at τ 5.58 and 5.44 to collapse to a broad singlet while irradiation at τ 7.16 (quartet) causes the original multiplet at τ 5.95 to simplify to a doublet at τ 5.96 (J = 4 cps). Double irradiation at τ 8.63 causes the multiplet at τ 5.04 to simplify and the multiplets at τ 5.44 and 5.58 now appear as a broad doublet at τ 5.51 (J = 13 cps). Similarly, irradiation at τ 8.15 greatly simplifies the C-methyl region to a broad singlet.

Figure 11a



Scan (a) represents the original spectrum of lycolucine. Scans (b), (c) and (d) result from double irradiation at τ8.63, 7.50 and 7.16 respectively.

Treatment of lycolucine with boiling dilute aqueous sulphuric acid yields desacetyllycolucine as a yellow gum which, like lycolucine, is dextrorotatory. The expected molecular formula $C_{28}H_{41}N_{3}$ was confirmed by mass spectrometry. Its i.r. spectrum determined in chloroform retains the 2780, 1580 and 1560 cm.$^{-1}$ absorption while the removal of the intense 1620 cm.$^{-1}$ band (N-acetyl) allows the detection of medium intensity absorption at 1665 cm.$^{-1}$, typical of a trisubstituted double bond. That the double bond is not that of an imine function is shown by the inertness of the compound to sodium borohydride reduction. The u.v. spectra in both neutral and acidic media are identical with those of lycolucine.

The n.m.r. spectrum is uncomplicated by rotational isomers. In deuterochloroform a six-proton multiplet is observed at τ 9.06 which, on closer inspection, appears to be an overlapping singlet and triplet. The N-CH_3 signal resonates at τ 7.89 and as in the case of lycolucine, a doublet (J = 6 cps) is observed at τ 7.48. The singlets which appeared at τ 7.01 and 6.89 in lycolucine now appear at τ 7.26 and 7.17. The signals attributed to the vinyl proton and the aromatic AB quartet are identical with those of lycolucine.

The n.m.r. spectrum of desacetyllycolucine in deuterochloroform, in contrast to that of lycolucine (figure 11), is devoid of peaks in the τ 6.80 to 4.90 region. The protons responsible for the multiplets in this region in lycolucine are therefore markedly influenced by the acetyl function. For this reason we suspect that these multiplets are associated with methylene and/or methine protons adjacent to the acetylated nitrogen atom in lycolucine.

When the n.m.r. spectrum of desacetyllycolucine is determined in perdeuteroacetic acid, the N-methyl signal shifts to τ 7.10 and two broad singlets, most likely due to methine or methylene protons adjacent to the N-methyl nitrogen, appear at τ 6.78 and 6.22 respectively. The AB quartet is now centered at τ 2.41 and 1.76 while the vinyl proton resonates at τ 3.04.

When desacetyllycolucine is treated with acetic anhydride crystalline lycolucine is regenerated.

Catalytic hydrogenation of lycolucine at room temperature and one atmosphere yields dihydrolycolucine, $C_{30}H_{45}N_3O$, the i.r. spectrum of which is indistinguishable from that of lycolucine above 1200 cm.$^{-1}$.

However, appreciable changes are observed both in the u.v. and n.m.r. spectra. In methanol the u.v. spectrum of dihydrolycolucine shows absorption maxima at 216 mμ and 276 mμ (ε = 8,500). Upon acidification the latter absorption is replaced by a more intense peak at 279 mμ (ε = 13,400). This behavior is typical of a trisubstituted pyridine[106]. A comparison of behavior of lycolucine and dihydrolycolucine in the u.v. confirms that in the former a vinyl group is conjugated with the pyridine ring. A comparison of the u.v. spectra of dihydrolycolucine in neutral and acidic media with the observed values of several known alkylpyridines, indicates that dihydrolycolucine contains a 2,3,6-trialkylpyridine moiety. By study of the behavior of various alkylpyridines in the u.v., Andon, Cox and Herington[110] proposed the following correlations for the absorption maxima of alkylpyridines at different pH.

In neutral or basic solution $\lambda max = 257 + 5a + 6b - 2c$

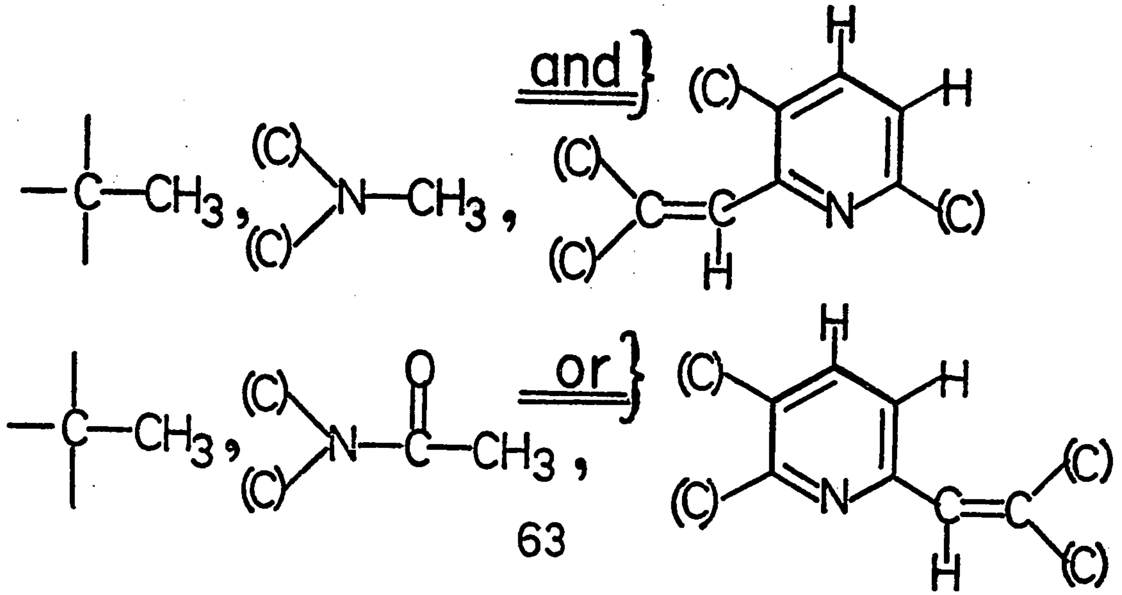
In acidic solution $\lambda max = 256 + 7a + 6.5b - 3c$

In these relationships a, b and c refer to the number of α, β and γ substituents. The calculated values of 2,3,6-trisubstituted pyridines are 273 mμ (in neutral or basic solution) and 276.5 mμ (in acidic solution).

The n.m.r. spectrum of dihydrolycolucine in deuterochloroform shows a small rotomeric effect. The C-methyl region is a little different from that observed for lycolucine and consists of two main signals at τ 9.12 and 9.06 (total 6H), each of which show a small secondary splitting (1 cps). Four signals, due to the N-acetyl and N-methyl groups, appear at τ 7.92, 7.91, 7.89 and 7.82. At lower field the signal attributed to the vinyl proton in lycolucine is now absent, and the AB quartet is centered at τ 3.14 and 2.70.

The foregoing evidence infers that lycolucine contains N-methyl, N-acetyl and two C-methyl groups, together with a 2,3,6-trialkylpyridine system with either the 2- or 6-substituent being a β-disubstituted vinyl group, as shown in partial structure 63.

Nkunika[111] had been able to show, by the following sequence of reactions, that lycolucine possessed the same carbon skeleton as the lucidine alkaloids. Lycolucine was converted into dihydrolycolucine which, on acidic hydrolysis gave a moderate yield of desacetyldihydrolycolucine, whose



63

expected molecular formula $C_{28}H_{43}N$, was confirmed by high resolution mass spectrometry. The compound showed absorption at 2780, 1580 and 1565 cm.$^{-1}$ in the i.r. which confirmed the absence of the olefinic and amido functions. Its u.v. spectrum was very similar to that of dihydrolycolucine and showed an absorption maximum at 275 mμ (ε = 6,300) in neutral solution and at 279 mμ (ε = 9,800) in acidic solution^{110}. The n.m.r. spectrum was consistent with the absence of the N-acetyl and vinyl protons. The N-methyl signal appeared at τ 7.84 and the AB quartet was now centered at τ 3.28 and 2.88. A comparison of this n.m.r. spectrum with those of lycolucine and desacetyllycolucine reveals that the doublet which resonates in the τ 7.50 region in the latter two components, is now absent. Nkunika then reduced desacetyldihydrolycolucine with sodium in isoamyl alcohol and obtained a mixture (t.l.c.) of two major and at least two minor components. The mass spectrum of the crude mixture showed a parent peak at m/e 427, $C_{28}H_{47}N_3$ and a base peak at m/e 261. The parent peak indicated that some octahydrodesacetyllycolucine had been formed, while the base peak was characteristic of compounds containing the dihydrolucidine-A or -B skeleton which, as mentioned earlier, all exhibit a base peak at m/e 261. Nkunika discovered that one of the minor components appeared to be identical with desacetyldihydrolucidine-A, formed from lucidine-A by sodium borohydride reduction, followed by acidic hydrolysis of the resultant dihydrolucidine-A.

The results indicate therefore that one of the isomers of octahydrodesacetyllycolucine is identical with desacetyldihydrolucidine-A and imply that lucidine-A is simply a hexahydro derivative of lycolucine. The observed similarities between the n.m.r. spectra of lucidine-A and lycolucine in the C-methyl region are consistent with this correlation.

An attempt to learn more about the environment around the olefinic double bond in lycolucine by treatment of the latter with ozone at low temperature[112] was unsuccessful and the only compound isolated and characterized was unchanged lycolucine.

However, treatment of lycolucine with osmium tetroxide, followed by sodium metaperiodate was more successful[113]. We were able to isolate a small amount of material whose infrared spectrum, determined in chloroform, besides showing the $N-CH_3$,$N-COCH_3$ and aromatic absorptions, shows a band of medium intensity at 1680 cm.$^{-1}$ typical of an α,β-unsaturated carbonyl compound. The mass spectrum of this product shows a parent peak at m/e 493, amounting to the addition of 32 atomic mass units (a.m.u.) to lycolucine (mw 461). High resolution mass spectrometry reveals that the parent peak of this product has the molecular composition $C_{30}H_{43}N_3O_3$, which corresponds to the addition of two oxygen atoms to lycolucine. Accordingly, this product has been named dioxolycolucine. The results of this oxidative cleavage indicate at least that the vinyl system, attached to the pyridine nucleus in lycolucine, cannot be a simple unbridged side-chain. If it had been, then two products would have been formed.

Like the lucidine series of alkaloids, the lycolucine based compounds generally show few intense peaks in their mass spectra. The fragmentation patterns of the latter compounds similarly indicate that upon electron impact, a facile fragmentation of the molecules into two major portions takes place, as exemplified for lycolucine in figure 12.

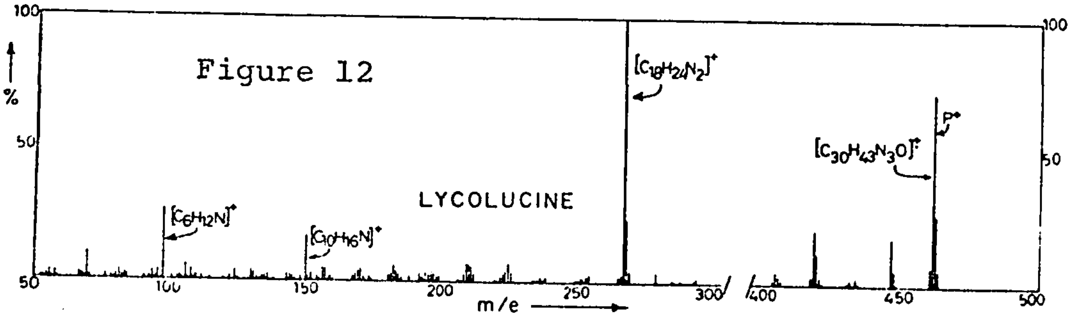


Figure 12

The table overleaf outlines the general features of these mass spectra. The figures in the columns refer to the relative intensities of peaks at various m/e values.

COMPOUND	mw	M+	m/e 300	m/e 273	m/e 270	m/e 268	m/e 261	m/e 257	m/e 255	m/e 150	m/e 98
Lycolucine	461	71		4		100				22	34
Dihydro "	463	46		12	100	6				18	13
Desacetyl "	419	23		9	39	83			55	100	21
Desacetyl-dihydro "	421	55		7	100	11		47		48	12
Octahydro-desacetyl "	427	36		20			100			24	7
Dioxo "	493	100	100							100	47

% intensities of base peak (100%)

A feature of all the compounds above is a peak at $M^+ - 43$ formed by loss of both $C_3H_7\cdot$ and $CH_3CO\cdot$ from the parent peak. The base peak at m/e 261 for octahydrodesacetyllycolucine, as mentioned earlier, implies that lycolucine and lucidine alkaloids possess a similar carbon skeleton. That the dioxolycolucine shows an intense peak at m/e 300, (i.e., 32 a.m.u. higher than the base peak of lycolucine) infers that the ion at m/e 268 in lycolucine contains the double bond. Unlike all the other compounds dioxolycolucine did show several intense peaks suggesting that the presence of the two extra oxygen atoms leads to several new fragmentation pathways. Many of these peaks were subjected to high resolution mass spectrometry and the results are as follows.

a) Lycolucine, $C_{30}H_{43}N_3O$, mw 461

m/e Found	m/e Calculated	Molecular composition
418.3226	418.3222	$C_{28}H_{40}N_3$
418.2862	418.2860	$C_{27}H_{36}N_3O$
268.1935	268.1939	$C_{18}H_{24}N_2$
150.1284	150.1283	$C_{10}H_{16}N$
98.0970	98.0970	$C_6H_{12}N$

b) Dihydrolycolucine, $C_{30}H_{45}N_3O$, mw 463

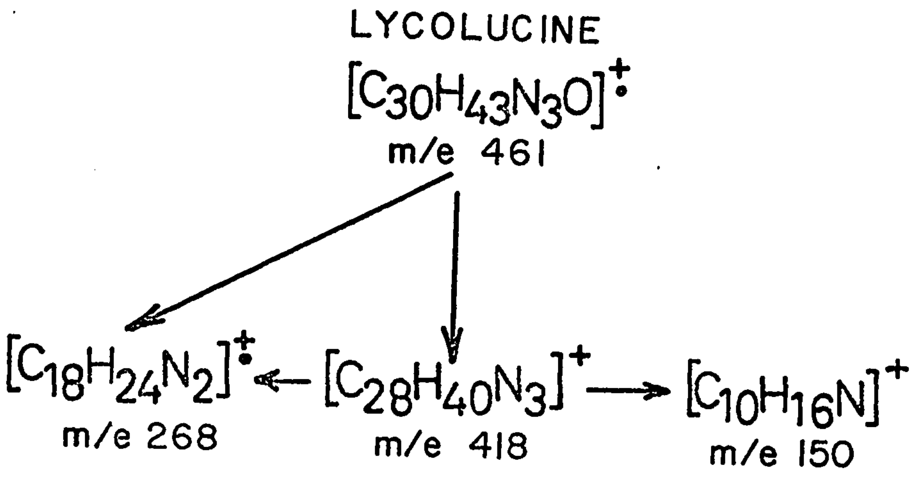
m/e Found	m/e Calculated	Molecular composition
463.3562	463.3563	$C_{30}H_{45}N_3O$
420.3378	420.3379	$C_{28}H_{42}N_3$
420.3014	420.3015	$C_{27}H_{38}N_3O$
270.2095	270.2096	$C_{18}H_{26}N_2$
150.1283	150.1283	$C_{10}H_{16}N$
98.0972	98.0970	$C_6H_{12}N$

c) Dioxolycolucine, $C_{30}H_{43}N_3O_3$, mw 493

m/e Found	m/e Calculated	Molecular composition
493.3304	493.3304	$C_{30}H_{43}N_3O_3$
465.3356	465.3355	$C_{29}H_{43}N_3O_2$
369.2417	369.2416	$C_{22}H_{31}N_3O_2$
300.1838	300.1838	$C_{18}H_{24}N_2O_2$
192.1750	192.1752	$C_{13}H_{22}N$
192.1386	192.1388	$C_{12}H_{18}NO$

A metastable peak at m*/e 156 in the mass spectrum of lycolucine indicates that the base peak at m/e 268 is formed directly from the molecular ion. Since the base peak is devoid of oxygen the nitrogen in the remaining portion of the molecule must be the one which is acetylated in lycolucine. The addition of the molecular composition values of the ions at m/e 150 and 268 in lycolucine give a hypothetical ion at m/e 418 ($C_{28}H_{40}N_3$) analogous to the peak at m/e 418 actually observed. This idea is reinforced by an inspection of the mass spectrum of dioxolycolucine which shows a strong m/e 192 peak corresponding possibly to an N-acetyl derivative of the m/e 150 ion. It follows then that the nitrogen atoms contained in the base peak of lycolucine are the ones associated with the pyridine and N-methyl groups.

A probable fragmentation scheme of lycolucine is outlined (64).



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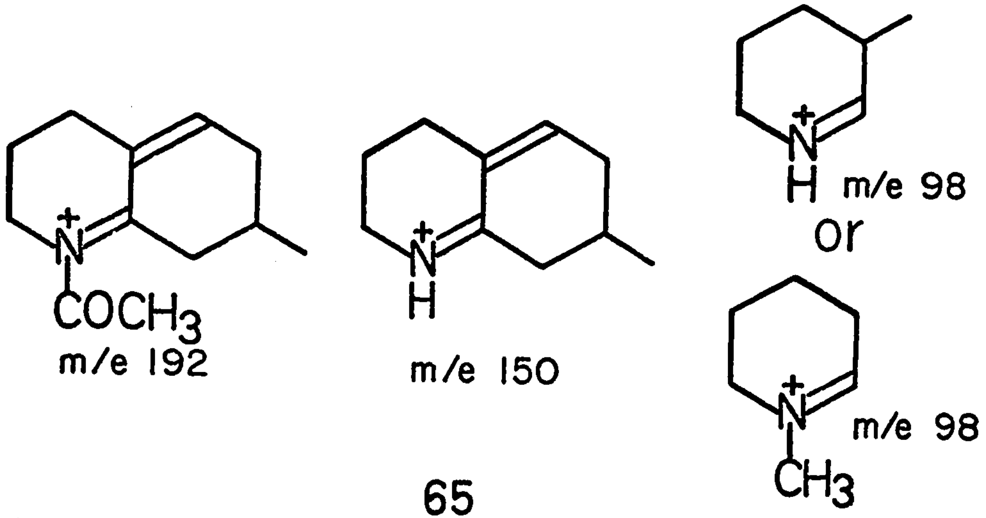
Since the major dehydrogenation products of many _Lycopodium_ alkaloids possess a 7-methylquinoline nucleus and it will be shown later that this is the case with these C_{30} compounds, it may be speculated that the ion at m/e 150 is related to this nucleus. Accordingly, possible structures for the ions at m/e 192, 150 and 98 are presented below (65).



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Whether or not the peak at m/e 98 is derived from the m/e 150 or the m/e 268 fragment is unknown. It is interesting to note that the spectrum of desacetyllycolucine, in which the >NH moiety pertaining to the above ions is already present, has a base peak at m/e 150.

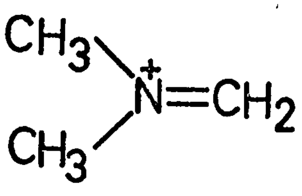
Analogy with the dihydrolucidine-A and -B alkaloids leads to the tentative assignment that the peaks at m/e 261 257 and 255 in octahydrodesacetyllycolucine, desacetyldihydrolycolucine and desacetyllycolucine respectively, have the composition $C_{17}H_{29}N_2$, $C_{17}H_{25}N_2$ and $C_{17}H_{23}N_2$.

At this stage in the characterization of lycolucine we began to doubt that its structure would be solved quickly through chemical methods alone. We therefore set out to prepare a crystalline derivative of lycolucine which would be suitable for x-ray analysis. However, repeated reactions of lycolucine with perchloric acid, hydrogen bromide, iodomethane and chloroplatinic acid have failed to provide us with even one crystalline sample. In all cases reaction does appear to take place since i.r. spectra of the products reveal no N-methyl absorption inferring that this nitrogen has been protonated or alkylated.

In one typical reaction a solution of iodomethane and lycolucine was heated under reflux for several days. By chromatography the separation of unreacted lycolucine from a more polar component could be accomplished. The i.r. spectrum of the polar component in chloroform showed the typical amide and aromatic absorption peaks but lacked a peak attributable to an N-methyl group. It was suspected then that this nitrogen had been quaternized. Mass spectrometry indicated a base peak at m/e 58, a parent peak at m/e 476 and intense peaks at m/e 461, 268, 254, 237 and 150. The molecular composition of the ions at m/e 254 and 58 was determined by high resolution mass spectrometry.

m/e Found	m/e Calculated	Molecular composition
254.1785	254.1783	$C_{17}H_{22}N_2$
58.0656	58.0657	C_3H_8N

Since lycolucine itself does not exhibit an unusually intense peak at m/e 58 and because it is reasonable to assume that the iodomethane reacted with either the N-methyl or pyridine nitrogen of lycolucine in preference to the acetylated nitrogen, it is considered that of the possible structures (66) and (67) for the ion at m/e 58, the former is the more likely and could arise *via*[114] a Hofmann-type elimination, as shown (68).



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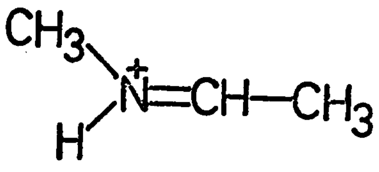

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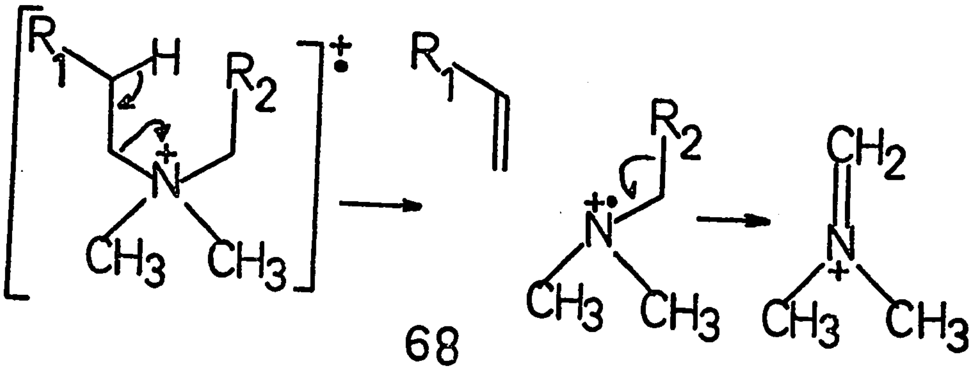

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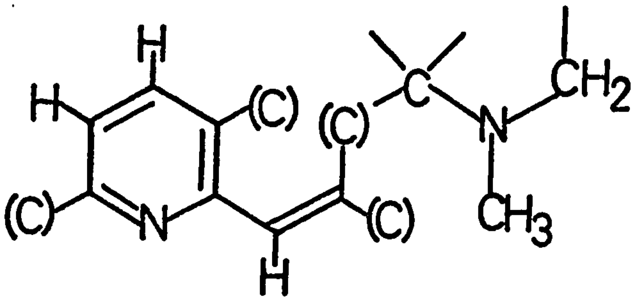
The u.v. spectra of the sample in neutral and acidic media were identical with that of lycolucine, indicating that the pyridine nitrogen was not quaternized, but the u.v. spectrum of a sublimed sample of this product in acidic methanol shows a shoulder at 360 mμ. This value corresponds to an extension of the original chromophore of lycolucine, which shows a corresponding maximum at 319 mμ, by one alkylated double bond. It appears as if the process of sublimation has brought about a degradation. Although it is perhaps premature to speculate, since this reaction has not been investigated further, a partial structure such as



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for example 69, may be contemplated. Further discussion of structural hypothesis is delayed until we have dealt with the other alkaloids.

The Alkaloids of Fractions A and B

The only difference between Fraction A (tubes 1-12) and Fraction B (13-25) is the presence of luciduline (33) in the latter fraction. However, the luciduline is easily separated from the other components of Fraction B by elution chromatography and this separation essentially leads to a second batch of the components of Fraction A.

Fraction A comprises the most weakly basic alkaloids of L. lucidulum. Its original characterization suggested the presence of only one component (t.l.c.) which was subsequently called the 1620H compound. However, it soon became apparent by comparison of the u.v., i.r. and mass spectral data that more than one compound was present. The ultraviolet spectrum was identical to that of the dihydrolycolucine formed from lycolucine and the suspicion that this constituted one of the components was reaffirmed by the presence of many peaks in the mass spectrum of the 1620H compound which corresponded to those of dihydrolycolucine. It therefore seemed likely that the 1620H compound consisted of two components whose Rf values on t.l.c. were very similar.

Following extensive chromatographic work employing a wide variety of alumina adsorbents in conjunction with various elution solvents, the separation of the compound into its two components has been achieved.

The less polar component is found to be identical in all respects with the dihydrolycolucine formed by catalytic hydrogenation of lycolucine at one atmosphere.

The major and more polar component, isolated as a yellow gum, has a molecular weight of 483 and molecular composition $C_{30}H_{49}N_3O_2$. Like the other weakly basic alkaloids already mentioned, its i.r. spectrum in chloroform (figure 13) suggests the presence of N-methyl (2780 cm.$^{-1}$) and N-acetyl (1620 cm.$^{-1}$) groups. However, it also shows an intense absorption at 1710 cm.$^{-1}$ typical of an aldehydic or ketonic carbonyl function, which therefore accounts for the second oxygen function of the molecule. Since the component is resistant to Jones' oxidation and does not show absorption in the n.m.r. below τ6.0 it is believed that the

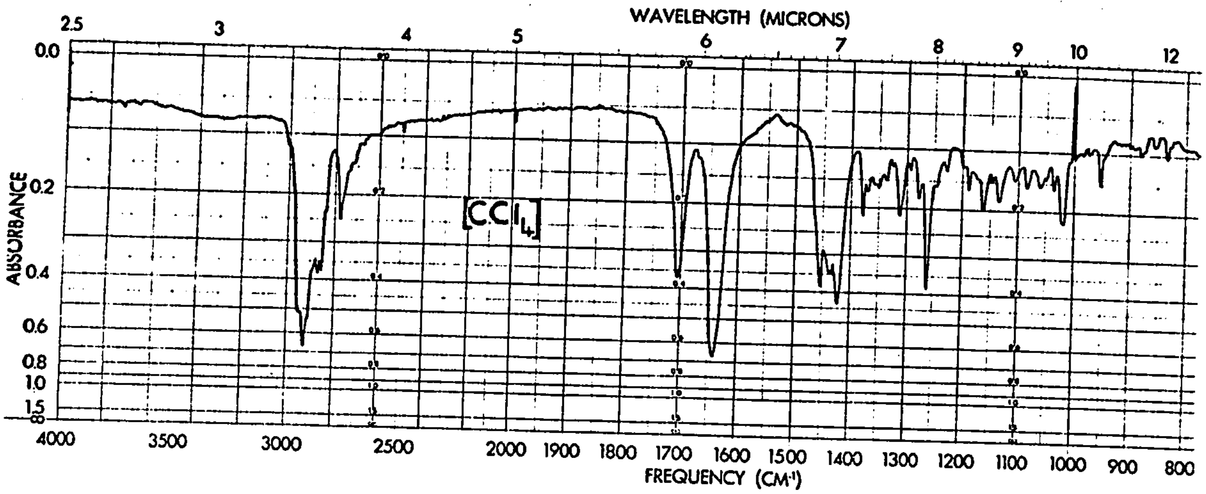


Figure 13

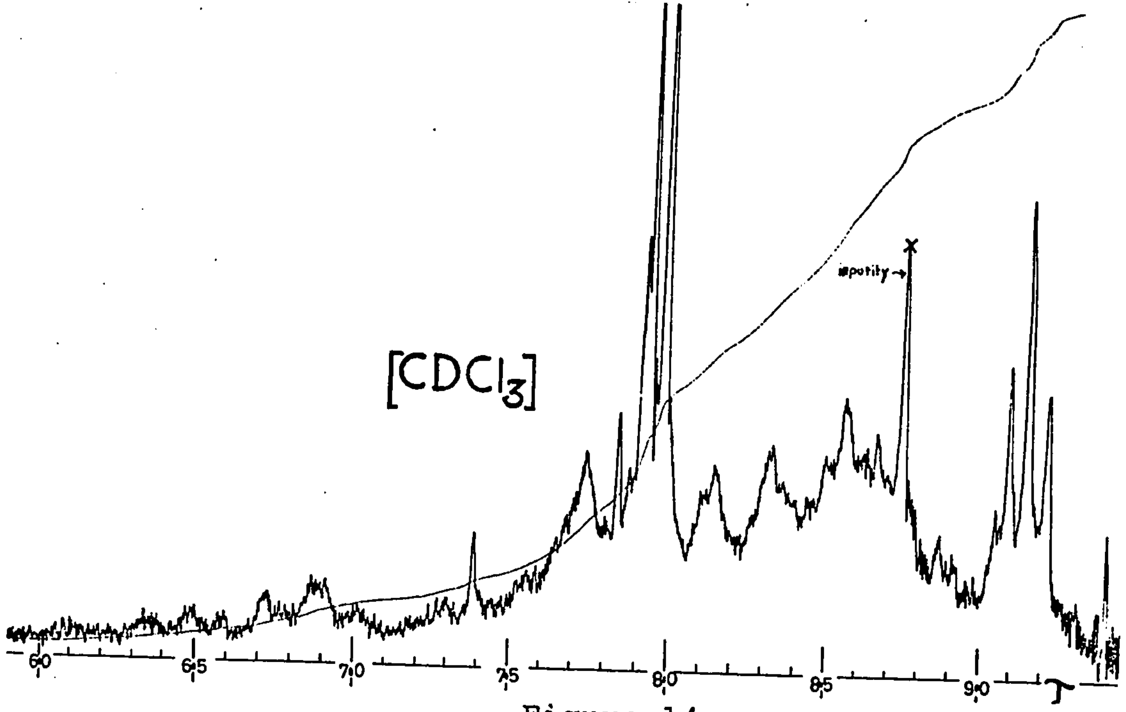


Figure 14

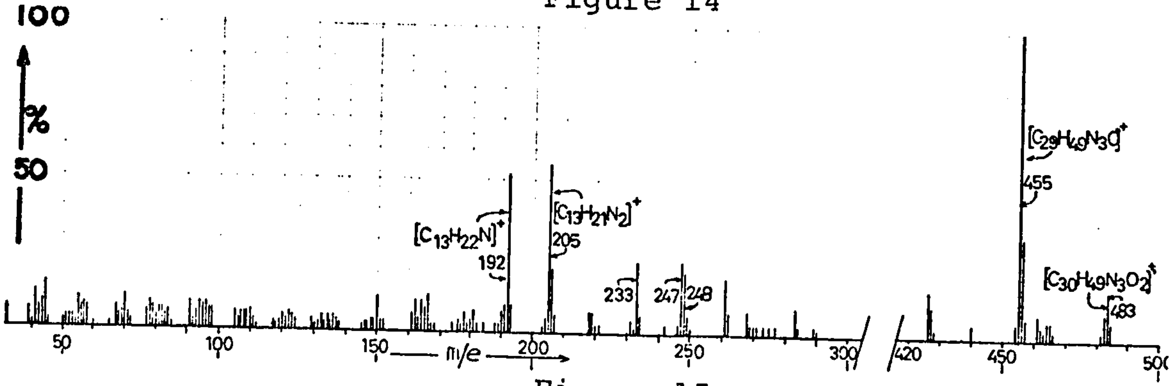


Figure 15

latter carbonyl function is ketonic. Accordingly, the component has become known as the 483-Ketone. A characteristic feature of this compound in the infrared is the appearance of medium intensity peaks at 1020 and 955 cm.$^{-1}$.

The n.m.r. of the 483-Ketone in deuterochloroform (figure 14) shows an apparent six-proton triplet at τ 9.15 ($J = 6$ cps). However, the appearance of a shoulder on the central peak of the triplet infers that two overlapping doublets at τ 9.12 and 9.18 (each $J = 6$ cps) may be present. Intense three-proton singlets ascribed to the N-acetyl and N-methyl groups are located at τ 7.98 and 7.93 respectively, while singlets of moderate intensity, possibly due to the methine and methylene protons adjacent to nitrogen or the ketonic carbonyl group, are observed at τ 7.92, 7.84 and 7.38. If, like the other compounds already discussed, the 483-Ketone is hexacyclic, then its molecular formula requires the absence of double bonds. By analogy with the lucidine series of alkaloids, the possibility arose that an $>$NH group might be present in the molecule. Attempts to establish this by acetylation have been unsuccessful but the possibility is not ruled out.

In methanol, the 483-Ketone displays a positive[115] Cotton effect in its optical rotatory dispersion (o.r.d.) curve with a peak at 344 mμ and a trough at 296 mμ. The curve, however, shows another minimum at 247 mμ (see figure 16). The circular dichroism curve shows a positive Cotton effect

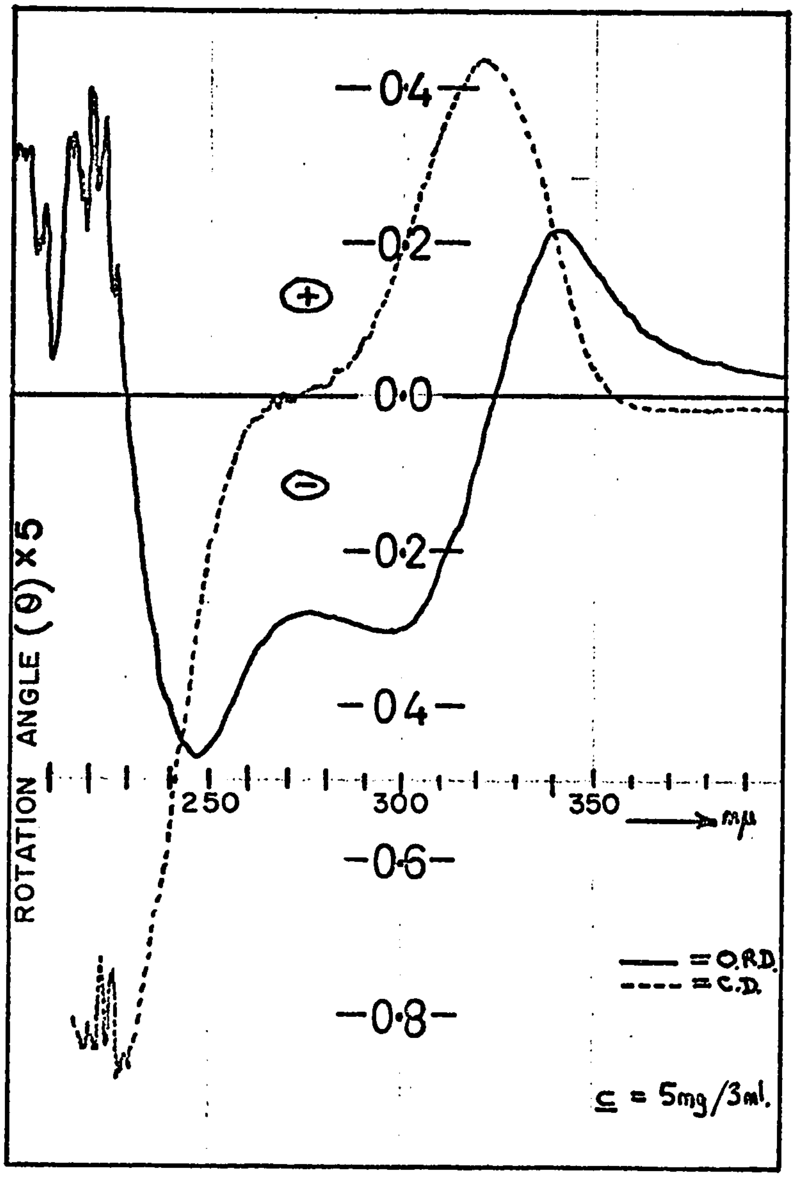
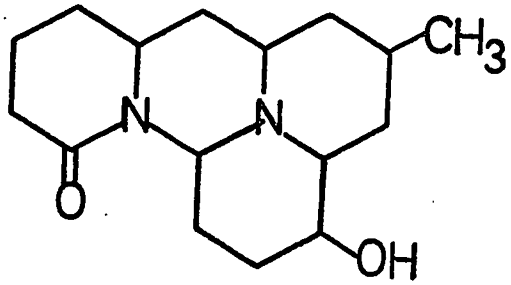


Figure 16

at 322 mμ and a negative Cotton effect below 230 mμ, thus accounting for the second minimum in the o.r.d. The Cotton effect due to the ketone n→π* transition must be that at 322 mμ. This bathochromic shift from the normal

(<300 mμ) position for the absorption band due to the ketone is possibly indicative of an α-aminoketone. Dihydrolycocernuine (70), the ketone formed by Jones' oxidation[65] of the alkaloid lycocernuine[50] (24), obtained from L. cernuum shows, for example, a Cotton effect centered at 316 mμ.



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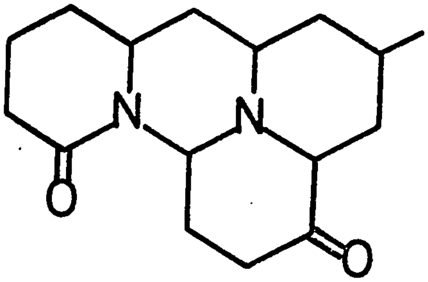
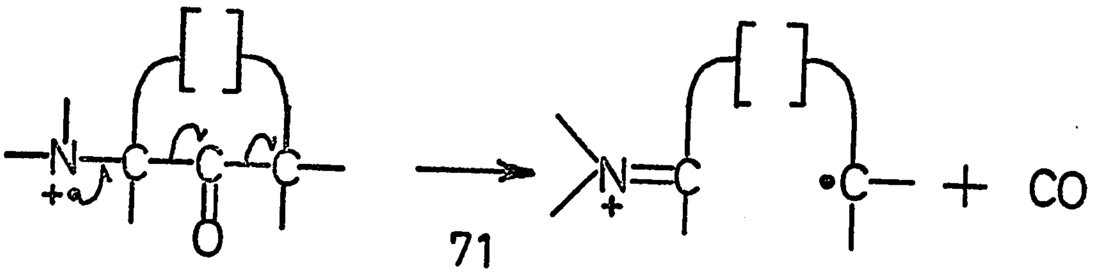


70

Mass spectrometry of the 483-Ketone has been very informative (figure 15). Apart from a parent peak at m/e 483 and a base peak at m/e 455, intense peaks are observed at m/e 261, 248, 247, 233, 205, 192 and 150. The peak at m/e 261 correlates the spectrum with the spectra of the dihydrolucidine-A and -B alkaloids. High resolution mass spectrometry has served to determine the molecular composition of these peaks.

m/e Found	m/e Calculated	Molecular composition
483.3828	483.3825	$C_{30}H_{49}N_3O_2$
455.3875	455.3876	$C_{29}H_{49}N_3O$
261.2334	261.2331	$C_{17}H_{29}N_2$
248.2254	248.2253	$C_{16}H_{28}N_2$
247.2172	247.2174	$C_{16}H_{27}N_2$
233.2010	233.2017	$C_{15}H_{25}N_2$
205.1704	205.1707	$C_{13}H_{21}N_2$
192.1750	192.1752	$C_{13}H_{22}N$
150.1284	150.1283	$C_{10}H_{16}N$

It may be seen that the base peak is formed by loss of carbon monoxide from the parent peak, a feature sometimes indicative of an α-aminoketone[116] (71).



Treatment of the 483-Ketone with sodium borohydride affords an alcohol (mw 485) which on acetylation yields O-acetyldihydro-483-Ketone (mw 527) as indicated by the presence of an intense absorption at 1732 cm.$^{-1}$ in the infrared. This reaction indicates that if a double bond is present then it cannot be of the imino type.

The n.m.r. spectrum of the alcohol reveals a six-proton C-methyl region which appears to consist of two overlapping doublets (J = 6 cps) each centered at τ9.12. A doublet (J = 4 cps) and singlet are located at τ8.70 and 8.56 respectively, while the N-acetyl and N-methyl signals occur at τ7.94 and 7.89. Many of these peaks show a small secondary splitting which is interpreted as being due to a rotomeric effect.

A comparison of the mass spectra of the 483-Ketone and its alcohol shows that the latter exhibits a base peak at m/e 207, together with reasonably intense peaks at m/e 192, 205, 235, 277, 278 and 291. The latter three peaks may be related to the peaks found at m/e 247, 248 and 261 in the spectrum of the 483-Ketone. Their presence in the alcohol can possibly be explained by the presence of the hydroxylated carbon atom (30 a.m.u.) relative to the case in the ketone where this carbon is lost as carbon monoxide. If this is true, then it infers that the original ketonic function is present in the major ($C_{17}N_2$)portion of the molecule rather than in that portion which is believed to contain the 7-methylquinoline skeleton.

The exact mass of the base peak ion of the alcohol is 207.1858 which corresponds to $C_{13}H_{23}N_2$ (calculated value: 207.1861).

From the reaction of the alcohol with iodomethane we were able to isolate a trace of material, less polar than the alcohol, whose parent peak in the mass spectrum is at m/e 499. The spectrum also shows an intense peak at m/e 481 (M^+ - 18) and a base peak at m/e 291. It appears that N-methylation has taken place (the loss of 18 mass units infers the presence of OH) and indicates that the alcohol and the original ketone contain an $>$NH group.

The mass spectrum of O-acetyldihydro-483-Ketone reveals a parent peak at m/e 527, a base peak at m/e 205 and prominent peaks at m/e 467, 273, 259, 217, 207, 192 and 150.

A comparison of the mass spectral pattern of the 483-Ketone (I) and its dihydro- and O-acetyldihydro-derivative (II) and (III) respectively, is shown in the following table, where the figures represent the relative peak intensities.

	M^+	m/e 467	m/e 455	m/e 277	m/e 273	m/e 261	m/e 259	m/e 247	m/e 235	m/e 233	m/e 217	m/e 207	m/e 205	m/e 192	m/e 150
I	15		100			35		38		30			75	56	14
II	35	8		15	5		4		9	9		100	51	29	10
III	34	71			69		61				41	62	100	40	25

It is seen that although the dihydro compound does not show an intense M^+ - 18 peak (i.e., loss of H_2O) electron impact upon the O-acetyldihydro compound causes a facile loss of 60 mass units (CH_3COOH). The peaks at m/e 273, 261 and 259 indicate that the compounds possess a similar carbon skeleton and fragmentation pathway to the lucidine-A and -B alkaloids and their derivatives.

The Alkaloids of Fraction D (part 2)

The 481/483 mixture

Earlier the characterization of the two least polar alkaloids of Fraction D, namely lucidine-A and -B, was described. The discussion now will deal with the two most polar alkaloids found in this same fraction.

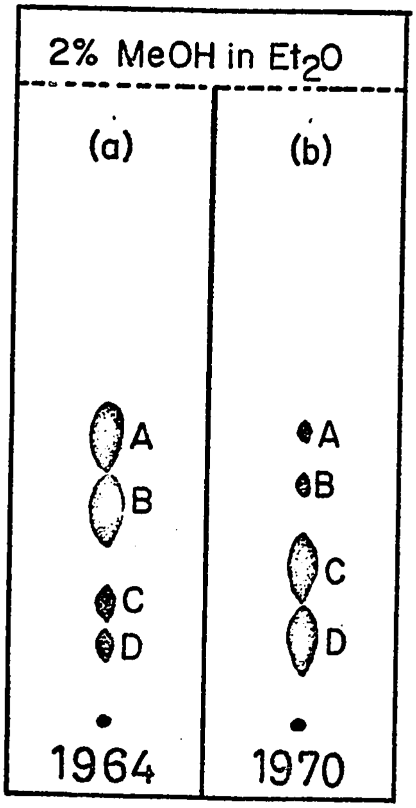


Figure 17

Original extractions of L. lucidulum, beginning in 1964 led to the isolation of the corresponding Fraction D in which lucidine-A and lucidine-B (i.e., A and B in figure 17a), were the major components. Recent extractions on more of the plant material collected in 1964, reveal that the more polar components denoted as C and D on the accompanying chromatogram (figure 17b), so dominate the fraction that the isolation of the lucidine-A and -B alkaloids (i.e., A and B in figure 17b), has become increasingly difficult.

The initial characterization of the more polar components of Fraction D was carried out in 1964 following their separation from the lucidine alkaloids by repeated elution and dry-column chromatography. The presence of N-methyl and N-acetyl groups was revealed by n.m.r. and i.r. spectra. The i.r. in chloroform also revealed an intense but broad absorption at 3300 cm.$^{-1}$ which was attributed to the presence of a hydroxyl group. Accordingly, the components became known as the 1620-C (less polar) and 1620-D compounds.

Most of the early research on these samples was carried out on the more polar 1620-D compound obtained from the mixture by repeated dry-column chromatography and preparative t.l.c. The n.m.r. of this component in deuterochloroform showed C-CH_3 multiplets at τ9.10 and 8.85. Strong singlets were present at τ7.93, 7.90 and 7.75 due to the N-acetyl, N-methyl and possibly methine or methylene protons adjacent to a nitrogen atom. The mass spectrum showed a parent peak at m/e 483, a base peak at m/e 271 and a strong peak at m/e 465. A metastable peak at m*/e 448 corresponded to the direct m/e 483→465 fragmentation. High resolution mass spectrometry was applied to three of the prominent peaks.

m/e Found	m/e Calculated	Molecular composition
483.3809	483.3825	$C_{30}H_{49}N_3O_2$
465.3704	465.3719	$C_{30}H_{47}N_3O$
271.2167	271.2174	$C_{18}H_{27}N_2$
271.1797	271.1810	$C_{17}H_{23}N_2O$

The compound thus is isomeric with the 483-Ketone described above. The loss of H_2O from the parent peak suggests the presence of a hydroxyl function in the molecule. The ion at m/e 271 ($C_{18}H_{27}N_2$)correlates the compound with the lucidine alkaloids which show a base peak at m/e 273 ($C_{18}H_{29}N_2$).

Since the behavior of the 1620-C and 1620-D compounds in their i.r., n.m.r. and mass spectra is very similar, it seemed appropriate at that time to consider that 1620-C was a stereoisomer of 1620-D.

The nature of the alcoholic function in 1620-D was uncertain. The alkaloid was recovered unchanged after treatment with Jones' reagent. Acetylation, however, led to a mixture of four components, one of which exhibited an absorption at 1732 cm.$^{-1}$ in the i.r. and gave a mass spectrum having a parent peak at m/e 525, a base peak at m/e 258 and a prominent $M^+ - 60$ peak at m/e 465.

Treatment of the 1620-D with sodium borohydride gave a dihydro compound whose mass spectrum showed a parent peak at m/e 485, a base peak at m/e 467 and other intense peaks at m/e 291, 277, 273, 235 and 192. Since the infrared spectra of the 1620-D compound showed that the only carbonyl function present in the molecule was that of the N-acetyl group, the formation of a dihydro compound must have occurred via reduction of an imine function.

The work reported to this point was carried out on Fraction D from our early isolations (pre-1967), in which the 1620-A and 1620-B components predominated in the fraction. Further work has been carried out on Fraction D rich in 1620-C and 1620-D.

Although the more polar components (C and D) now isolated from Fraction D bear strong similarity to the characterization of the 1620-C and 1620-D, there are some discrepancies and it is possible that the present components are different from the ones previously isolated.

It should be emphasized again that we are dealing with non-volatile, non-crystalline materials and criteria of absolute purity are difficult to establish. The appearance of a single spot on t.l.c. (in more than one solvent system) is used as a criterion of purity, although we recognize that this is not a fool-proof criterion.

Recent studies show that the two more polar components of Fraction D (tubes 41-85) have molecular weights of 483 and 481. The component of molecular weight 483 probably corresponds to one of the 1620-C or -D alkaloids but this is not certain.

Much of the recent research has been carried out on mixtures of these two components since their separation is very tedious. The infrared spectrum of the mixture shows absorptions at 3300 (broad), 2790 and 1620 cm.$^{-1}$, typifying the presence of hydroxyl, N-methyl and N-acetyl groups. The mass spectrum shows prominent peaks at m/e 483, 481, 465, 287, 276, 271, 233, 205, 192 and 164. The $M^+ - 18$ peak at m/e 465 is very intense. It was mass spectrometry on a mixed sample which gave the first indications that a component of molecular weight 481 was present in Fraction D.

By repeated dry-column chromatography and preparative t.l.c. a small amount of the less polar component of the mixture has been obtained. Its infrared spectrum cannot be distinguished from that of the mixture. Mass spectrometry reveals a parent peak at *m/e* 483 and prominent peaks at *m/e* 465, 289, 271, 233, 205, 166 and 164. Its n.m.r. spectrum in deuterochloroform (figure 18) shows two three-proton overlapping doublets (J = 6 cps) at τ 9.12 and 9.06 somewhat similar to those in the spectrum of lucidine-A. Two sharp signals at τ 7.94 and 7.91 are ascribed to the rotomers of the N-acetyl group while the N-methyl singlet appears at τ 7.78.

The mass spectrum (figure 19) shows metastable peaks at *m*/e* 448 and 254 corresponding to the *m/e* 483→465 and *m/e* 289→271 fragmentations respectively. The fragmentation pattern of this compound is similar to those of the dihydrolucidine alkaloids and suggests that a similar hexacyclic carbon skeleton is present in this alkaloid. This necessitates the presence of a double bond in the molecule and this was shown to be a $>$C=N double bond by sodium borohydride reduction.

The more polar component of the mixture has a molecular weight of 481 and has been shown by high resolution mass spectrometry to have the molecular formula $C_{30}H_{47}N_3O_2$. The mass spectrum (figure 20) shows a base peak at *m/e* 287 and prominent peaks at *m/e* 246, 231, 203, 164, 162 and 124. The presence of the peaks at *m/e* 205 and 203 in the less polar and more polar components of Fraction D suggests that they may

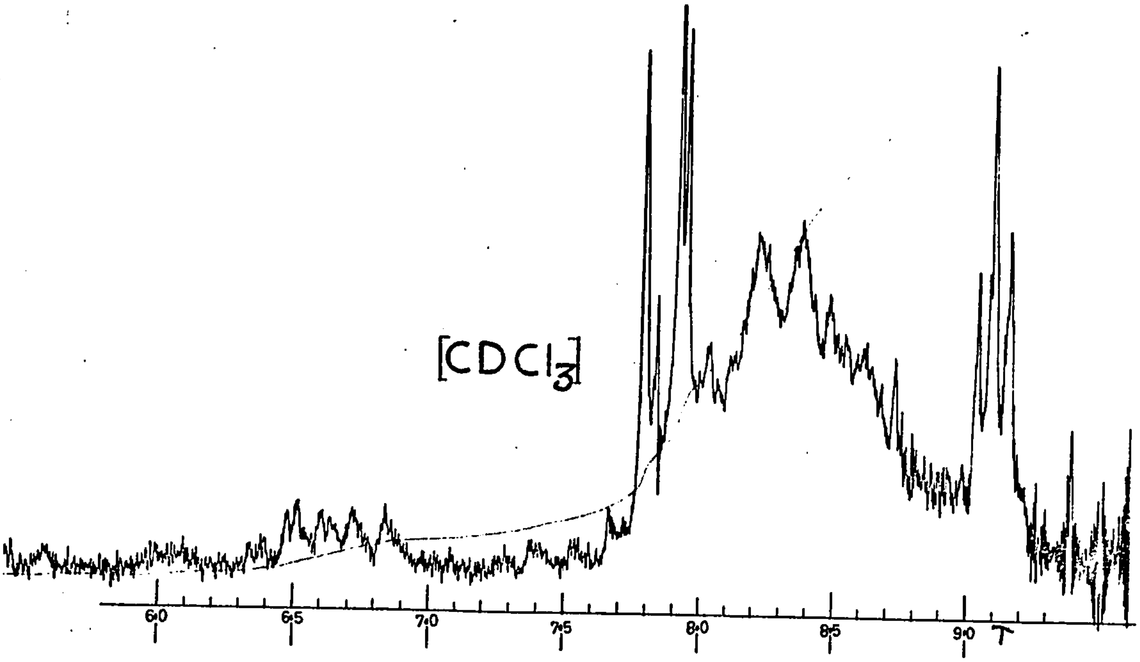


Figure 18

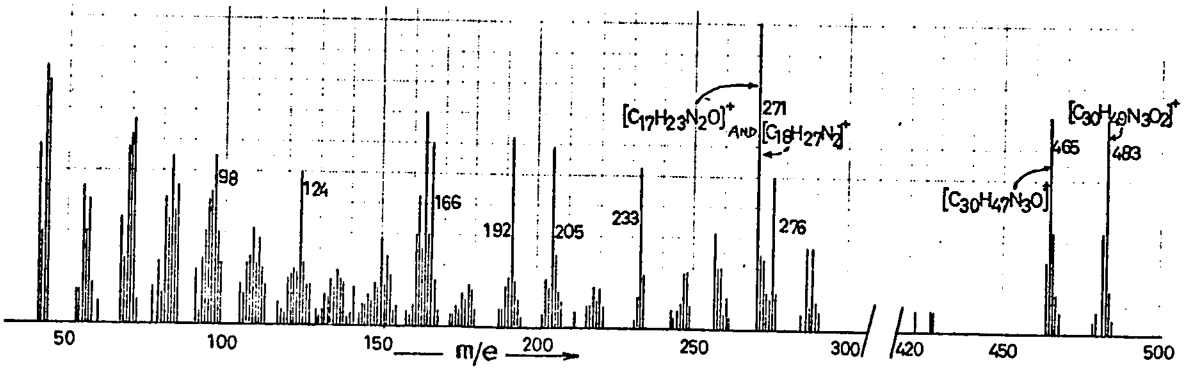


Figure 19

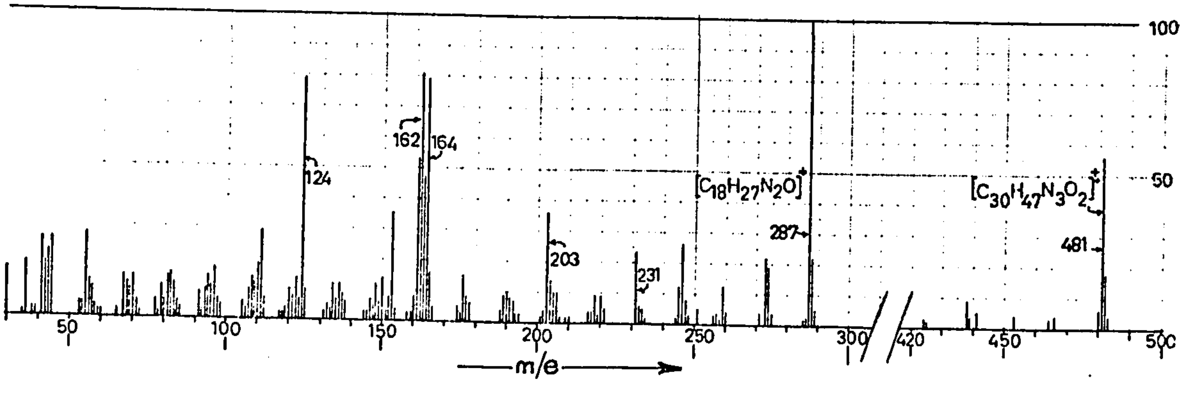


Figure 20

be closely related to the 483-Ketone and its dihydro derivative which show intense peaks at m/e 205 and 207 respectively.

The 481-compound has been further characterized following its isolation from a very recent countercurrent experiment. In chloroform its i.r. spectrum shows bands at 3350 and 2790 cm.$^{-1}$ which are less intense than those of the 483-compound.

The molecular composition of several peaks in the mass spectrum of the 481-compound has been determined.

m/e Found	m/e Calculated	Molecular composition
481.3676	481.3678	$C_{30}H_{47}N_3O_2$
392.2712	392.2702	$C_{25}H_{34}N_3O$
330.2670	330.2671	$C_{21}H_{34}N_2O$
287.2132	287.2129	$C_{18}H_{27}N_2O$

If we consider that the second oxygen function in the 481-compound is attached to the carbon skeleton in such a way that it displaces two hydrogen atoms, then the correlation of the peak at m/e 287 with the base peak of lucidine-A or -B at m/e 273 ($C_{18}H_{29}N_2$) is possible. The base peak of the lucidine-A and -B alkaloids and the 481-compound are all produced from the parent peak via a loss of 194 atomic mass units ($C_{12}H_{20}NO$) which in turn may be related to the ion at m/e 192 ($C_{12}H_{18}NO$) in many of these alkaloids.

The n.m.r. of the 481-compound in deuterochloroform shows a six-proton multiplet at τ9.16 while the peaks of the

N-acetyl group appear at τ 8.04 and 8.02. A singlet at τ 7.80 corresponds to the N-methyl group and broad singlets are also observed at τ 7.48 and 7.63. When the spectrum is determined in perdeuteroacetic acid the N-acetyl signals appear at τ 7.88 and 7.84 while the singlet of the N-methyl group shifts downfield to τ 7.18. The latter spectrum also exhibits a broad singlet at τ 6.25 while the C-methyl region now consists of one broad peak at τ 9.12.

Both the 481- and the 483-compounds exhibit N-methyl absorption at somewhat higher frequency (2790 cm.$^{-1}$) than other weakly basic alkaloids of the plant (2780 cm.$^{-1}$). Similarly, they exhibit the N-methyl singlet (τ 7.80) at slightly lower field (0.05 ppm) in the n.m.r., determined in deuterochloroform, than these other alkaloids. The C-methyl region of the 481-compound, which shows a six-proton multiplet at τ 9.16, i.e., somewhat similar to lucidine-A and lycolucine, is more complex than that of the 483-compound.

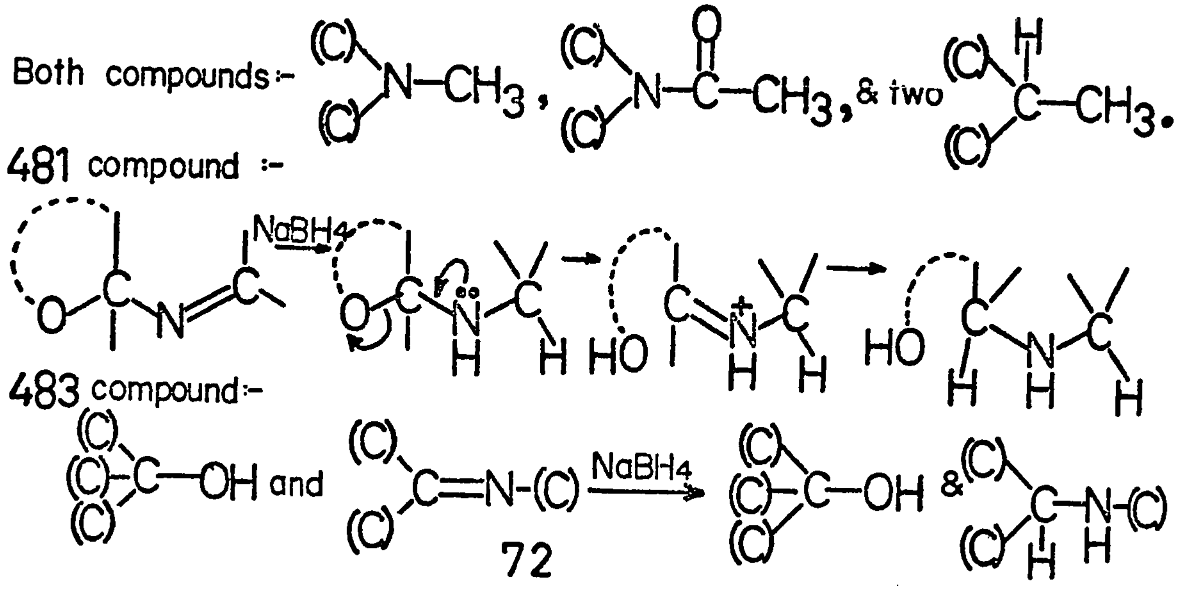
Neither the 483-compound nor the 481-compound yield O-acetyl derivatives on reaction with acetic anhydride at room temperature. The former compound, however, does appear to yield an N-acetyl derivative. The product exhibits an increased amide absorption in the infrared at 1620 cm.$^{-1}$ in chloroform and its mass spectrum reveals a parent peak at *m/e* 525, but exhibits no loss of 60 mass units which is typical of O-acetyl derivatives[117]. This result is difficult to rationalize unless, as is thought to be the case in the lucidine series, acetylation occurs via rearrangement of the imine function[104].

When a mixture of the 481- and 483-compounds was treated with acetic anhydride under more vigorous conditions, a compound of molecular weight 507 was formed. This has been tentatively interpreted as being derived by the loss of water from an initially formed N-acetyl derivative of the 483-compound.

The breakthrough in the characterization of these alkaloids followed the reaction of the mixture with sodium borohydride. Two compounds were formed, one less polar and the other more polar than either of the starting materials. The difference in their polarity is sufficient to allow their easy separation via elution chromatography. It was surprising at first to discover that both reduction products have the molecular formula $C_{30}H_{51}N_3O_2$ (mw 485) and since neither component contains a ketonic or aldehydic carbonyl group, it was particularly difficult to interpret this fact in terms of the 481-compound.

Unlike the 483-compound, the 481-compound does not show an appreciable absorption at 3350 $cm.^{-1}$ in the i.r. or an $M^+ - 18$ peak in its mass spectrum. The latter compound then probably lacks a hydroxyl group. Since spectral evidence rules out the presence of a second carbonyl moiety the 481-compound, if hexacyclic, must contain two double bonds both of which are reducible by sodium borohydride. The presence of one imino function accounts for one unsaturation but the presence of two such functions is improbable. One possibility is that the compound contains a seventh ring, perhaps involving oxygen in an ether, which could account for its behavior towards sodium borohydride. Normally, ethers are inert to sodium borohydride, but a carbinolamine ether could be reduced by this reagent[118, 119].

The following functional groups are tentatively assigned to the 481- and 483-compounds (72).



72

At this stage, since all reduction experiments had been carried out on mixtures of the two starting materials, it was impossible to state which of the latter gave which reduction product. A comparison of the behavior of the starting materials and products towards acetic anhydride was very informative.

Neither of the starting materials gave an O-acetyl derivative on reaction with acetic anhydride at room temperature. One of these, the 483-compound showed a strong $M^+ - 18$ peak in its mass spectrum, inferring that it possessed a hydroxyl group which, because of its inertness to acetylation, is most likely tertiary. This compound would still contain a tertiary hydroxyl group after reduction with sodium borohydride and would still resist O-acetylation. However, the more polar reduction product readily forms an O-acetyl derivative, and it therefore must be derived from the 481-compound.

The reduction products yield very similar i.r., n.m.r. and mass spectra. For the sake of the ensuing discussion they will be termed the less polar (derived from the 483-compound) and the more polar (derived from the 481-compound) reduction products.

Both reduction products exhibit absorption at 3300 (broad), 2790 and 1640 $cm.^{-1}$ in the infrared, determined in carbon tetrachloride. They exhibit well-defined fingerprint regions and the spectrum of the less polar reduction product in particular, is very distinct between 1450 and 800 $cm.^{-1}$ (figure 21).

The reduction products each show a parent peak in their mass spectra at m/e 485, a base peak at m/e 467 and prominent peaks at m/e 277, 260, 259, 235, 192, 163 and 150. A metastable peak at m*/e 450 corresponds to the m/e $485 \rightarrow 467$

fragmentation. The mass spectrum of the less polar reduction product is illustrated in figure 23.

(i) The more polar reduction product.

Treatment of this compound with acetic anhydride and pyridine led to an O-acetyl derivative, characterized by an intense signal at 1735 cm.$^{-1}$ in the i.r. of the product in chloroform.

(ii) The less polar reduction product.

The n.m.r. of this component in deuterochloroform (figure 22) reveals $\text{CH-}\underline{\text{CH}_3}$ doublets at τ9.19 (J = 6 cps) and 8.93 (J = 7 cps) respectively. The latter signal appears to be somewhat further split possibly due to the N-acetyl rotomers. Sharp singlets at τ7.98 and 7.95 are attributed to the N-acetyl group while a singlet at τ7.84 is assigned to the N-methyl group. Several signals accounting for four protons appear in the region τ7.7 to 6.6.

The reaction of this component with acetic anhydride at room temperature yields a product whose mass spectrum reveals that it is a monoacetyl derivative. The i.r. spectrum of the product shows no absorption typical of an O-acetyl group but displays an enhanced amide absorption, which appears as a shoulder to the original amide peak, at 1640 cm.$^{-1}$

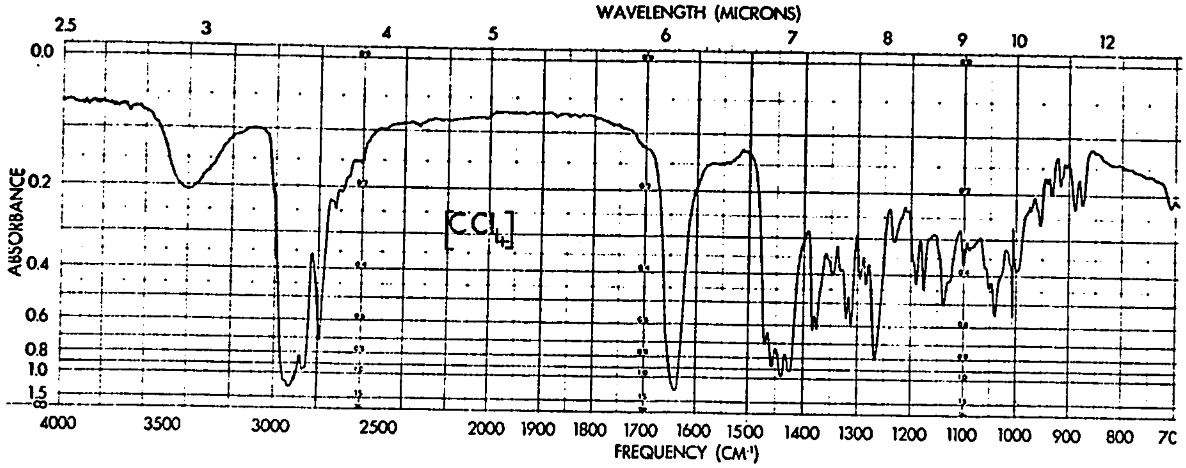


Figure 21

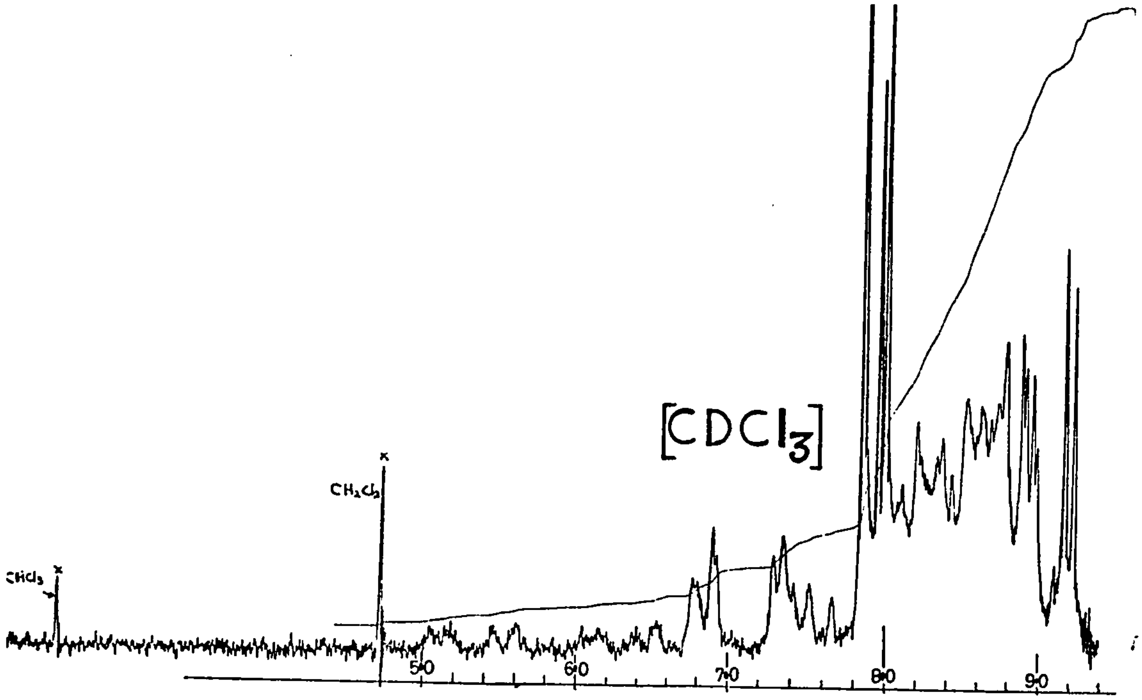


Figure 22

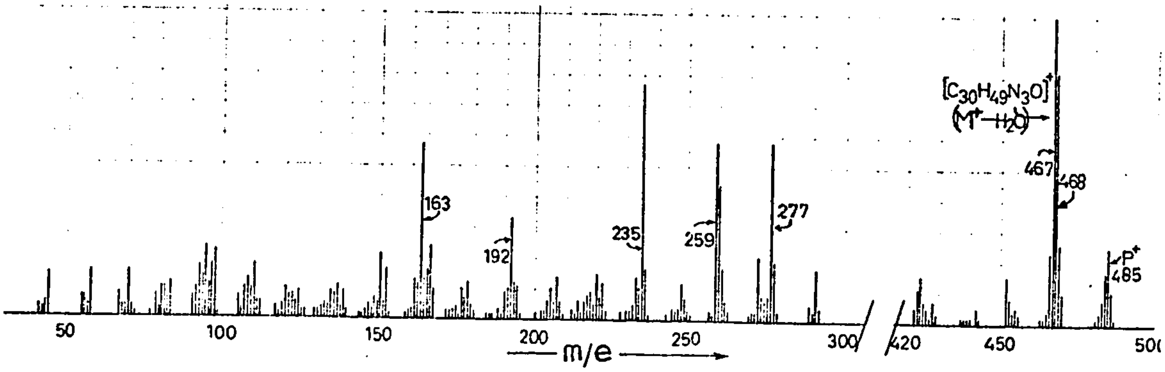


Figure 23

in carbon tetrachloride. In the mass spectrum intense peaks are observed at m/e 527, 509 (base peak), 466, 423, 259, 192 and 43. Metastable peaks at m*/e 492, 427 and 385 correspond to direct m/e 527→509, 509→466, 466→423 fragmentations respectively. The formation of the base peak by loss of 18 mass units (H_2O) infers the presence of an OH group in the product, which in turn is most likely an N-acetyl derivative of the less polar reduction product.

Treatment of this N-acetyl derivative with thionyl chloride in pyridine led to a product of dehydration (mw 509), whose i.r. spectrum lacks the typical band at 3400 $cm.^{-1}$ associated with the hydrogen-bonded hydroxyl group of its precursors. The i.r. in carbon tetrachloride also reveals strong bands at 888 and 872 $cm.^{-1}$. The mass spectrum indicates a parent peak at m/e 509 which is also the base peak and other prominent peaks at m/e 466, 423, 259 and 257. An attempt to purify this compound by molecular distillation in readiness for the determination of its n.m.r. spectrum, led to its decomposition.

The mass spectra of the N-acetyl and N-acetylanhydro derivatives above also show peaks at m/e 301 and 302, corresponding to the addition of 42 mass units (i.e., acetyl derivative) to the peaks at m/e 260 and 259, which are prominent in the spectra of the original reduction products. It is reasonable to presume then that this N-acetylation has taken place on one of the nitrogen atoms contained in the major

($C_{17}N_2$)portion of the molecule. Since in all the described cases so far one nitrogen is methylated and tertiary, it is inferred that the acetylation has occurred on the nitrogen present as the imino function in the 483-compound.

The less polar reduction product yields crystalline derivatives with a variety of reagents. This property has been exploited in the search for a crystalline derivative suitable for x-ray analysis.

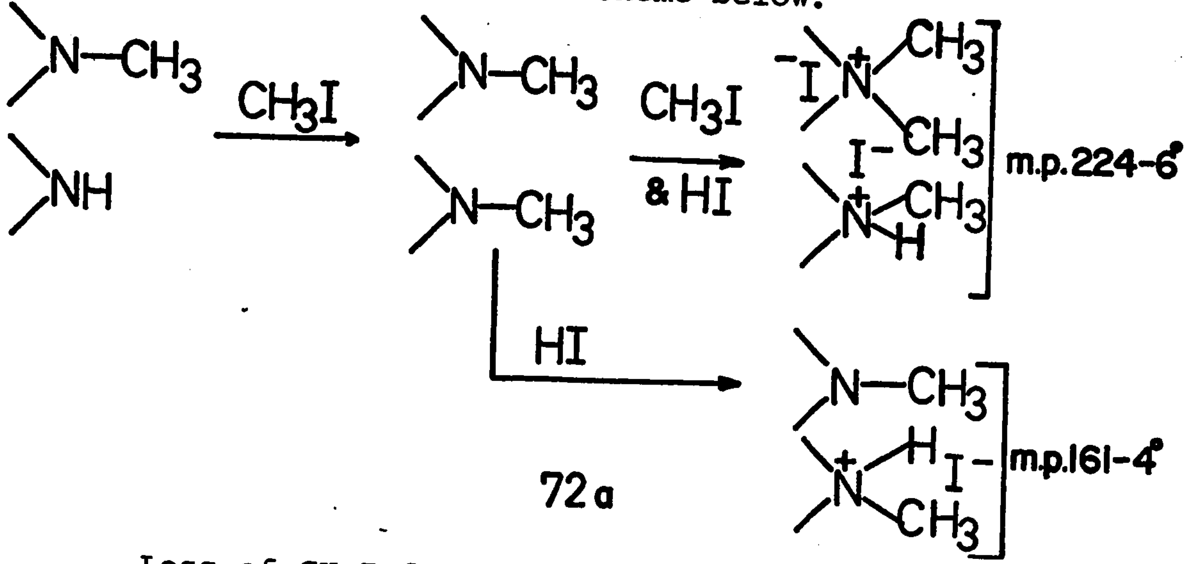
Treatment of the less polar reduction product with hydrogen bromide yielded a white crystalline solid, m.p. 227-230°, and similar treatment with perchloric acid yielded a crystalline hydroperchlorate salt m.p. 256-9°. The i.r. spectra of these derivatives lack the typical N-CH_3 absorption, indicating quaternization. The spectrum of the hydrobromide salt shows peaks at 3320, 3105 cm.$^{-1}$ and several of moderate intensity in the region 1650-1550 cm.$^{-1}$. The hydroperchlorate salt absorbs at 3207, 3160, 1620 and 1583 cm.$^{-1}$.

When the less polar reduction product was treated with iodomethane at room temperature two crystalline products, m.p. 224-6° and 161-4° respectively were isolated, which were separated by fractional crystallization. The i.r. spectrum of the higher melting derivative shows absorption at 3430 (broad), 3150 (broad), 1605 and 1593 cm.$^{-1}$. The highest peaks in the mass spectrum of this derivative are at <u>m/e</u> 500 and 499, but whether these represent the mass of the actual cation is disputable. The spectrum shows a base peak at m/e 481, possibly due to loss of H_2O from an ion at m/e 499 and prominent peaks at m/e 466, 291, 275 and 273. This derivative was so very insoluble in a variety of solvents that its characterization by n.m.r. was impossible.

The lower melting and more soluble derivative shows a very weak band at 2790 $cm.^{-1}$ ($N-CH_3$) in its i.r. and exhibits other absorption bands at 3350 and 1615 $cm.^{-1}$. Its mass spectrum is identical with that of the higher melting component. Metastable peaks at m*/e 463, 256 and 156 infer direct 499→481, 291→273 and 483→273 fragmentations respectively. The n.m.r. spectrum in deuterochloroform reveals C-methyl doublets at τ9.10 ($J = 6$ cps) and 8.93 ($J = 7$ cps). Two sharp singlets are present at τ7.97 and 7.89 while a broad multiplet is observed at τ7.0. When the spectrum is determined in trifluoroacetic acid the C-methyl doublets appear at τ8.96 and 8.80, and a singlet, believed to be due to the N-acetyl group, resonates at τ7.37. Two intense singlets, typical of methyl groups attached to a quaternized nitrogen are observed at τ6.86 and 6.72. The spectra suggest the presence of a second N-methyl function.

Chemical analyses of the two iodomethane derivatives were carried out. Although the results are not sufficiently accurate to unequivocally state a molecular formula for the two derivatives, they indicated that the higher melting derivative contains two iodine atoms, while the lower melting derivative possesses only one.

The analytical data agrees best with the formation of the salts depicted in the scheme below.



Loss of CH_3I from the higher melting derivative would yield a cation at *m/e* 500 while further loss of HI would give rise to a compound of molecular weight 499.

Since better results are obtained from x-ray analysis if the heavy atom is bromine rather than iodine, we set out to form the corresponding bromide salt of the more abundant iodomethane derivative by use of an anion exchange resin. The slow elution of an aqueous solution of the higher melting iodomethane derivative through a bromide loaded resin column, led to the isolation of a crystalline derivative, m.p. 198-203°. This derivative shows absorption at 3400 (broad) and 1600 cm.$^{-1}$ in the i.r. determined in nujol. A chemical analysis indicated the presence of two bromine atoms.

This dibromide derivative of the less polar reduction product has been submitted for x-ray analysis.

Treatment of the lower melting derivative with base followed by extraction into chloroform yields a component whose spectral properties imply it is the N-methyl derivative of the less polar reduction product. In the i.r. determined in chloroform a very intense N-methyl absorption appears at 2780 while the N-acetyl peak appears at 1620 cm.$^{-1}$. The n.m.r. of this component in deuterochloroform (figure 24) shows C-methyl doublets at τ 9.16 (J = 6 cps) and 8.92 (J = 7 cps), while the region τ 8.0 to 7.8 is more complex than observed for the less polar reduction product. Sharp signals appear at τ 7.96, 7.92, 7.91, 7.89 and 7.81 due to N-acetyl and possibly two N-CH_3 groups. When determined in perdeuteroacetic acid (figure 25), signals appear at τ 7.88 and 7.86 attributable to the N-acetyl group, while intense signals appear at τ 6.97 and 6.90, typical of methyl groups attached to a protonated nitrogen atom.

That this component is a monomethylated derivative of the less polar reduction product (i.e., $>NH \rightarrow >N-CH_3$) is confirmed by the presence in the mass spectrum (figure 26) of a parent peak at m/e 499. The spectrum also shows a base peak at m/e 291, prominent peaks at m/e 481, 174 and 273, and metastable peaks at m*/e 463, 452 and 256, which correspond to the m/e 499 → 481, 481 → 466 and 291 → 273 fragmentations.

A comparison of the mass spectra of the two most polar components of Fraction D and their reduction products and

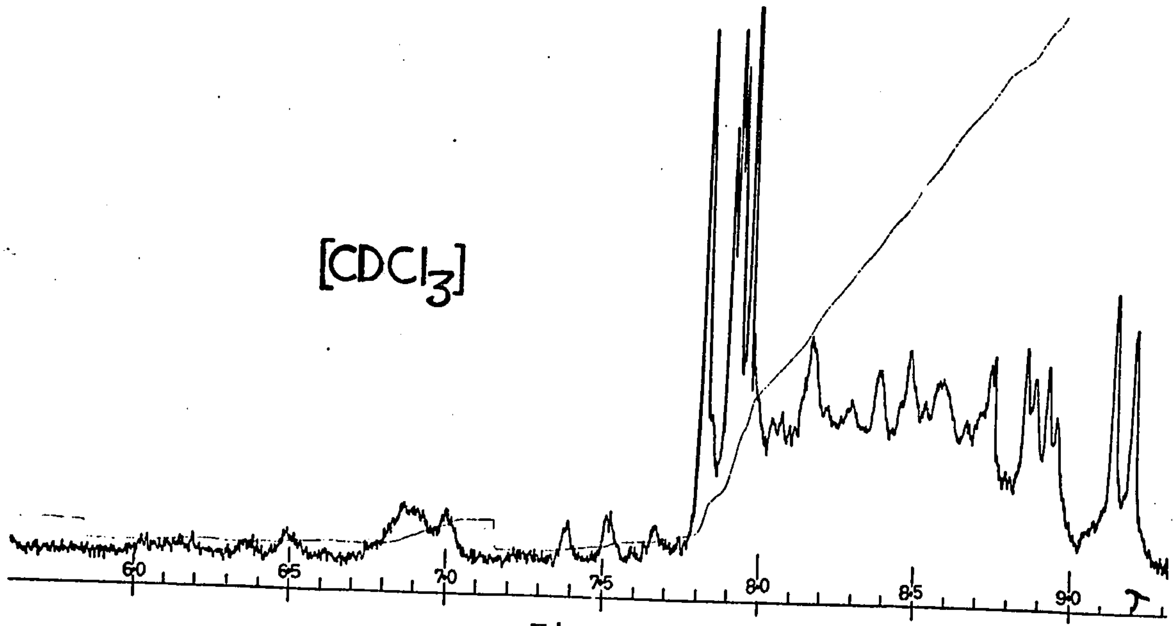


Figure 24

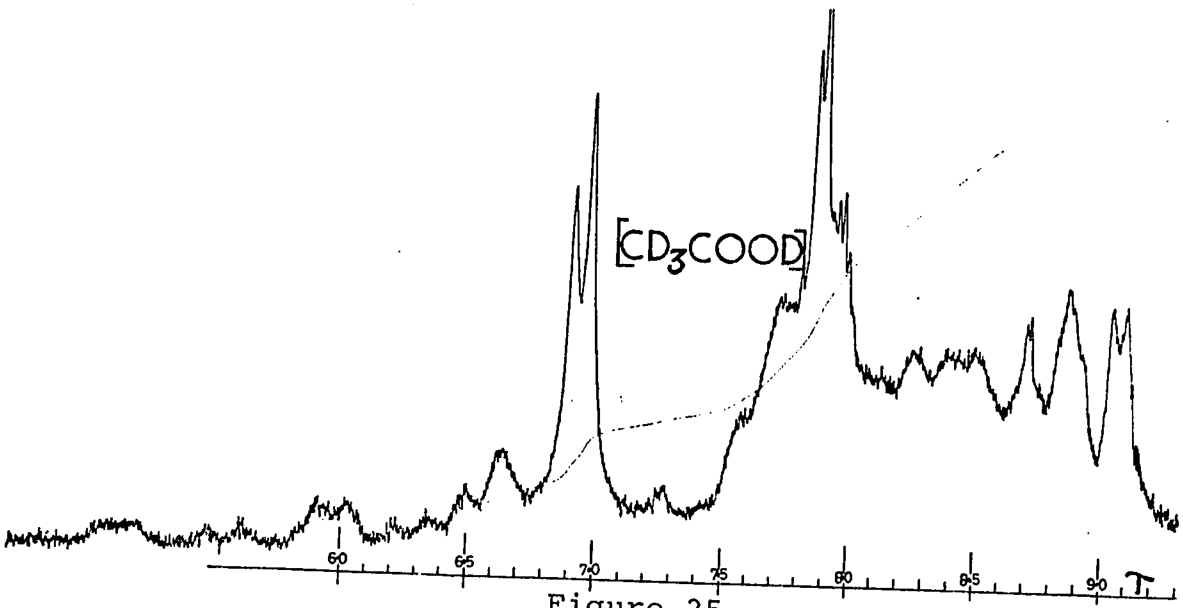


Figure 25

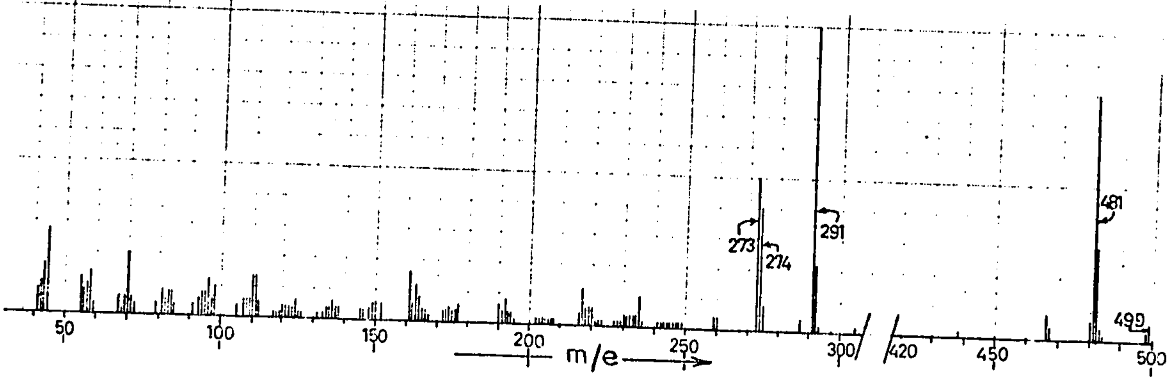
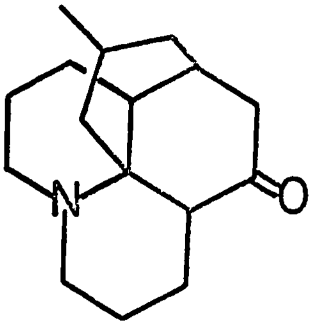


Figure 26

derivatives, suggests that the imino and second oxygen functions present in the original Fraction D components are both incorporated in the major ($C_{17}N_2$) portion of the molecules.

The Alkaloids of Fraction E

This fraction, made up of tubes 86-100 from the countercurrent experiment, consists of two major and two minor components. The characterization of the four components followed purification by dry-column chromatography. The less polar and more polar of the major components were found to be identical in all respects with lycopodine (2) and lycodine (12) respectively.



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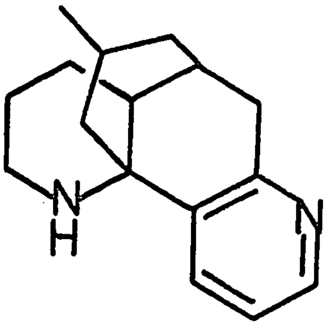

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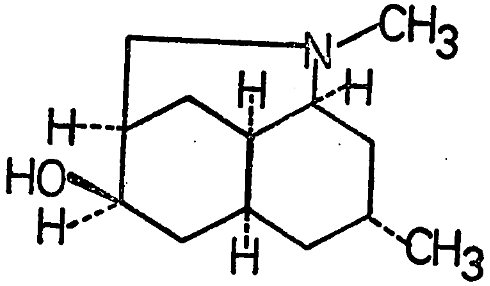
The more polar minor component shows intense absorption at 3580 and 2780 $cm.^{-1}$ in the i.r. determined in chloroform. Mass spectrometry showed it to have molecular formula $C_{13}H_{23}NO$, corresponding to a dihydroluciduline. The compound was shown to be identical in all respects with an authentic sample of dihydroluciduline (73) prepared from luciduline (33) by reduction with sodium borohydride[19].

The less polar minor component was isolated in trace amounts following a ten-funnel countercurrent experiment on Fraction E. Its i.r. spectrum in carbon tetrachloride showed an intense carbonyl peak at 1700 $cm.^{-1}$. Its identity was established after it was transformed into the hydrochloride salt which was identical in all respects with an authentic sample of the hydrochloride salt of anhydrolycodoline [33] (50).



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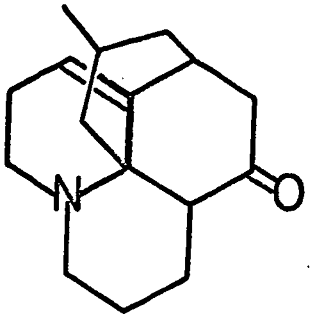


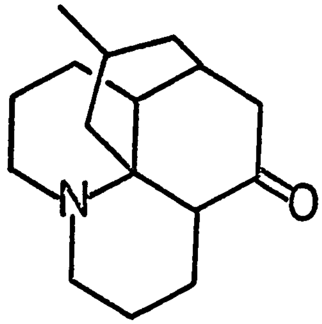
50

None of the C_{30} alkaloids were found in this fraction.

Dehydrogenation Studies

Dehydrogenation studies have contributed to the structure elucidation of many alkaloids from a wide variety of plant species. The isolation of quinoline derivatives from the dehydrogenation of various Lycopodium alkaloids has inferred the presence of a reduced quinoline type of carbon skeleton in these alkaloids.

The structure elucidation[17] of lycopodine (2) was aided by the isolation of 7-methylquinoline (74) and 5,7-dimethylquinoline (75) from the products of its dehydrogenation with selenium or palladium[22].



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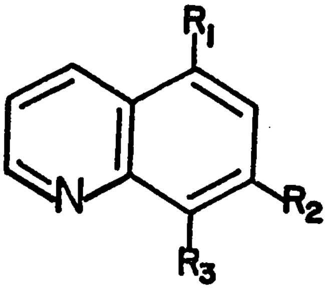
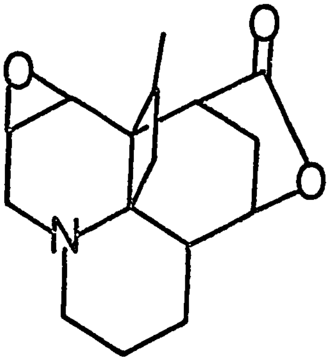


74. $R_1 = R_3 = H, R_2 = CH_3$
75. $R_1 = R_2 = CH_3, R_3 = H$
76. $R_1 = R_2 = H, \; R_3 = n\text{-}C_3H_7$

Likewise, the isolation of 8-n-propylquinoline[120] (76) and the quinolone (78) from the dehydrogenation of annotine (1) and one of its derivatives (77) respectively[121,122], played a large part in the structural solution[15] of the alkaloid.



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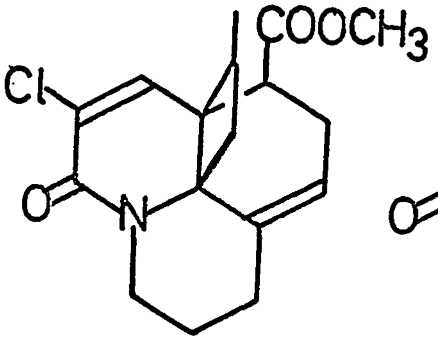

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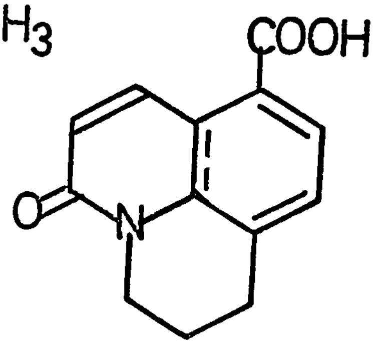
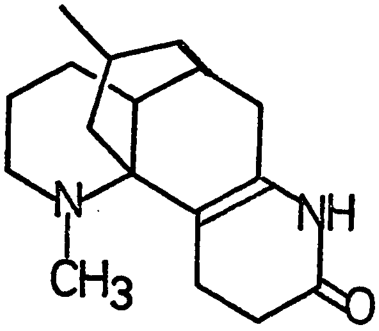


78

A most striking example of the utility of dehydrogenation involves the early investigations of the obscurine alkaloids. The foundation for the subsequent structure elucidation[36,37] of α-obscurine (13) and related alkaloids was based upon the isolation of 7-methylquinoline (74) and 6-methyl-2-pyridone (79) from the dehydrogenation of α-obscurine[123]. These two account for all of the carbon and nitrogen atoms in the skeleton of the alkaloid.



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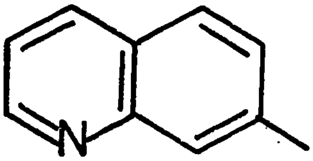

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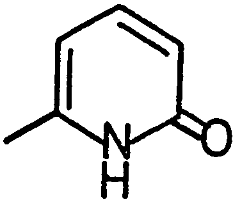
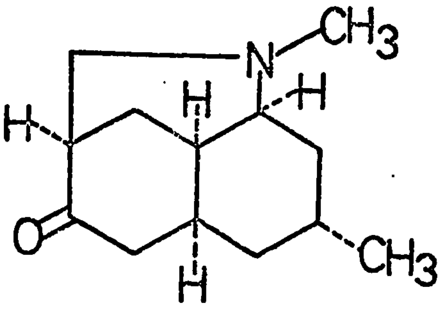
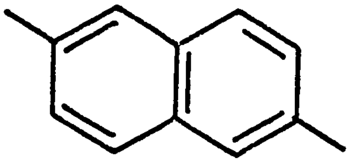


79

The dehydrogenation experiments carried out on the weakly basic alkaloids of Lycopodium lucidulum have utilized both selenium and zinc dust. Luciduline (33), which represents the only mononitrogenous weakly basic alkaloid, was studied by Nkunika. Dehydrogenation with selenium produced one major and several minor components detectable via t.l.c. or by g.l.p.c. Mass spectrometry revealed that the major component had a molecular weight of 156 and composition $C_{12}H_{12}$, and it was identified as 2,6-dimethylnaphthalene (80).



33



80

Initial studies by Nkunika[124] also showed that when the other weakly basic alkaloids were subjected to dehydrogenation with selenium, a complex mixture of products was obtained. Gas liquid phase chromatographic studies indicated that this mixture was the same irrespective of whether lucidine-A, lucidine-B, lycolucine, or a mixture of the crude 1620 compounds was used as the substrate. The dehydrogenation products were separated initially into basic, acidic and neutral fractions and the basic fraction was further subdivided into strongly and weakly basic fractions in the following manner.

A solution of the basic fraction in methylene chloride was repeatedly extracted with dilute hydrochloric acid (10%). Removal of the solvent from the methylene chloride layer yielded the weakly basic fraction while basification of the aqueous acid layer, followed by extraction with chloroform, afforded the strongly basic fraction.

From the strongly basic fraction Nkunika isolated and identified 7-methylquinoline, a 7-methyldecahydroquinoline, a methyl-5,6,7,8-tetrahydroquinoline which, according to its mass spectrum, must have the methyl group in either the 6 or 7 position (presumably the 7 position), and a dimethylquinoline.

We have repeated the dehydrogenation using both selenium and zinc dust[125] and have found that the change of dehydrogenating agent does not appreciably alter the nature of the basic fraction. G.l.p.c. of the strongly basic fraction enabled us to isolate, though in very small quantities, four components A to D. These components were characterized according to their behavior in the ultraviolet[106,110] and mass spectra, and their molecular formulae were determined by high resolution mass spectrometry.

Component A, $C_{10}H_9N$ shows absorption maxima at 220, 273, 281, 305 and 318 mμ in the u.v. determined in methanol, while the mass spectrum (figure 27) shows a parent peak at m/e 143 (base peak) and other prominent peaks at m/e 142, 119 and 115. The spectra suggest that A is either a 6- or 7-methylquinoline[126].

Component B, $C_{10}H_{13}N$, the major component, exhibits absorption maxima in the u.v. at 210, 268 and 276 mμ (methanol) and its mass spectrum (figure 28) shows a parent peak at m/e 147 and prominent peaks at 132, 118, 117 and 105.

The ultraviolet spectrum of component C, $C_{11}H_{11}N$, suggests it is a dimethylquinoline rather than an ethylquinoline. In methanol the spectrum shows absorption maxima at 220, 276, 309 and 322 mμ. The mass spectrum (figure 29) shows a parent (and base) peak at m/e 157 and prominent peaks at m/e 156 and 142.

Combined gas liquid phase chromatography-mass spectrometry allowed the detection of component D, whose mass spectrum (figure 30) shows a parent peak at m/e 161, which is also the base peak, and other prominent peaks at

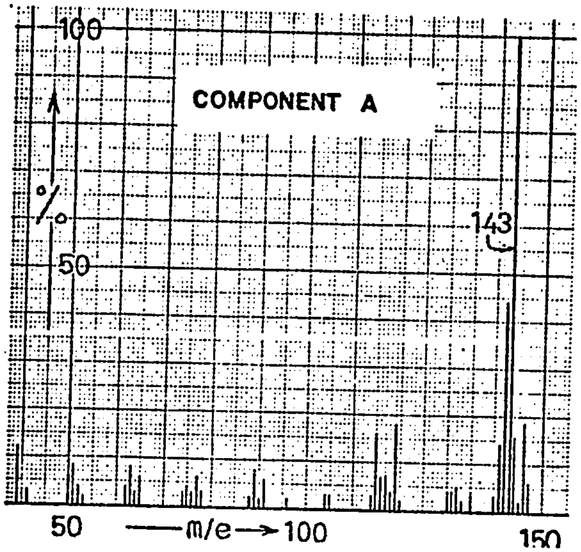


Figure 27

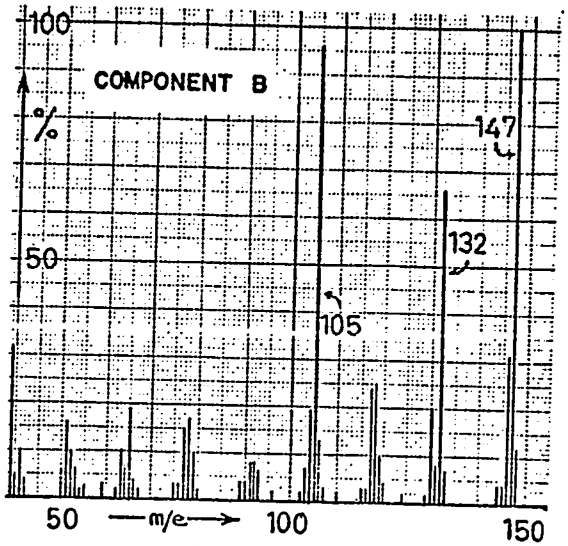


Figure 28

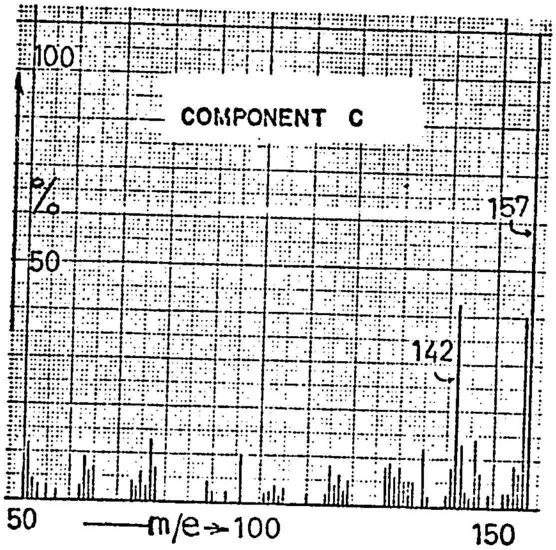


Figure 29

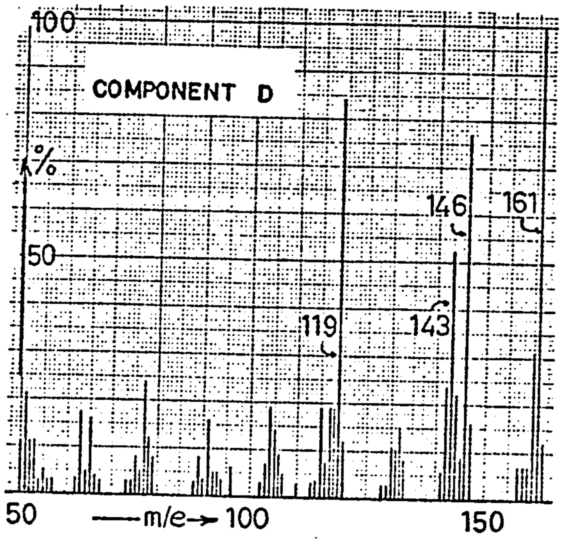
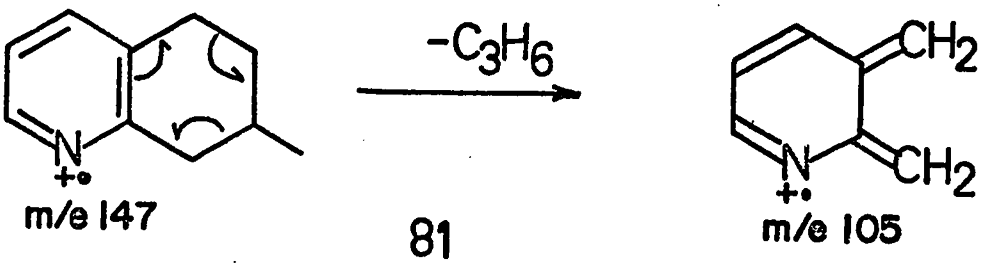


Figure 30

m/e 146, 143 and 119. The peak at m/e 143 was attributed to the presence of a methylquinoline. The mass spectra of both component B and component D show a prominent peak at $M^{+}-42$ (i.e., at m/e 105 and 119 respectively), which can be due to the loss of C_3H_6 as a result of a reverse Diels Alder reaction of a 5,6,7,8-tetrahydroquinoline which contains a methyl group at either the 6 or 7 position[127], as illustrated below (81).



81

The information from the ultraviolet and mass spectra therefore suggests that component B is a 6- or 7-methyl-5,6,7,8-tetrahydroquinoline, and also that one of the methyl groups of component D, believed to be a dimethyl-5,6,7,8-tetrahydroquinoline, is at either the 6 or 7 position.

Although a methyldecahydroquinoline was not detected in our experiments, the spectral characteristics of components A, B and C parallel the results previously obtained by Nkunika. It is reasonable to consider that the dimethylquinoline (component C) might be related to the dimethyl-5,6,7,8-tetrahydroquinoline isolated as component D. Component C then possibly contains a methyl group at either the 6 or 7 position. When Nkunika isolated a dimethylquinoline he considered that it might be 5,7-dimethylquinoline, a known dehydrogenation product of lycopodine[22]. However, comparison of the n.m.r. spectrum of the isolated dimethylquinoline in deuterochloroform, which shows methyl singlets at τ7.46 and

and 7.52, with the n.m.r. of an authentic sample of 5,7-dimethylquinoline showed that this was not the case.

From the weakly basic fraction Nkunika isolated two pyridine derivatives, named GC-17 and GC-18 on account of their location on g.l.p.c., for which he was unable to deduce structures. These compounds contain seventeen carbon atoms and it is possible that they are related to the ion at m/e 261, ($C_{17}H_{29}N_2$) , found in the mass spectra of several of the weakly basic alkaloids of L. lucidulum. Since GC-17 and GC-18 therefore may represent the "non-7-methylquinoline" portion of the original alkaloids, we had hoped to study these compounds further. However, we were only able to isolate sufficient amounts of these compounds for the determination of their ultraviolet and mass spectra.

The summary of Nkunika's evidence, together with our current interpretations of all the evidence available, is outlined below.

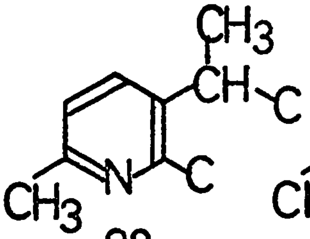
In carbon tetrachloride each compound shows infrared absorption at 3040, 1580 and 1565 $cm.^{-1}$, indicative of an aromatic system. The u.v. spectra of GC-17 and GC-18 suggest that they both possess a 2,3,6-trialkylpyridine skeleton[110] and show absorption maxima in methanol at 221 mμ (ε = 4,700), 272 mμ (ε = 5,720) and 278 mμ (shoulder, ε= 5,090). The spectra closely resemble that of the alkaloid dihydrolycolucine and it is possible that these dehydrogenation products represent a major portion of this alkaloid.

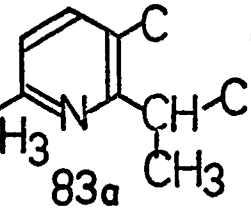
Upon electron impact (figure 31) the compounds exhibit a parent peak at m/e 243, a base peak at m/e 135 and other prominent peaks at m/e 187 and 146. A metastable peak at m*/e 75.0 indicates that the m/e 243→135 fragmentation is direct. The molecular composition of the three most prominent peaks was determined by high resolution mass spectrometry.

m/e Found	m/e Calculated	Molecular composition
243.1989	243.1987	$C_{17}H_{25}N$
187.1358	187.1361	$C_{13}H_{17}N$
135.1046	135.1048	$C_9H_{13}N$

The n.m.r. of GC-17 in deuterochloroform (figure 32) shows a C-methyl doublet at τ9.11 (J = 6 cps), an intense doublet at τ8.70 (J = 8 cps) and a strong singlet at τ7.53 ascribed to a methyl group attached to an α-position of the pyridine ring[128]. The aromatic region shows an AB quartet (J_{AB} = 8 cps) whose sectors are centered at τ3.16 and 2.76 respectively. The chemical shift and coupling constant associated with this quartet suggest that it is due to the β and γ protons[108] of the pyridine ring, which is consistent with the notion that the molecule is a 2,3,6-trialkylpyridine. A comparison of the integration factor of the singlet methyl at τ7.53 with that of the doublet at τ8.70 indicates that the latter is due to one secondary methyl group rather than an isopropyl group, while its chemical shift suggests that it is situated on a carbon atom which is itself attached to the pyridine ring.

From the foregoing evidence the following partial structures can be tentatively proposed for GC-17.


82a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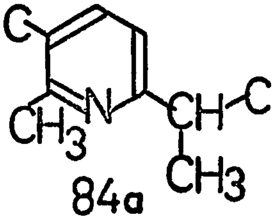
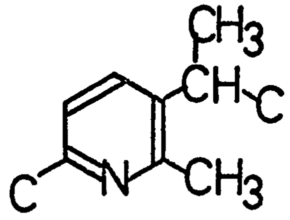
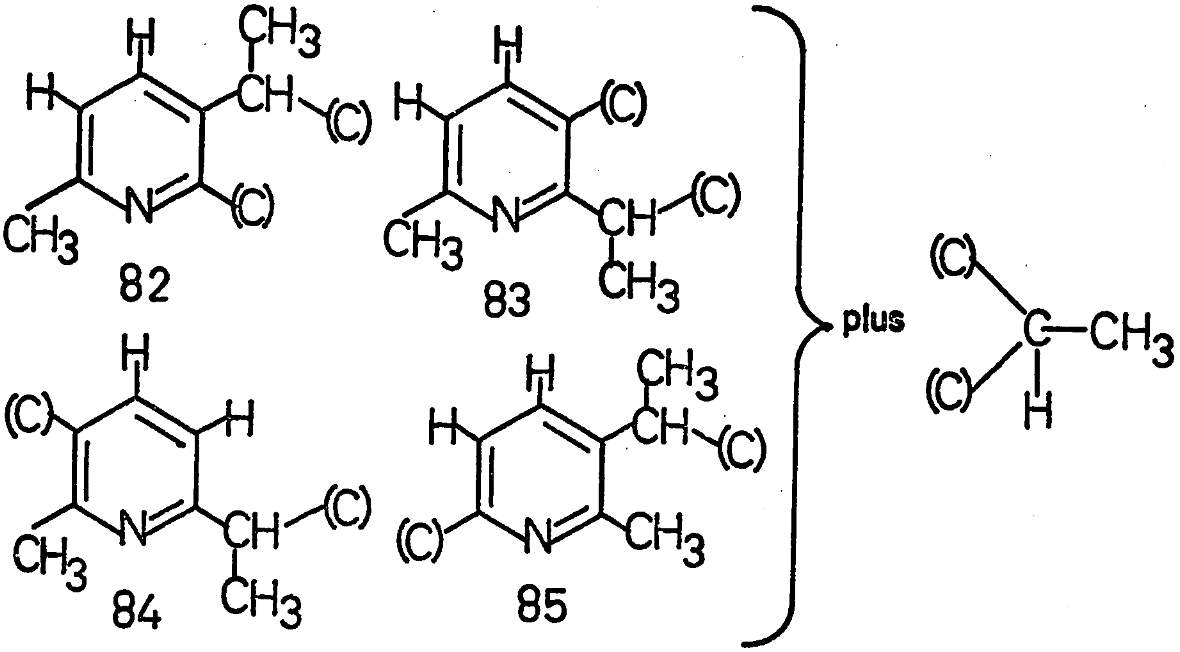

84a


85a

The absorption pattern of GC-18 in the n.m.r. is very similar to that of GC-17. In deuterochloroform (figure 33) the spectrum of GC-18 shows doublets at τ9.16 (J = 6 cps) and 8.74 (J = 7 cps), a singlet at τ7.56 and an AB quartet (J_{AB} = 8 cps) whose sectors are centered at τ3.11 and 2.69 respectively. When the spectrum is determined in perdeuteroacetic acid it shows a broad singlet at τ9.07, a doublet at τ8.59, a singlet at τ7.32, while the sectors of the AB quartet are now centered at τ2.47 and 1.92.

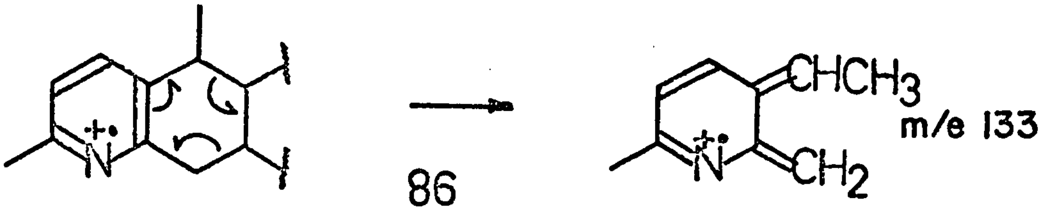
The i.r., u.v., n.m.r. and mass spectral evidence presented so far suggests that GC-17 and GC-18 may be stereoisomers and that they have one of the partial structures depicted overleaf.

The molecular formula $C_{17}H_{25}N$ indicates that GC-17 and GC-18 contain six sites of unsaturation. The pyridine moiety accounts for four of these and since spectral evidence does not suggest the presence of other double bonds, and as GC-18 is



82 83 84 85

recovered unchanged following catalytic hydrogenation over[129] Adams' catalyst at atmospheric pressure for two days, the compounds are therefore tricyclic. The mass spectra of these compounds were difficult to interpret. For some time we were unable to account for the formation of the most abundant fragment, m/e 135 ($C_9H_{13}N$) from the molecular ion, m/e 243 ($C_{17}H_{25}N$). It was first considered that the necessary loss of a neutral C_8H_{12} fragment could arise via a reverse Diels Alder reaction upon a possible 5,6,7,8-tetrahydroquinoline moiety in GC-17 and GC-18. However, an examination of this possibility reveals that the base peak (m/e 135) cannot be formed in this manner unless a subsequent transfer of two hydrogen atoms takes place, as shown in scheme 86.



86

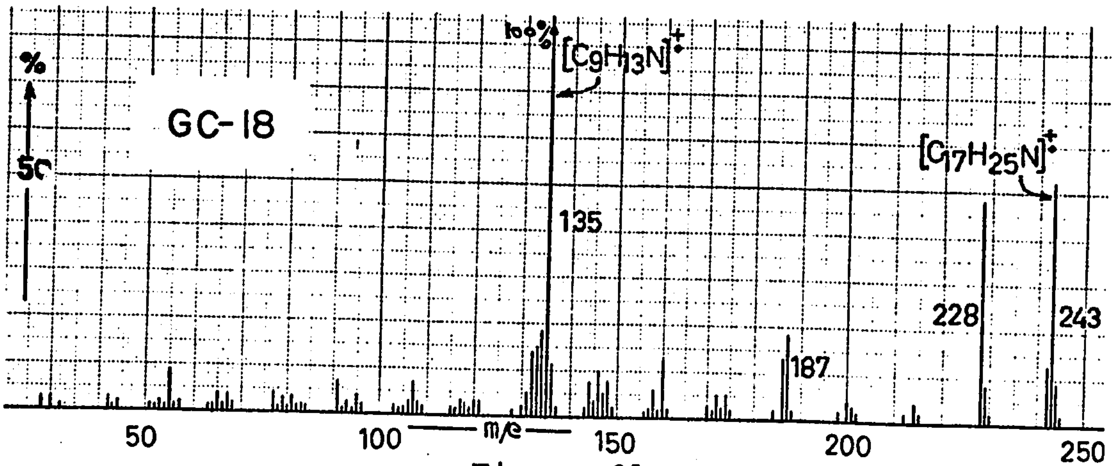


Figure 31

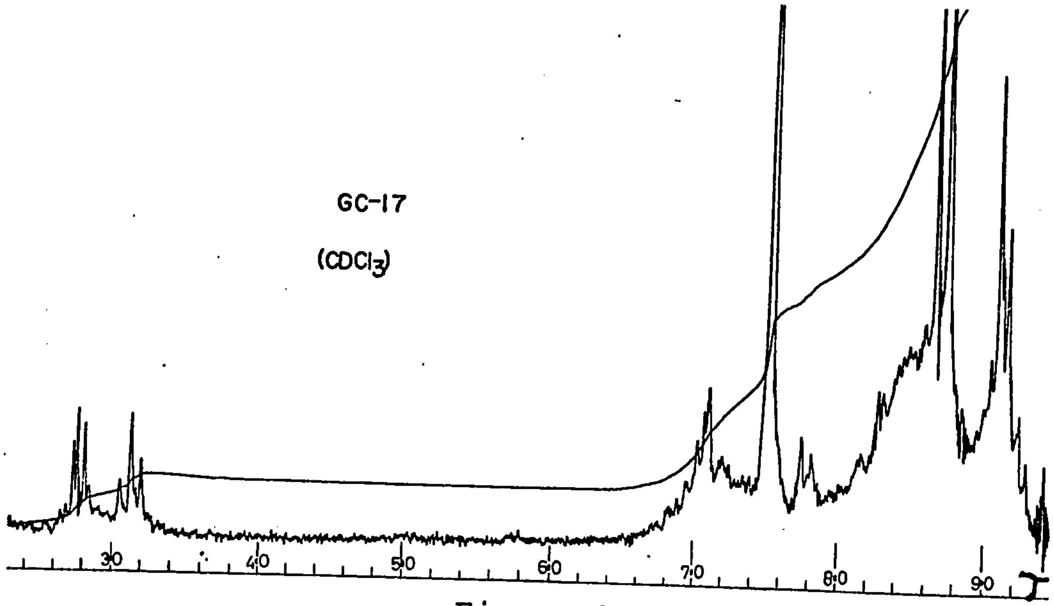


Figure 32

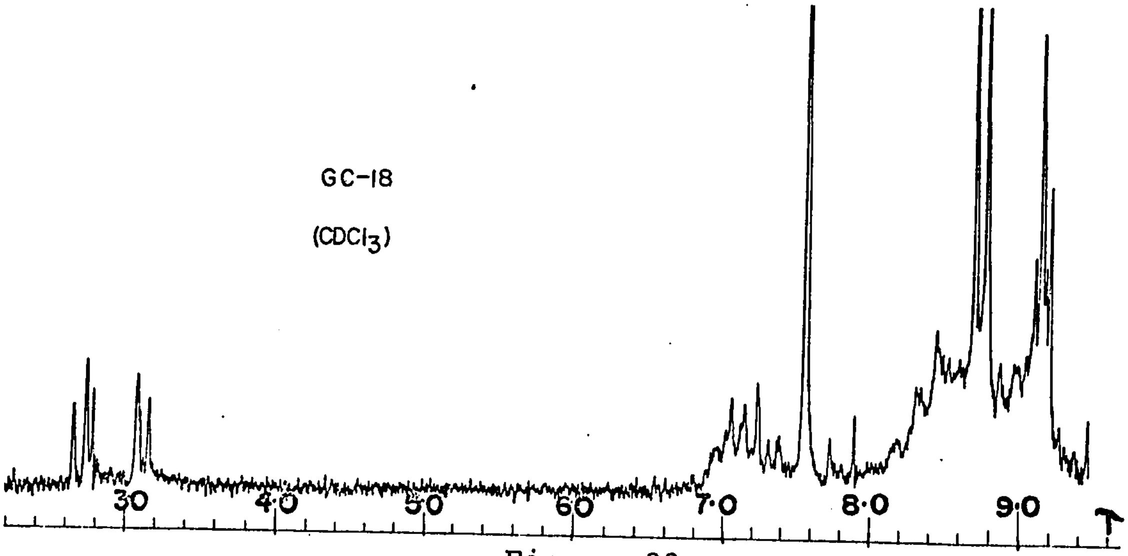


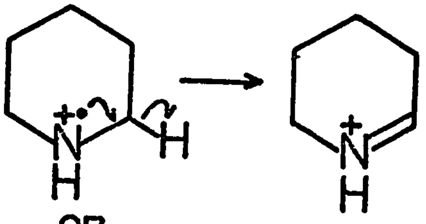
Figure 33

Since mass spectrometry also indicates that the formation of the base peak from the molecular ion is a direct process, the foregoing type of fragmentation has been discounted.

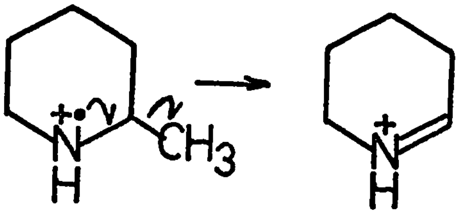
Nkunika found that when GC-18 was treated with a large excess of sodium in ethanol it formed a hexahydro derivative which in turn could be transformed into the corresponding N-acetylhexahydro derivative by reaction with acetic anhydride in pyridine. The mass spectrum of the hexahydro derivative shows no peak at m/e 249 corresponding to the expected parent peak but shows a base peak at m/e 124. However, the spectrum of the N-acetylhexahydro derivative does show a parent peak at the anticipated value (m/e 291), a base peak at m/e 166 and a prominent peak at m/e 124. Metastable peaks which are observed at m*/e 94.6 and 92.8 indicate respectively that the m/e 291→166 and m/e 166→124 fragmentations are direct. The molecular composition of the following ions was determined mass spectrometrically.

m/e Found	m/e Calculated	Molecular composition
291.2560	291.2562	$C_{19}H_{33}NO$
166.1229	166.1232	$C_{10}H_{16}NO$
124.1124	124.1126	$C_8H_{14}N$

The formation of these ions can be explained by analogy with the mass spectral behavior of piperidines[130]. The most abundant ion in the mass spectrum of both piperidine (87) and 2-methylpiperidine (88) is formed by α-cleavage. The fragmentation of the former compound involves cleavage of an α-C-H bond while the latter compound undergoes cleavage of the α-C-CH_3 bond as shown below.

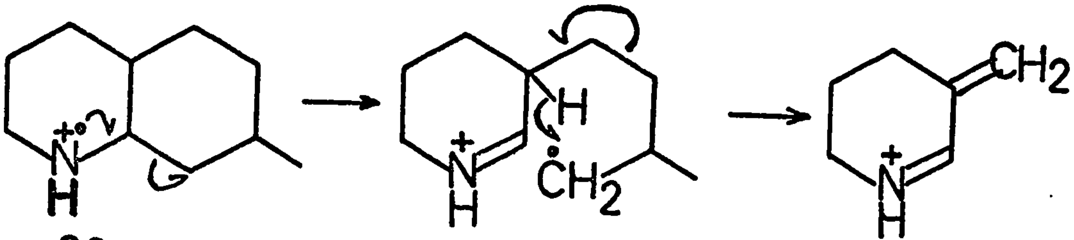


87



88

Similarly, the most abundant ions in the mass spectra of decahydroquinolines arise from an ion formed by an initial α-cleavage, e.g., for 7-methyldecahydroquinoline (89).

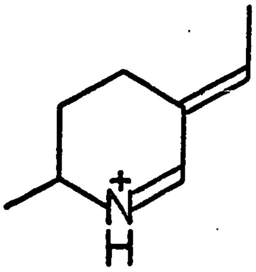


89

m/e 153

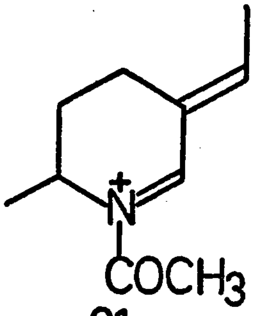
m/e 96

In accordance with this concept the ion at m/e 124 in the mass spectra of hexahydro-GC-18 and N-acetylhexahydro-GC-18 is possibly a simple dimethyl homologue of the ion above at m/e 96, as depicted in structure 90. Also the ion at m/e 166 derived from N-acetylhexahydro-GC-18, can be regarded as the corresponding N-acetyl derivative (91) of the ion at m/e 124.



90

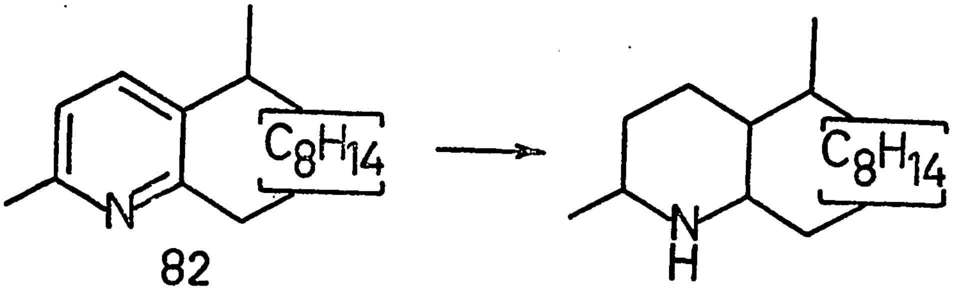
m/e 124



91

m/e 166

If this type of fragmentation process is occurring in the hexahydro derivatives of GC-18 and the initial step involves only cleavage of an α-carbon-carbon bond, then of the four possible partial structures which were postulated for GC-18 (82-85), partial structure 82 becomes the most favored. Accordingly, the hexahydro GC-18 can be represented by partial structure 92.

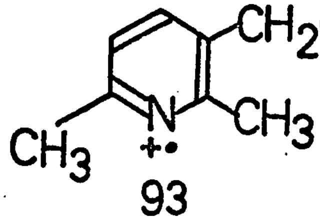


82

92

Although the interpretation of the foregoing data has not led to the structure elucidation of GC-17 and GC-18, some concept of their fundamental carbon skeleton has been obtained. The information available has formed the basis of frequent attempts to derive structures which satisfy the spectral properties.

If it is assumed that the 2,3,6-trisubstituted pyridine skeleton remains intact in the base peak ion at m/e 135 ($C_9H_{13}N$), then the following three structures represent some of the possible structures for this ion.



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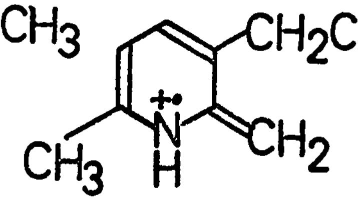

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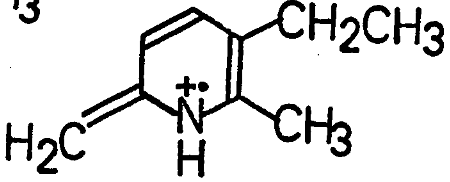


95

Earlier in this discussion it was shown that the weakly basic alkaloids of L. lucidulum (apart from luciduline) fragment into two portions upon electron impact. The larger fragment, usually either a $C_{17}N_2$ or a $C_{18}N_2$ unit is probably derived from the part of the alkaloids that give rise to the GC-17 and GC-18 dehydrogenation products, whereas the smaller fragment, a $C_{10}N$ or a $C_{12}N$ unit most likely originates from that portion of the alkaloid which yields the methylquinolines upon dehydrogenation.

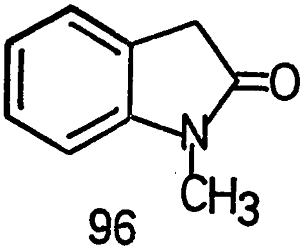
Support for this idea oomes from the fact that Nkunika was able to isolate both a 7-methyldecahydroquinoline and a 7-methyl-5,6,7,8-tetrahydroquinoline from the selenium dehydrogenation of lycolucine which itself is believed to possess a 2,3,6-vinyldialkylpyridine system.

From the neutral portion of the selenium dehydrogenation products Nkunika isolated and identified biphenyl. We have not examined the neutral fraction from the selenium dehydrogenation but have isolated from the neutral portion of the zinc dust distillation products a semi-crystalline compound. The mass spectrum (figure 34) of this compound shows a parent peak (also the base peak) at m/e 147 and other prominent peaks at m/e 146, 119, 118 and 91. Although the fragmentation pattern is somewhat similar to that of 7-methyl-5,6,7,8-tetrahydroquinoline, high resolution mass spectrometry has established that the compound has a molecular formula C_9H_9NO, and has identified the molecular composition of several of the prominent peaks as follows :

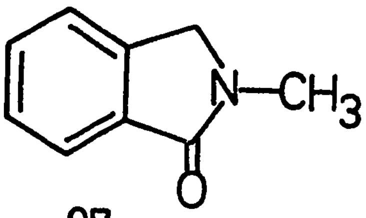
m/e Found	m/e Calculated	Molecular composition
147.0682	147.0684	C_9H_9NO
119.0497	119.0497	C_8H_7O
118.0654	118.0657	C_8H_8N
91.0543	91.0547	C_7H_7

The i.r. spectrum of the compound in carbon tetrachloride exhibits absorption at 3040, 1700 (strong), 1620 (weak), 1483, 1396, 1277 and 1050 cm.$^{-1}$. When the u.v. spectrum is determined in methanol, absorption maxima are observed at 219 mμ (ε = 11,400), 226 mμ (ε = 11,300), 240 mμ (shoulder, ε = 7,500), 266 mμ (shoulder, ε = 2,400) and 277 mμ (ε = 1760), which show no change on acidification. The n.m.r. spectrum in deuterochloroform (figure 35) shows absorption at τ 6.89 (3H, singlet), τ 5.72 (2H, singlet), τ 2.59 (3H, 3 singlets or triplet), and a multiplet at τ 2.21 (1H).

The preliminary interpretation of the foregoing spectra led us to suspect the compound to be either N-methyloxindole (96), or N-methylphthalimidine (97).



96



97

The three-proton singlet in the n.m.r. at 6.89, ascribed to an N-methyl group is consistent with either structure. The two-proton singlet at τ 5.72 attributed to the methylene group at C-3 is at considerably lower field than the value (τ 6.6) usually associated with compounds possessing an oxindole nucleus[131]. However, it is consistent with the chemical shift expected for the methylene protons of N-methylphthalimidine.

We have recently found that in carbon tetrachloride the i.r. spectrum of the isolated compound is identical with that of an authentic sample of N-methylphthalimidine[132] (figure 36).

That N-methylphthalimidine is an actual dehydrogenation product of the weakly basic alkaloids of L. lucidulum is questionable. Rather we suspect that it is an artifact which originates from the phthalic esters that are present as

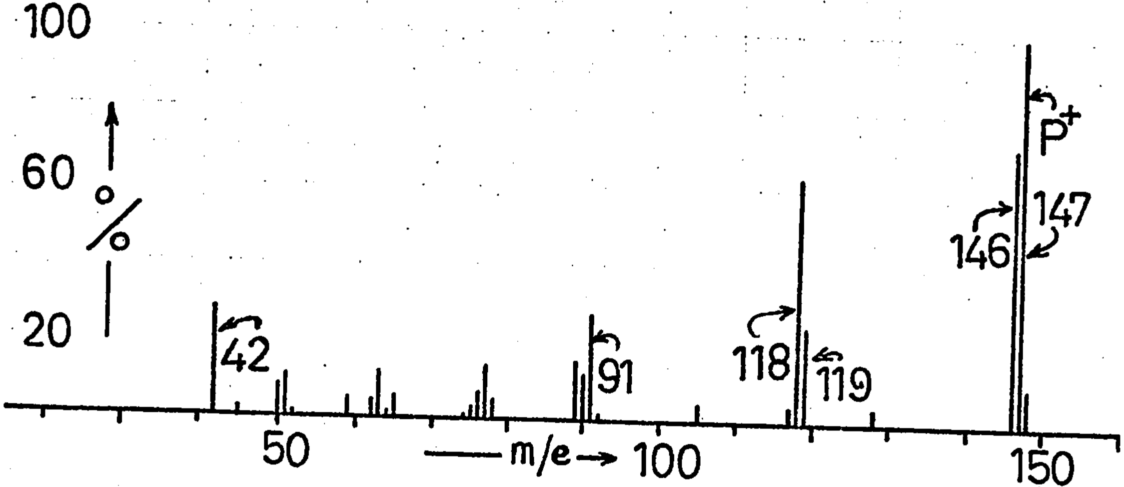


Figure 34

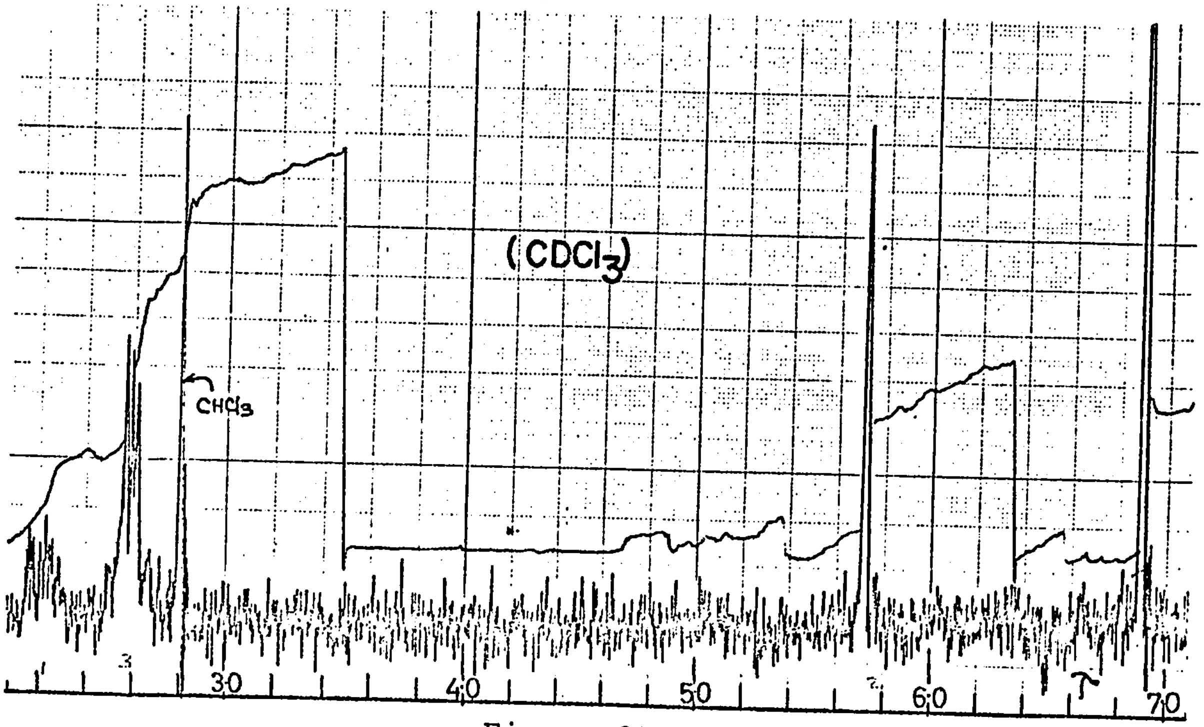


Figure 35

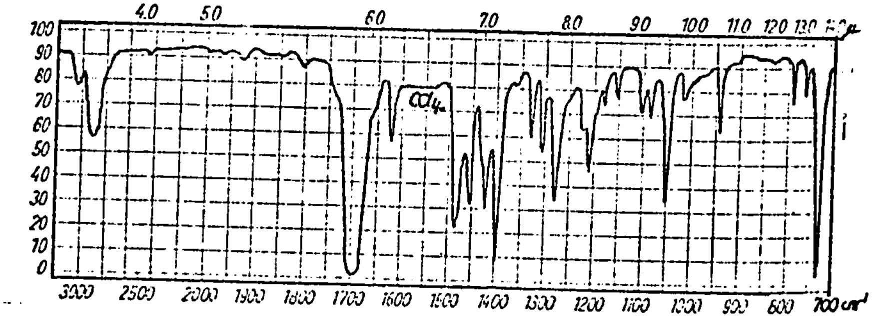
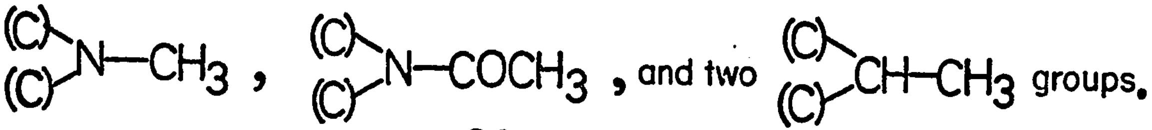


Figure 36

plasticizers in many polymeric materials used as containers of various solvents and reagents. They are frequently present in small quantities in our alkaloid samples. We suggest that under the conditions of zinc dust distillation either the phthalic esters or the phthalic anhydride derived from them react with methylamine, which has been generated by degradation of the alkaloids to initially form N-methylphthalimide. This is then reduced by the zinc dust[133] to yield N-methylphthalimidine.

Before any discussion concerning the possible structures of the weakly basic alkaloids can be made, it is appropriate to summarize the information that has been presented so far. The evidence suggests that all the alkaloids are $C_{30}N_3$ compounds and that they contain the following functional groups.

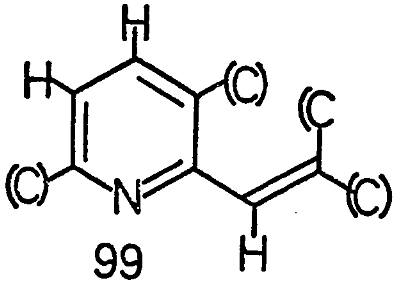


98

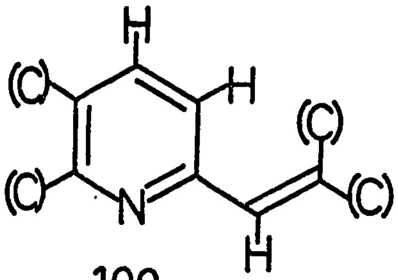
The third nitrogen is present as a pyridine moiety in lycolucine and dihydrolycolucine and possibly as an $>$NH group in the 483-Ketone. In the other alkaloids it exists as an imino function. Lucidine-A, which is isomeric with lucidine-B, is a hexahydro derivative of lycolucine. The behavior of all the compounds upon electron impact or towards dehydrogenating agents suggests that they possess a similar carbon skeleton, and furthermore, infers that they readily cleave into two fragments, the minor of which is a $C_{12}N$ or $C_{10}N$ unit that contains the original amido nitrogen, while the major is usually a $C_{18}N_2$ or $C_{17}N_2$ unit.

Since it is believed that all the alkaloids possess a similar carbon skeleton to lycolucine and dihydrolycolucine it is appropriate to present the immediate discussion in terms of these two alkaloids.

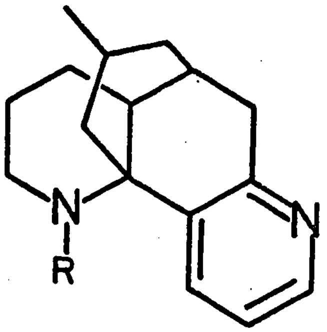
Both lycolucine, $C_{30}H_{43}N_3O$, and dihydrolycolucine possess a 2,3,6-trisubstituted pyridine moiety. In the former alkaloid the substituent at either the 2 or the 6 position of the pyridine ring is a β-disubstituted vinyl group, as depicted in partial structures 99 and 100. In dihydrolycolucine this vinyl group has been reduced.


99

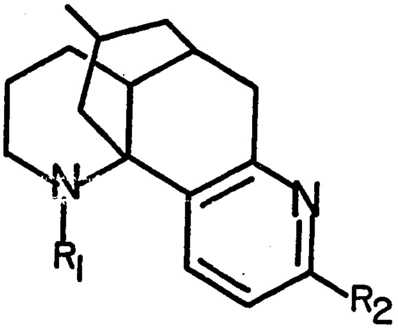
or


100

Since lycodine (12), $C_{16}H_{22}N_2$, which is the only structurally known Lycopodium alkaloid to possess a pyridine ring, is present in L. lucidulum it seemed natural to initially suspect that either an N-acetyllycodine (101) or an N-methyllycodine (102) moiety might be incorporated into the structure of dihydrolycolucine as depicted in partial structures 103 and 104.



12. R=H
101. R=COCH$_3$
102. R=CH$_3$

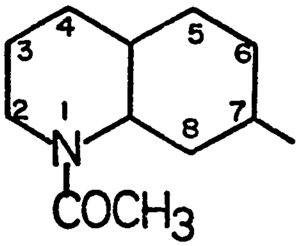


103. R$_1$ =COCH$_3$, R$_2$=C$_{12}$H$_{22}$N
104. R$_1$ =CH$_3$, R$_2$=C$_{13}$H$_{22}$NO

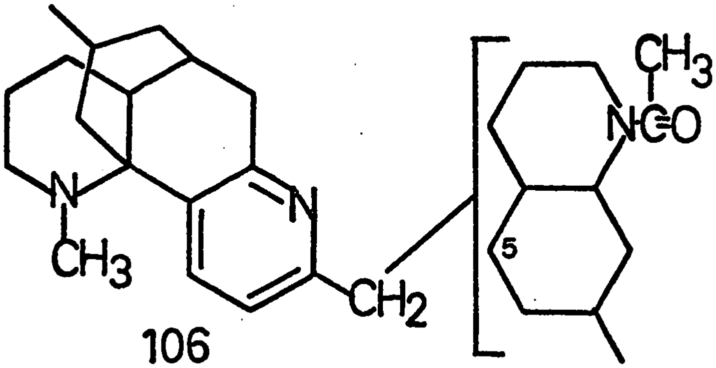
At first structure 103 was considered for dihydrolycolucine since we anticipated that such a system would exhibit a rotomeric effect in the n.m.r. However, we discovered that when the n.m.r. of N-acetyllycodine (101) was determined in deuterochloroform no rotomeric effect was present. The N-acetyl protons resonate as a singlet at τ7.72, approximately 0.2 ppm downfield from the respective signal in lycolucine.

Dehydrogenation and mass spectral studies favor structure 104 more than 103 since together they suggest that the minor fragment initially formed upon electron impact contains an N-acetyl-7-methyldecahydroquinoline system (105). The alkaloids usually show peaks at m/e 194 and 192 ($C_{12}H_{18}NO$) in which the latter corresponds to a dehydro derivative of the m/e 194 fragment. The loss of ketene from the N-acetyl group then leads to the ions at m/e 152 and 150 ($C_{10}H_{16}N$) which are also characteristic of many of these alkaloids.

If the N-acetyl-7-methyldecahydroquinoline system (105) is linked through an external methylene group to the 6 position of the pyridine ring in N-methyllycodine (102) then 106 emerges as a possible partial structure for dihydrolycolucine.

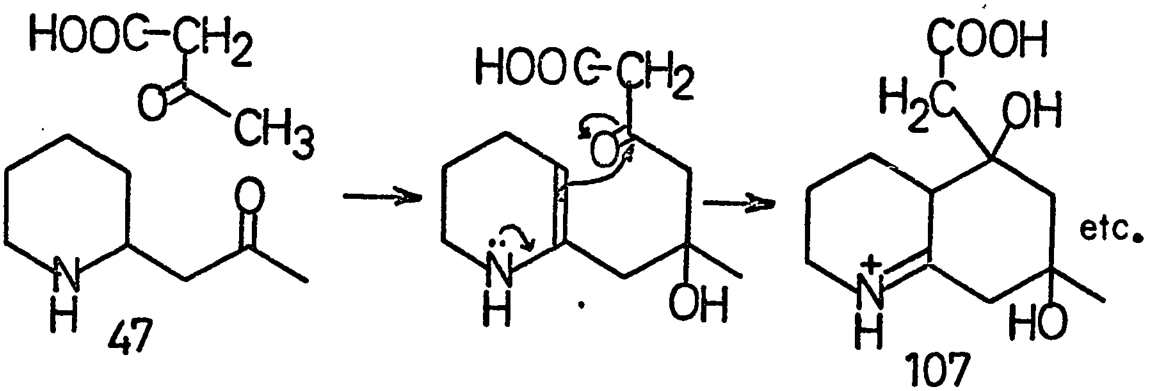


105

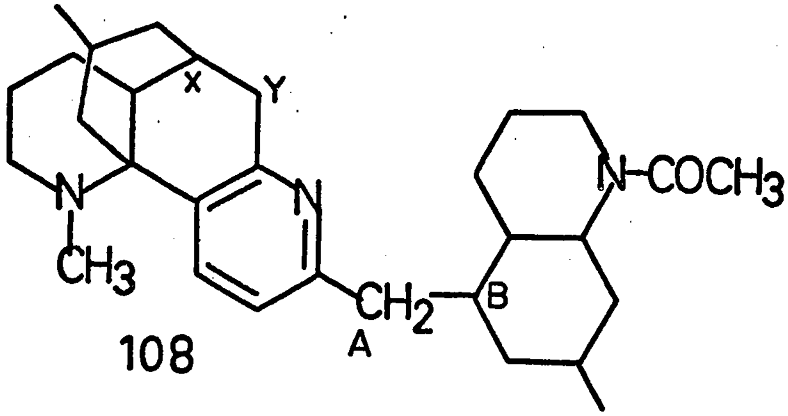


106

From a consideration of the currently accepted mode of biosynthesis of the Lycopodium alkaloids[74,75] the most probable point of attachment of this methylene group to the 7-methyldecahydroquinoline moiety is at C_5 as exemplified below, utilizing pelletierine (47) and acetate.



In terms of the above facts dihydrolycolucine can possibly be represented by structure 108.

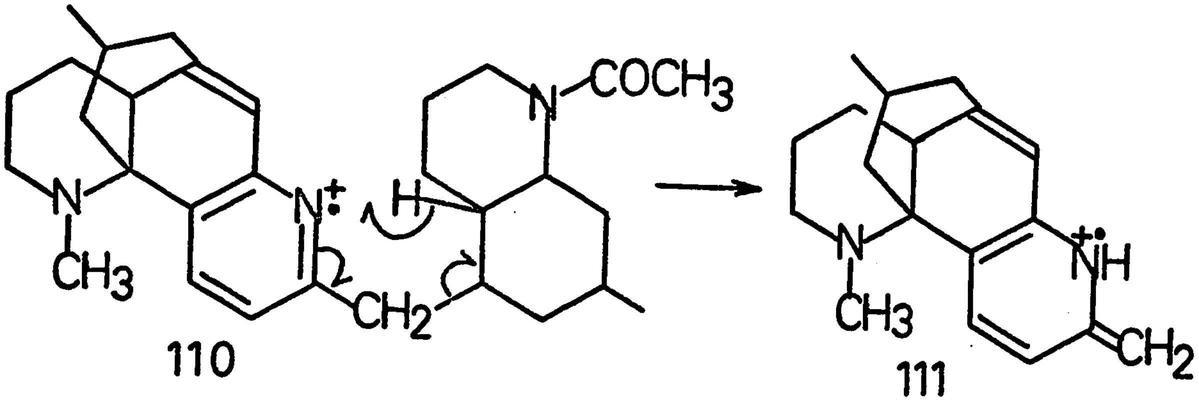


108

109. Double bond linking C_A to C_B.
110. Double bond linking C_X to C_Y.

The above structure 108 gives rise to two possibilities for the structure of lycolucine. From the reaction of lycolucine with osmium tetroxide followed by[113] sodium metaperiodate, a dicarbonyl derivative was obtained mw 493, $C_{30}H_{43}N_3O$. Therefore the structure 109, having a double bond linking C_A to C_B was eliminated since such a molecule would cleave into two pieces. The base peak in the mass spectrum of lycolucine, m/e 268, ($C_{18}H_{24}N_2$) was replaced by an intense peak in the spectrum of the product at m/e 300 ($C_{18}H_{24}N_2O_2$) implying that the original double bond was internally situated in the dinitrogenous portion of the lycolucine molecule.

The alternative structure 110 having a double bond linking C_X to C_Y is consistent with the i.r., u.v., and n.m.r. data of lycolucine, and it is possible to write plausible fragmentation pathways to account for the formation of the more prominent peaks in the mass spectrum of lycolucine, in terms of this structure (110) as exemplified below for the formation of the base peak at m/e 268 ($C_{18}H_{24}N_2$) in the mass spectrum of lycolucine.

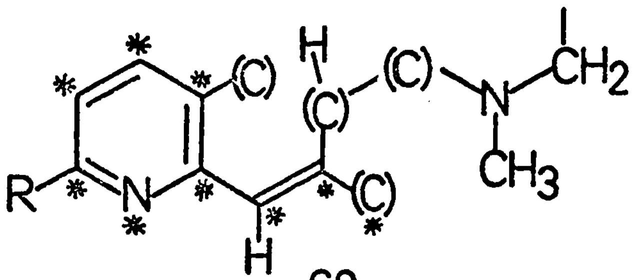


110

111

The unsatisfactory feature of structure 110 is that it incorporates a double bond at the bridgehead of a bicyclo [3.3.1] nonane system. Recently it has been shown that such compounds may be prepared[134], but an inspection of models indicates that structure 110 is very highly strained and it is therefore doubtful that it represents the alkaloid lycolucine.

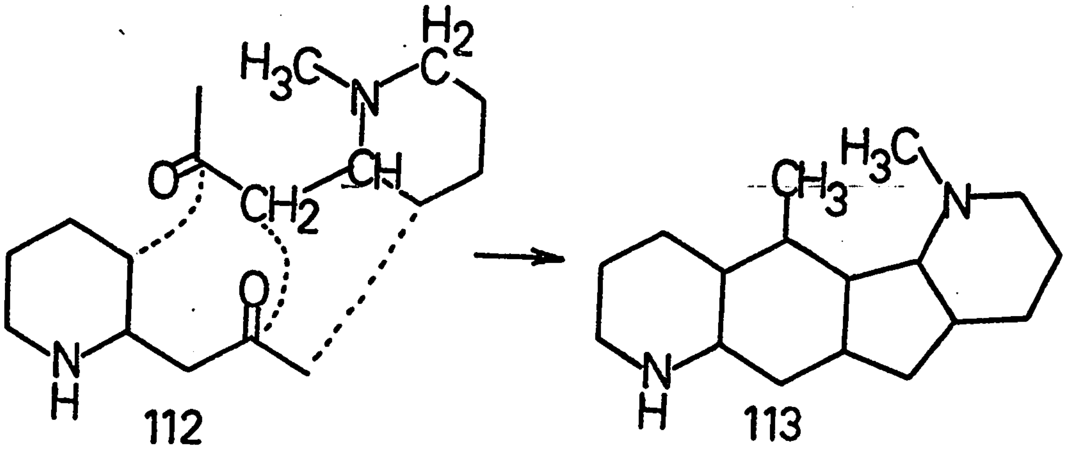
Earlier in this thesis (page 73) it was mentioned that there is some evidence for the partial structure 69 in lycolucine. Assuming that this moiety is indeed present



69

in the $C_{17}N_2$ or $C_{18}N_2$ portion of the molecule and that the $C_{17}N_2$ portion may be derived from pelletierine units, it is possible to derive a structure which accounts for most of the properties of these alkaloids. This structure is suggested as a working hypothesis which may form the basis for further study.

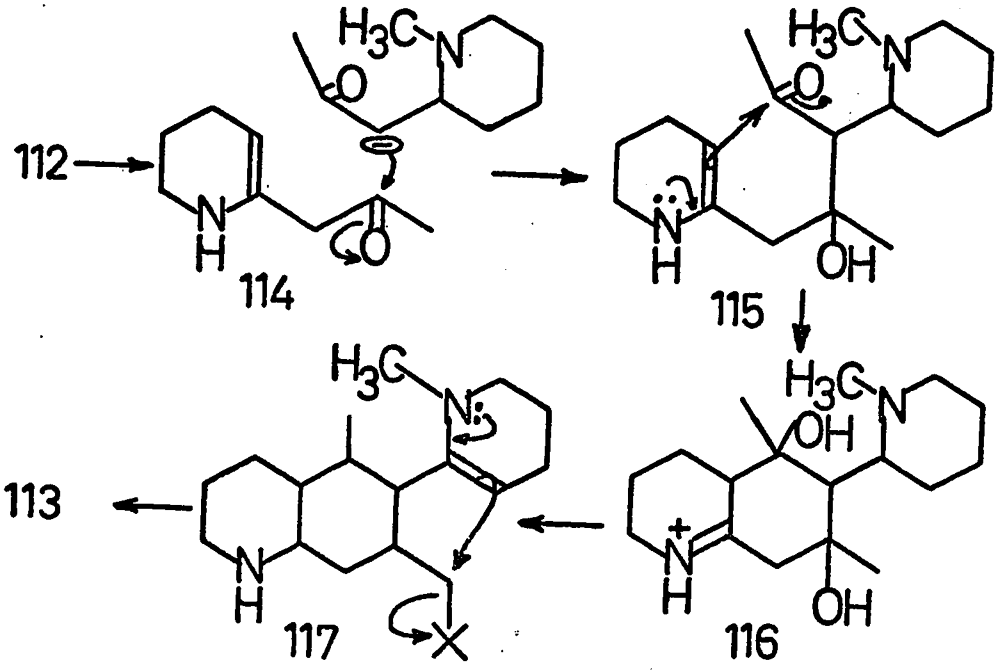
If the starred atoms in 69 represent one pelletierine unit and that the remaining atoms are part of an N-methylpelletierine unit, then the connections shown by the dotted lines in partial structure 112 lead to skeleton 113. One possible sequence for the condensation of the two units is outlined overleaf.



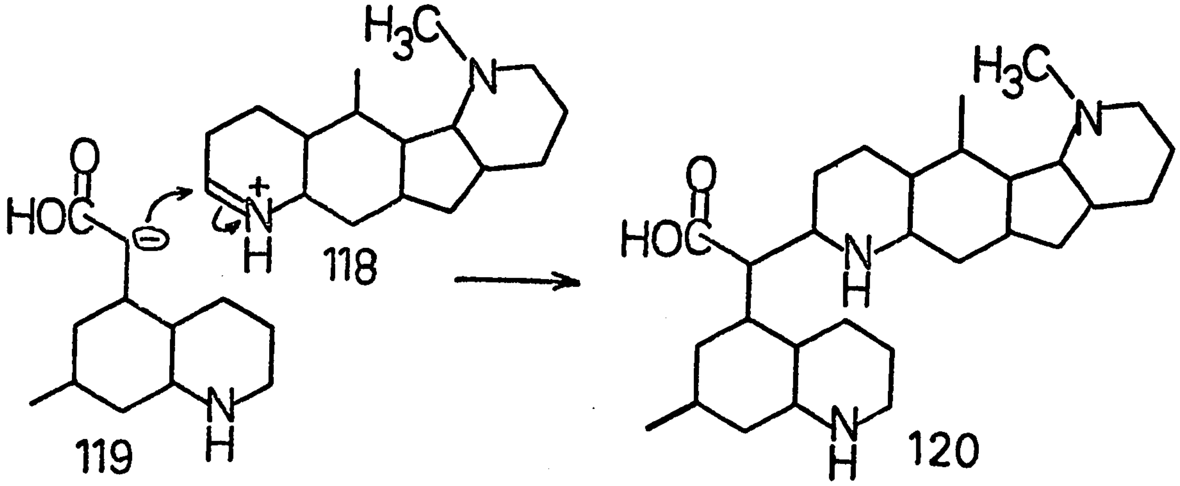
112

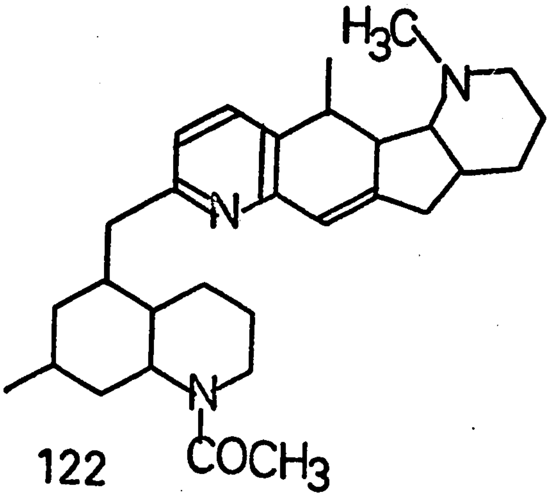
113

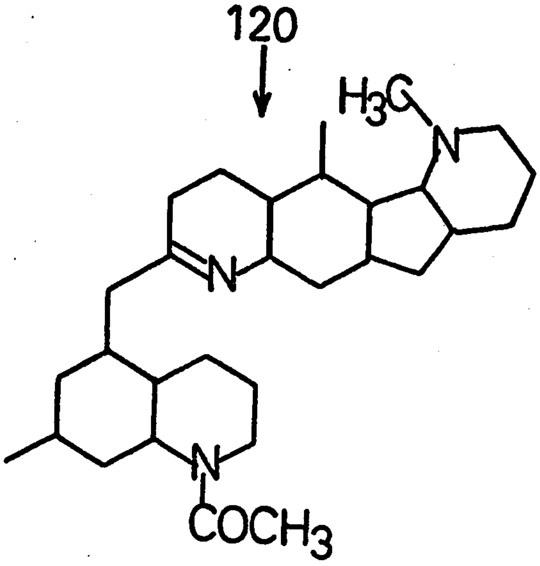
As depicted in structure 117, a suitable leaving group (X) has to be introduced at some stage in order to make a ring closure involving the enamine of the N-methylpelletierine unit a feasible process.



If then the pelletierine unit in 113 by way of its imine salt (118) is condensed with unit 119, a possible intermediate in the biosynthesis of luciduline, and the product (120) is then oxidatively decarboxylated and acetylated, structure 121 emerges as a possible representation of the skeleton of these alkaloids.



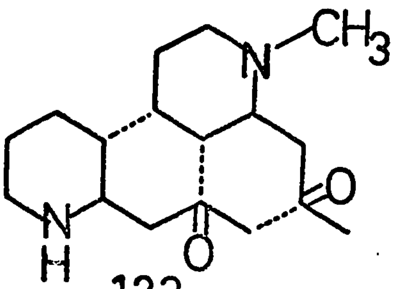

122

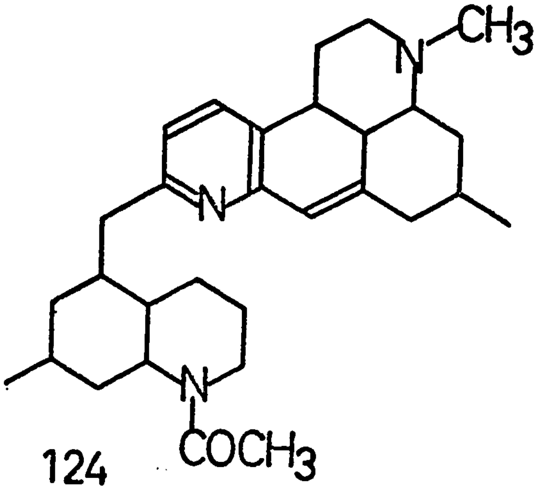


121

In terms of skeleton 121 lycolucine would have structure 122. An inspection of models of structure 122 reveals that the N-methyl group is very hindered.

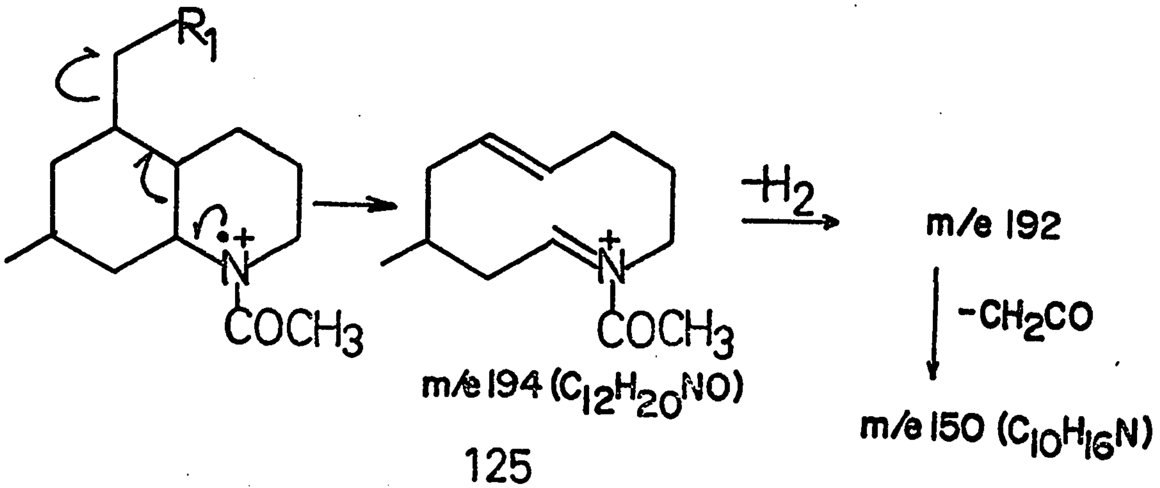
By varying the biosynthetic scheme so as to bring about the ring closures depicted by the dotted lines in 123, it is possible to arrive at structure 124 for lycolucine, in which the N-methyl group is relatively unhindered.


123


124

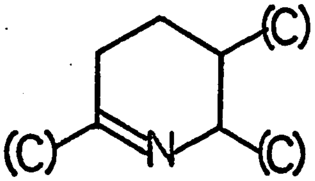
Of these two possible structures for lycolucine (122 and 124) the former is favored since the sterically hindered nature of the N-methyl group may partially account for the weakly basic nature of lycolucine and the other C_{30} alkaloids. The possible structures of the other C_{30} alkaloids will be presented in terms of the skeleton 121. The brief discussion about each of the alkaloids will center around probable fragmentation pathways to account for the formation of the major peaks in their mass spectra. In the fragmentations R_1 represents the $C_{17}N_2$ unit while R_2 represents the $C_{12}N$ (or $C_{10}N$) unit of the alkaloid under discussion.

Most of the alkaloids show peaks in the mass spectrum at m/e 192 and 150, which are believed to represent the $C_{12}N$ (or $C_{10}N$) portion of the molecules, and whose formation can be accounted for in the following way.



125

The possible structures of lucidine-A and -B are denoted by skeleton 121. It is doubtful that the differences between these two components are due solely to the position of the imino double bond, as depicted in partial structures 126 and 127. Instead it is suspected that the differences are due to differing configurations at one or more of the asymmetric centers of skeleton 121.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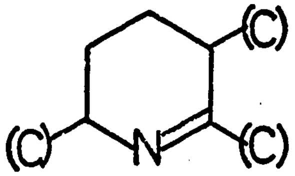

127

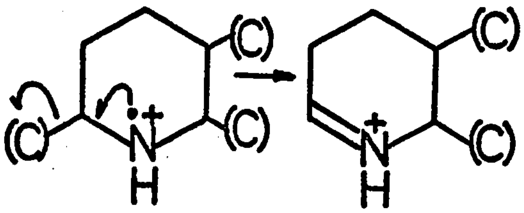
The formation of some of the prominent ions in the mass spectra of the C_{30} alkaloids can be explained in terms of three distinct fragmentation pathways :-

(a) α-cleavage[130] of a piperidine moiety exemplified by the 483-Ketone and the dihydrolucidines.

(b) McLafferty rearrangement involving abstraction of hydrogen atom from the γ-carbon of an alkyl pyridine[135] moiety and subsequent β-cleavage, exemplified by lycolucine.

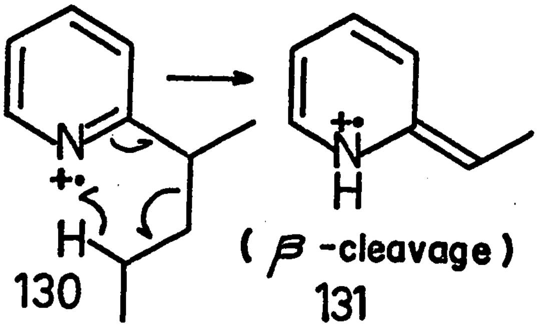
(c) β-cleavage of an imine moiety without hydrogen transfer, as indicated in the fragmentation of lucidine-A and -B, the 483 compound and the 481 compound.

Pathways (a) and (b) are illustrated as follows.



(α-cleavage)

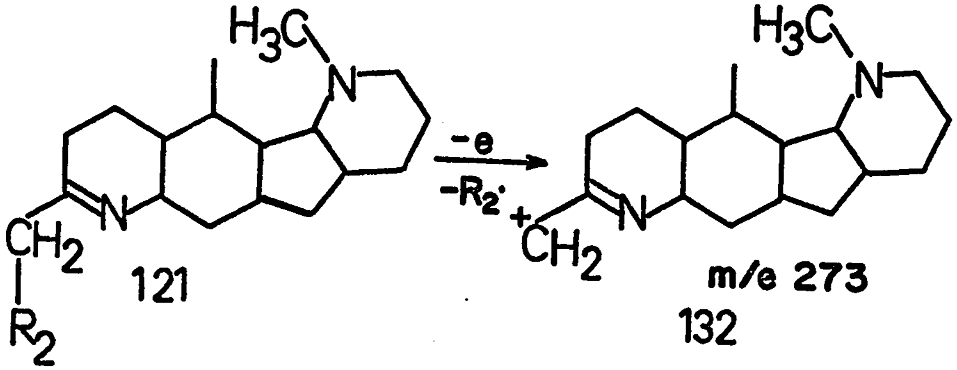
128 129



(β-cleavage)

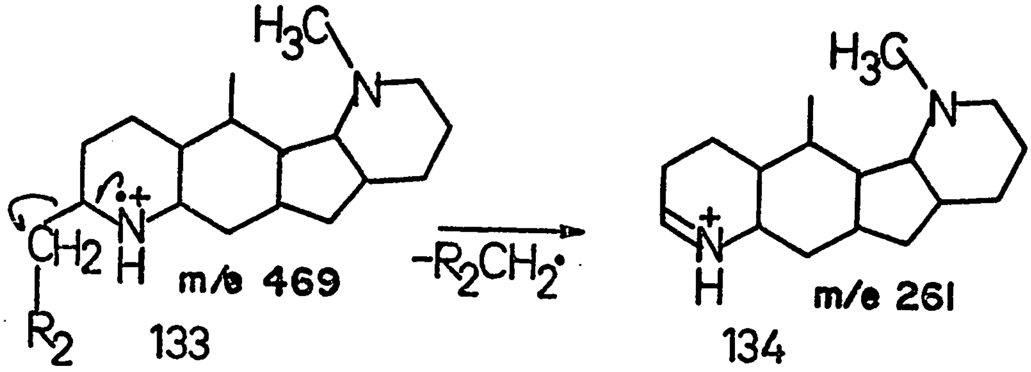
130 131

A possible fragmentation to account for the formation of the base peak of the lucidine-A and -B alkaloids (121) at <u>m/e</u> 273 ($C_{18}H_{29}N_2$) is :-



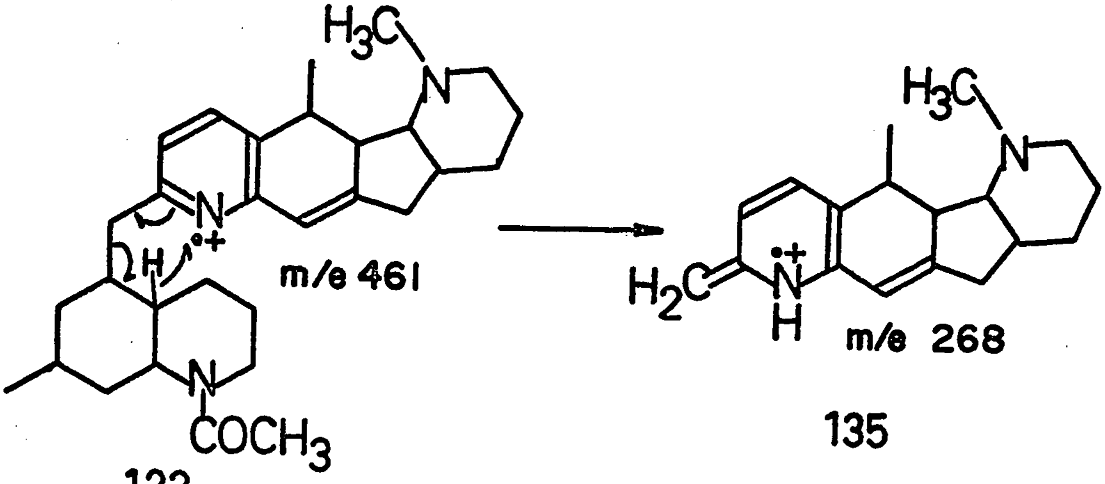
121 132

The base peak in the mass spectra of the dihydrolucidine-A and -B compounds (133) at <u>m/e</u> 261 ($C_{17}H_{29}N_2$) can be explained in terms of an α-cleavage.



133 134

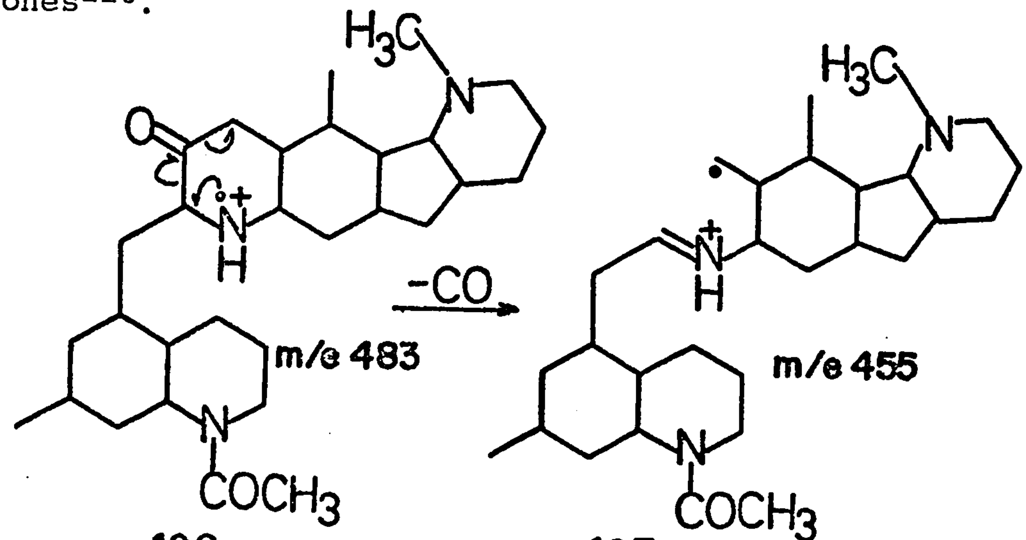
The mass spectrum of lycolucine, represented by structure 122, shows a base peak at m/e 268 ($C_{18}H_{24}N_2$) whose formation can be explained by means of a McLafferty rearrangement.



122

135

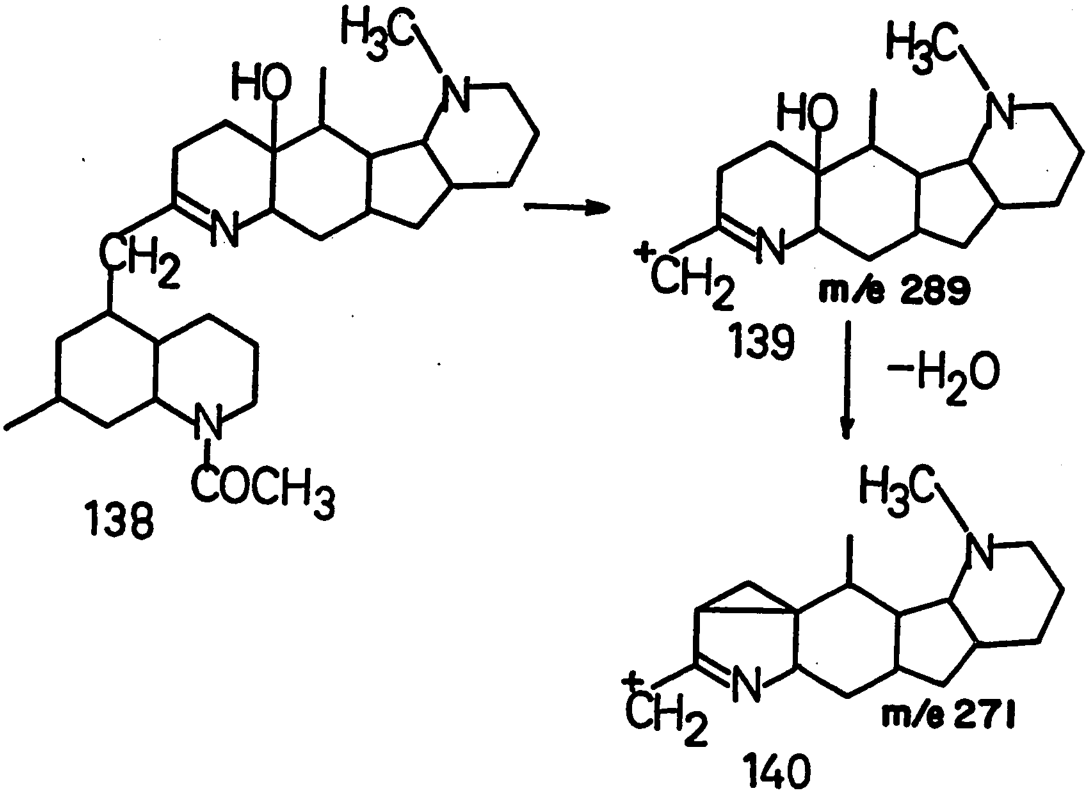
A possible structure for the 483-Ketone, which is believed to possess an >NH group and an α-aminoketone moiety is 136. The appearance of a strong peak at m/e 455 ($C_{29}H_{43}N_3O$) corresponding to expulsion of carbon monoxide from the molecular ion is characteristic of α-aminoketones[116].



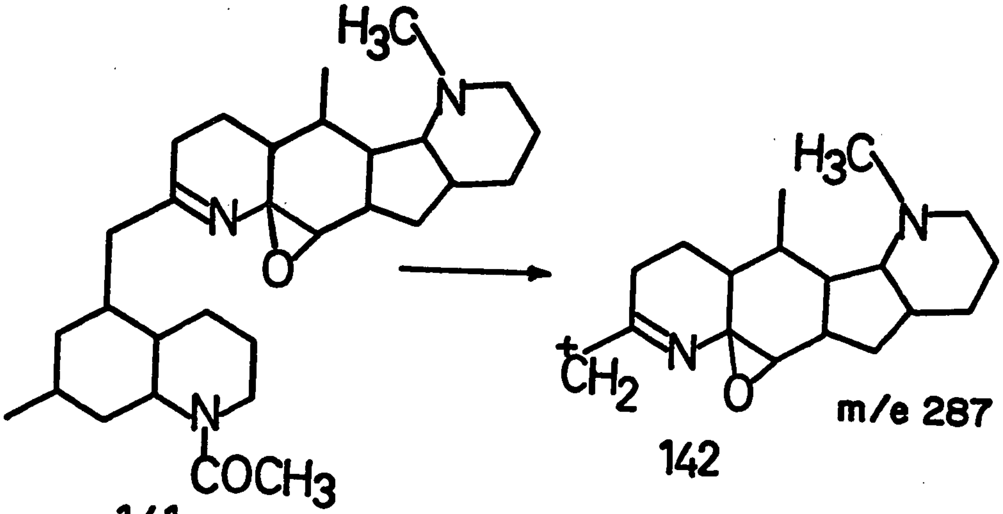
136

137

A possible structure for the other component of molecular weight 483 (i.e., the 483 compound), which is believed to contain an imino double bond and a tertiary alcohol, is denoted by 138. The formation of the base peak at m/e 271 ($C_{18}H_{17}N_2$) in its mass spectrum is probably analogous to the fragmentation shown earlier for lucidine-A and -B.



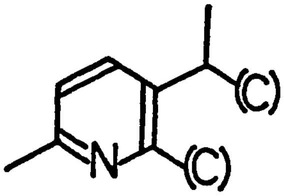
One example of the structures possible for the 481 compound, which contains an imino double bond and a carbinolamine ether, is 141. In its mass spectrum the compound shows a base peak at m/e 287 ($C_{18}H_{27}N_2O$) whose mode of formation is also probably analogous to the scheme outlined for lucidine-A and -B.



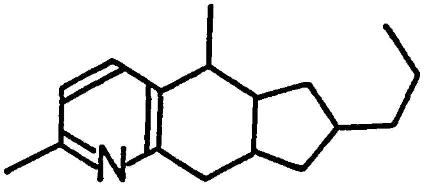
141

142

The structural skeleton 121 is also consistent with the results of dehydrogenation studies described earlier which indicated that GC-18 ($C_{17}H_{25}N$) contains the partial structure 82. Accordingly, a possible structure for GC-18 is 143.

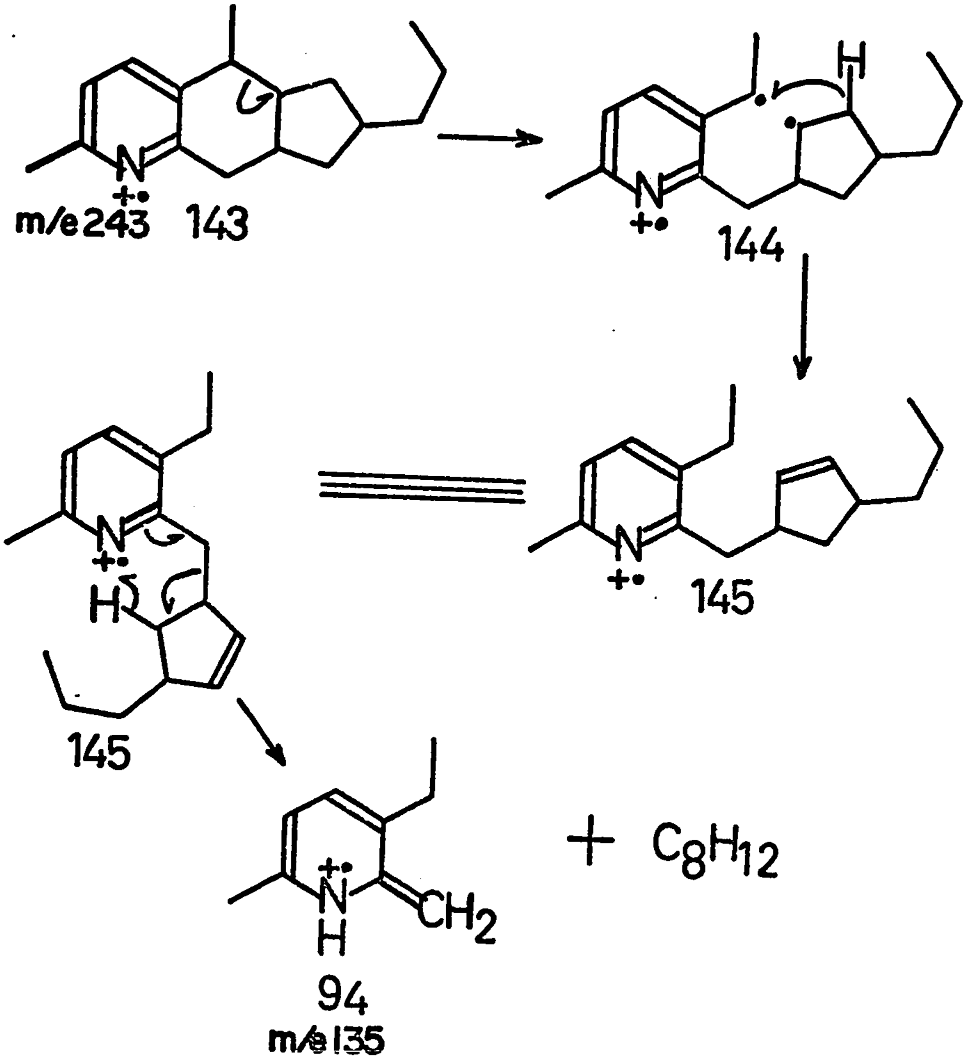


82



143

The following mass spectral fragmentation pathway, involving a McLafferty rearrangement accounts for the direct formation of the base peak at m/e 135 ($C_9H_{13}N$) from the molecular ion.



During recent isolation studies on the weakly basic alkaloids of L. lucidulum two hitherto unknown alkaloids have been isolated. One of these, which is mononitrogenous, appears to possess an oxygenated lycopodine (2) skeleton while the other, which is dinitrogenous, shows an intense band at 1620 cm.$^{-1}$ in the i.r., characteristic of the weakly basic alkaloids just discussed.

The dinitrogenous alkaloid has been isolated from the most weakly basic fractions of recent countercurrent distribution experiments. It has been obtained as a gum and exhibits the highest mass in its mass spectrum (figure 37) at m/e 292, molecular composition $C_{17}H_{28}N_2O_2$. The base peak is at m/e 84 while other prominent peaks are seen at m/e 249, 195, 194, 166, 152, 110 and 96. Several of these peaks have been subjected to analysis via high resolution mass spectrometry.

m/e Found	m/e Calculated	Molecular composition
292.2156	292.2151	$C_{17}H_{28}N_2O_2$
249.1978	249.1967	$C_{15}H_{25}N_2O$
152.1435	152.1439	$C_{10}H_{18}N$
152.1081	152.1075	$C_9H_{14}NO$
84.0444	84.0449	C_4H_6NO

Metastable peaks are observed at m*/e 212 and 119 which correspond to the direct m/e 292→249 and 194→152 fragmentations respectively.

The infrared spectrum of the compound determined in chloroform (figure 38) shows strong absorption at 3420, 1695 and 1620 cm.$^{-1}$ which, by correlation with the molecular composition, are interpreted to represent the presence of a secondary $>$NH group and two carbonyl moieties.

The facile loss of $CH_3CO\cdot$ from the parent peak together with the absorption in the i.r. at 1620 cm.$^{-1}$ leads us to suspect that one of the carbonyl groups is an N-acetyl function.

The n.m.r. of the compound in deuterochloroform (figure 39) exhibits two overlapping doublets ($J_1 = J_2 = 7$ cps) centered at τ 8.95 and 8.93 respectively. Since the integration infers the presence of only three protons it is suspected that the doublets represent one secondary methyl group which is being influenced by a rotomeric effect elsewhere in the molecule. Since the i.r. of the compound lacks a typical N-methyl absorption at 2780 cm.$^{-1}$ the singlets in the n.m.r. determined in deuterochloroform at τ 7.98 and 7.94 are attributed to an N-acetyl function, which in turn is responsible for the presence of the rotomers.

A sharp signal at τ 7.75 is attributed to the protons of methylene or methine groups attached to nitrogen. The spectrum shows an AB quartet ($J_{AB} = 14$ cps) with centers at τ 5.55 and 5.13, while a doublet centered at τ 2.85 ($J = 22$ cps) whose chemical shift is concentration dependent, is believed to be due to the $>$NH proton. When the spectrum is deter-

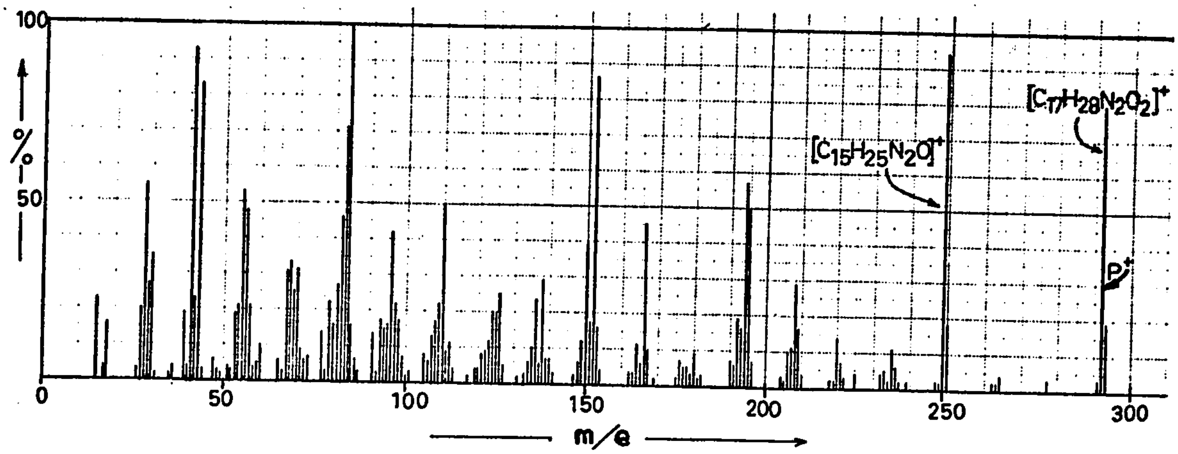


Figure 37

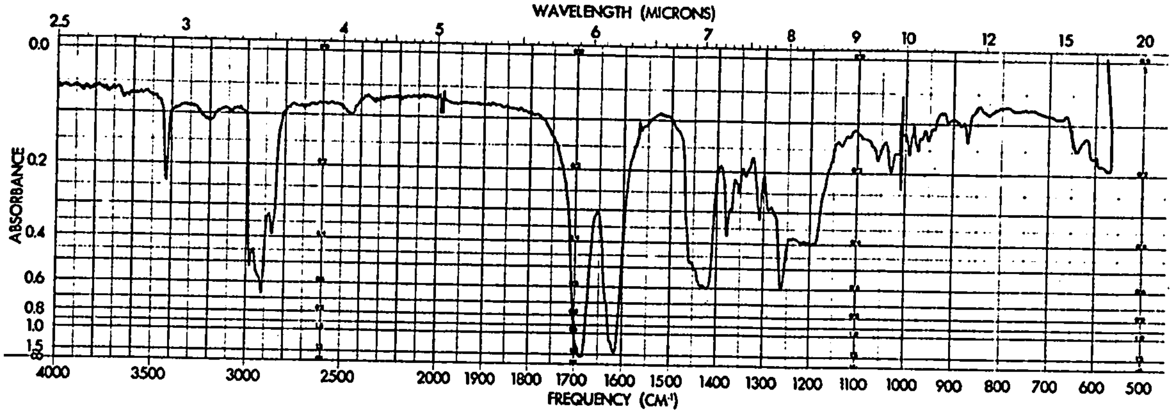


Figure 38

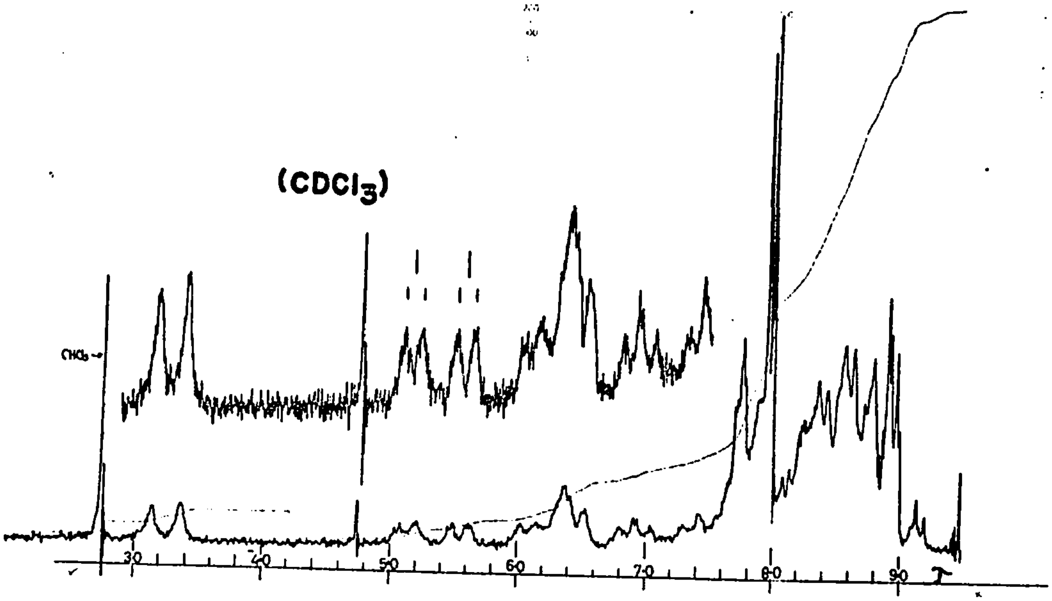
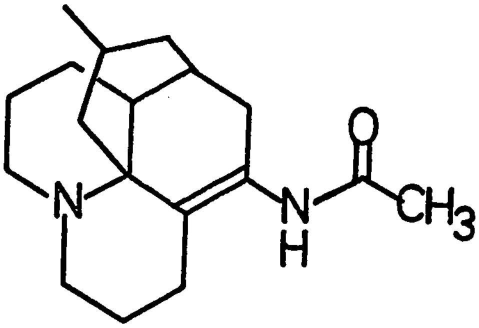


Figure 39

mined in perdeuteroacetic acid the signals attributed to the $>$N-COCH$_3$, $>$N-CH$_2$- and $>$N-CH- protons show a downfield shift of between 0.06 and 0.14 ppm. In these circumstances, the signal attributed to the $>$NH proton appears as a broad singlet at τ2.02. The n.m.r. of the Lycopodium alkaloid flabelline (7) $C_{18}H_{28}N_2O$, similarly shows a concentration[27] dependent signal attributable to its $>$NH proton.



7

The compound shows only end absorption in the ultraviolet. It is recovered unchanged following treatment with hydrogen over a catalyst at one atmosphere or with sodium borohydride. The spectral data and relevant chemical data suggest that the compound contains no carbon-carbon nor carbon-nitrogen double bonds and, since two carbonyl moieties are present, the molecular formula requires that the molecule be tricylic. The failure of the compound to be reduced with sodium borohydride led us to consider that the carbonyl function responsible for the band at 1695 cm.$^{-1}$ in the i.r. might be due to the presence of a γ-lactam in the molecule as well as an N-acetyl function. This would also explain the low basicity.

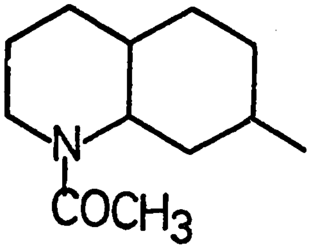
Support for the idea of a γ-lactam comes from an attempted acid hydrolysis of the compound. The usual procedures led to the isolation of only trace amounts of substance which proved to be starting material. Apparently the remainder had stayed behind as a water-soluble amino acid.

Attempts to form a crystalline methiodide or hydroperchlorate derivative were unsuccessful. Reaction of the compound with acetic anhydride and pyridine led to a small amount of product, whose infrared spectrum lacks typical O-acetyl absorption but whose mass spectrum shows a weak parent peak corresponding to monoacetylation. Reaction with sodium nitrite in dilute hydrochloric acid led to a product whose infrared spectrum in chloroform shows strong absorption at 3420, 1765, 1690 and 1620 $cm.^{-1}$. The highest peak in the mass spectrum of the product is at *m/e* 322, which probably represents the cation formed by protonation of an initially formed N-nitroso derivative.

When the alkaloid was treated with hydrogen bromide a crystalline derivative (m.p.115-119°) was isolated whose infrared spectrum in nujol shows an absorption at 1650 $cm.^{-1}$ of moderate intensity.

Lack of material prevented further study of this alkaloid.

One possible structure for this compound has been formulated in the following manner from a consideration of the mass spectral evidence. It was mentioned earlier that the peaks at m/e 194, 166, 152 and 150 are found in the mass spectra of many of the weakly basic alkaloids of *L. lucidulum* and it was suggested that these peaks originated from that portion of the C_{30} alkaloids which incorporates an N-acetyl-7-methyldecahydroquinoline moiety (105). The presence of peaks at m/e 194, 166 and 152 in the mass spectrum of the compound under discussion leads us to suspect that its structure similarly contains N-acetyl-7-methyldecahydroquinoline.



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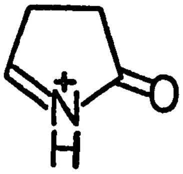

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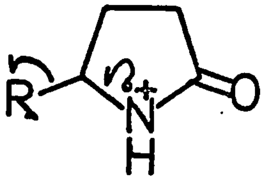
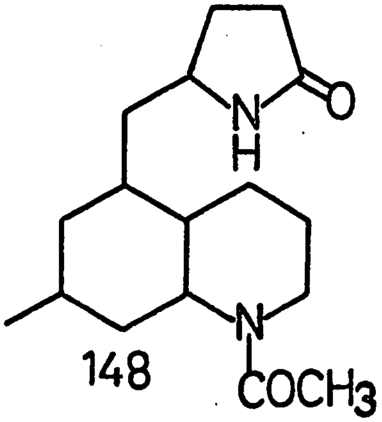


147

The infrared spectrum of the compound indicates the presence of a γ-lactam and it is possible that this γ-lactam might be incorporated into the most abundant fragment, at m/e 84 (C_4H_6NO), in the mass spectrum of this compound, as depicted in 146. If this is indeed the structure of the ion at m/e 84 then its formation has probably arisen *via* an initial α-cleavage of a structure such as 147.

Structures 105 and 147 account for all but one of the carbon atoms of the alkaloid. By analogy with the structures proposed for the C_{30} alkaloids it is feasible to consider that in this alkaloid the units 105 and 147 might be linked through a methylene group which is attached to both the C_5 position of the decahydroquinoline moiety and the γ position of the γ-lactam (147). Accordingly, the structure 148 is a possibility for this alkaloid.



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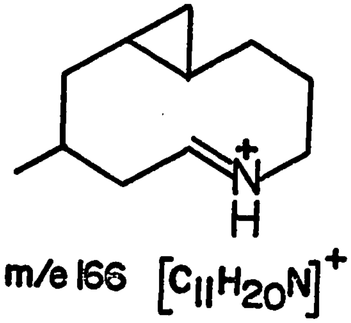
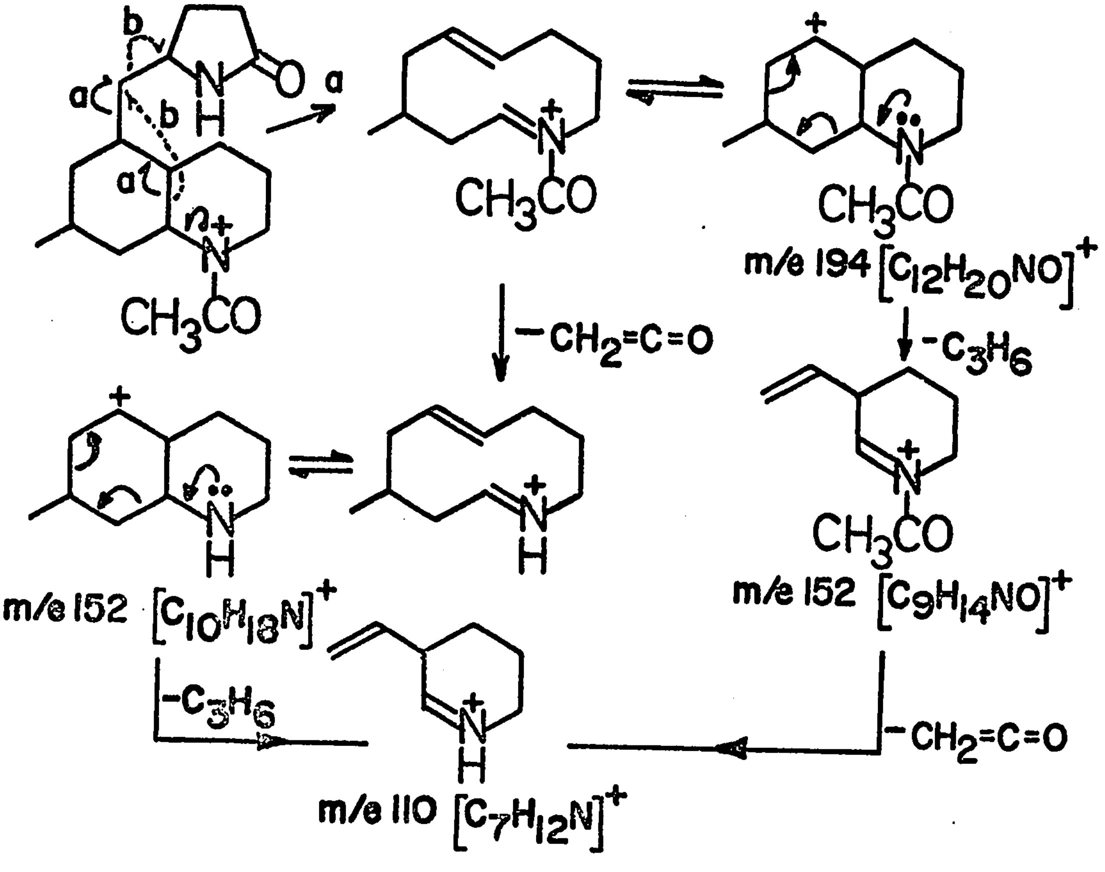


149

The possible mass spectral fragmentation pathways of



150

this alkaloid are outlined on the previous page (150) in accord with the proposed structure (148).

It can be seen that an initial α-cleavage according to pathway (a) readily leads to structures for the ions at m/e 194, 152 and 110. If on the other hand, the α-cleavage follows pathway (b), i.e. dotted arrow, then the initial fragment formed corresponds to m/e 208 ($C_{13}H_{22}NO$), which on loss of ketene yields an ion at m/e 166 ($C_{11}H_{20}N$) of structure 149. Both this alkaloid and the C_{30} alkaloids show a strong $M^+ - 43$ peak corresponding to loss of $CH_3CO\cdot$ from the molecular ion. The normal fragmentation of an N-acetyl compound is to lose ketene and it is suspected[136] that in these alkaloids such a loss is either preceded by or followed by loss of a hydrogen atom via α-cleavage. This accounts for the presence of the peak at m/e 249 in the mass spectrum of this alkaloid.

The mononitrogenous alkaloid has been isolated from the strongly basic fractions of Lycopodium lucidulum.

Many various separation procedures have been designed to achieve a reasonable supply of weakly basic alkaloids from the total crude alkaloids of L. lucidulum. One of these involved the extraction of a methylene chloride solution of the crude alkaloids with increasingly strong aqueous acid solutions beginning with potassium hydrogen phthalate (pH 4). On one occasion the further purification by chromatography and countercurrent distribution of the bases extracted by the initial partition with potassium hydrogen phthalate solution led to the isolation of a very small amount of the mononitrogenous alkaloid.

The crystalline alkaloid, m.p. 166-168°, rapidly discolored on exposure to air. Mass spectrometry revealed that the alkaloid has the molecular composition $C_{16}H_{21}NO_2$ and molecular weight 259. Also the molecular compositions of three of the fragmentation ions were determined.

m/e Found	m/e Calculated	Molecular composition
259.1571	259.1572	$C_{16}H_{21}NO_2$
230.1548	230.1545	$C_{15}H_{20}NO$
216.1028	216.1025	$C_{13}H_{14}NO_2$
202.0861	202.0868	$C_{12}H_{12}NO_2$

The mass spectrum (figure 40) shows a base peak at m/e 202 ($M^+ - 57$) and strong peaks at m/e 230 ($M^+ - 29$), 216 ($M^+ - 43$), 188 ($M^+ - 71$) and 174 ($M^+ - 85$) and exhibits the typical fragmentation pattern of a lycopodine-type (2) alkaloid[28]. Metastable peaks, consistent with the direct m/e 259⟶202 and m/e 202⟶174 fragmentations are observed at m*/e 157.5 and 150 respectively.

The i.r. spectrum of the alkaloid determined in nujol (figure 41) shows absorption at 3038, 1662 (shoulder), 1654, 1611 and 1405 $cm.^{-1}$ together with a very sharp fingerprint region. When the spectrum is determined in chloroform intense additional absorptions are observed at 3440 and 1620 cm.$^{-1}$. The latter peak may correspond to the vibration responsible for either the shoulder at 1662 cm.$^{-1}$ or the weak peak at 1611 cm.$^{-1}$ in the original nujol spectrum, while the former infers the presence of an $\rangle$NH or -OH group.

The ultraviolet spectrum is very informative and shows absorption maxima at 212 and 321 mμ in methanol which, after acidification, are observed at 212 and 314 mμ respectively. When the spectrum is determined in a weakly basic solution of methanol the maxima appear at 220 and 364 mμ. This large bathochromic shift on basification of the solution is a characteristic of diosphenols[137].

In deuterochloroform the n.m.r. spectrum (figure 42) shows a doublet at τ9.17 (J = 6 cps). Double irradiation experiments show that the proton to which the methyl is coupled appears as a multiplet at τ8.40. The distinguishing features of the n.m.r. spectrum between τ8.0 and 3.0 are an AB quartet (2H), a symmetrical octet at τ4.17 (1H) and a doublet of doublets at τ3.29 (1H, J_1 = 10 cps, J_2 = 2 cps). The centers of the AB quartet appear at τ7.00 and 6.05 respectively (J_{AB} = 19 cps). The initial four signals of the AB system are each further split, the upfield signals into a doublet of doublets (J_1 = 5 cps, J_2 = 1 cps) and the downfield signals into a doublet of triplets

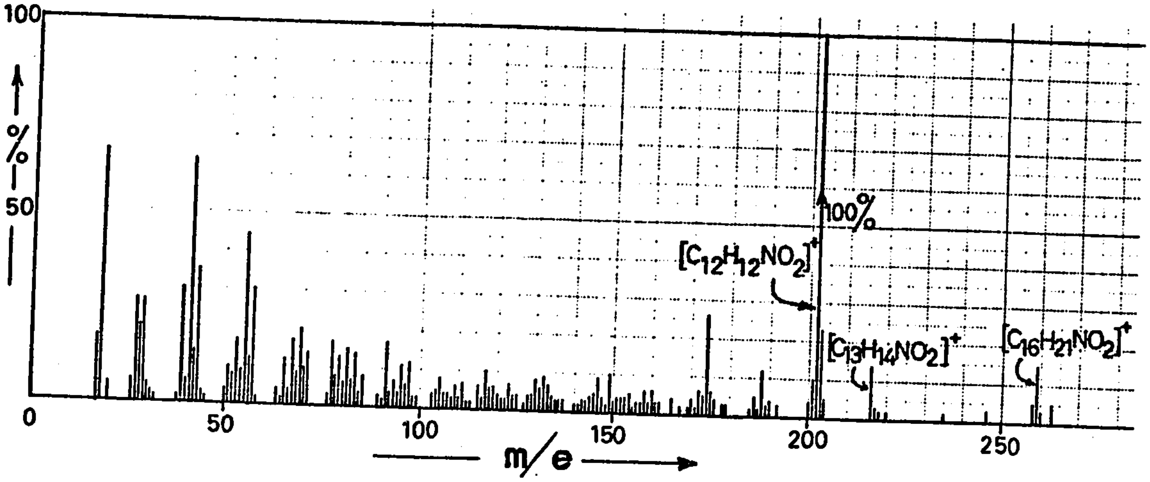


Figure 40

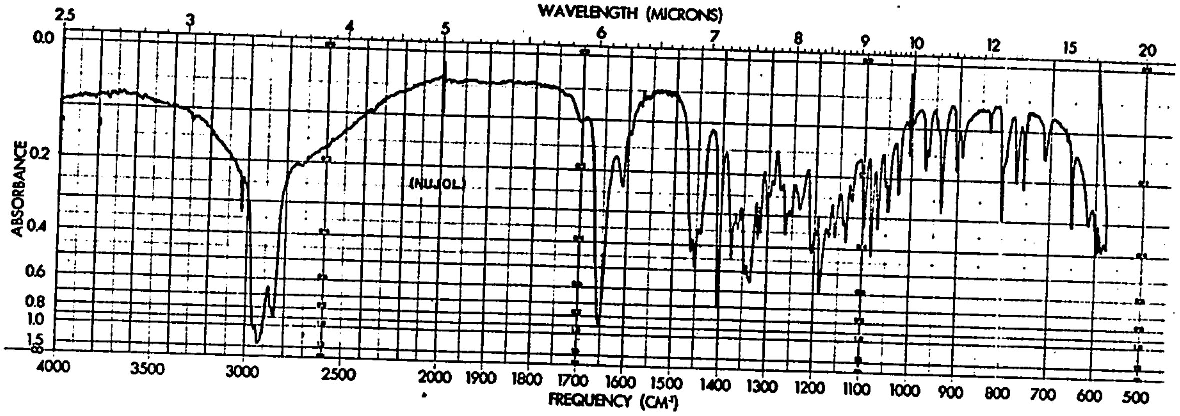


Figure 41

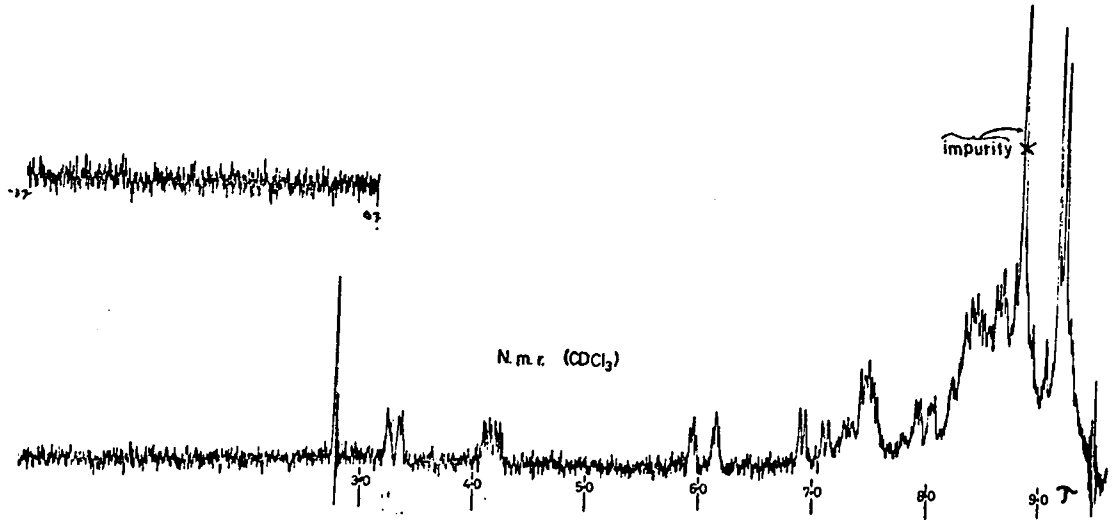


Figure 42

($J_1 1$ = 3 cps, $J_2 1$ = 1 cps).

Double irradiation at τ4.12 causes the collapse of the doublet at τ3.29 and greatly simplifies the AB system, confirming that the protons associated with these signals are each coupled to the proton which appears as the octet at τ4.17. Double irradiation at τ5.97, i.e., in the center of the most downfield signal of the AB quartet, causes a collapse of the complete AB system.

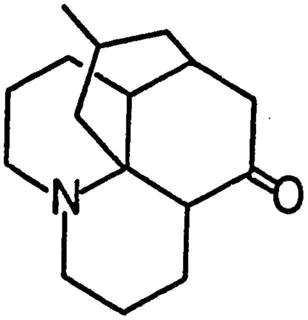
The reaction of the alkaloid with acetic anhydride in pyridine gave a product whose infrared spectrum in chloroform indicates the presence of a vinyl ester (1760 cm.$^{-1}$) and also shows peaks at 1640 and 1615 cm.$^{-1}$. This reaction infers that the alkaloid contains an enolic hydroxyl group and is consistent with the belief that a diosphenol system is present. Attempts to purify this product by the usual acid-base extraction procedures led to rapid hydrolysis to starting material.

Catalytic hydrogenation of the alkaloid gave a product whose ultraviolet spectrum in methanol shows absorption maxima at 210 and 282 mμ. Acidification causes a slight hypsochromic shift of the latter peak to 278 mμ, while basification causes a large bathochromic shift (40 mμ) to 322 mμ, indicating that a diosphenolic system was still present even though the spectrum displayed an overall hypsochromic shift of 40 mμ relative to the original alkaloid.

This behavior suggested that the alkaloid contains an α-hydroxy conjugated dienone system (151) and that catalytic hydrogenation had reduced the double bond to yield the α-hydroxy enone system (152).


151
152

If this assignment is correct the molecular formula $C_{16}H_{21}NO_2$ requires that the molecule be tetracyclic. The similarities between its mass spectral fragmentation pattern and those of known lycopodine-type alkaloids led us to consider that this alkaloid contained a lycopodine skeleton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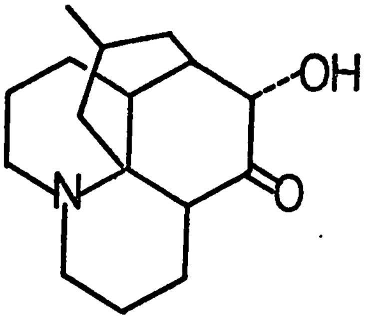
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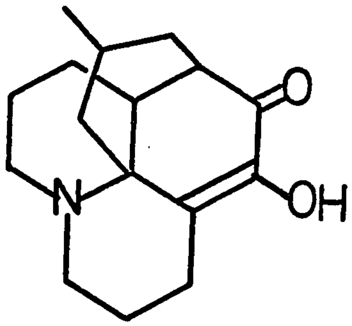
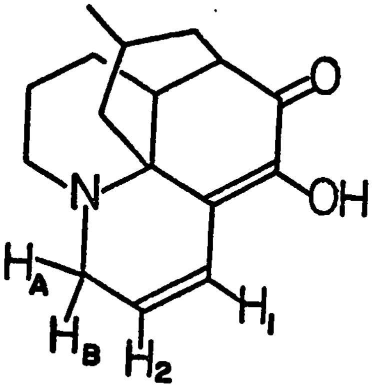

52

This suggestion is reinforced by the knowledge that the diosphenol (52), which is readily formed[92] by aerial oxidation in basic medium of alkaloid L.20 (9), or by selenium dioxide oxidation of lycopodine (2), had previously been isolated in these laboratories from L. lucidulum[91].

It is possible then that the diosphenol (52) is identical with the one obtained by the catalytic hydrogenation experiment just described. The theoretical absorption maximum of the diosphenol in neutral solution would be at 279 mμ which is in close agreement with the 282 mμ figure obtained for the hydrogenation product. Lack of material prevented a direct comparison of the two compounds.

After consideration of all the chemical properties and spectral data we have tentatively assigned the following structure (153) to the mononitrogenous alkaloid of molecular formula $C_{16}H_{21}NO_2$.

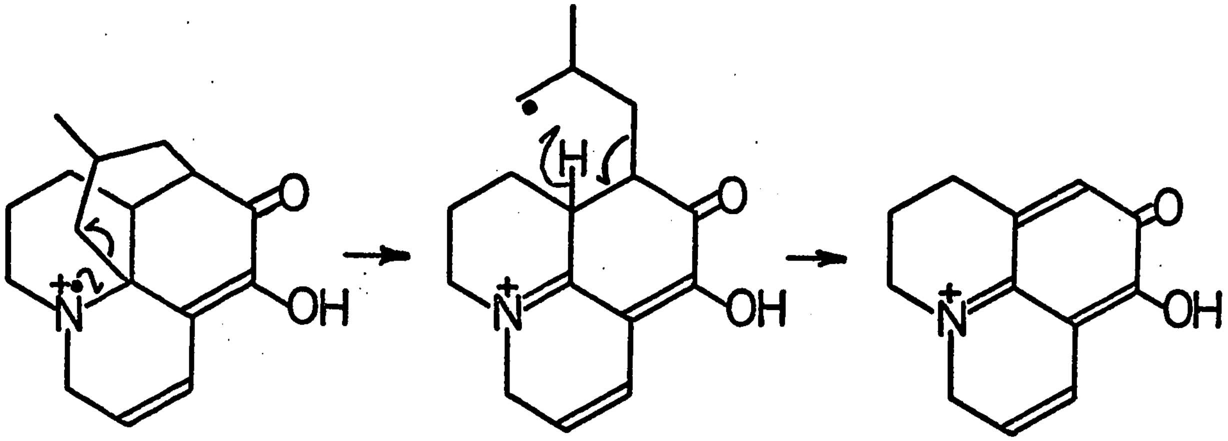


153

For this structure the predicted u.v. absorption[138] maximum in methanol solution would be at 315 mμ which is reasonably close to the observed value at 321 mμ.

The protons H_1, H_2 and H_AH_B are then believed to be responsible for the appearance in the n.m.r. of the doublet at τ3.29, the multiplet at τ4.17, and the AB quartet (with centers at τ6.05 and 7.00) respectively.

The formation of base peak in the mass spectrum at <u>m/e</u> 202 (M^+ - 57) is then explained by the following scheme.

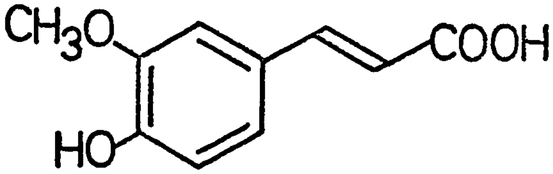


153 m/e 259

154

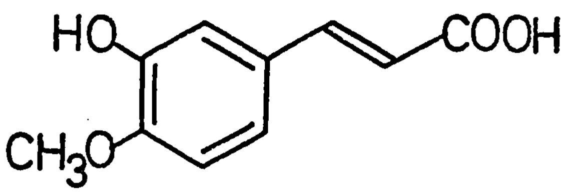
155 m/e 202

The presence of phenolic acids in various Lycopodium species has been known for some time. In 1958 Achmatowicz and Werner-Zamojska[139] reported the isolation of ferulic acid (156), isoferulic acid (157) and vanillic acid (3-methoxy-4-hydroxybenzoic acid) from L. annotinum, L. selago and L. clavatum. Several years later Towers and Maass[140], during their investigations of twenty-one Lycopodium species,



156

$C_{10}H_{10}O_4$ (m.p. 168-9°)



157

$C_{10}H_{10}O_4$ (m.p. 228-9°)

including L. lucidulum, found that the major component among the several phenolic acids which they isolated was ferulic acid.

On the one occasion that we studied the acidic constituents of L. lucidulum, a colorless crystalline component was isolated, m.p. 165-6°. In nujol the i.r. spectrum of the components (figure 43) shows many intense peaks including those at 3440, 1693, 1666, 1623, 1603 and 1520 $cm.^{-1}$. The u.v. spectrum determined in methanol shows absorption maxima at 218 $m\mu$ ($\varepsilon = 15{,}500$), 228 $m\mu$ (shoulder, $\varepsilon = 13{,}460$), 288 $m\mu$ ($\varepsilon = 13{,}820$), 303 $m\mu$ (shoulder, $\varepsilon = 13{,}580$), and 313 $m\mu$ ($\varepsilon = 14{,}680$). Upon basification the maxima are observed at 218 $m\mu$ ($\varepsilon = 14{,}190$), 242 $m\mu$ ($\varepsilon = 9{,}458$), 290 $m\mu$ (shoulder, $\varepsilon = 8{,}245$), 305 $m\mu$ ($\varepsilon = 10{,}670$), and 347 $m\mu$ ($\varepsilon = 16{,}730$). In pyridine-d_5 the n.m.r. of the component (figure 44) shows a three-proton doublet at τ 6.26 (J = 1 cps), an AB quartet (J_{AB} = 16 cps) with centers at τ3.23 and 1.95, each peak of which is further split (J = 1 cps). In the region τ 2.83 to 2.69 is a three-proton multiplet, while at τ -1.91 is a broad singlet, for which the integration suggests the presence of two protons.

The parent (and base) peak in the mass spectrum (figure 45) is at m/e 194. Other prominent peaks are observed at m/e 179, 133, 77 and 51. The molecular composition of the following ions was determined by high

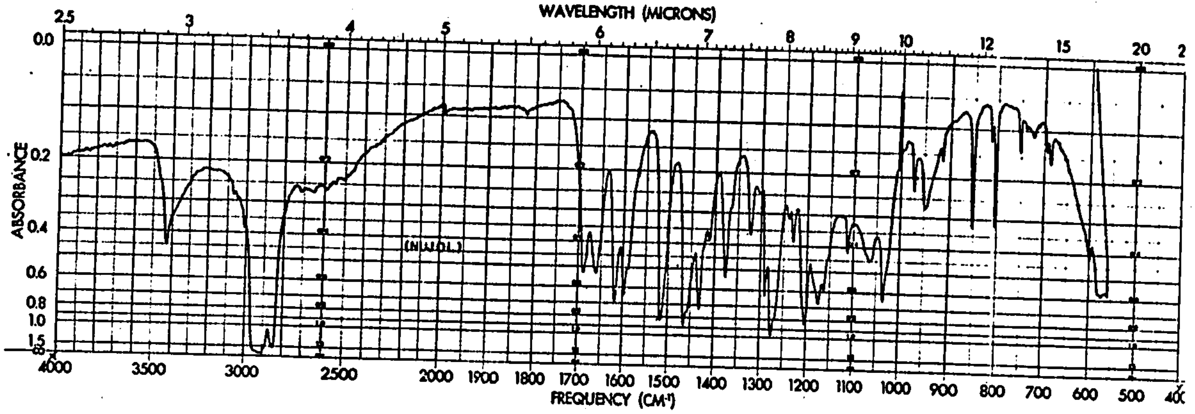


Figure 43

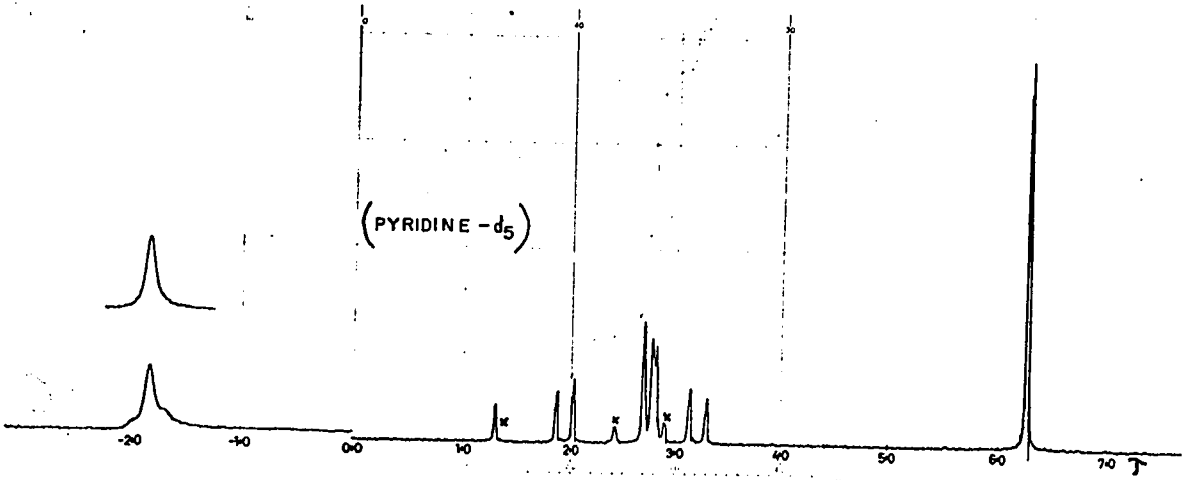


Figure 44

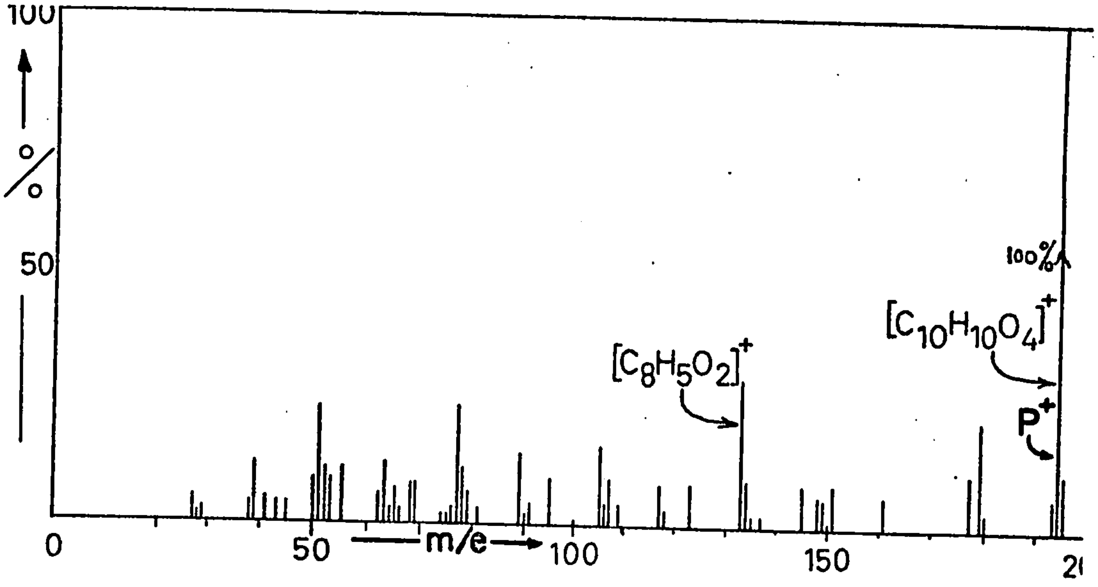


Figure 45

resolution mass spectrometry.

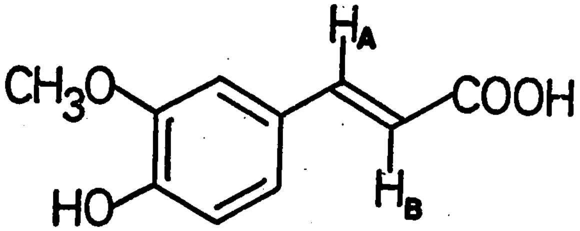
m/e Found	m/e Calculated	Molecular composition
194.0578	194.0579	$C_{10}H_{10}O_4$
133.0289	133.0290	$C_8H_5O_2$

The evidence of the i.r., u.v., n.m.r. and mass spectra led us to suspect that the isolated component was either ferulic acid (156) or isoferulic acid (157).

It was found that the mixing of an authentic sample of ferulic acid (m.p. 168-9°) with the isolated component caused no depression of the melting point. The treatment of the component with acetic anhydride in pyridine afforded a compound whose melting point (194-7°) corresponded[141] to the value reported for O-acetylferulic acid. The infrared spectrum of the product in nujol indicated the presence of a vinyl ester group (1770 $cm.^{-1}$).

The u.v. and i.r. spectra of the isolated component were identical with those of authentic ferulic acid. The n.m.r. spectra are very similar and it is suspected that the small difference in chemical shift of the signals is due to their concentration dependence.

All the evidence presented suggests that the isolated component is ferulic acid (156). The AB quartet is attributed to the vinyl protons H_A and H_B.



156

Ferulic acid has recently been isolated in these laboratories from *Lycopodium thyoides* by Dr. H. Katayama.

GENERAL EXPERIMENTAL

The isolation of the total crude alkaloid mixture was carried out by Mr. J. McCutcheon using L. lucidulum Michx collected near Fredericton, New Brunswick, Canada, and identified by Mr. M.A. Stillwell.

Infrared spectra were recorded on a Perkin-Elmer Model 421 dual grating or a Perkin-Elmer Model 337 grating infrared spectrophotometer.

Most of the ultraviolet spectra were determined on a Perkin-Elmer Model 202 spectrophotometer. Some of the ultraviolet spectra and all the optical rotatory dispersion and circular dichroism spectra were measured on a Durrum-Jasco Recording Spectropolarimeter Model 5.

The 220Mc nuclear magnetic resonance spectrum of lycolucine was measured on the 220Mc Proton Nuclear Magnetic Resonance Spectrometer at the Ontario Research Foundation, Sheridan Park, Ontario, through the courtesy of Dr. A.A. Grey. The other nuclear magnetic resonance spectra were measured on a Varian Associates Model A-60 or HR-100 spectrometer. Tetramethylsilane was used as an internal standard unless otherwise stated. The multiplicity of the signals is denoted by: s = singlet; d = doublet; t = triplet; q = quartet; and m = multiplet.

Most of the mass spectra were determined on an A.E.I. Model MS-9 or an A.E.I. Model MS-12 mass spectrometer and were recorded as a percentage of the most intense peak (base peak). The remaining mass spectra were determined on an A.E.I. Model MS-2H mass spectrometer. Unless otherwise stated the mass spectra were determined by using a heated inlet or direct probe (170-200°) and energy of 70 electron volts.

Gas liquid phase chromatographic measurements and separations were carried out on a Varian Aerograph A-90-P3 gas chromatograph.

The microanalyses were performed by A.B. Gygli, Toronto, Canada.

The biological testing of luciduline was performed at the Merck, Sharp and Dohme Research Laboratories, New Jersey, U.S.A., through the courtesy of Dr. N.G. Brink.

The alumina utilized for column and dry-column chromatography refers to British Drug Houses (B.D.H.) Alumina of activity III-IV (Brockmann scale).

Research Specialty Company aluminum oxide G was used for most of the thin layer (0.25 mm thickness) and preparative thin layer (0.5 mm) chromatography. More recently, Merck aluminum oxide G has been used. The thin layer chromatograms were visualized in an iodine chamber or with Dragendorff's reagent[144].

Melting points were determined on a Leitz-Wetzlar hot-stage melting point apparatus and are uncorrected.

EXPERIMENTAL

Isolation of Total Crude Alkaloids

In a typical procedure, finely ground *Lycopodium lucidulum* (5.8 kg) was percolated with methanol in a Soxhlet extractor for three days. After removal of most of the methanol by evaporation under reduced pressure, the residue was digested with aqueous hydrochloric acid (6%) and filtered. The insoluble portion was then digested with aqueous hydrochloric acid (10%) and again filtered. The combined filtrates were washed with ether, basified to pH 10-11 with ammonium hydroxide solution and then extracted with chloroform. Removal of the solvent from the dried ($MgSO_4$) chloroform solution yielded the total crude alkaloids (59g).

Isolation of the Weak and Strong Bases

The total crude alkaloids (59g) were dissolved in aqueous hydrochloric acid (10%) and the pH of the solution was adjusted to 5.5 by the addition of a saturated solution of disodium hydrogen phosphate. Extraction with methylene chloride yielded the weak bases (38g). The pH of the solution was then raised to 10-11 by the addition of ammonium hydroxide. Extraction with chloroform yielded the strong bases (14g).

The weak bases showed the following spectral characteristics: i.r. ($CHCl_3$) 2780 (N-methyl), 1700 (weak), 1620 (intense,amide) 1455, and 1380 cm.$^{-1}$; n.m.r. (CDCl$_3$) τ 9.05 (6H, m, C-CH$_3$) and 7.90 to 7.75 (6H, N-CH$_3$ and N-CO-CH$_3$).

Further Purification of the Weak Base Sample

1. Dry-column chromatography[99].

A length of polythene tubing (120 cm) with internal diameter 19 mm was clamped vertically and plugged at its lower end with a cork fitted with a cotton-packed open glass tube. Alumina (300g) was added to the tubing such that its upper level was about 10-15 cm from the top of the tubing. The weak base sample (3.0g) was introduced onto the upper surface of the adsorbent in the minimum volume of methylene chloride and then covered with a thin layer of alumina (about 1 cm), followed by a similar quantity of sand. The column was eluted with ethyl acetate-benzene (3:1) until the solvent front just reached the lower level of the column.

During the elution process the head of solvent in the tube above the upper surface of the adsorbent was kept at about 5 cm. The tubing was then cut into 33 equal sections and the alumina from each was transferred to an appropriately numbered flask and stored overnight in chloroform-methanol (19:1, 25 ml).

The contents of each section were monitored by t.l.c. and those sections containing a similar distribution of components were combined. In this way five fractions (1 to 5) were obtained. Fraction 1, containing the least polar material, consisted of a yellow oil (85 mg), which was found to be identical with an authentic sample of luciduline (33). Fraction 2 was crystalline and consisted of two components. Following recrystallization from ether a component (122 mg) was isolated which melted at 113-115°. The component was identical with an authentic sample of lycopodine (2).

The infrared spectra of the more polar fractions 3, 4 and 5, each of which consisted of a multicomponent mixture indicated the presence of the C_{30} weakly basic alkaloids; i.r.($CHCl_3$) 3350 (broad), 2780, 1700 (weak), and 1620 (intense) $cm.^{-1}$. No attempt was made to separate the components of these three fractions.

2. Column chromatography.

A sample of the weak bases (9.7g) was chromatographed over alumina (300g). Elution with benzene (900 ml) gave, after removal of the solvent, an oil (130 mg) which was identical with an authentic sample of luciduline. Elution with ether (1500 ml) gave a crystalline component (750 mg) which, after recrystallization from acetone, melted at 114-115°. The behavior of this component on t.l.c. and the appearance of its infrared spectrum determined in nujol, revealed it to be identical with lycopodine. Further elution with ether (1800 ml) gave fractions containing a component (243 mg) which, after recrystallization from ether-acetone (1:1), melted at 114-117° (i.e., similar to lycopodine), but whose spectral properties were vastly different from those of lycopodine. This component was found to be identical with an authentic sample of lycodine (12).

All the remaining fractions, which were isolated by elution with increasingly polar solvents, were non-crystalline and consisted of many components.

These fractions contained the C_{30} weakly basic alkaloids: i.r. ($CHCl_3$) 3350 (broad), 2780, 1700 (weak) and 1620 cm.$^{-1}$. They were combined so as to give a weakly basic sample from which most of the luciduline, lycopodine and lycodine had been removed.

3. Ten funnel countercurrent distribution.

This experiment was not designed to effect a complete separation of the components comprising the C_{30} bases, but rather with the intention of obtaining a supply of the latter from which most of the "non-C_{30}" alkaloids had been removed.

A sample of the weak bases (115g) was subjected to a ten funnel countercurrent distribution between chloroform (900 ml aliquots, stationary phase) and a saturated aqueous solution of potassium hydrogen tartrate (900 ml aliquots, moving phase, pH 3.0). On completion of the distribution, sodium chloride (50g) was added to the contents of each funnel so as to further saturate the aqueous phase, which was then basified to pH 10-11 with ammonium hydroxide. Extraction with chloroform and removal of the solvent under reduced pressure yielded ten samples which were then monitored by t.l.c. The combination of samples of similar composition led to the isolation of four fractions (<u>1</u> to <u>4</u>), as tabulated below.

Funnel samples	Fraction	Quantity (g)
1	<u>1</u>	36 (most weakly basic)
2-6	<u>2</u>	36
7-9	<u>3</u>	19
10	<u>4</u>	8 (most strongly basic)

The major component of fraction <u>1</u> was luciduline. The infrared spectra of fractions <u>3</u> and <u>4</u> in chloroform were characterized by intense absorption at 1700 cm.$^{-1}$ and somewhat less prominent absorption at 1620 cm.$^{-1}$. Fractions <u>3</u> and <u>4</u> each contained at least four components. The bulk of the C_{30} weakly basic alkaloids appeared to be in fraction <u>2</u> whose behavior on t.l.c. indicated the presence of at least six components. The infrared spectrum of fraction <u>2</u> determined in chloroform showed strong peaks at 2780 (N-methyl) and 1620 (amide) cm.$^{-1}$, indicating that it contained the C_{30} weakly basic alkaloids.

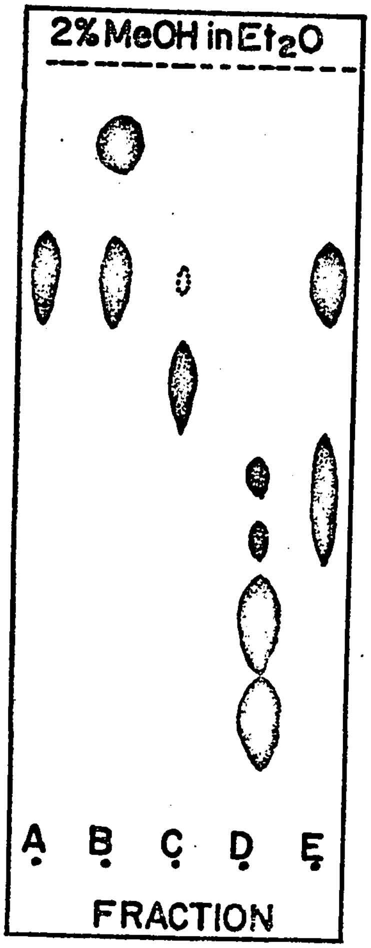
<u>100-Tube Countercurrent Distribution</u>[100]

<u>Isolation of Fractions A, B, C, D and E</u>.

This procedure has been applied either to weak base samples from which most of the luciduline, lycopodine and lycodine have been removed by one of the previously described processes or, directly to the original weak base sample. The experimental details that follow apply to the latter case.

The first 100 tubes of a Craig-Post 200 tube automatic countercurrent apparatus were used. Chloroform was used as the lower stationary phase and a saturated aqueous solution of potassium hydrogen tartrate (pH 3.0) comprised the upper moving phase. Equal volumes of chloroform and saturated potassium hydrogen tartrate (1,000 ml) were shaken together in a separatory funnel until the necessary volumes of each phase (5,000 ml) had been pre-equilibrated. The total volume of the aqueous solution was added to the large reservoir, while a standard volume (40 ml) of the organic phase was added to each of the tubes 2 to 99. A further quantity (500 ml) of the organic phase was added to the smaller reservoir. The weak base sample (8.0g) was dissolved in the pre-equilibrated chloroform and transferred to the tube zero. The washings from this initial transfer were made up to 40 ml and added to tube 1. The instrument which is designed to introduce a fresh supply (40 ml) of aqueous phase into tube 0 at the beginning of each cycle, was adjusted so as to ensure that the two phases were shaken together 20 times during each cycle and so as to allow a two minute interval for the phases to separate prior to the commencement of the next cycle. After the distribution was complete the contents of the 100 tubes were transferred to appropriately numbered flasks.

Sodium chloride (10g) was added to each flask and the contents were basified to pH 10-11 with ammonium hydroxide, then extracted with chloroform. The chloroform extracts were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The 100 alkaloid samples so obtained were examined by t.l.c. and similar samples were combined (see page 42). In this way five distinct fractions were obtained, named Fractions A, B, C, D and E, whose constituency and t.l.c. behavior is depicted below.



Tubes	Fraction	Components	Quantity (g)
0-12	A	2	0.7
13-25	B	3	0.4
26-40	C	1	0.3
41-85	D	4	5.5
86-99	E	2	1.1

The infrared spectra of Fractions A to E, determined in chloroform, can be summarized as follows :

Fractions A to D all exhibit prominent absorption at 2780 (N-methyl) and 1620 (amide) cm.$^{-1}$. Fractions A and B also show an intense band at 1700 cm.$^{-1}$. Fraction E shows an intense band at 1700 cm.$^{-1}$ but only weak absorption at 2780 and 1620 cm.$^{-1}$. The latter spectrum inferred that Fraction E contained a negligible amount of the weakly basic alkaloids.

Subsequent work also revealed that the five fractions contained the following distribution of alkaloids.

Fraction A	Dihydrolycolucine and the 483-Ketone.
Fraction B	Luciduline, dihydrolycolucine, and the 483-Ketone.
Fraction C	Lycolucine.
Fraction D	Lucidine-A, lucidine-B, the 481-compound, and the 483-compound.
Fraction E	Lycopodine, lycodine, anhydrolycodoline, and dihydroluciduline.

Before describing in more detail the procedure by which pure samples of all the weakly basic alkaloids have been obtained, it is appropriate to outline the most efficient method available for the isolation of luciduline and lycopodine.

Isolation of Luciduline and Lycopodine

Reasonable amounts (1g) of both of these alkaloids were needed so that their ^{13}C n.m.r. spectra could be taken. An abundant supply of lycopodine was also in constant demand for studies related to the synthesis of other *Lycopodium* alkaloids. Another reason for requiring an abundant quantity of luciduline, whose structure elucidation had been reported in 1968 by Ayer, Masaki, and Nkunika[19], was so that it could be sent for biological assay tests.

A sample of the weak bases (42g) was subjected to column chromatography over alumina (1 kg) according to the manner described earlier. From the fractions eluted with benzene a pure sample of luciduline (440 mg) was obtained.

The fractions eluted with ether gave, after removal of the solvent by evaporation under reduced pressure, a crystalline sample of lycopodine (1.2g). From the intermediate fractions a mixture (2.9g) of luciduline and lycopodine was isolated.

The mixture was subjected to a six funnel counter-current experiment, utilizing the usual organic and aqueous phases (200 ml aliquots), which led to the isolation of further amounts of pure luciduline (740 mg) and lycopodine (1.72g).

A portion of the luciduline sample (1.0g) was further purified by molecular distillation at 120° under vacuum (0.5 mm), and submitted for biological testing. The results of these tests are shown below.

Biological activity of luciduline (x = negative)

Parasitology in Vivo	x	Nervous System assays	x
Parasitology in Vitro	x	Cardiovascular studies	x
Agricultural Screening	x	Enzyme studies	x
Renal System assays	x	Mental Health	x
Reproductive Physiology	x	Chemotherapy	x

Luciduline was found to be inactive in all of the biological tests performed.

Isolation of Lucidine-A and Lucidine-B

Since 1967, extractions of more of the *L. lucidulum* plant material collected in 1964 have yielded samples of Fraction D containing such small quantities of lucidine-A and -B that we have been unable to isolate sufficient material to allow the continuation of chemical studies on these two components. A full account of the isolation of lucidine-A and -B from earlier extractions of the same plant material, in which they constituted the major components of the corresponding Fraction D, has been described by Nkunika[95]. Their isolation was then accomplished by repeated dry-column chromatography.

The lucidine-A so obtained was a white foam. Found: optical rotation $[\alpha]_D$ -21^o (C, 0.20, $CHCl_3$); mass spectrum *m/e* 467 (molecular ion,relative intensity 17), 273 (100), 260 (14), 259 (12), 218 (8), 217 (10), 166 (8), 164 (12), 163 (13); infrared ($CHCl_3$) 2780, 1620 cm.$^{-1}$; n.m.r. ($CDCl_3$) τ9.15 (6H, m, $-CH-\underline{CH_3}$), 7.97 and 7.94 ($N-COCH_3$), 7.87 and 7.86 ($N-CH_3$); n.m.r. (CD_3COOD) τ7.20 ($N-CH_3$).

Lucidine-B was similarly isolated by Nkunika as a white foam. Found: optical rotation $[\alpha]_D$ -46^o (C, 0.23, $CHCl_3$); mass spectrum *m/e* 467 (molecular ion, 44), 452 (5), 273 (100), 260 (81), 218 (23), 217 (26), 164 (17); infrared ($CHCl_3$) 2780, 1620 cm.$^{-1}$; n.m.r. τ 9.16 (3H, d, J = 6 cps, $-CH-\underline{CH_3}$) 8.93 (3H, d, J = 7 cps, $-CH-\underline{CH_3}$), 7.95 and 7.91 ($N-CO-CH_3$), 7.83 ($N-CH_3$).

Isolation of Lycolucine

The detection of lycolucine was facilitated by the fact that the alkaloid had a very characteristic u.v. spectrum. This showed an absorption maximum at 300 mμ when determined in methanol, and at 319 mμ when determined in acidified methanol.

The countercurrent fraction which contained lycolucine (Fraction C) was subjected to dry-column chromatography in the usual manner. In a typical procedure an amount of Fraction C (195 mg), obtained from 8.1 grams of weak bases, gave rise to a pure sample of lycolucine (40 mg).

Lycolucine was isolated as a colorless crystalline solid which, after recrystallization from acetone, melted at 198-200°. Found: optical rotation $[\alpha]_D$ +399° (C, 0.73, $CHCl_3$); mass spectrum m/e 461 (molecular ion, 71) 446 (19), 418 (23), 268 (100), 150 (22), 98 (34); infrared ($CHCl_3$) 2780, 1620, 1580, 1565 $cm.^{-1}$; infrared (nujol) 2780, 1630, 1580, 1560 $cm.^{-1}$; n.m.r. ($CDCl_3$) τ 9.10 (6H, m, $C-CH_3$) at 7.93, 7.89, 7.87 and 7.83 (total 6H, $N-CO-CH_3$ and $N-CH_3$, rotomeric effect), 7.50 (d, J = 6 cps), 7.16 (q, J = 4 cps), 7.01 (s), 6.89 (s), (6.53 (m), 6.39 (m),5.95 (m), 5.58 (m), 5.44 (m), 5.04 (m), 3.40 (1H, Ar-CH=C), 3.26 and 2.80 (2H, AB quartet, J_{AB} = 8 cps); u.v. (MeOH) 217 mμ (ϵ = 14,500) 261 mμ (ϵ = 8,800), 272 mμ (ϵ = 7,800) and 300 mμ (ϵ =10,200); u.v. (MeOH-HCl) 212, 258, 268, (each less intense than 217, 261 and 272 mμ maxima of previous spectrum respectively) and 319 mμ (ϵ = 15,000).

Isolation of Dihydrolycolucine and the 483-Ketone

The initial examination of Fraction A from the countercurrent distribution experiment indicated that it contained just one component. This same component appeared to be present in Fraction B (t.l.c.) together with a less polar component. The initial procedure was therefore designed to effect the removal of the less polar component from Fraction B so that the remaining material would essentially constitute a second batch of Fraction A.

Fraction B material (900 mg) from two countercurrent experiments was subjected to dry-column chromatography over alumina (200g) using ethyl acetate-benzene (3:1) as eluent. The usual procedure led to the isolation of two major fractions. Fraction 1, made up of the less polar material, consisted of a yellow oil (89 mg) which was found to be identical with an authentic sample of luciduline. Fraction 2, containing the more polar material (33 mg) was identical (t.l.c.) with the material of Fraction A.

Fraction 2 was combined with the Fraction A material from two countercurrent experiments and the total amount (1.34g) was subjected to dry-column chromatography under the same conditions as described above. A quantity of yellow gum (72 mg) was isolated, which was originally named the 1620H compound. Found: mass spectrum *m/e* 483 (13), 463 (86), 455 (100), 270 (78), 261 (29), 248 (33), 247 (33), 233 (36), 205 (96), 192 (63) and 150 (32); infrared ($CHCl_3$)

2780, 1703, 1620, 1580, 1560 cm.$^{-1}$; u.v. (MeOH) 216 and 276 mμ; u.v. (MeOH-HCl) 217 and 279 mμ.

Several features about the spectra of the 1620H compound led us to suspect that it was a mixture of two components, even though preliminary t.l.c. studies had only indicated the presence of one component. The spectra bore a strong resemblance to those of a sample of dihydrolycolucine that had been prepared by catalytic hydrogenation of lycolucine at one atmosphere. The u.v. spectra in methanol and acidified methanol of the dihydrolycolucine so prepared were identical with those of the 1620H compound. The dihydrolycolucine also exhibited intense absorption in the i.r. at 1580 and 1560 cm.$^{-1}$ and showed parent and base peaks in its mass spectrum at m/e 463 and 270 respectively.

Accordingly the 1620H compound (72 mg) was subjected to column chromatography over alumina (4g) in an attempt to isolate any naturally occurring dihydrolycolucine. The first 18 fractions (75 ml each) eluted with Skelly B-benzene (1:1), benzene, and benzene-ether (99:1) yielded a small amount of material (3 mg) whose ultraviolet and mass spectra were identical with those of a dihydrolycolucine sample prepared from lycolucine. Found: u.v. (MeOH) 216 mμ and 275 mμ (ε = 8,000); u.v. (MeOH-HCl) 215 mμ and 280 mμ (ε = 12,500); mass spectrum m/e 463 (molecular ion, 47), 448 (18), 420 (19), 270 (100), 150 (20); infrared ($CHCl_3$)

2775, 1620, 1580, 1565 cm.$^{-1}$

Further elution with benzene-ether (99:1) and benzene-ether (19:1) gave, upon evaporation of the solvent, a yellow gum (25 mg) which was slightly more polar on t.l.c. than the dihydrolycolucine. Found: mass spectrum m/e 483 (molecular ion, 16), 455 (100), 426 (15), 261 (18), 248 (20), 247 (23), 233 (23), 205 (55), 192 (52), 166 (13), 164 (10), 150 (12); infrared ($CHCl_3$) 2780, 1710, 1620, 1450, 1020, 955 cm.$^{-1}$; infrared (CCl_4) 2780, 1705, 1640, 1440, 1020, 955 cm.$^{-1}$; n.m.r. ($CDCl_3$) τ 9.18 (3H, d, J = 6 cps, $-CH-\underline{CH_3}$), 9.12 (3H, d, J = 6 cps , $-CH\ \underline{CH_3}$), 7.98 (3H, s, $N-COCH_3$), 7.93 (3H, s, $N-CH_3$); o.r.d. (C, 0.17, MeOH) $[\Phi]_{344\ m\mu} = +12,500^\circ$, $[\Phi]_{296\ m\mu} = -17,050^\circ$, $[\Phi]_{247\ m\mu} = -26,120^\circ$.

This component was named the 483-Ketone.

Isolation of the 481 and 483 Compounds

As mentioned earlier, the 481 and 483 compounds now completely dominate the constituency of the Fraction D, obtained by countercurrent distribution of the weak bases. Most of the chemical studies related to these compounds have been associated with their respective sodium borohydride reduction products which, unlike the parent compounds, are readily separated via column chromatography.

Before it was realized that the reduction products were so easily separated, many attempts had been made to separate pure amounts of the parent alkaloids and a typical procedure is described overleaf.

The spectral properties of the two component mixture contained in Fraction D can be summarized as follows; infrared (CCl_4) 3300 (broad), 2790, 1645, 1380 cm.$^{-1}$; mass spectrum 483 (45), 481 (100), 465 (78), 287 (69), 276 (69), 271 (74), 233 (81), 205 (78), 192 (69), 164 (94).

The Fraction D mixture (1.0g) was subjected to dry-column chromatography over alumina (250g), using ethyl acetate-benzene (3:1) as solvent. The sections from the column were analyzed by t.l.c. in the usual manner and two fractions (1 and 2) were obtained. Fraction 1 (520 mg) consisted mainly of the unseparated components of the original mixture, while the content of fraction 2 (120 mg) was dominated by the more polar component of the original sample

Fraction 1 was subjected to a further dry-column chromatography and the usual work up gave a small amount of material (26 mg) which appeared to consist solely of the less polar component (t.l.c.). Found: infrared ($CHCl_3$) 3350 (broad), 2790, 1620, 1450, 1375, 1135, 1020, 990, 957 cm.$^{-1}$; mass spectrum m/e 483 (molecular ion, 70), 465 (71), 289 (27), 287 (27), 276 (50), 271 (100), 233 (52), 205 (59), 192 (62), 166 (60), 164 (70), 124 (50); n.m.r. ($CDCl_3$) τ 9.09 (6H, two overlapping doublets, J = 6 cps, 2-CH-CH_3), 7.94 and 7.91 (total 3H, N-$COCH_3$, rotomeric effect), 7.78 (3H, s, N-CH_3). The spectra indicated that fraction 1 contained the 483 compound.

After fraction 2 had been subjected to a further dry-column chromatography a trace of material (5 mg) was obtained, whose t.l.c. indicated that it represented the more polar alkaloid of the original mixture. Found: infrared 3400 (broad), 2795, 1620, 1450, 1370, 1130, 1090, 970, 950, 910 cm.$^{-1}$; mass spectrum *m/e* 481 (molecular ion, 54), 287 (100), 273 (22), 246 (26), 231 (24), 203 (36), 164 (79), 162 (81), 124 (79). These spectra indicated that fraction *2* consisted of the 481 compound.

A further supply of this compound (15 mg) was obtained recently from the contents of one tube in a 100 tube countercurrent experiment. This experiment had been performed on a weak base sample which had initially been partially separated *via* a ten funnel countercurrent experiment. Found: n.m.r. ($CDCl_3$)τ 9.16 (6H, m, CH-$\underline{CH_3}$), 8.04 and 8.02 (3H, N-$COCH_3$), 7.80 (3H, s, N-CH_3).

Lucidine-A and Lucidine-B

Lack of material prevented the continuation of experimental work on lucidine-A and -B. The discussion of these alkaloids in this thesis was based upon experimental studies performed earlier and described by Nkunika[95].

In summary it will suffice to mention that the acetylation, hydrolysis, dehydrogenation and sodium borohydride reduction of these alkaloids were carried out by methods analogous to those utilized in the following text.

Rotomerism of Lycolucine

A comparison of the 100 Mc and 220 Mc n.m.r. spectra of lycolucine (15 mg) in deuterochloroform was undertaken to obtain conclusive evidence that the signals at τ 7.93, 7.89, 7.87 and 7.83 in the former spectrum, ascribed to the N-COCH$_3$ and N-CH$_3$ groups, are associated with a rotomeric effect.

That the chemical shifts of the four signals are unchanged when the spectrum is determined in 220 Mc is consistent with this hypothesis. N.m.r. spectroscopy is therefore detecting differences in chemical environment of the N-COCH$_3$ and N-CH$_3$ groups associated with two rotomeric forms. The four signals are then interpreted as being due to two overlapping pairs of singlets.

Further evidence to support this concept results from a study of the τ 8.0 to 7.5 region of the 100 Mc spectrum at various temperatures. Found: n.m.r. (CDCl$_3$ at + 25°) τ 7.93, 7.89, 7.87 and 7.83 (all of similar intensity); n.m.r. (CDCl$_3$ at -20°) τ 7.89 and 7.79 (intense singlets), 7.85 (broad peak of weak intensity). The temperature study suggested that in the preferred rotomeric form of lycolucine the N-COCH$_3$ and N-CH$_3$ groups absorb at τ 7.89 and 7.79 respectively.

Preparation of Dihydrolycolucine

Lycolucine (12 mg) in methanol (10 ml) was subjected to catalytic hydrogenation at atmospheric pressure over Adams' catalyst (platinum oxide, 10 mg) for 24 hours. The catalyst was removed by filtration. After removal of the solvent by evaporation under reduced pressure the product, dihydrolycolucine, was isolated as a pale yellow gum (10 mg). On t.l.c. (ether-methanol, 99:1) dihydrolycolucine was slightly less polar than lycolucine. Found: infrared ($CHCl_3$) 2780, 1620, 1580, 1565, 1450, 1370 cm.$^{-1}$; u.v. (MeOH) 229 mμ, 274 mμ (ε = 8,500); u.v. (MeOH-HCl) 223 mμ, 280 mμ (ε = 13,400); mass spectrum 463 (molecular ion, 48) 448 (18), 420 (18), 270 (100), 150 (20), 98 (14).

Preparation of Desacetyllycolucine

Lycolucine (18 mg) was refluxed with aqueous sulphuric acid (15% by volume 12 ml) for 72 hours. The reaction mixture was diluted with cold water (30 ml), basified to pH 11 with ammonium hydroxide and extracted with methylene chloride (3 x 20 ml). The combined methylene chloride extracts were dried ($MgSO_4$) and yielded, after removal of the solvent, a yellow gum (16 mg). The desacetyllycolucine so produced was homogeneous by t.l.c. (ethyl acetate) and of lower R_f (more polar) than lycolucine. Found: infrared ($CHCl_3$) 2780 (N-methyl), 1665 (double bond), 1585 and 1565 (aromatic), 1470, 1380, 890 cm.$^{-1}$; infrared (CCl_4) 2775, 1660, 1585, 1560, 1470, 1380, 890, 878 cm.$^{-1}$; u.v. (MeOH) 219, 262, 273 and 299 mμ; u.v. (MeOH-HCl) 216, 262, 273 and 319 mμ; mass spectrum 419 (molecular ion, 22), 404 (2), 295 (9), 270 (42), 268 (80), 255 (56), 152 (10), 150 (100), 98 (18); n.m.r. ($CDCl_3$) τ 9.06 (6H, m, C-CH_3), 7.89 (3H, s, N-CH_3), 7.48 (d, J = 6 cps), 7.26(s), 7.17(s), 3.39 (1H,

$ArCH{=}C$), 3.29 and 2.80 (2H, AB quartet, $J_{AB} = 8$ cps).

Regeneration of Lycolucine from Desacetyllycolucine

A solution of desacetyllycolucine (14 mg) in pyridine (1 ml) and acetic anhydride (1 ml) was magnetically stirred for 24 hours at room temperature. After removal of the excess reagents by evaporation under reduced pressure, the residue was basified with aqueous sodium bicarbonate solution (10%) and extracted with chloroform (3 x 20 ml). The combined chloroform extracts were dried ($MgSO_4$) and yielded, after removal of the solvent, a crystalline material (4 mg) which was identical with an authentic sample of lycolucine.

Reaction of Lycolucine with Hydrobromic Acid

Lycolucine (4 mg) was dissolved in acetone (1 ml). Hydrobromic acid (1% by volume) in acetone was added until the solution showed red on litmus paper. The solvent was removed and the precipitate was dissolved in methanol (2 drops). Acetone was added until the solution remained turbid. The solution was stored at 0° for one week. The material could not be induced to crystallize and was not examined further.

Reaction of Lycolucine with Hydroperchloric Acid

Hydroperchloric acid (1% by volume) in acetone was added to a solution of lycolucine (4 mg) in acetone (1 ml) until the solution showed red on litmus paper. The usual work up gave a yellow gum (4 mg) which could not be induced to crystallize.

Reaction of Lycolucine with Iodomethane

Lycolucine (10 mg) was dissolved in acetone (2 ml). After the addition of an excess of iodomethane (5 drops) the mixture was refluxed for three days. Removal of the solvent and excess reagent led to the isolation of a yellow gum which, by its appearance on t.l.c., consisted of a mixture of unchanged lycolucine and a more polar component. The separation of the mixture was achieved by preparative t.l.c. over alumina utilizing benzene-ethylacetate-methanol (2:2:1) as the developing solvent system. By this process the more polar material (5 mg) was isolated as a yellow oil. Found: infrared ($CHCl_3$) 3400 (broad), 1620, 1590, 1565 cm.$^{-1}$, i.e., no absorption corresponding to an $N-CH_3$ group; u.v. (MeOH) 220, 260, 283, 302 (shoulder) mμ; u.v. (MeOH-HCl) 220, 263, 320 mμ, i.e., similar to lycolucine.

In an attempt to purify the component the sample was subjected to evaporative distillation at 260° under vacuum (0.1 mm). The distillate (1 mg) was isolated as a yellow oil. Found: mass spectrum <u>m/e</u> 476 (highest peak in spectrum, 2), 475 (4), 461 (17), 268 (23), 254 (9), 235 (8), 150 (10), 98 (7), 58 (100); u.v. (MeOH) 218, 270 and 303 mμ; u.v. (MeOH-HCl) 216, 273, 326, 360 (shoulder) mμ;

The u.v. spectra indicated that the original chromophore of lycolucine had been extended. It was considered that the process of evaporative distillation had brought about a Hofmann degradation upon the originally formed methiodide salt.

Reaction of Lycolucine with Ozone[112].

Ozonized oxygen generated by a Welsbach T-23 Laboratory Ozonator was bubbled (rate 0.02 $ft^3 \cdot min^{-1}$) into ethyl acetate (40 ml) at -78° until the solvent acquired a deep blue color. This solution was then added in small quantities to lycolucine (30 mg) in ethyl acetate (20 ml) at -78° until the reaction mixture attained a permanent blue color. After standing at -78° for one hour the mixture was allowed to warm up to room temperature. Palladinized charcoal (50 mg of 5%) was added and the sample was hydrogenated for 12 hours at room temperature. The usual work up yielded a quantity (22 mg) of material which, by its appearance on t.l.c., was mainly unchanged starting material. This material in methylene chloride (30 ml) was filtered through alumina (2g). Evaporation of the solvent yielded crystalline material which was identified as lycolucine. No other products were isolated.

Preparation of Dioxolycolucine

Lycolucine (50 mg) was dissolved in a magnetically stirred mixture of dioxane (50 ml), acetic acid (10 ml) and water (15 ml). To the resultant solution at 0° was added osmium tetroxide (5 mg). Over the duration of 1 hour an aqueous solution of sodium metaperiodate (200 mg in 2 ml H_2O) was added. The solution was then stored for 3 hours after which the remainder of the osmium tetroxide (5 mg) was added.

After the reaction mixture had been stood for a further 18 hours it was poured into water (100 ml). The solution was basified with sodium carbonate containing a trace of sodium hydroxide, then extracted with methylene chloride (4 x 50 ml). Removal of the solvent from the dried ($MgSO_4$) extracts afforded a dark brown oil (93 mg). An inspection of the material by t.l.c. (ethyl acetate-methanol 99:1) indicated that two components were present, the less polar of which was unchanged lycolucine.

The black residue which was present in the crude product was removed _via_ filtration of the sample in chloroform through alumina (1g). The resultant product, isolated as a yellow gum, was then chromatographed over alumina (2g). The first five 5 ml fractions eluted with benzene yielded unchanged lycolucine (28 mg). From the next two 5 ml fractions, also eluted with benzene, a yellow oil was obtained (6 mg) which was homogeneous on t.l.c. and corresponded to the more polar component of the original crude product. Found: infrared ($CHCl_3$) 3570 (weak), 2790 ($N-CH_3$), 1680 (C=O), 1618 ($N-COCH_3$), 1590, 1560, 1450, 1020 cm.$^{-1}$; u.v. (MeOH) 218, 276 and 282 (shoulder) mμ; u.v. (MeOH-HCl) 217 and 282 mμ; mass spectrum _m/e_ 493 (molecular ion, 100), 475 (83) 369 (75), 300 (100), 192 (50), 185 (55), 150 (100), 98 (47), 70 (100). This compound was named dioxolycolucine.

The preparations of desacetyldihydrolycolucine and octahydrodesacetyllycolucine, originally carried out by

Nkunika[95] were not repeated.

Preparation of N-acetyllycodine (101)

This synthesis was undertaken at a time when it was contemplated that the structures of lycolucine and dihydrolycolucine might incorporate an N-acetyllycodine system. We were particularly interested to discover whether the n.m.r. spectrum of such a system would exhibit a rotomeric effect.

A sample (45 mg) of lycodine (12) was dissolved in a mixture of pyridine (2 ml) and acetic anhydride (1 ml). After the solution had been standing at room temperature for 12 hours the solvents were evaporated under reduced pressure. The usual work up afforded N-acetyllycodine (101) as a colorless gum (44 mg), which was less polar than lycodine on t.l.c. (benzene-ethyl acetate 3:1). Found: infrared ($CHCl_3$) 1635, 1575, 1570, 1400, 1375, 960, 870 $cm.^{-1}$; u.v. (MeOH) 224, 269 and 276 (shoulder) $m\mu$; u.v. (MeOH-HCl) 224 and 273 $m\mu$; mass spectrum m/e 284 (molecular ion, 100), 269 (10), 241 (38), 227 (76), 199 (13), 185 (93), 157 (13); n.m.r. ($CDCl_3$) τ 9.16 (3H, d, J = 5 cps, -CH-$\underline{CH_3}$), 7.76 (3H, s, N-$COCH_3$, no rotomeric effect), at 2.90, 2.42 and 1.60 (three quartets associated with β, γ and α protons of the pyridine ring, $J_{\alpha\gamma}$ = 2 cps, $J_{\alpha\beta}$ = 5 cps, $J_{\beta\gamma}$ = 8 cps).

Attempted Oxidation of the 483-Ketone

The 483-Ketone (10 mg) was dissolved in acetone (5 ml). To the magnetically stirred solution was added Jones' reagent

(6 drops). Methanol (10 drops) was then added and the solution evaporated to near dryness. The residue was partitioned between dilute aqueous sodium bicarbonate (10%, 20 ml) and chloroform (25 ml). The dried ($MgSO_4$) chloroform solution was evaporated to yield a yellow gum (6 mg) which was found to be unchanged starting material (t.l.c., i.r. and mass spectrum).

Reaction of the 483-Ketone with Sodium Borohydride

To a magnetically stirred sample of the 483-Ketone (73 mg) in methanol (10 ml) at 0^o was added sodium borohydride (100 mg) over a period of 1 hour. The solution was then stirred for a further 15 hours, after which most of the methanol was removed by evaporation. Water (20 ml) was added to the residue and the resultant mixture was extracted with methylene chloride (3 x 25 ml). Removal of the solvent afforded a colorless gum (40 mg), which was more polar than the starting material on t.l.c. (ethyl acetate). Found: infrared (CCl_4) 2780, 1632, 1440, 1380, 1040, 1016 $cm.^{-1}$; n.m.r. ($CDCl_3$) τ9.12 (6H, 2d, J = 6 cps, 2-CH-$\underline{CH}_3$ groups), 7.93 and 7.92 (total 3H, N-$COCH_3$), 7.87 (3H, s, N-CH_3); mass spectrum m/e 485 (molecular ion, 35) 468 (5), 467 (5), 429 (9), 428 (9), 278 (10), 277 (15), 235 (10), 207 (100), 205 (51), 192 (30), 150 (10). This product was named the dihydro-483-Ketone.

Acetylation of the Dihydro-483-Ketone

A sample of the dihydro-483-Ketone (30 mg) in pyridine

(1 ml) and acetic anhydride (1 ml) was kept overnight at room temperature. The usual work up afforded a colorless gum (20 mg) which consisted predominantly of a component which was less polar on t.l.c. (benzene-ethyl acetate, 1:2) than the dihydro-483-Ketone. Following column chromatography over alumina (1g) a pure sample (t.l.c.) of the component was obtained. Found: infrared (CCl_4) 2780, 1734 (OAc), 1640 (amide), 1380, 1025 cm.$^{-1}$; mass spectrum m/e 527 (molecular ion, 34), 467 (71), 273 (69), 259 (61), 217 (41), 207 (62), 205 (100), 192 (40), 150 (25). This product was named the O-acetyldihydro-483-Ketone.

Reaction of the Dihydro-483-Ketone with Iodomethane

A sample of the dihydro-483-Ketone (55 mg) was dissolved in acetone (2 ml) containing an excess of iodomethane (0.05 ml). The reaction mixture was stood at room temperature for 4 days, after which the solvent and excess reagent were removed by evaporation. An inspection of the gummy product on t.l.c. (ether-methanol 49:1) revealed the presence of two components. One of these was less polar than the starting material, while the other was more polar than the latter. They were separated by column chromatography over alumina (2g). Found, for the less polar product: infrared (CCl_4) 2780, 1645 cm.$^{-1}$; mass spectrum m/e 499 (molecular ion, 43) 481 (26), 291 (100), 273 (66), 221 (92), 220 (85), 219 (85). The spectra suggest that an N-methyl derivative of the dihydro-483-Ketone has been formed. The more polar product was not further characterized.

Acetylation of a Mixture of the 481 and 483 Compounds

After a mixture (10 mg) of the 481 and 483 compounds in pyridine (0.5 ml) and acetic anhydride (0.5 ml) had been stirred overnight at room temperature, the usual work up yielded only unchanged starting material (t.l.c. and mass spectrum).

When the reaction, involving similar quantities, was performed under refluxing conditions, a quantity (4 mg) of a yellow gum was isolated which was more polar on t.l.c. (ethyl acetate) than either of the original components. Found: infrared ($CHCl_3$) 2780 (v. weak), 1665 (shoulder), 1620 cm.$^{-1}$; mass spectrum, prominent peaks at *m/e* 507, 464, 205, 177, 161, 155, 133, 71, 43.

Sodium Borohydride Reduction of a Mixture of the 481 and 483 Compounds

Over a period of 2 hours sodium borohydride (4g) was added to a magnetically stirred solution of the 481 and 483 compounds (1.60g) in ice-cold methanol (25 ml). After the solution had been stored at 0° for a further 12 hours most of the solvent was removed by evaporation under reduced pressure. Water (50 ml) was added and the resultant mixture was extracted with methylene chloride (4 x 35 ml). The combined extracts were dried ($MgSO_4$) and the solvent removed in the usual manner. A colorless gum (1.45g) was obtained, which consisted of two components (t.l.c. ethyl acetate) one of which was less polar and the other more polar than either of the starting materials. The mixture of products was adsorbed onto alumina (60g) and subjected to column chromatography. Elution with ether-chloroform (19:1, 1500 ml) afforded the less polar reduction product (422 mg). Found: infrared (CCl_4) 3400 (broad), 2790, 1640, 1440, 1380 cm.$^{-1}$; mass spectrum m/e 483 (molecular ion, 25), 468 (82), 467 (100), 277 (58), 260 (44), 259 (58), 235 (77), 192 (33), 163 (57), 150 (21), 98 (22); n.m.r. ($CDCl_3$) τ 9.19 (3H, d, J = 6 cps, $-CH-\underline{CH_3}$), 8.95 and 8.92 (total 3H, two overlapping doublets, $-CH-CH_3$, possible rotomeric effect), 7.98 and 7.95 (total 3H, $N-COCH_3$), 7.84 (3H, s, $N-CH_3$), prominent multiplet peaks at 7.35, 7.29, 6.91 and 6.78. This component was termed the less polar reduction product.

Continued elution with ether-chloroform (3:1, 600 ml) and chloroform (300 ml) yielded the more polar component (258 mg). Found: infrared (CCl_4) 3420 (broad), 2780, 1640, 1440, 1370 cm.$^{-1}$; mass spectrum m/e 485 (molecular ion, 6), 468 (37), 467 (100), 291 (24), 277 (50), 273 (50), 260 (29), 259 (78), 235 (29), 192 (32), 164 (39), 163 (48), 150 (25). The component was termed the more polar reduction product.

Acetylation of the More Polar Reduction Product

A solution of the more polar reduction product (20 mg) in pyridine (1 ml) and acetic anhydride (1 ml) was stirred for 24 hours at room temperature. The reaction product (24 mg) was isolated in the usual manner and appeared as a less polar spot on t.l.c. (ethyl acetate-methanol, 49:1) than the starting material. Found: infrared (CCl_4) 3350 (v.weak), 2775 (N-methyl), 1735 (O-acetyl), 1640 (N-acetyl) $cm.^{-1}$. The spectrum suggests the presence of an O-acetyl derivative.

Acetylation of the Less Polar Reduction Product

The less polar reduction product (20 mg) was dissolved in a mixture of pyridine (1 ml) and acetic anhydride (1 ml) and the resultant solution was stirred for 24 hours at room temperature. The usual work up yielded a pale yellow gum (20 mg) which was more polar on t.l.c. (ethyl acetate) than the starting material.

Found: infrared (CCl_4) 3400 (broad), 2775, 1640, 1624 (shoulder) $cm.^{-1}$; mass spectrum 527 (molecular ion, 4), 526 (6), 510 (55), 509 (100), 484 (8), 466 (18), 423 (4). 259 (11), 192 (55), 164 (24). The spectra suggest that an N-acetyl derivative of the less polar reduction product has been formed.

Reaction of the N-acetyl Derivative of the Less Polar Reduction Product with Thionyl Chloride

This experiment, designed to effect the dehydration of the N-acetyl derivative, followed the method described by Iverach[142]. Thionyl chloride (1 ml) was added to a stirred solution of the N-acetyl derivative (20 mg) in methylene chloride (20 ml) and the mixture was stood at room temperature for 3 hours. The solvent and excess reagent were removed by evaporation under reduced pressure, after which the gummy residue was dissolved in aqueous hydrochloric acid (25 ml, 10%). After extraction with ether (1 x 25 ml) had removed non-basic material the aqueous solution was basified with ammonium hydroxide and then extracted with chloroform (3 x 25 ml). After removal of the solvent from the combined and dried ($MgSO_4$) extracts, a yellow gum (25 mg) was isolated which, on t.l.c. (ethyl acetate) appeared to be a mixture of the starting N-acetyl derivative and a less polar component. Column chromatography over alumina (1g) effected a separation and the less polar component was isolated as a yellow oil (12 mg). Found: infrared (CCl_4) 2780, 1640, 1380, 1030, 885, 870 $cm.^{-1}$; mass spectrum m/e 509 (molecular ion, 100) 467 (12), 466 (33), 259 (12), 257 (10).

Attempted Oxidation of the Less Polar Reduction Product

A sample of the less polar reduction product (10 mg) was dissolved in acetone (2 ml). Jones' reagent (4 drops) was added, followed 10 minutes later by methanol (4 drops). The solution was evaporated almost to dryness, after which it was basified with aqueous sodium bicarbonate (5%) and extracted with chloroform (3 x 20 ml). The yellow gum so obtained (10 mg) was identical with the starting material (t.l.c. and i.r.).

Reaction of the Less Polar Reduction Product with Hydrobromic Acid

Hydrobromic acid (1% solution in acetone) was added to a solution of the sample (10 mg) in acetone (2 ml) until the solution showed red on litmus paper. The solvent was removed and the residual white solid was dissolved in methanol (2 drops). Acetone was added until the solution remained turbid and the mixture was kept overnight. A colorless crystalline product (10 mg) was formed which, after recrystallization from acetone-methanol, melted at 227-230°. Found: infrared (nujol) 3320 (broad), 3105 (broad), 1650, 1555 cm.$^{-1}$.

Reaction of the Less Polar Reduction Product with Hydroperchloric Acid

By a method analogous to that described above for the reaction with hydrobromic acid, a colorless crystalline product (11 mg) was obtained, m.p. 256-259°. Found: infrared (nujol) 3470 (broad), 3205, 3150, 1620 (shoulder), 1584 (intense), 1100 (broad, intense, Cl=O).

Reaction of the Less Polar Reduction Product with Iodomethane

To a sample of the less polar reduction product (213 mg) in acetone (2 ml) was added an excess of iodomethane (0.5 ml). The solution was stored at room temperature for 4 days. Globular crystals formed which were separated by filtration and washed with acetone. The crystals so obtained (97 mg) melted at 198-201°. Further evaporation of the solvent from the original mother liquor led to the isolation of a further quantity (45 mg) of crystalline material, which melted at 159-164°. Recrystallization of the former crystalline sample from methanol-acetone (2:1) afforded crystals (58 mg) which melted at 221-224°. Recrystallization of the original sample melting at 159-164°, from acetone-ether (3:1) afforded crystals which melted at 161-164°.

(In the following text the recrystallized samples melting at 221-224° and 161-164° shall be referred to as the higher melting and lower melting iodomethane derivatives respectively).

The Lower Melting Iodomethane Derivative

This compound, which is the more soluble of the two derivatives, showed the following spectral characteristics:

Infrared (nujol) 3330 (broad), 2780 (weak), 1610, 1460, 1380 cm.$^{-1}$; mass spectrum m/e 499 (highest peak, 3) 482 (40), 481 (100), 466 (20), 464 (20), 291 (94), 275 (20), 274 (59), 273 (65), 190 (23); n.m.r. ($CDCl_3$) τ 9.09 (3H, d, J = 5 cps, CH-$\underline{CH_3}$), 8.93 (3H, d, J = 7 cps, CH-$\underline{CH_3}$), 7.97 (3H, s, N-$COCH_3$), 7.89 (6H, s, possibly two N-CH_3 groups); n.m.r. (CF_3COOH) τ 8.96 (3H, d, J = 5 cps), 8.80 (3H, d, J = 7 cps), 7.41 (3H, s, N-$COCH_3$), 6.90 (3H, s, N-CH_3), 6.76 (3H, s, N-CH_3).

It was mentioned in the discussion (page 98) that this compound was possibly the hydroiodide salt of an initially formed N-methyl derivative of the less polar reduction product ($C_{30}H_{51}N_3O_2$). The compound was submitted for elemental analysis.

Calculated for $C_{31}H_{54}N_3O_2I$: C,59.31; H,8.69; N,6.69.
Found: C, 58.15, 57.94; H, 9.02, 8.74; N,6.58.

Reaction of the Lower Melting Iodomethane Derivative with Potassium t-Butoxide

This reaction, which was undertaken in an attempt to degrade the compound, was carried out according to the procedure reported by French and MacLean[143]. The iodomethane derivative (98 mg) was dissolved in a mixture of t-butanol (12 ml) and benzene (4 ml). Potassium t-butoxide (250 mg) was added and the solution was refluxed for 18 hours. After cooling, water (20 ml) was added and the organic solvents were removed by evaporation under reduced pressure. Extraction with chloroform (3 x 30 ml) afforded a yellow gum (48 mg). Found: infrared (CCl_4) 3400 (broad) 2790 (v.intense, N-CH_3), 1640 (amide) $cm.^{-1}$. The spectrum which was very well defined in the region 1460 to 850 $cm.^{-1}$ closely resembled that of the original less polar reduction product. N.m.r. ($CDCl_3$) τ 9.16 (3H, d, J = 6 cps) 8.92 and 8.89 (total 3H, two overlapping doublets, J = 7 cps, rotomeric effect), intense signals at 7.96, 7.92, 7.91, 7.89 and 7.82 (total 9H, N-$COCH_3$ and two N-CH_3 groups); n.m.r. (CD_3COOD) τ 9.07 (3H, d, J = 5 cps), 8.86 (3H, m), 7.88 and 7.86 (total 3H, N-$COCH_3$), 6.97 (3H, s, N-CH_3), 6.90 (3H, s, N-CH_3); mass spectrum *m/e* 499 (molecular ion, 6), 481 (80), 291 (100), 274 (40), 273 (50).

The spectra indicated that instead of causing degradation, the potassium t-butoxide had simply liberated the free base of the lower melting iodomethane derivative, and that this, in turn, corresponded to the N-methyl derivative of the original less polar reduction product.

The Higher Melting Iodomethane Derivative

The spectral characteristics of this compound can be summarized as follows: infrared (nujol) 3430 (broad), 1605, 1594 (shoulder) cm.$^{-1}$; mass spectrum m/e 499 (highest peak, 2), 481 (100), 466 (18), 291 (46), 275 (36), 273 (26); also peaks at m/e 128 (49) and 127 (25) ascribed to (HI) and (I) species. The very low solubility of this derivative prevented its further characterization by n.m.r.

In the discussion it was suggested (page 98) that this compound corresponded to the methiodide salt of the lower melting iodomethane derivative just described. The sample was submitted for elemental analysis.

Calculated for $C_{32}H_{57}N_3O_2I_2$: C, 49.96; H, 7.47; N, 5.46.

Found: C,47.84, 48.33; H,7.44, 7.58; N,5.59.

Preparation of the Corresponding Dimethobromide Derivative

The preparation of the dimethobromide compound corresponding to the higher melting iodomethane derivative above was carried out with the intention of preparing a crystalline heavy atom derivative which would be suitable for x-ray analysis.

An aqueous solution of sodium bromide (10%, 40 ml) was slowly drained through a chloride-loaded Dowex 2-X8 anion exchange resin (1.0g) contained in a burette. By this process the original anion was replaced with bromide. Once the elution of the sodium bromide solution was complete, the excess ions were eluted from the column with freshly distilled water until the eluate gave a negative silver nitrate test (for Br).

A solution of the alkaloid derivative (25 mg) in freshly distilled water was then slowly eluted through the bromide loaded column. From the first 10 fractions (total volume 200 ml), 29 mg of a white solid was obtained which, after crystallization from a hot acetone-methanol (10:1) solution, melted at 198-203°. After recrystallization from a similar solvent system the compound (12 mg) melted at 201-204°. Found: infrared (nujol) 3400 (broad), 1600 (strong), 1460, 1375 cm.$^{-1}$

The sample was submitted for an elemental analysis of the bromine content.

Calculated for $C_{32}H_{57}N_3O_2Br_2$: Br, 23.64%

Found: Br, 22.46%.

The specific gravity of the crystals was determined by the neutral buoyancy method utilizing a mixture of chlorobenzene (s.g. 1.107, in which the crystals sank) and carbon tetrachloride (s.g. 1.595, in which the crystals floated). The proportions of each solvent were adjusted until the crystals attained an equilibrium after which the specific gravity (s.g.) of the solution was measured with a hydrometer. Found: specific gravity of crystals, 1.33 ± 0.01.

Selenium Dehydrogenation of a Weak Base Sample

Into each of 4 thick-walled Carius tubes was placed a thoroughly mixed sample of weak bases (200 mg) and selenium powder (800 mg). The tubes were sealed under vacuum and heated at 300° for 4 hours. After cooling the tubes were crushed and the fragments transferred into the thimble of a Soxhlet extractor, wherein they were extracted with methylene chloride for 6 hours. After removal of the solvent by evaporation the residue (747 mg) was dissolved in benzene (25 ml). The benzene solution was extracted with several portions (4 x 25 ml) of aqueous hydrochloric acid (8%), after which the aqueous solution was basified with ammonium hydroxide and extracted with chloroform (3 x 20 ml). Removal of the solvent from the dried ($MgSO_4$) chloroform extracts afforded the basic dehydrogenation products (216 mg). Found: infrared ($CHCl_3$) 3400 (broad), 1690, 1665, 1620, 1575 cm.$^{-1}$; u.v. (MeOH) 228, 271, 277 (shoulder) mμ; u.v. (MeOH-HCl) 219, 242, 277 mμ.

The non-basic products contained in the original benzene solution were not examined further.

Separation of the Basic Dehydrogenation Products into Weakly Basic and Strongly Basic Fractions

This separation was carried out in a manner similar to that described by Nkunika[95]. The total basic dehydrogenation (216 mg) was dissolved in methylene chloride (50 ml) and the solution was extracted with portions (7 x 20 ml) of aqueous hydrochloric acid (4%). The remaining organic layer was dried (K_2CO_3-$MgSO_4$) and afforded, after removal of the solvent, the weakly basic fraction (65 mg). The aqueous layer was basified with ammonium hydroxide and then extracted with methylene chloride (4 x 30 ml). Removal of the solvent from the dried ($MgSO_4$) solution by evaporation, yielded the strongly basic fraction (135 mg).

The Weakly Basic Fraction

Preliminary studies by t.l.c. (benzene) indicated the presence of at least 5 components. The two least polar components of the mixture were separated by chromatography over alumina (2.5g) and elution with hexane (50 ml). The less polar of the two components was identical on t.l.c.with a sample of GC-18 isolated by Nkunika. The two component mixture showed the following spectral behavior. Found: infrared (CCl_4) 3035, 1590, 1575, 1450 and 1370 $cm.^{-1}$; u.v. (MeOH) 220, 233 (shoulder), 271 and 276 (shoulder) mμ; u.v. (MeOH-HCl) 215, 244, 278, 283 (shoulder) mμ; mass spectrum m/e 243 (molecular ion, 50), 228 (48), 187 (50), 146 (60), 135 (100), 121 (44). The mass spectrum confirmed the presence of GC-18 ($C_{17}H_{25}N$).

When Nkunika had carried out this procedure both GC-17 and GC-18 had been detected in the fraction eluted with hexane. Then the separation *via* g.l.p.c. on a column 16' x ¼", 15% Apiezon L, on Chromosorb W, 60-80 mesh, at 240° with helium flow rate of 1 ml/sec., gave sufficient quantities of GC-17 (retention time 38 mins.) and GC-18 (retention time 44 mins), to allow their further characterization by n.m.r. Found for GC-17: n.m.r. (CDCl$_3$)τ9.10 (d, J = 6 cps, CHCH$_3$),8.70 (3H, d, J = 8 cps), 7.52 (3H, s, Ar-CH$_3$), 7.10 (m), centered at 3.16 and 2.76 (total 2H, ABq, J_{AB} = 8 cps). Found for GC-18: n.m.r. (CDCl$_3$)τ9.16 (d, J = 6 cps, CH-CH$_3$), 8.74 (3H, d, J = 7 cps), 7.56 (3H, s, Ar-CH$_3$), centered at 3.11 and 2.69 (total 2H, ABq, J_{AB} = 8 cps); n.m.r. (CD$_3$ COOD) τ9.07 (s, broad), 8.59 (3H, d), 7.32 (3H, s), centered at 2.47 and 1.92 (total 2H, ABq).

The Strongly Basic Fraction

Gas liquid phase chromatographic analysis of this fraction on 25% SE-30, on Chromosorb W, 60-80 mesh, (5' x ¼") at 200° and helium flow rate of 0.5 ml/sec., allowed the isolation of three components, A, B and C (see pages 106 and 107), having retention times of 4.0, 3.1 and 4.7 mins. respectively.

Component A. Found: u.v. (MeOH) 220, 273, 281, 305 and 318 mμ; mass spectrum *m/e* 143 (molecular ion, 100), 142 (44), 119 (18), 115 (16).

Component B. Found: u.v. (MeOH) 210, 268 and 276 mμ; mass spectrum *m/e* 147 (molecular ion, 100), 146 (31), 132 (66), 118 (25), 117 (24), 105 (96).

Component C. Found: u.v. (MeOH) 220, 276, 309 and 322 mμ; mass spectrum *m/e* 157 (molecular ion, 100) 156 (40) 142 (43).

The ultraviolet and mass spectra outlined in the foregoing inferred the following: A was either a 6- or 7-methylquinoline; B, the major component, was either a 6- or 7-methyl-5,6,7,8-tetrahydroquinoline; C was a dimethylquinoline.

Also the spectra of components A and B closely resembled the ones which Nkunika had assigned to 7-methylquinoline and 7-methyl-5,6,7,8-tetrahydroquinoline.

When, some time later, the strongly basic fraction was subjected to an analysis using the MS-12 mass spectrometer, in conjunction with a gas liquid phase chromatographic column consisting of 3% OV-17 on Gas Chrom Q, 100-120 mesh, (10' x ¼") at 208°, and helium flow rate of 1 ml/sec., a fourth component, D, was detected. Found: mass spectrum <u>m/e</u> 161 (molecular ion, 100), 160 (31), 146 (77), 143 (possibly 7-methylquinoline as impurity, 52), 119 (84). The mass spectrum suggested that D was a dimethyltetrahydroquinoline. Furthermore, the facile loss of 42 a.m.u. inferred that D contained a 5,6,7,8-tetrahydroquinoline skeleton with one of the methyl groups at either the 6 or 7 position.

<u>Zinc Dust Distillation of a Weak Base Sample</u>[125]

A thoroughly mixed sample of weak bases (4g) and zinc dust (25g) was placed into a glass tube which was held in a sublimation block. The protruding length of tube was bent into a U-shape and surrounded by a dry-ice acetone bath at -78°, so as to trap any volatile products. The contents of the tube were heated at 300° for 36 hours. The tube was cooled then rinsed several times with methylene chloride. The methylene chloride extracts were extracted several times (6 x 25 ml) with aqueous hydrochloric acid (15%), after which the aqueous solution was basified with ammonium hydroxide and extracted with methylene chloride (4 x 30 ml). Removal of the solvent from the dried ($MgSO_4$) solution afforded the total basic dehydrogenation products (198 mg).

The total basic product was separated into strongly basic and weakly basic fractions as described under selenium dehydrogenation. T.l.c. examination of the weakly basic fraction failed to detect any GC-17 or 18 and the fraction, which was only minor, was not examined further. When the strongly basic fraction was subjected to combined g.l.p.c.-MS 12 analysis using the OV-17 column, the resultant mass spectra indicated the presence of four dehydrogenation products corresponding to the compounds A,B,C and D from the selenium dehydrogenation

By g.l.p.c. alone, using the OV-17 column under the conditions described earlier for the selenium dehydrogenation products, small amounts of components A,B and C could be isolated, as indicated by their ultraviolet spectra.

The Non-basic Zinc Dust Distillation Products

The methylene chloride solution which remained following the repeated extractions with aqueous hydrochloric acid (15%), was dried (K_2CO_3-$MgSO_4$) and extracted with aqueous sodium hydroxide solution (10%, 3 x 20 ml). The residual organic layer was washed with a little dilute hydrochloric acid and dried (K_2CO_3-$MgSO_4$). After evaporation of the solvent the neutral fraction (320 mg) was isolated as a brown gum. The sample was chromatographed over alumina (5g) and eluted with benzene (100 ml), followed by similar quantities of ether, then chloroform. The major fraction, which was eluted with ether, was shown by g.l.p.c. to consist mainly of one component. Preparative g.l.p.c. using 20% DEGS, on Chromosorb W, 60-80 mesh, (5' x 1/8") at 184° and helium flow rate of 50 ml/min., allowed the separation of this major component (retention time 6.6 mins) as a colorless semi-crystalline solid (7 mg).

Found: infrared (CCl_4) 3040, 1700 (intense), 1620 (weak), 1483, 1396, and 1050 cm.$^{-1}$; u.v. (MeOH) 219 mμ (ε = 11,400), 226 mμ (ε =11,300), 240 mμ (shoulder, ε =7,500), 266 mμ (shoulder, ε = 2,400), 277 mμ (ε =1,760), no change on acidification; n.m.r. ($CDCl_3$) τ 6.89 (3H, s), 5.72 (2H, s), 2.59 (3H), 2.21 (1H, m); mass spectrum m/e 147 (molecular ion, 100), 146 (72), 119 (24), 118 (63), 91 (27).

The infrared spectrum of this component in carbon tetrachloride was found to be identical with that of an authentic sample of N-methylphthalimidine.

Isolation of Lycopodine, Lycodine, Dihydroluciduline and Anhydrolycodoline

A sample of Fraction E (1.5g) obtained from the tubes 86-100 of the 100 tube countercurrent distribution of a weak base mixture (8g) was subjected to dry-column chromatography over alumina (60g), contained in a length of polythene tube (20 cm) of internal diameter 19 mm. After elution with ethyl acetate the column was cut into 10 sections (section 1 containing the least polar material). The usual work up afforded four fractions. Fraction 1(90mg) consisted of lycopodine and a slightly less polar component (t.l.c.). From fraction 2, made up of sections 2 and 3, a crystalline compound was obtained (140 mg) which, after recrystallization from acetone, melted at 114-115°. This compound was found to be identical with an authentic sample of lycopodine. Fraction 3 (480g), made up of sections 4, 5 and 6, contained 3 components, the least polar and most polar of which (t.l.c.) were lycopodine and lycodine. Fraction 4 (130 mg), which consisted of sections 7 and 8, was crystalline and found to be identical with an authentic sample of lycodine.

Attention was turned to fraction 1, which appeared to rapidly discolor on exposure to air. G.l.p.c. analysis on 10% QF-1, on Chromosorb W, 60-80 mesh, (5' x ¼") at 210°, with a helium flow rate of 1 ml/sec., indicated the presence of two components, of retention times 4.8 and 7.2 mins. respectively. The material having the longer retention time was identified as lycopodine by g.l.p.c. analysis of an authentic sample. A pure sample of the other component was obtained by preparative g.l.p.c., dissolved in ether and treated with hydrogen chloride gas. The hydrochloride salt so produced was found to be identical in all respects with an authentic sample of the hydrochloride salt of anhydrolycodoline.

Fraction 3 was subjected to column chromatography over alumina (20g). Elution with benzene (400 ml) yielded lycopodine (135 mg). Elution with benzene-ether (2:1, 1,000 ml) afforded material which consisted of three components. Further elution with ether yielded pure lycodine (28 mg). After the three component mixture had been re-chromatographed it was possible to isolate a component of polarity intermediate between that of lycopodine and lycodine.

Found: infrared ($CHCl_3$) 3580 (strong), 2780 (intense), 1450, 1130, 1010 cm.$^{-1}$.

The component was found to be identical with a sample of dihydroluciduline prepared by sodium borohydride reduction of luciduline.

Isolation of the 292 Compound (molecular formula $C_{17}H_{28}N_2O_2$)

A weak base sample (8.6g) was subjected to a ten funnel countercurrent distribution experiment utilizing chloroform (400 ml fractions) as the lower stationary phase and a saturated aqueous solution of potassium hydrogen tartrate (400 ml fractions) as the upper moving phase. On completion of the ten cycles the bulk of the alkaloids was found in the chloroform layer of funnel 1, containing the more weakly basic alkaloids of the original mixture.

The chloroform layer was extracted with aqueous hydrochloric acid (0.1N, 400 ml) and then with progressively more concentrated hydrochloric acid solutions. The fraction obtained by extraction with acid solutions of concentration 2N and 8N gave, after basification (NH_4OH) and extraction with chloroform, a brown gum (1.2g). On t.l.c. this material appeared to consist of mainly one component (R_f 0.1, ether-methanol 99:1).

Found, after partial purification by dry-column chromatography over alumina (100g): infrared ($CHCl_3$) 3420. 1690 and 1620 cm.$^{-1}$; mass spectrum m/e 292 (molecular ion, 80), 249 (94), 166 (45), 152 (86), 84 (100), 83 (72), 43 (83), 41 (93); n.m.r. ($CDCl_3$) τ 8.95 and 8.93 (total 3H, 2d, J = 7 cps, rotomeric effect), 7.98 and 7.94 (total 3H, possibly N-$COCH_3$, rotomers), at 5.55 and 5.13 (total 2H, centers of AB quartet, J_{AB} = 14 cps), 2.85 (d, J = 22 cps, or two singlets). The latter signal is concentration dependent. High resolution mass spectrometry revealed that the molecular ion had the composition $C_{17}H_{28}N_2O_2$.

Hydrogenation of the 292 Compound

The 292 compound (10 mg) in ethyl acetate (10 ml) was hydrogenated at atmospheric pressure over palladinized charcoal (10 mg, 5% Pd on C) for 12 hours. The catalyst was removed by filtration and the solvent evaporated under reduced pressure.

The sample isolated (10 mg) was identical with the starting material (t.l.c., and i.r.).

Reaction of the 292 Compound with Sodium Borohydride

Sodium borohydride (30 mg) was added to an ice-cold solution of the compound (20 mg) in methanol (10 ml) over a period of two hours, after which the mixture was left standing for 15 hours. The usual work up afforded only starting material as identified by t.l.c., i.r. and mass spectra.

Attempted Acidic Hydrolysis of the 292 Compound

A solution of the compound (24 mg) in dilute sulphuric acid (25%, 30 ml) was heated under reflux for three days. The usual work up led to the isolation of only trace amounts of material which proved to be starting material. The unusually low recovery of material by the usual procedure was tentatively interpreted to mean that the hydrolysis had generated a water soluble amino acid.

Reaction of the 292 Compound with Nitrous Acid

The compound (38 mg) was dissolved in ice-cold aqueous hydrochloric acid (12%, 2 ml). A cooled (0^{o}) solution of sodium nitrite in water (1%) was added until no further precipitation occurred. The yellow precipitate was separated from the aqueous solution by extraction with chloroform (20 ml). Found: infrared ($CHCl_3$) 3430 (broad), 1765 (intense) 1695 (medium intensity) and 1620 (intense) cm.$^{-1}$. The absorption at 1765 cm.$^{-1}$ is much higher than the value usually associated with N-nitroso derivatives, and in fact, is more typical of an azlactone moiety.

The mass spectrum shows a peak at m/e 321, i.e., consistent with the value expected for the N-nitroso derivative. However, a peak at m/e 322 of almost equal intensity, is observed which might be due to the cationic species formed by protonation of the initially formed N-nitroso derivative. Found: mass spectrum m/e 322 (highest peak in spectrum, 2), 321 (2), 292 (75), 249 (97), 208 (35), 195 (47), 194 (80), 166 (45), 152 (100), 150 (60), 110 (35), 96 (85) and 84 (90).

Reaction of the 292 Compound with Hydrobromic Acid

The compound (16 mg) was dissolved in acetone (1 ml). Hydrobromic acid (1% in acetone) was added until the solution showed red on litmus paper. The resultant precipitate was dissolved in methanol (1 drop) and chloroform was added until the solution attained a slight turbidity. The solution was kept at 0° for two days, after which pale yellow crystals (16 mg) were isolated m.p. 116-120°.

Found: infrared (nujol) 3200 (broad), 1645 (broad), 1460, 1410, 1380 $cm.^{-1}$.

Attempts to form crystalline derivatives by treatment of the 292 compound with iodomethane or hydroperchloric acid were unsuccessful.

Isolation of the 259 Compound (molecular formula $C_{16}H_{21}NO_2$)

A solution of the weak bases (86g) in methylene chloride (500 ml) was repeatedly extracted with an aqueous solution of potassium hydrogen phthalate (0.05M, pH 4.0, 500 ml). From the initial extraction 12.5 grams of alkaloid material was isolated which consisted mainly of lycopodine and one other more polar component. The sample (12.5g) was subjected to column chromatography over alumina (500g) and those fractions whose infrared spectra in chloroform showed an intense absorption at 1620 cm.$^{-1}$, i.e., typifying the presence of the C_{30} amide containing alkaloids, were combined. The resultant material (5.3g) was subjected to a ten funnel countercurrent distribution experiment involving similar volumes (300 ml) of chloroform, the stationary phase, and of a saturated aqueous solution of potassium hydrogen tartrate as the upper phase. After the usual work up, the material isolated from funnels 6 and 7 was partially crystalline. The addition of small quantities of ether or methylene chloride readily dissolved and effected the separation of the non-crystalline constituents, allowing the isolation of a small amount of the crystals (10 mg) which, after recrystallization from acetone, melted at 166-169°, with decomposition. The spectral properties revealed that the component was not a C_{30} alkaloid despite the presence of an intense peak at 1620 cm.$^{-1}$ in the infrared spectrum, determined in chloroform. Found: infrared (nujol) 3038, 1662 (shoulder), 1654 (intense), 1611 and 1405 cm.$^{-1}$; mass spectrum <u>m/e</u> 259 (molecular ion, 15), 216 (14), 202 (100), 174 (27); u.v. (MeOH) 212 and 314 mμ (slight bathochromic

shift, i.e., 2 mμ on acidification); u.v. (MeOH-NaOH) 220 and 364 mμ; n.m.r. ($CDCl_3$) τ 9.17 (3H, d, J = 6 cps, CH-$\underline{CH_3}$) centered at 7.00 and 6.05 (2H, ABq, J_{AB} = 19 cps), 4.17 (1H, symmetrical octet) and a doublet of doublets at 3.29 (1H, J_1= 10 cps, J_2= 2 cps).

The behavior of this component in the ultraviolet suggested that it contained a diosphenolic type of skeleton.

Acetylation of the 259 Compound

A solution of the 259 compound (8 mg) in pyridine (0.2 ml) and acetic anhydride (0.2 ml) was stirred for 18 hours at room temperature. The usual work up yielded a yellow gum. Found: infrared ($CHCl_3$) 1760 (vinyl ester). 1640 and 1615 cm.$^{-1}$.

Attempts to purify this derivative by the usual acid-base extraction procedure led to rapid hydrolysis to starting material.

Catalytic Hydrogenation of the 259 Compound

A solution of the 259 compound (5 mg) in ethanol (98%, 10 ml) was subjected to catalytic hydrogenation over palladinized charcoal (5%, 10 mg). After the catalyst had been removed by filtration the solution was evaporated to dryness under reduced pressure. The product was obtained as a pale yellow oil (4 mg). Found: u.v. (MeOH) 210 and 282 mμ; u.v. (MeOH-NaOH) 217 and 322 mμ.

A comparison of the u.v. spectra of this product with those of the original alkaloid indicated that the 259 compound contains an α-hydroxy conjugated dienone system which, on catalytic hydrogenation, is transferred into the α-hydroxy-enone system.

Isolation of Ferulic Acid

The extraction of the methylene chloride solution of the weak base sample (86g) from which the 259 compound had been isolated, was continued with increasingly more concentrated aqueous acid solutions until no more basic components could be extracted. The methylene chloride solution at this stage essentially contained just neutral and acidic components.

The latter solution was extracted with aqueous sodium hydroxide (4 x 25 ml, 5%). The basic extracts were acidified with dilute hydrochloric acid and extracted with methylene chloride (4 x 25 ml). After removal of the solvent from the dried (K_2CO_3-$MgSO_4$) a semi-crystalline gum (210 mg) was isolated. The gum was dissolved in acetone and left standing at 0° for two days, after which crystallization had occurred. After recrystallization from acetone the isolated crystals melted at 163-164°. Found: infrared (nujol) 3440, 1693, 1666, 1623, 1603 and 1520 cm.$^{-1}$; u.v. (MeOH) 218 mμ (ε = 15,500), 228 mμ (shoulder,ε = 13,460), 288 mμ (ε = 13,820), 303 mμ (shoulder,ε = 13,580), and 313 mμ (ε = 14,680; u.v. (MeOH-NaOH) 218 mμ (ε = 14,190), 242 mμ (ε = 9,458), 290 mμ (shoulder,ε = 8,245), 305 mμ (ε=10,670) and 347 mμ (ε = 16,730); n.m.r. (pyridine -d_5)τ 6.26 (3H, d, J = 1 cps), at 3.23 and 1.95 (total 2H, ABq, J_{AB} = 16 cps), 2.83 to 2.69 (3H, Ar-H), -1.91 (2H); mass spectrum m/e 194 (molecular ion, 100), 179 (20), 133 (26), 77 (21), 51 (20). High resolution mass spectrometry revealed that the molecular composition of the ion at m/e 194 was $C_{10}H_{10}O_4$.

The spectra indicated that the isolated sample was ferulic acid. The mixing of a sample of the isolated compound with authentic ferulic acid (m.p. 168-169°) caused no depression in the melting point of the latter. The ultraviolet and infrared spectra of the isolated and authentic samples were identical. The only differences between their n.m.r. spectra concerned the signal associated with the two acidic protons. In the authentic sample this signal was at slightly higher field.

Acetylation of the Isolated Ferulic Acid

The isolated ferulic acid (15 mg) was dissolved in pyridine (0.2 ml) and acetic anhydride (0.2 ml) and the solution was left standing for 12 hours. The excess reagents were removed by evaporation under reduced pressure and the gummy residue (16 mg) was crystallized from benzene. Sublimation of this residue yielded white crystals (m.p. 194-197°) whose infrared spectrum, determined in nujol, showed intense absorption at 1770 cm.$^{-1}$ (vinyl ester), corresponding to O-acetyl ferulic acid.

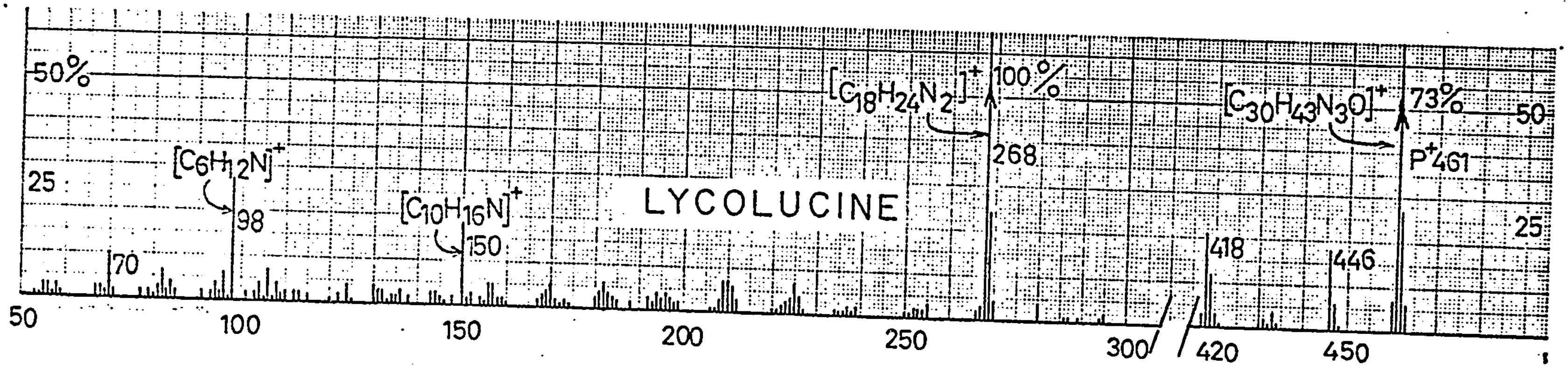


Figure 46

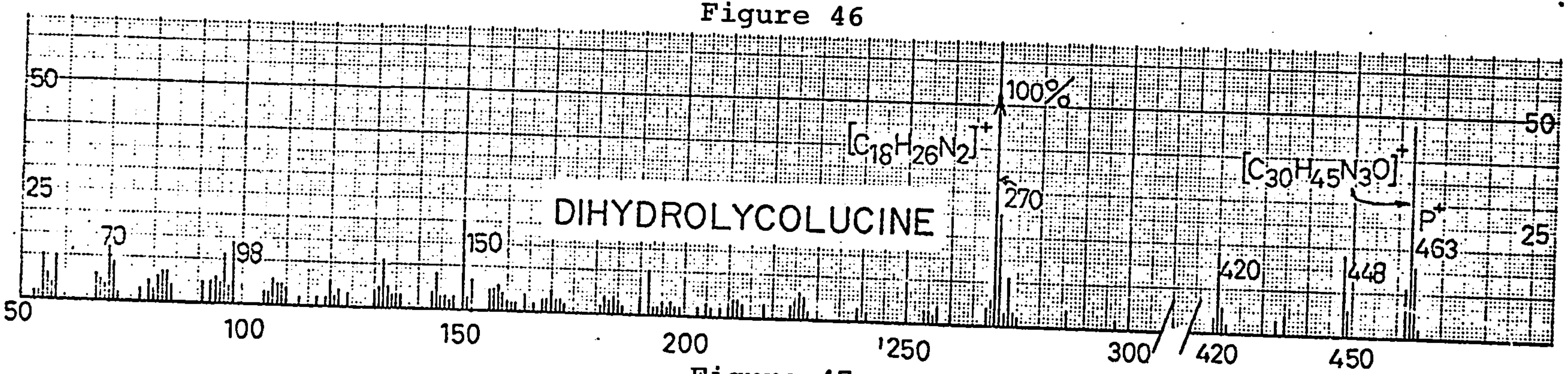


Figure 47

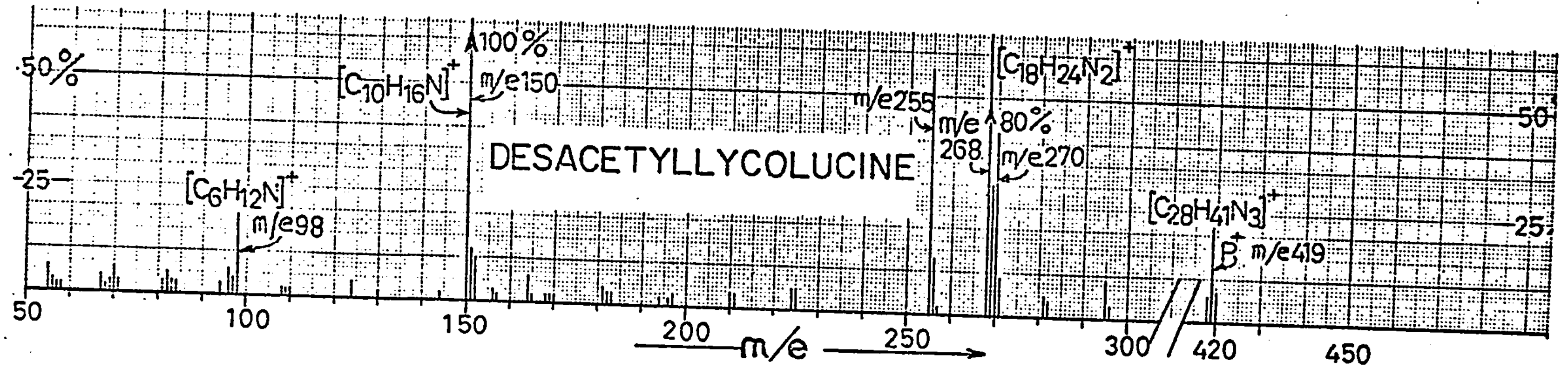
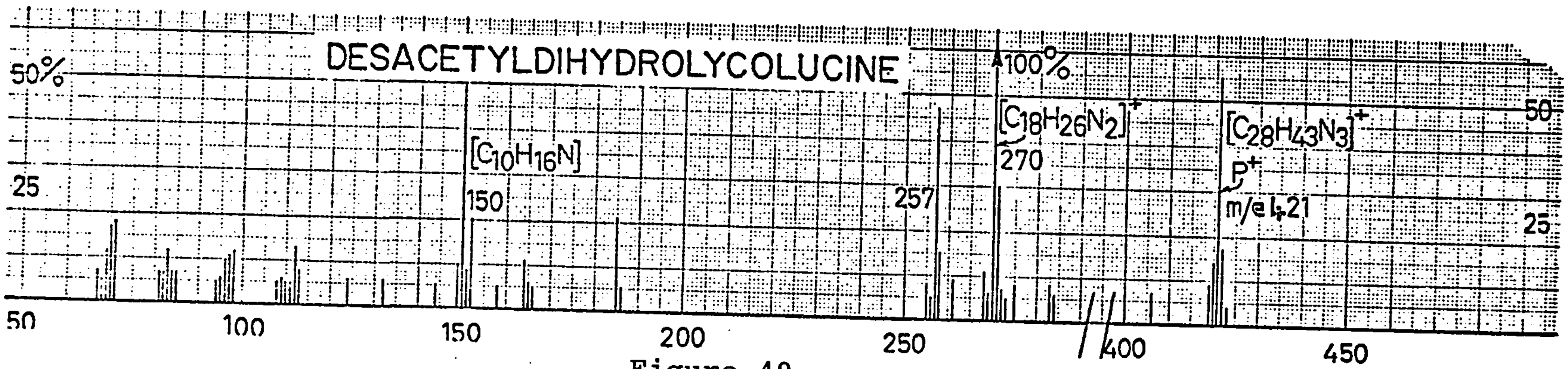


Figure 48



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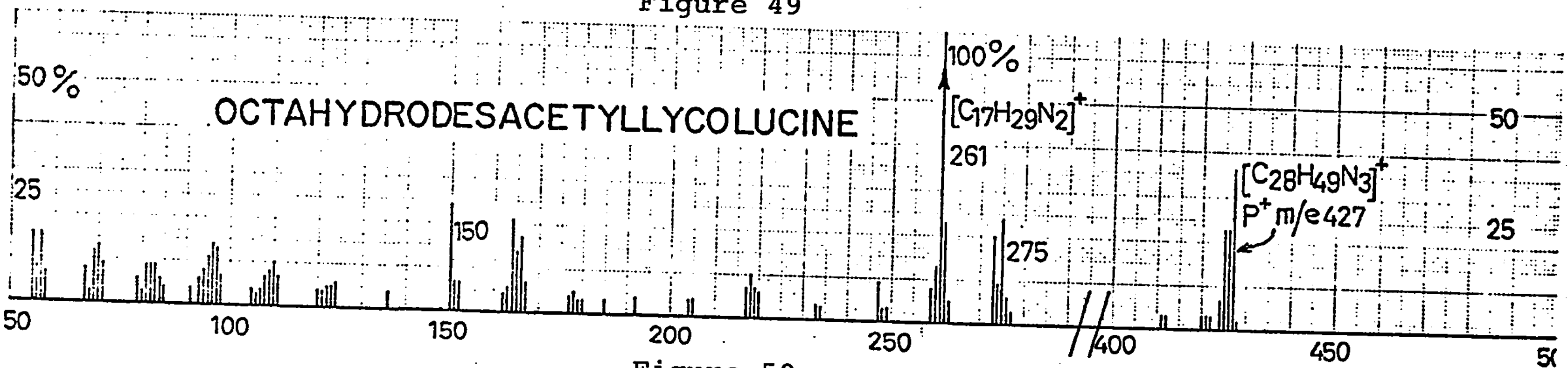

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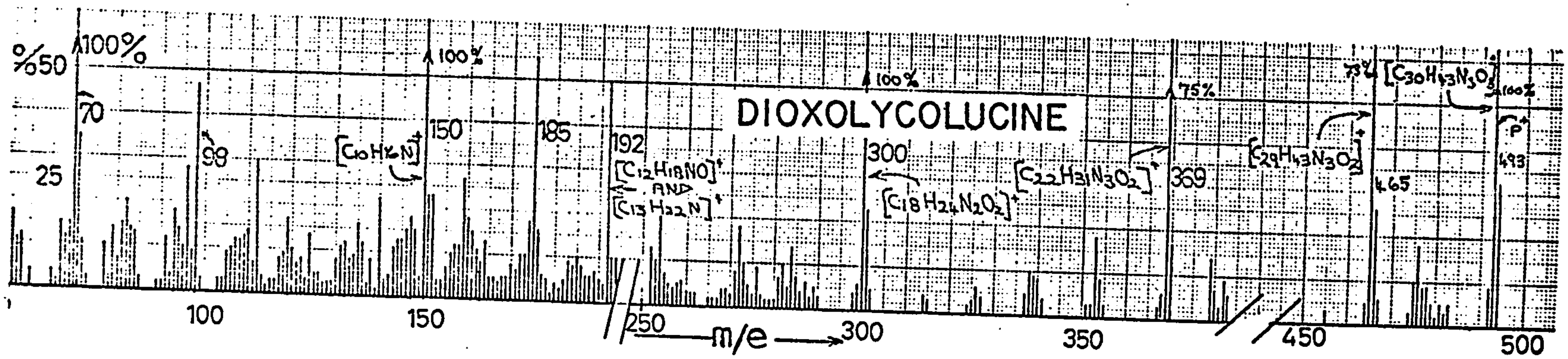

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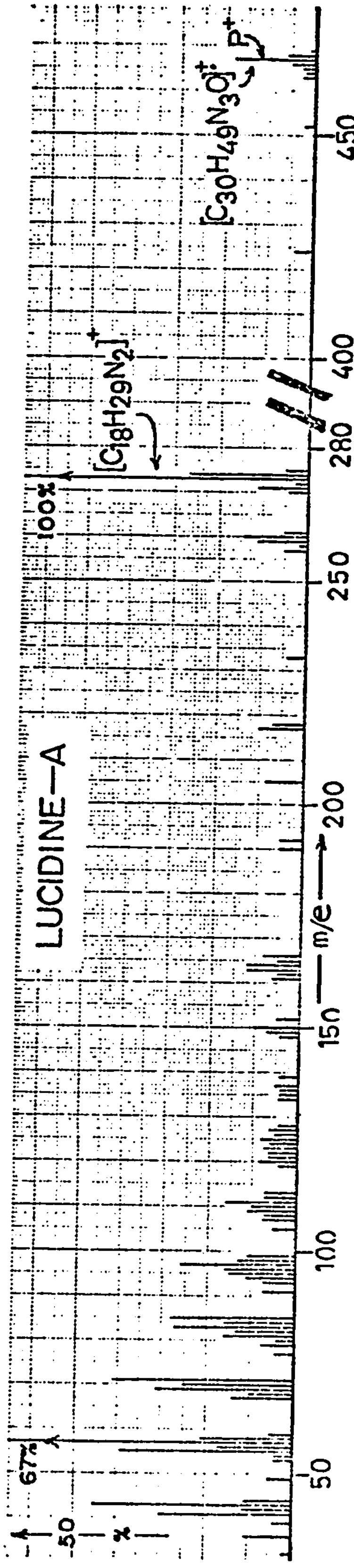
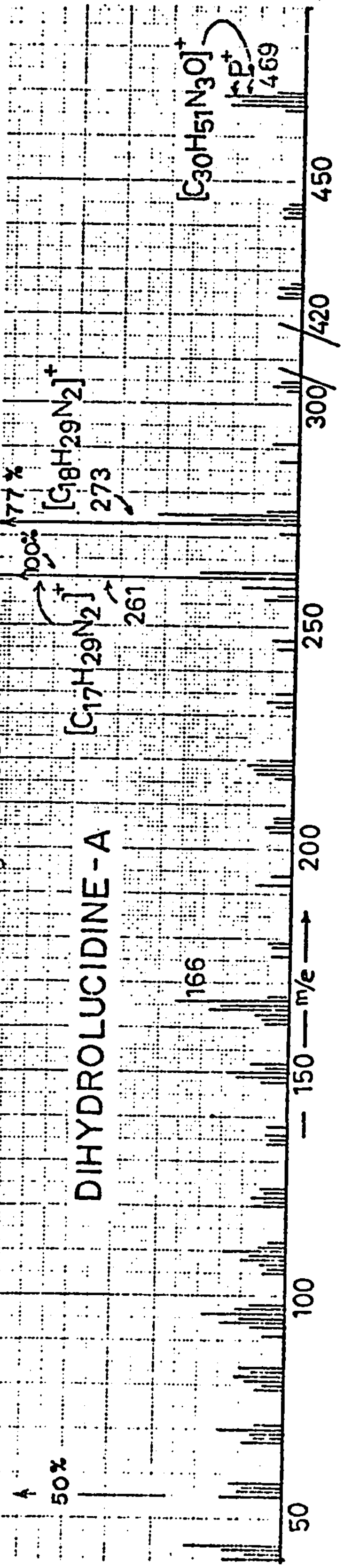


Figure 52



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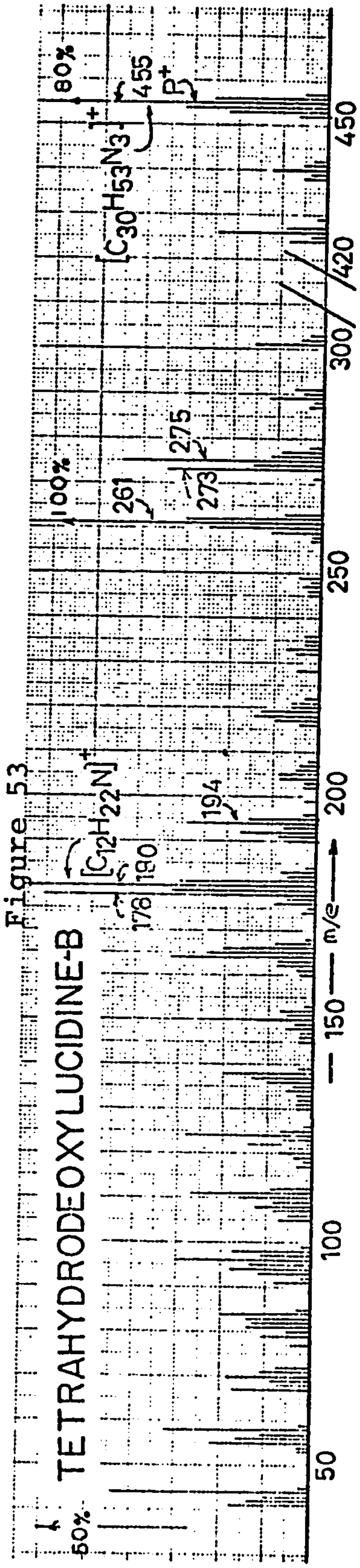

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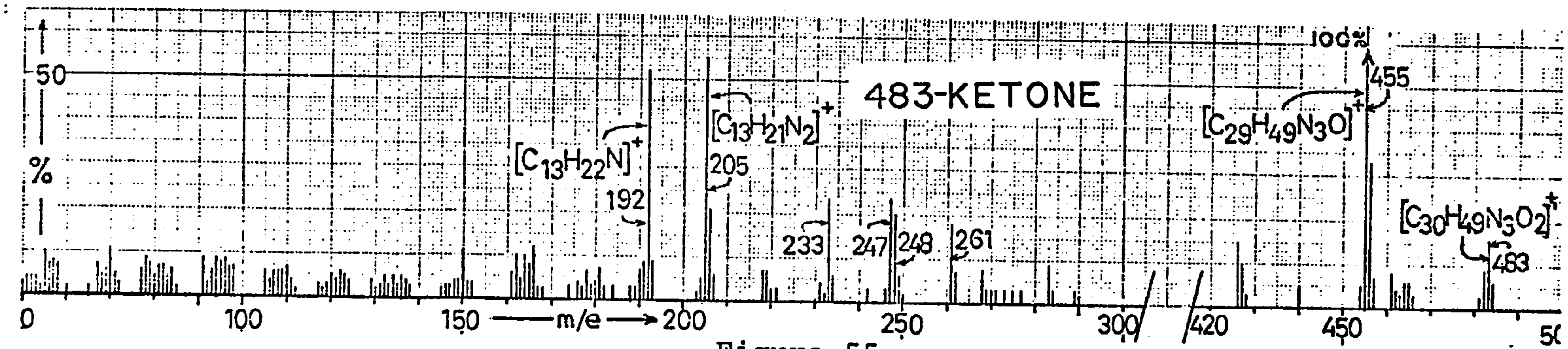

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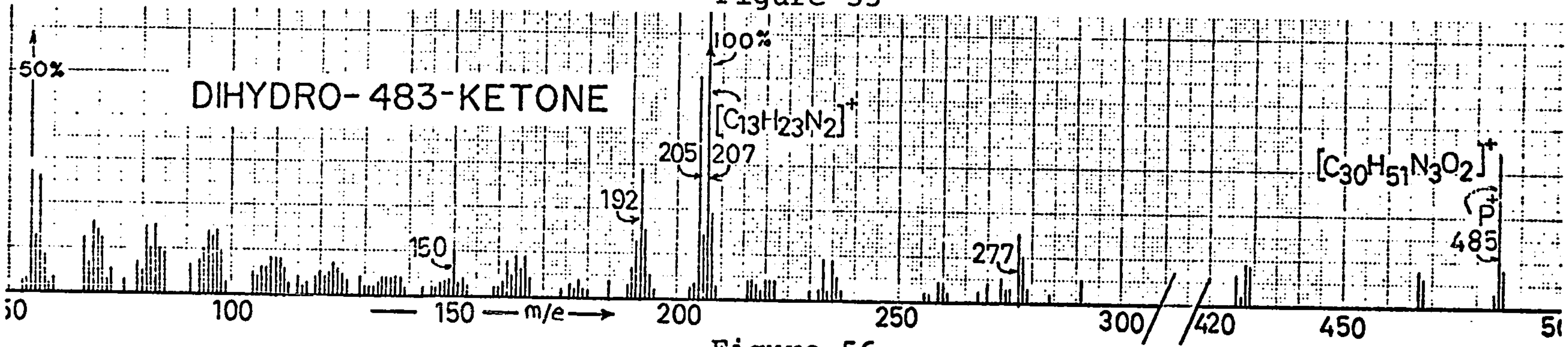

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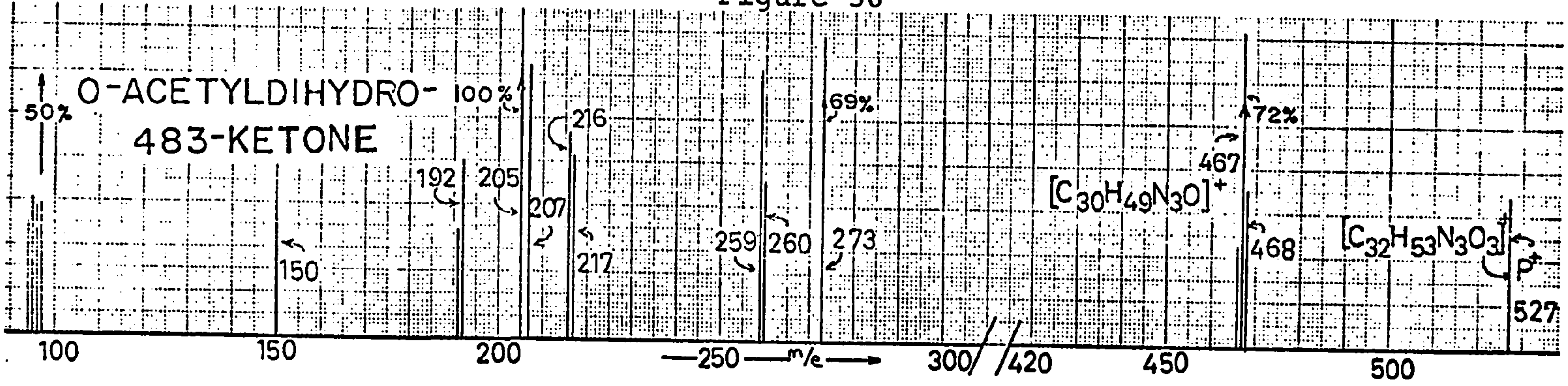
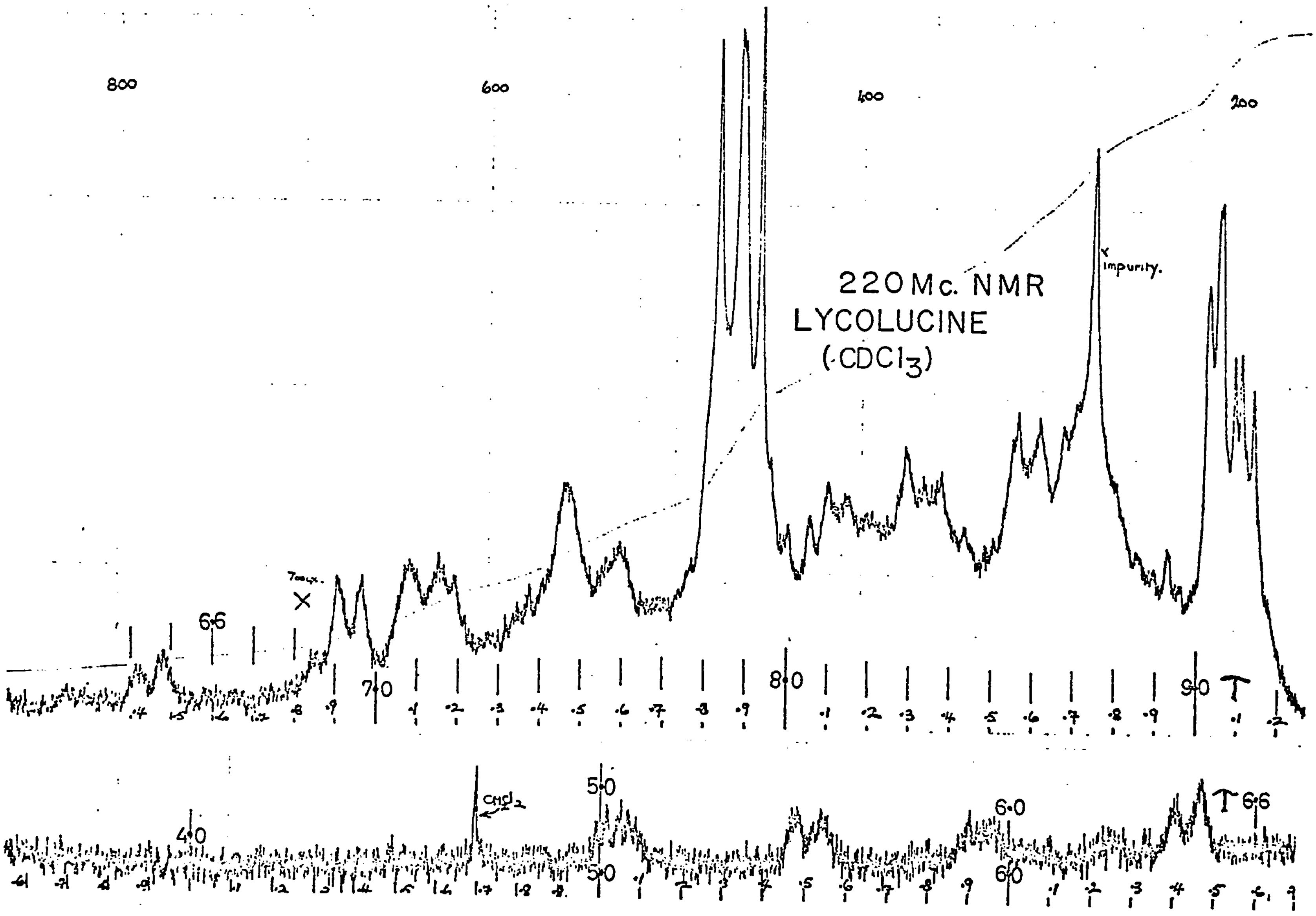


Figure 57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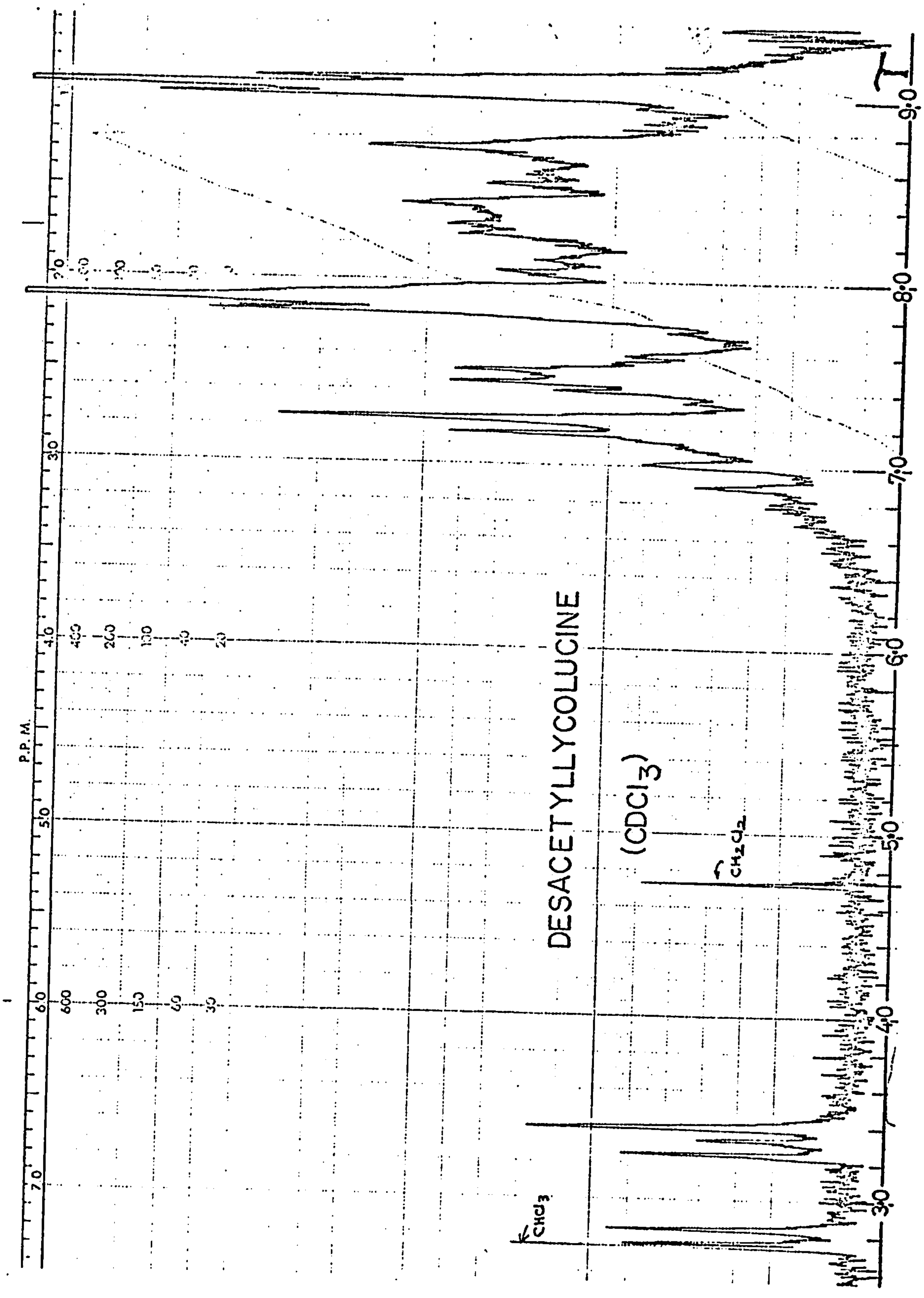
(CDCl3)
800
600
400
200
impurity.
6.6
7.0
8.0
9.0
τ
CHCl3
4.0
5.0
6.0
τ 6.6

Figure 58


DESACETYLLYCOLUCINE
(CDCl3)
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CHCl3
P.P.M.
2.0
3.0
4.0
5.0
6.0
7.0
400
200
100
40
20
600
300
150
60
30
9.0
8.0
7.0
6.0
5.0
4.0
3.0

Figure 59

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