THE UNIVERSITY OF CALGARY

ALKALOIDS OF DELPHINIUM MACROCENTRUM

by

FRANCIS INYANGALA OKANGA

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF CHEMISTRY

CALGARY, ALBERTA

JULY, 1987

ISBN 0-315-38069-1

THE UNIVERSITY OF CALGARY

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Alkaloids of Delphinium Macrocentrum", submitted by Francis Inyangala Okanga in partial fulfillment of the requirements for the degree of Master of Science.

M.H. Benn (Supervisor)
Chemistry

T.G. Back
Chemistry

T.S. Sorensen
Chemistry

M.M. Moloney
Biology

ABSTRACT

A comprehensive study of the alkaloidal constituents of Delphinium macrocentrum Oliv., collected on Mt. Kenya was undertaken. The air-dried and ground aerial parts of D. macrocentrum plants were extracted with 95% EtOH. Subsequent employment of pH-gradient extraction and chromatographic separation resulted in the isolation of six known and five new diterpenoid alkaloids, which were characterized by spectroscopic methods (MS, IR, ^{1}H-NMR, and ^{13}C-NMR) and chemical transformations. The known alkaloids were identified as 14-acetyldelcosine, deltatsine, methyllycaconitine, browniine, delcosine, and 13-O-acetylhetisine, while the five novel alkaloids were designated as deacetylnudicauline, macrocentridine, macrocentrine, hetisine-13-O-benzoate, and a dehydrohetisine.

ACKNOWLEDGEMENTS

I wish to express my most profound gratitude to my supervisor, Dr. M.H. Benn, for his excellent supervision and patience through this programme.

I am also indebted to Dr. M.H. Benn and the entire staff in the Instrumental Laboratory for running numerous NMR and mass spectra, and to Mrs. Christine Schill for typing this thesis. Special thanks to Dr. R.M. Munavu and Mr. S.G. Mathenge for assisting in collecting the plant material from Mt. Kenya, to Kenya Industrial Research and Development Institute (K.I.R.D.I.) for granting study leave and to my family for their support.

I wish also to thank the Canadian International Development Agency (CIDA) for a scholarship and financial support.

To My Family

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

The abbreviations used in this thesis are those commonly employed by the Canadian and American Chemical Journals. Less commonly encountered, or otherwise exceptional abbreviations are identified in the text at their first appearance.

CHAPTER 1. INTRODUCTION

1.1. General Objectives

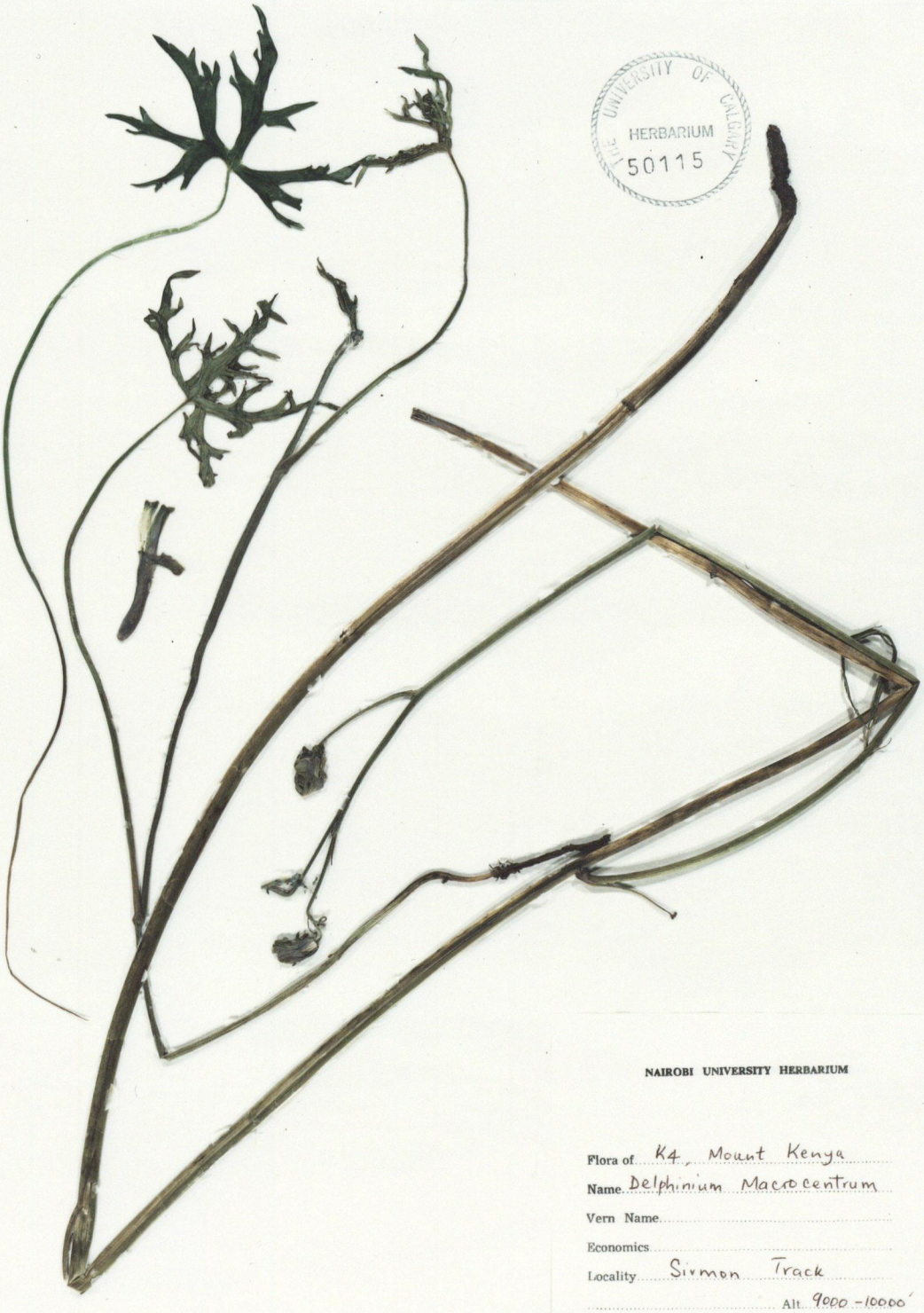
This thesis describes the results of an investigation of the alkaloids of Delphinium macrocentrum Oliv. (Figure 1), a plant native to Kenya, Uganda and Malawi.

Little is known about Kenyan delphiniums, apart from their descriptions as part of the flora of the region[1,2] by naturalists, whose collections were subsequently deposited in the National Herbarium, and the herbarium of the University of Nairobi. No phytochemical studies have been carried out, and virtually nothing is known about their toxicological and pharmacological properties.

Introduced, horticultural species of delphinium are fairly popular with Kenyan florists, and chances are high that one will find them in flower gardens, especially around Nairobi and in the Kenya Highlands. Apart from these introductions, three species of delphinium have been listed as being native to Kenya.[1,2] D. macrocentrum Oliv., D. leroyi Hutch., and D. wellbyi Hemsl. Of these D. macrocentrum is found in moist, and often rocky grassland above 5000 ft in highland areas around Mt. Kenya, Mt. Elgon, Mau hills, the Aberdare mountains, and in the Nairobi area. Delphinium leroyi is to be found around Mumias, and Kajiado, while D. wellbyi has been collected in Nyambeni hills, and Embu.

In view of the fact that some delphiniums are highly toxic to livestock, especially in North America,[3,4] and that certain species exhibit insecticidal properties,[5,6] both of which effects are attributed to

Figure 1: Delphinium macrocentrum



NAIROBI UNIVERSITY HERBARIUM

Flora of K4, Mount Kenya

Name Delphinium Macrocentrum

Vern Name

Economics

Locality Sirmon Track

Alt 9000 - 10000'

Habitat Associated with Stoebe - Artemisia, Philippia Erica Woodland.

Description Herb to 1-2 m high with purple blue flowers

Date 8·8·86

Collected by F.I. Okanga, R.M. Munavu and S.G. Mathenge

No. 86/1

Figure 1

their containing complex diterpenoid alkaloids,[7] it was decided to commence a study of the species endemic to Kenya. Because of its relatively ready availability, D. macrocentrum was selected as the starting point for this work, in which it was planned to compare the alkaloidal content with that found for European, North American, and Asian species. No East African delphiniums have previously been subjected to such an analysis. It was hoped that new diterpenoid alkaloids would be discovered whose structures might shed light on the biosynthesis of this group of natural products. As well the study might allow an assessment to be made of the toxicology and pharmacology of the native Kenyan delphinium species, particularly the possibility of harnessing them in the economically important area of insect pest control.

1.2 The Genus Delphinium

The taxonomic status of the genus Delphinium is non-controversial. There is general agreement that it is a member of the order Ranunculales (formerly Ranales), family Ranunculaceae,[8] where it is very closely related phylogenetically to the genus Aconitum. The name Delphinium comes from the Greek delphinion, which, derived from delphis, a dolphin, alludes to the shape of the flower. Common names also based on fanciful views of the shape of the flower are: in English, Larkspur, with the equivalent Pieds d'alouette in French, while in German and Spanish the allusion is to a Knight's Spur, Rittersporn, and Espuela de Caballero, respectively. Of the more than two hundred species of delphinium, all are herbaceous, and they are almost exclusively endemic to the temperate zones of the Northern Hemisphere.[9] Although a matter of speculation

rather than established fact, the global distribution of these plants is consistent with the origin of the genus in what is now China followed by evolutionary diversification as it spread: Westwards across Asia into Europe and Africa; Eastwards, via the Bering bridge, into North America. The southern limit of its distribution range in the New World is found in Mexico, but in Africa delphiniums extend from Egypt down through the highlands in the East to reach below the equator.[9] Delphinium macrocentrum is one of four species found in this Southernmost extension of the global range of the genus.

Delphiniums are conspicuous plants when in bloom, most having panicled racemes of showy blue flowers, though in a few species they are red, white or yellow. For this reason the plants are widely grown for ornamental purposes.[10]

Delphinium species have a long and well recorded history as having served as sources of herbal preparations used to treat a variety of afflictions, in particular as analgesics, sedatives, emetics, anthelmintics, and as pediculicides.[5,6,11-15] For example, fleas and lice in animal coats can be treated very easily and successfully with a decoction of D. staphisagria seed, soaked overnight.[5] In India, the ripe seeds of this plant are used locally against Pediculis capitis or P. pubis.[6]

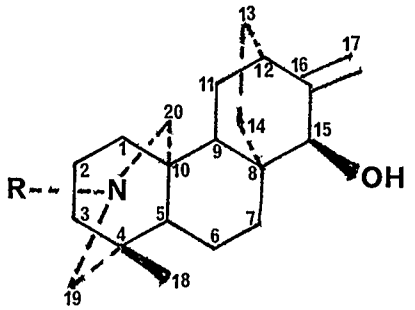
Various species of delphinium have been recognised as dangerously poisonous to man and cattle.[6,12] The poisoning of cattle is still a problem for ranchers in the foothills of the Rocky Mountains.[3,16-17] Minor stock loss is, however, attributed to delphinium in countries other than the United States and Canada. Most cases of poisoning occur

before the plants flower, in early spring. This is due to uptake of the plants by the stock, and not to a maximization of toxin production. Studies indicate that the lethal amount of alkaloids requires the animal to eat plants equal to about 2-3% of its body weight.[16,18-19] Apparently cattle at times actually seek out these dangerous plants, being attracted by the flavour of the leaves. Curiously, sheep are unaffected, and have been used to eradicate the plants in restricted areas. Game animals (deer, moose) are likewise apparently unaffected by ingestion of delphinium plants.

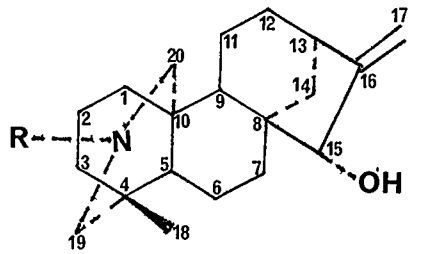
The toxicological and pharmacological properties of delphinium species have been shown in several cases (and may be confidently inferred in others) to be due to the presence of alkaloids in the plants.[7] These alkaloids are closely related to those found in Aconitum, a genus notorious for its toxicity.[7]

1.3 Delphinium Alkaloids

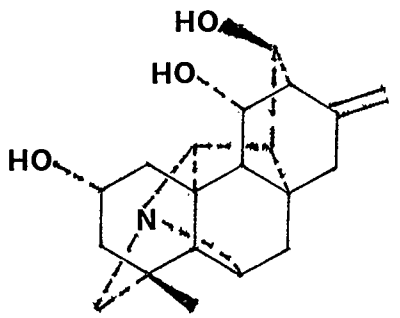
The genus Delphinium is a rich source of alkaloids. Typically these are distributed throughout the plant, and average about 0.3% on a dry weight basis. No species have yet been reported as alkaloid-free. Although traces of benzylisoquinoline-derived alkaloids have occasionally been reported,[20] and may be more generally present, vastly predominant are the so-called diterpenoid alkaloids.[21] These can be classified according to their carbon skeletons as C_{20}-, or C_{19}-diterpenoids. Of the former the types encountered in delphinium are atisines (1), or veatchines (2), and their derivatives, e.g. hetisine (3), denudatine (4) and delnudine (5); while the latter are aconitines (6) or lycoctonines (7).



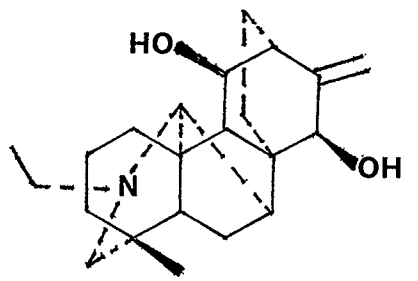
1



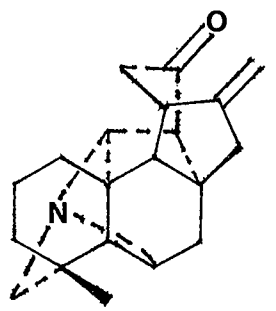
2



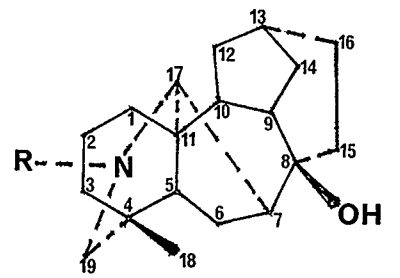
3



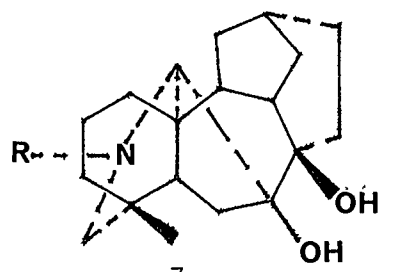
4



5



6



7

Figure 2: Skeletal Systems of Diterpenoid Alkaloids from Delphinium and Aconitum.

The same set of skeletons are encountered among the Aconitum alkaloids, together with further modifications. The numbering systems used for the atisine, veatchine and aconitane (lycoctonine and aconitine) skeletons are shown in (1), (2) and (6) respectively. To date, wherever their absolute stereochemistry has been established the alkaloids have corresponded to derivatives of ent-kaurene, as shown in Fig. 2 and elsewhere in this thesis.

The C_{20}-diterpene alkaloids[22] are comparatively simple and relatively non-toxic alkamines. These compounds are not extensively oxygenated and contain at most one methoxyl group. They usually possess an exocyclic methylene group.

The C_{19}-diterpene alkaloids[23] on the other hand are often extensively functionalized and some are highly toxic. They do not possess an exocyclic methylene group and are heavily substituted by hydroxyls, some of which may be esterified, and methoxyl groups. Both the aconitine and lycoctonine-type alkaloids have a hexacyclic skeleton. Typically they possess a tertiary nitrogen substituted with either an ethyl or methyl group. The key difference between the aconitine and lycoctonine-type skeletons is that the latter contains a hydroxyl functional group at C-7 whereas the former does not. The presence or absence of a ditertiary α-glycol system or derivative thereof is thus the determining factor in the classification, as well as differences in the chemistry of the two groups of alkaloids. Two further trends are to be noted. When hydroxylated or methoxylated at C-6 the orientation is typically α- in the aconitine system, but β- in the lycoctonine one. Also, whereas many of the aconitine-type alkaloids possess a bridgehead hydroxyl at C-13, none

of the lycoctonine-type alkaloids have been isolated with a substituent of any kind at this site.

1.4 The Biosynthesis of Diterpenoid Alkaloids

The identification of delphinium and aconitum alkaloids as diterpenoid is based upon the idea that the nitrogen-free framework can be visualized as formed from a classical terpene[24] (Figure 3). The presumed progenitor of the diterpene alkaloids is geranylgeranyl pyrophosphate which undergoes acid-catalysed cyclization to the carbocation (8), which generates a pimaradiene (9). When the diene is reprotonated and further isomerized, a cation results which may have the classical structure (10a), or a non-classical one (10b). When this ion undergoes a Wagner-Meerwein rearrangement, the known diterpene ent-kaurene (11), which resembles veatchine (12) is obtained. If the cation (10a or b) undergoes the equivalent of a 2,6-endo hydride shift in a norbonyl cation, a different cation (13) capable of a different Wagner-Meerwein rearrangement, is formed, and a hydrocarbon skeleton (14), resembling atisine (15) results.

The aconitane (lycoctonine and aconitine) skeleton may be derived formally from the atisine skeleton[26,27] by cleavage of the C(8)-C(9) bond, formation of new bonds between C(7) and C(20), C(9) and C(15), and loss of the C(17) exocyclic methylene group. As depicted in figure 4, assuming that atisine or some species closely resembling atisine were capable of being transformed into a more highly oxidized form, e.g. (16), then rearrangement and attack by water could potentially generate (17), which, further rearranging via the cyclization shown, produces

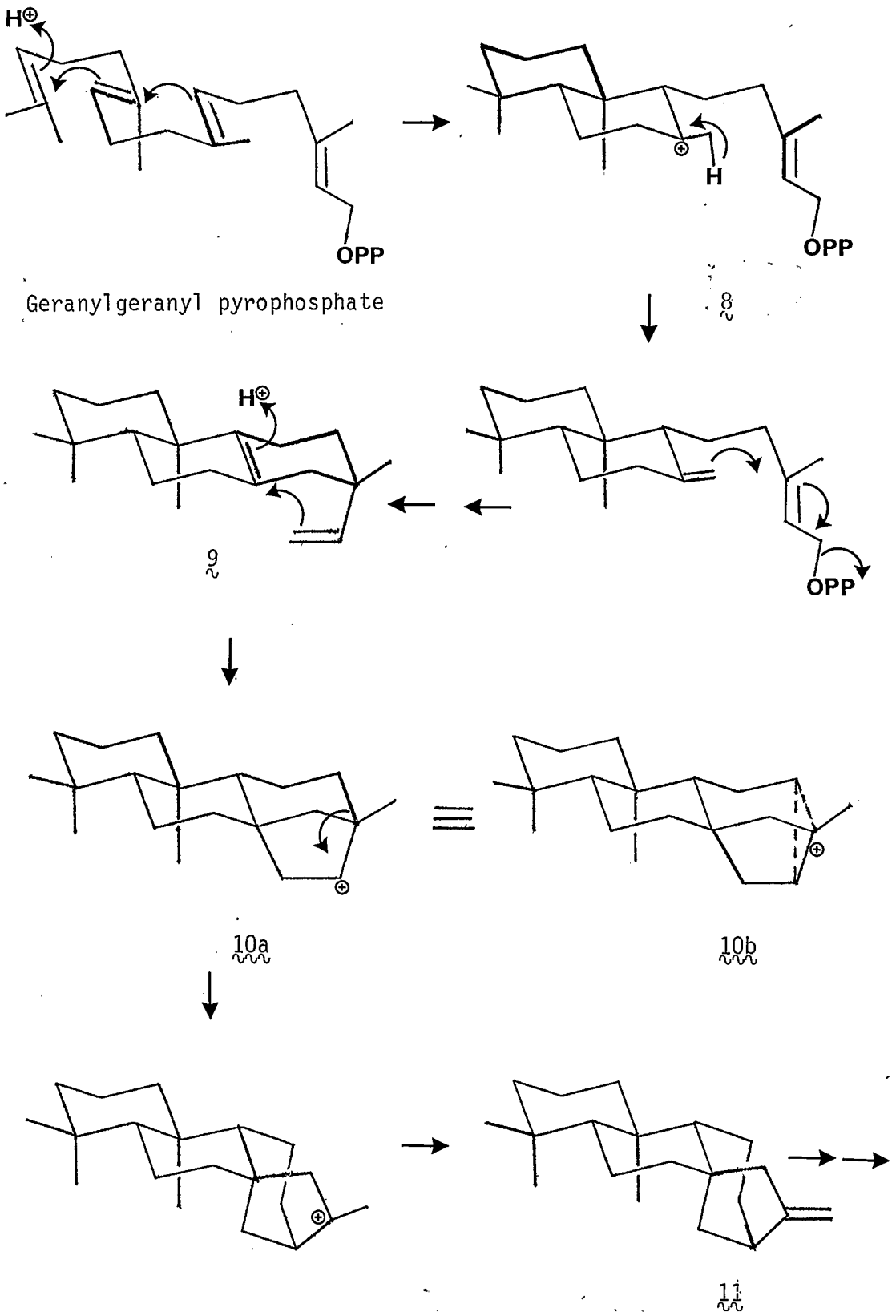


Figure 3: A possible Pathway to Diterpene Alkaloids Veatchine and Atisine.[25]

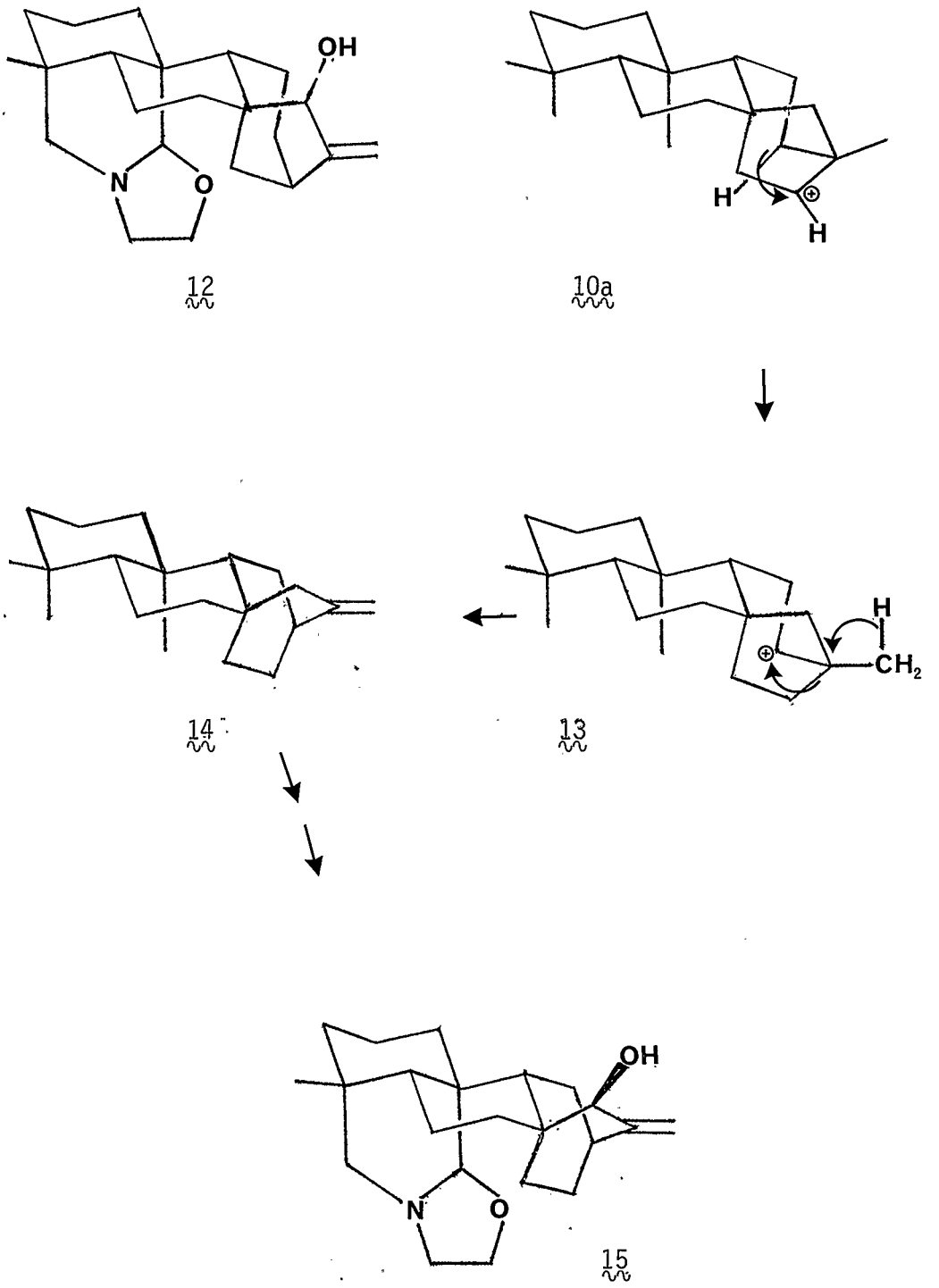


Figure 3: (continued)

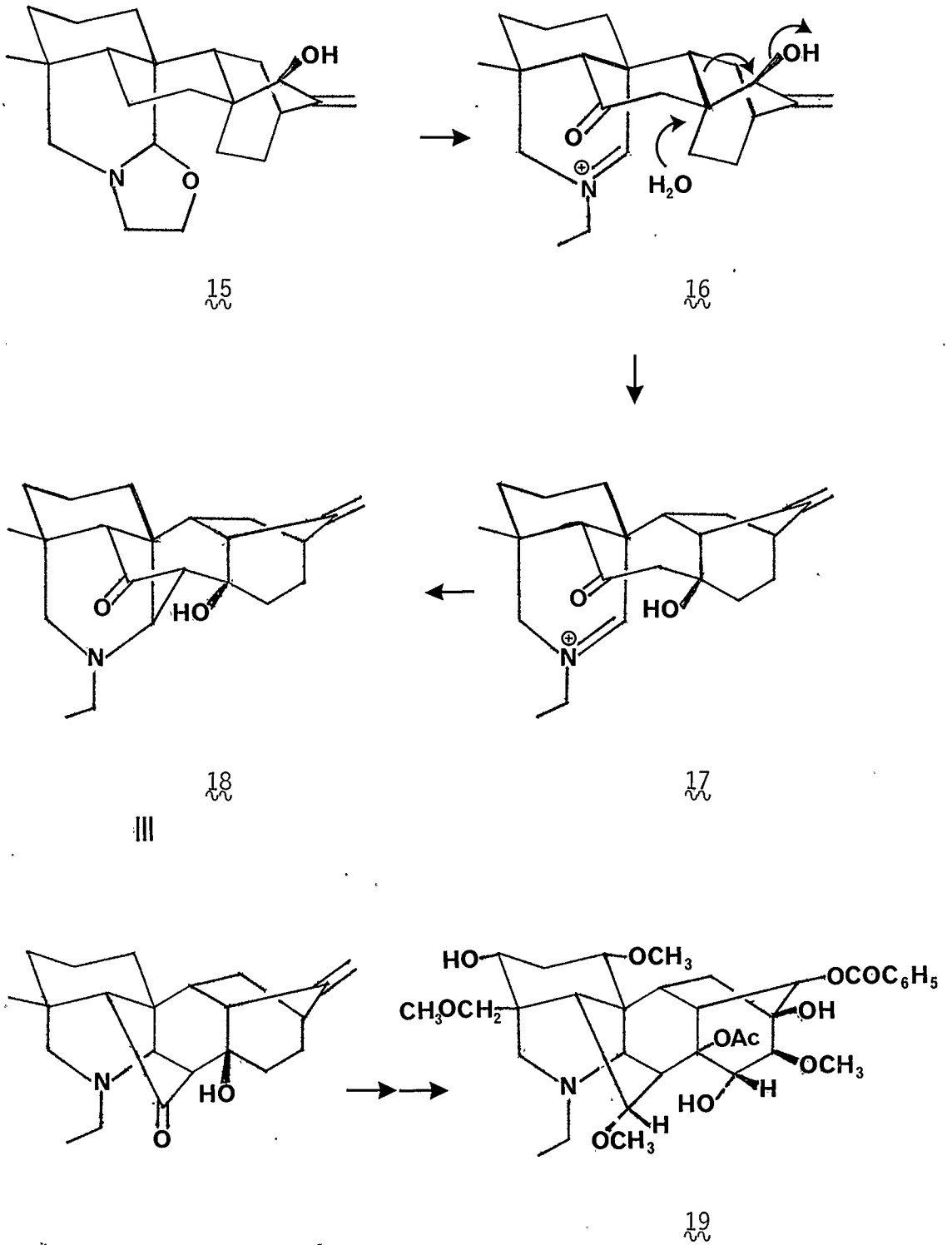


Figure 4: A possible Pathway to Aconitine from Atisine.[25]

(18), a species resembling aconitine (19). All of this is highly speculative, and likewise the source of the nitrogen atom and the way in which it is incorporated into the diterpenoid skeleton. It has been suggested[25] that early oxidation occurs in allylic positions and that nitrogen is introduced later.

In outline the hypothesis is thus that veatchine-like species lead to atisines, and that these are then the progenitors of aconitanes.

The terpenoid-derivation of the alkaloids, although very plausible, has only sketchy direct experimental support. Feeding experiments with labelled potential precursors, e.g. acetate, mevalonate, etc. have resulted in only miserably low incorporations into the alkaloids,[28-30] and no degradations have been performed to show that the labels were introduced into the specific sites predicted by the biosynthetic hypothesis.

CHAPTER 2. RESULTS AND DISCUSSION

2.1. Source of Plant Material, Extraction and Fractionation

Delphinium macrocentrum was collected just prior to blossoming on Mt. Kenya. Details of the processing of this material are provided in the experimental and summarised in Figure 5. Extraction with ethanol yielded on a dry weight basis, about 0.4% of a mixture of alkaloids which were initially roughly fractionated by exploiting differences in their base strengths (extractions over a pH-gradient). The TLC analytical results for the fractions obtained at pH 5 (A1), pH 7 (A2), pH 9 (A3) and pH 11 (A4) were as sketched in Figure 6.

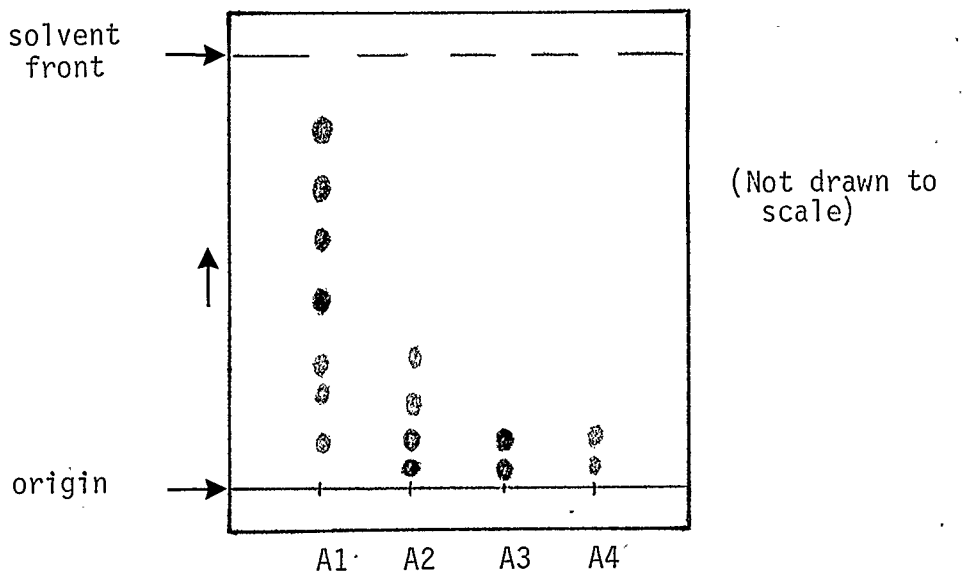


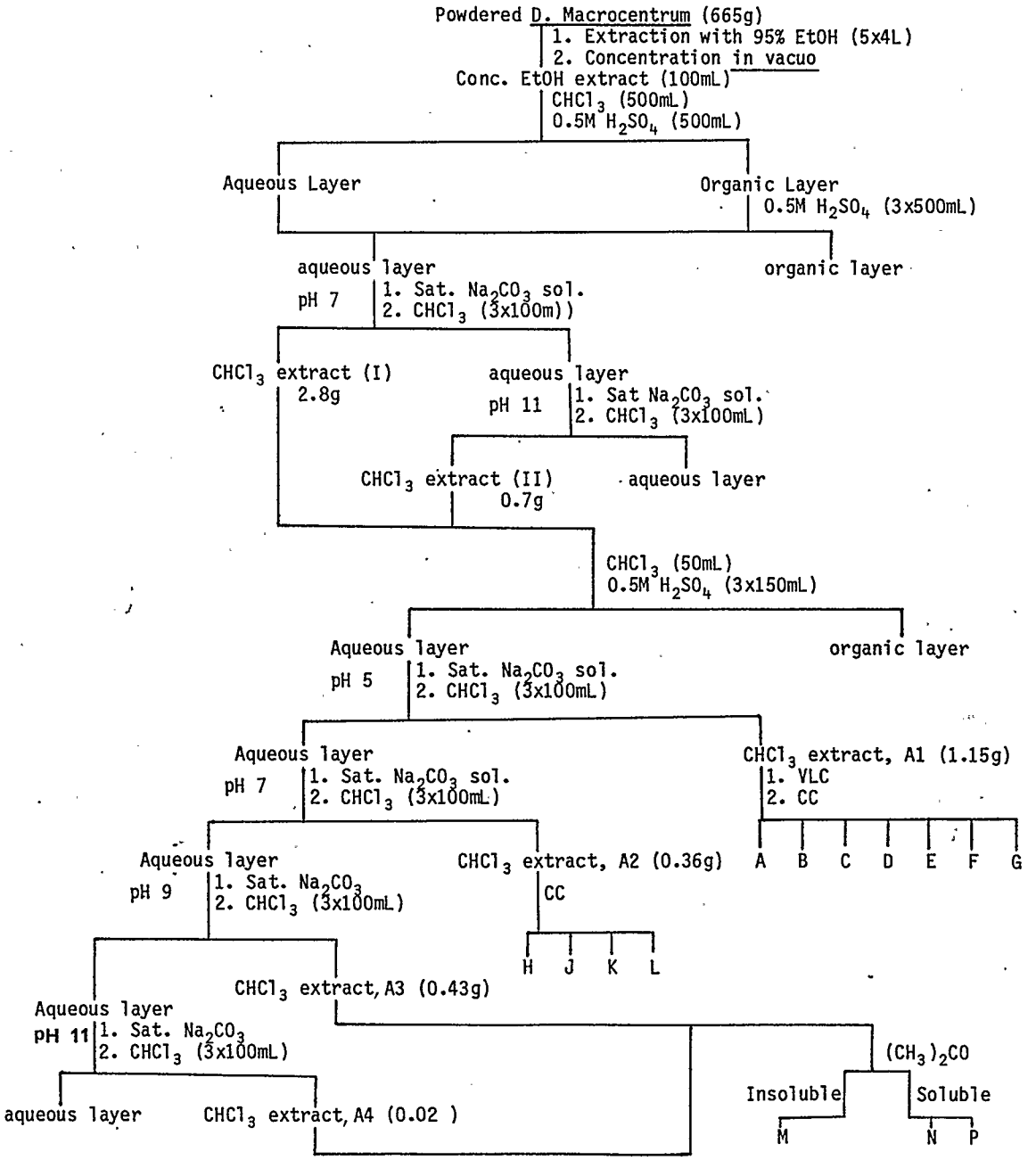
Figure 6: TLC Analysis of the pH-gradient-fractionated Alkaloids.

2.2 Alkaloids from the pH 5 (A1) Fraction

The bulk of the alkaloids were weakly basic and extracted into chloroform from an aqueous solution at pH 5. For the further fractionation of this material, short-column vacuum liquid chromatography (VLC)[31]

Figure 5

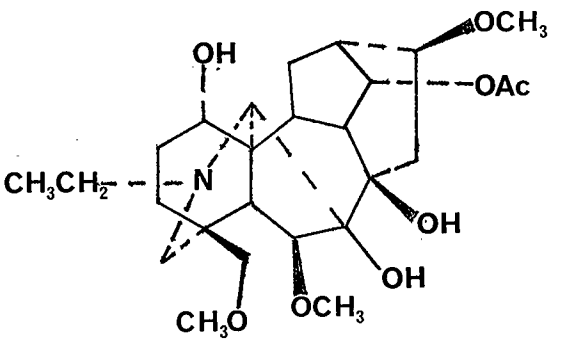
Summary of the Extraction and Separation Procedures



over TLC-grade basic alumina was compared with conventional, gravity flow, column chromatography (CC) over neutral alumina. The results of these separations are given in Tables 11 and 12 (see experimental section) and, as will become clear later, resulted in equivalent fractionations. VLC has been advertised[31] as faster and more powerful than CC. In this investigation, the relative speediness of VLC was apparent, but not the superiority of resolving power. Fractions of similar composition, as revealed by TLC analyses were pooled and further fractionated by VLC and, most usually, PTLC. By these means seven substances (A-G) were isolated, homogeneous, or nearly so, by TLC. These were then characterized by the usual spectroscopic procedures with the following results.

Alkaloid A

The MS revealed an apparent molecular ion at m/z 495 with intense fragment ions at 480 (M-15), 478 (M-17), 464 (M-31), 462 (M-33). As the ^{13}C-NMR spectrum revealed twenty-six carbon atoms, a molecular formula of $C_{26}H_{41}NO_8$ was inferred. The ^{1}H-NMR spectrum revealed the presence of an N-ethyl unit (3H, t, J=7Hz, centred at δ 1.09), an acetate (3H, s, δ 2.06), and three aliphatic methoxyl groups (each 3H, s, δ 3.32, 3.33, 3.34). As well there was an absorption (1H, dd, J=5Hz, δ 4.80) typical of a C(14)-β-H of a C_{19}-lycoctonine type alkaloid carrying an α-acetoxyl function at this position.[32] The intense M-17 ion in the MS suggested that a hydroxyl group was present at C(1),[33] and if the three methoxyl groups were then present at the usual sites and with usual configuration (6β, 16β and 18), the alkaloid would be 14-acetyldelcosine (20).



20 14-Acetyldelcosine

This tentative identification was confirmed by the perfect correspondence of the ^{13}C-NMR spectrum of the alkaloid with that reported for 14-acetyldelcosine [31,34] (see Table 1).

The recorded features of the ^{1}H-NMR spectrum[35] were similarly in accord with those observed for 20. The isolated yield of this alkaloid comprised about 4.2% of the mixed alkaloid fraction A1.

Alkaloid B

The ^{1}H-NMR spectrum revealed the alkaloid to contain an N-ethyl group (3H, t, J=7.3Hz, centred at δ 1.09) and four methoxyl groups (each 3H, s, 3.37, 3.39, 3.40 and 3.47). The mass spectrum exhibited a molecular ion peak at m/z 467, with high mass fragment ions at 452 (M-15), 436 (M-31), 420 (M-47), 404 (M-63). As the ^{13}C-NMR spectrum showed 25 signals, apparently corresponding to 25 carbon atoms, the molecular formula $C_{25}H_{41}NO_7$ was therefore deduced. Of the 25 carbons, six were seen in the ethyl unit and four methoxyl groups, leaving 19 i.e. it seemed likely that this alkaloid also belonged to the C_{19}-class. If this were the case, C(18) must be functionalised because neither the

TABLE 1

Carbon-13 Chemical Shifts and Assignments for Alkaloid A and 14-Acetyldelcosine (20)[32,34]

C-atom	Compound[a] A	20
C-1	72.6	72.6
2	27.2	27.2
3	29.9	29.9
4	37.5	37.5
5	43.5	43.5
6	90.1	90.1
7	87.7	87.6
8	78.3	78.4
9	44.8	44.9
10	38.0	38.0
11	49.2	49.2
12	29.3	29.4
13	42.5	42.6
14	76.3	76.3
15	33.8	33.8
16	82.6	82.7
17	66.1	66.1
18	77.3	77.3
19	57.3	57.2
NCH_2	50.3	50.3
CH_3	13.6	13.6
CH_3O-6	57.2	57.2
16	56.3	56.3
18	59.0	59.1
C=O	171.4	171.4
CH_3	21.5	21.4

[a] In $CDCl_3$

^{13}C-NMR nor the ^{1}H-NMR spectrum contained an absorption attributable to a tertiary C-methyl group. Of the common alternatives, hydroxylation or methoxylation at C(18), the presence of an absorption at 78.7 ppm (seen as a triplet in the ^{13}C-NMR off-resonance spectrum) was consistent with the latter. In the case of a C(18) bearing a hydroxyl, the signal appears at about 66.5-68.5 ppm.[36,37]

The C_{19}-diterpenoid alkaloids usually bear a hydroxyl or a methoxyl group on C(1), C(8), C(14) and C(16).[21,23,32] Making this assumption for B, only two oxygens of the seven present in the molecule remained to be located. Of the alternative sites, C(13) and C(15) oxygenation is common in the alkaloids of Aconitum, but not Delphinium. On the other hand C(7) is oxygenated in the lycoctonine-type alkaloids typically encountered in Delphinium, and C(6) is much more often similarly functionalised than C(9) or C(10). A singlet in the ^{13}C-NMR spectrum of B at 87.7 ppm due to a carbon atom bearing a hydroxyl/methoxyl, was in exactly the position expected for a lycoctonine type C(7)-β-OH.[32] A methoxyl at C(7) appeared unlikely since none of the naturally occuring diterpenoid alkaloids has hitherto been isolated bearing a methoxyl at this position. After making this decision, one oxygen remained to be located.

All the C_{19}-diterpenoid alkaloids having an OH group at C(9) are known to show a singlet at about 77.5-78.5 ppm.[38-39] As no such singlet was observed in this region, an oxygen function at C(9) was excluded. The signals in the ^{13}C-NMR at 37.2 and 48.6 ppm could be assigned to the non-oxygenated quarternary carbon atoms, C(4) and C(11). Since C(11) appears at 48.6 ppm and not around 55-56 ppm,[40,41] the C(10) position

does not bear an oxygen function.

In the case of aconitine-type C_{19}-diterpenoid alkaloids bearing a hydroxyl group at C(13), the C(12) signal (triplet) is observed in the region 33.5-38.0 ppm.[42] As there was no absorption (triplet) in this region of the spectrum of B, an OH at C(13) could be ruled out. Furthermore there were three upfield signals (triplets) at 27.1, 29.3 and 28.0 ppm which were assigned to C(2), C(3) and C(12), respectively. Carbon-6 was therefore considered as the most likely to bear the remaining oxygen function. An absorption at 90.7 ppm (doublet) was ascribed to this carbon, as it was in the expected position for a C(6) bearing a β-methoxyl in a lycoctonine-type skeleton.[32]

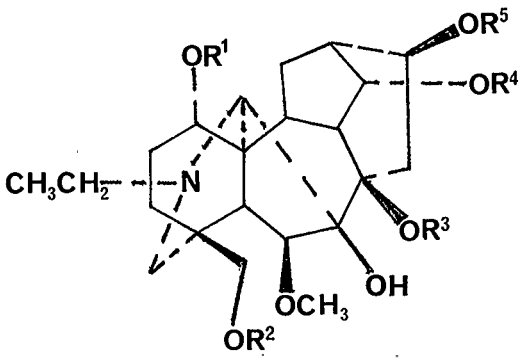
Most of the C_{19}-diterpenoid alkaloids bear a β-methoxyl at C(16), and the resonance for this carbon appears around 79.5-84.5 ppm.[32] An absorption at 82.1 ppm (doublet) in the spectrum of B was assigned to this functionality. It may be noted that if a C(15)-α-OH is present together with C(16) methoxylation, the C(16) absorption shifts to the region 89.5-92.0 ppm and that the methoxyl carbon appears as a signal in the region 60.5-62.5 ppm. Since none of the methoxyl carbons appeared in this region, a C(15)-hydroxyl group could be excluded.

It was therefore concluded that alkaloid B belongs to the lycoctonine-type, with oxygen functions at C(1)-α, C(6)-β-OCH_3, C(7)-β-OH, C(8)-β, C(14)-α, C(16)-β-OCH_3 and C(18)-OCH_3.

The molecular formula deduced for B, $C_{25}H_{41}NO_7$ corresponds to that of three well known alkaloids, browniine (21), lycoctonine (22) and delsoline (23). However, a comparison of the spectroscopic data for B with that reported for these alkaloids and in particular their ^{13}C-NMR

spectra (see Table 2) excluded these possibilities; while the ^{1}H-NMR spectrum of B was different from that reported for the rare alkaloid delbiterine (24), also of the same molecular formula. The ^{13}C-NMR data for delbiterine [32,44] was not available for comparison.

Returning to the ^{13}C-NMR spectrum of B, it was deduced that a methoxyl at C(1) was unlikely because the shifts attribted to C(2) and C(3), at 27.1 and 29.3 ppm correspond to C(1)-α-hydroxylation. Also, the chemical shift of C(14), when α-methoxylated, should be around 83.5-85.0 ppm.[32] Since no signal was observed in this region, C(14) must be substituted by a hydroxyl group and the absorption at 74.9 ppm (doublet) was in accord with this possibility. This led to structure (25) for alkaloid B, which corresponds to deltatsine, a compound which had been



21 $R^1=R^2=R^5=CH_3$, $R^3=R^4=H$; Browniine

22 $R^1=R^4=R^5=CH_3$, $R^2=R^3=H$; Lycoctonine

23 $R^1=R^3=H$, $R^2=R^4=R^5=CH_3$; Delsoline

24 $R^1=R^2=R^4=CH_3$, $R^3=R^5=H$; Delbiterine

25 $R^1=R^4=H$, $R^2=R^3=R^5=CH_3$; Deltatsine

TABLE 2

Carbon-13 Chemical Shifts and Assignments for Alkaloid B, Deltatsine (25),[43] Browniine (21),[32,34] Lycoctonine (22)[34,36] and Delsoline (23)[34]

	Compound[a]				
C-atom	B	25	21	22	23
C-1	72.3 d	72.3 d	85.2	84.2	72.6
2	27.1 t	27.2 t	25.5	26.1	27.2
3	29.3 t	29.3 t	32.5	31.6	29.3
4	37.2 s	37.1 s	38.4	38.6	37.4
5	39.8 d	39.9 d	45.1	43.3	43.9
6	90.7 d	90.6 d	90.1	90.6	90.4
7	91.4 s	91.2 s	89.1	88.3	87.8
8	81.1 s	81.2 s	76.3	77.5	78.5
9	48.9 d	48.9 d	49.6	49.7	44.9
10	45.3 d[b]	45.2 d[c]	36.4	38.0	37.7
11	48.6 s	48.6 s	48.2	48.9	49.3
12	28.0 t	28.5 t	27.5	28.8	30.5
13	39.6 d[b]	39.9 d[c]	46.1	46.1	43.3
14	74.9 d	74.7 d	75.3	84.0	84.5
15	31.2 t	30.9 t	33.1	33.7	33.5
16	82.1 d	82.4 d	81.7	82.7	82.9
17	66.7 d	66.5 d	65.4	64.8	66.0
18	78.7 t	78.6 t	78.0	67.6	77.3
19	57.3 t	57.3 t	52.7	52.9	57.2
NCH_2	50.5 t	50.3 t	51.3	51.1	50.3
CH_3	13.8 q	13.7 q	14.3	14.1	13.5
CH_3O-1	-	-	56.0	55.7	-
6	59.3 q	59.2 q	57.5	57.7	57.2
8	51.5 q	51.3 q	-	-	-
14	-	-	-	58.0	57.9
16	56.4 q	56.3 q	56.5	56.2	56.3
18	59.4 q	59.3 q	59.1	-	59.1

[a] In $CDCl_3$

[b,c] These assignments may be interchanged

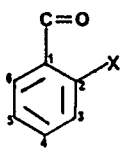
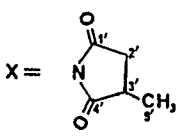
described once before.[43] The ^{13}C-NMR spectrum (Table 2) was in good agreement with that reported for deltatsine, thus providing support for this identification. The isolated yield of this alkaloid comprised 3.5% of the mixture A1.

Alkaloid C

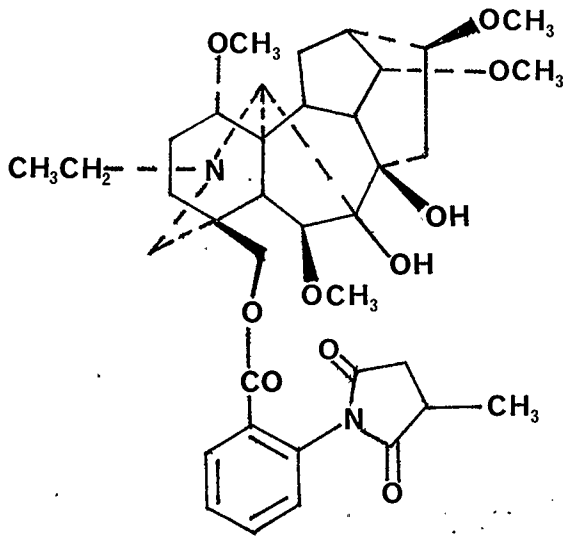
Unlike most of the others, this alkaloid was strongly UV-absorbing. The ^{1}H-NMR spectrum disclosed the presence of an N-ethyl (3H, t, J=7Hz, centred at δ 1.04), four methoxyls (each 3H, s, δ 3.26, 3.41 and 6H, s, δ 3.34) and four aromatic protons (1H, dd, J=7.5Hz, 2Hz, δ 7.34; 1H, dt, J=7.5Hz, 2Hz, δ 7.60; 1H, dt, J=7.5Hz, 2Hz, δ 7.74; 1H, dd, J=7.5Hz, 2Hz, δ 8.10) characteristic of an ortho-disubstituted benzene ring. This pattern is typical of C_{19}-diterpenoid alkaloids in which the C(18)-hydroxyl is esterified by an anthranilic acid moiety. The ^{13}C-NMR spectrum showed 36 signals. An absorption at 83.9 ppm was attributed to two carbon atoms since it corresponded to about twice the intensity of the other proton bearing carbon atoms, so a total of 37 carbons was inferred. Off-resonance decoupling experiments gave the multiplicity of each signal. The crystalline HI salt of alkaloid C showed strong signals in the IR spectrum at ν_{max} 3473 (OH), 1716 (Benzoyl C=O), 1773, 1695 (Imide C=O), 1396 (C-CH_3), 1256 (Ester C-O), 1085 (ether C-O). On the basis of this data the alkaloid was tentatively assigned the molecular formula $C_{37}H_{50}N_2O_{10}$, and suspected to be methyllycaconitine (26). Indeed, ^{13}C-NMR data was in full agreement with that previously reported for methyllycaconitine[32,34,45] (Table 3). Further confirmation of its identity was obtained from co-chromotography of C with an authentic specimen of methyllycaconitine. Finally the mp and IR spectrum of the

TABLE 3

Carbon-13 Chemical Shifts and Assignments for Alkaloid C and Methyllycaconitine (26)[32,34,45]

	Compound[a]	
C-atom	C	26
C-1	83.9 d	83.9
2	26.1 t	26.0
3	32.1 t	32.0
4	37.5 s	37.6
5	43.2 d	43.2
6	90.8 d	90.8
7	88.5 s	88.5
8	77.5 s	77.4
9	50.2 d	50.3
10	38.2 d	38.0
11	49.0 s	49.0
12	28.7 t	28.7
13	46.1 d	46.1
14	83.9 d	83.9
15	33.6 t	33.6
16	82.5 d	82.5
17	64.5 d	64.5
18	69.5 t	69.5
19	52.3 t	52.3
NCH_2	51.0 t	50.9
CH_3	14.0 q	14.0
CH_3O-1	55.8 q	55.7
6	57.8 q	57.8
14	58.1 q	58.2
16	56.3 q	56.3
	164.1 s	164.1
1	127.1 s	127.1
2	133.0 s	133.1
3	129.3 d	129.4
4	133.6 d	133.6
5	131.0 d	131.0
6	130.0 d	130.0
X =  1'	179.8 s	179.8
2'	37.0 t	37.0
3'	35.3 d	35.3
4'	175.8 s	175.8
5'	16.4 q	16.4

[a] In $CDCl_3$



26 Methyllycaconitine

crystalline HI salt of C were identical with those of an authentic sample of methyllycaconitine HI salt. The isolated yield of Alkaloid C amounted to 12.6% of A1.

Alkaloid D

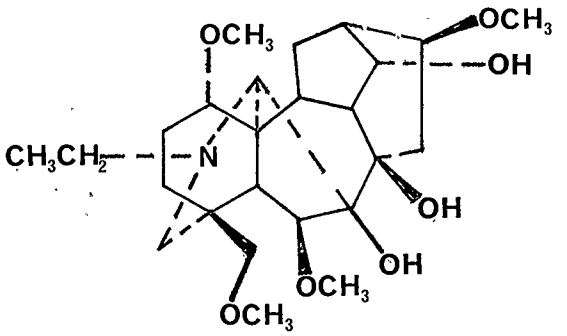
The ^{1}H-NMR spectrum showed signals from the methyl part of an N-ethyl group (3H, t, J=7Hz, centred at δ 1.04) and four methoxyl groups (each 3H, s, δ 3.25, 3.30, 3.36, and 3.41). The mass spectrum revealed an apparent molecular ion peak at m/z 467 with high mass fragment ions at 452 (M-15), 437 (M-30), 436 (M-31), 435 (M-32), 417 (M-50), 406 (M-61). The ^{13}C-NMR spectrum showed 25 signals, apparently corresponding to 25 carbon atoms. From this data a C_{19}-diterpenoid alkaloid with a lycoctonine-type skeleton, and formula $C_{25}H_{41}NO_7$ was again inferred. The ^{13}C-NMR spectral data of alkaloid D was in excellent agreement with that reported for browniine (27)[32,34] (see Table 4), and the ^{1}H-NMR spectrum was similarly in accord with that reported for browniine.[32,35,36]

TABLE 4

Carbon-13 Chemical Shifts and Assignments for Alkaloid D and Brownline (27)[32,34]

	Compound[a]	
C-atom	D	27
C-1	85.2	85.2
2	25.5	25.5
3	32.5	32.5
4	38.3	38.4
5	45.1	45.1
6	90.0	90.1
7	89.1	89.1
8	76.3	76.3
9	49.6	49.6
10	36.3	36.4
11	48.1	48.2
12	27.5	27.5
13	46.0	46.1
14	75.3	75.3
15	33.0	33.1
16	81.7	81.7
17	65.4	65.4
18	77.9	78.0
19	52.6	52.7
NCH_2	51.3	51.3
CH_3	14.3	14.3
CH_3O-1	56.0	56.0
6	57.4	57.5
16	56.5	56.5
18	59.1	59.1

[a] In $CDCl_3$



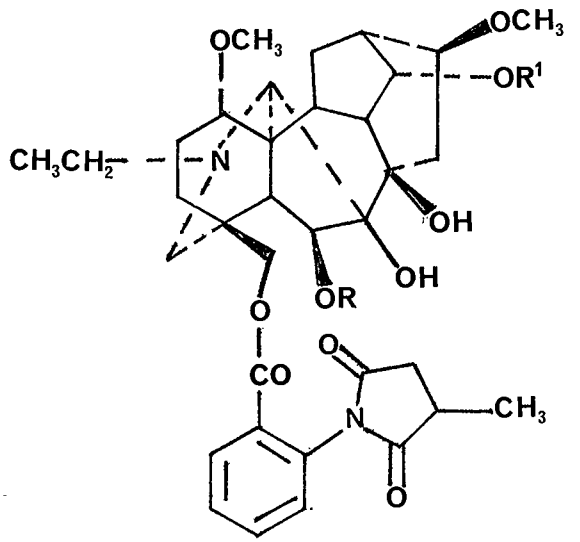
21 Browniine

Further confirmation of identity was made by direct comparison and co-chromatography of D with an authentic specimen of browniine. The isolated yield of Alkaloid D comprised about 4.5% of A1.

Alkaloid E

The ^{1}H-NMR spectrum revealed the alkaloid to possess an N-ethyl group (3H, t, J=7.2Hz, centred at δ 1.06), three methoxyl groups (3H, s, δ 3.25, and 6H, s, δ 3.36) and four aromatic protons (1H, dd, J=7.5Hz, 2Hz, δ 7.30; 1H, dt, J=7.5Hz, 2Hz, δ 7.57; 1H, dt, J=7.5Hz, 2Hz, δ 7.72; 1H, dt, J=7.5Hz, 2Hz, δ 8.06) characteristic of an ortho-disubstituted benzene ring. As for alkaloid C, earlier on identified as methyllycaconitine, this pattern is typical of C_{19}-diterpenoid alkaloids in which the C(18)-hydroxyl is esterified by an anthranilic acid moiety, most probably methylsuccinimidoanthranilic acid. Also like alkaloid C, this alkaloid was strongly UV-absorbing. The mass spectrum exhibited an apparent molecular ion peak at m/z 668, with high mass fragment ions at 651 (M-17), 637 (M-31), 583 (M-85), 436 (M-232). The ^{13}C-NMR spectrum showed 36 signals corresponding to 36 carbon atoms, so a molecular formula $C_{36}H_{48}N_2O_{10}$ was deduced. On the basis of this data, the

alkaloid was suspected to be a close relative of methyllycaconitine. The only other known diterpenoid alkaloid of molecular formula $C_{36}H_{48}N_2O_{10}$ and containing three methoxyl groups was found to be glaudelsine (27),[45] and the ^{13}C-NMR spectrum of alkaloid E closely resembled that reported [32,45] for 27 (see table 5). The main differences



26 R=R^1=CH_3; Methyllycaconitine

27 R=H, R^1=CH_3; Glaudelsine

28 R=CH_3, R^1=$COCH_3$; Nudicauline

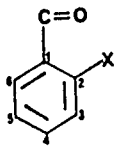
29 R=CH_3, R^1=H; Alkaloid E

were the signals at 75.2 ppm, 36.3 ppm and the appearance of signals corresponding to single carbon atoms each, at 37.0 and 84.9 ppm. The main difference in the ^{13}C-NMR spectrum of E in comparison with that of methyllycaconitine (Table 5) was the appearance of a signal at 75.2 ppm and a low-field signal at 84.9 ppm corresponding to a single carbon atom, as compared to the signal at 83.9 ppm for two carbons in methyl-

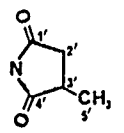
TABLE 5

Carbon-13 Chemical Shifts and Assignments for Alkaloid E (29), Glaudelsine (27),[32,45] and Methyllycaconitine (26)[32,34,45]

C-atom	Compound[a] 29	27	26
C-1	84.9 d	84.9	83.9
2	25.3 t	25.3	26.0
3	32.1 t	32.2	32.0
4	37.8 s	37.0	37.6
5	45.1 d	45.8	43.2
6	90.3 d	90.3	90.8
7	89.2 s	89.2	88.5
8	76.3 s	76.3	77.4
9	50.1 d	50.2	50.3
10	36.3 d	37.9	38.0
11	48.3 s	48.3	49.0
12	27.4 t	27.6	28.7
13	46.0 d	46.1	46.1
14	75.2 d	84.9	83.9
15	33.1 t	33.1	33.6
16	81.7 d	81.7	82.5
17	65.0 d	65.0	64.5
18	69.4 t	69.5	69.5
19	52.3 t	52.4	52.3
NCH_2	51.2 t	51.2	50.9
CH_3	14.2 q	14.3	14.0
CH_3O-1	56.1 q	56.1	55.7
6	58.3 q	-	57.8
14	-	58.3	58.2
16	56.5 q	56.5	56.3
C=O	164.2 s	164.2	164.1
1	127.0 s	127.1	127.1
2	133.1 s	133.1	133.1
3	129.4 d	129.5	129.4
4	133.7 d	133.7	133.6
5	131.0 d	131.0	131.0
6	130.0 d	130.1	130.0
1'	179.9 s	179.8	179.8
2'	37.0 t	37.0	37.0
3'	35.2 d	35.3	35.3
4'	175.9 s	175.9	175.8
5'	16.3 q	16.3	16.4



X =



[a] In $CDCl_3$

lycaconitine. It therefore appeared that the alkaloid had the basic structure of methyllycaconitine, but with the C(14)-α-methoxyl replaced by a hydroxyl. The signal at 75.2 ppm was attributed to C(14) bearing an α-oriented hydroxyl function, while the signal at 84.9 ppm was assigned to C(1). In the case of methyllycaconitine with C(1) and C(14) bearing α-methoxyls, both these atoms are observed as resonances at 83.9 ppm. On the basis of the above spectral evidence, alkaloid E was assigned structure (29).

In passing, the assignment of the resonance signal at 90.3 ppm to a C(6) bearing a β-OH in the ^{13}C-NMR spectrum of glaudelsine [32,45] appears to be in error. A C(6) with a β-OH would be expected to appear in the region 77.0-79.5 ppm,[32] for a lycoctonine-type alkaloid, as in the cases of delphinifoline[32] and delcorine.[32,46]

To test the proposed structure, alkaloid E was saponified to yield a product whose ^{1}H-NMR revealed the presence of an N-ethyl group (3H, t, J=7.1Hz, centred at δ 1.05), and three methoxyls (each 3H, s, δ 3.26, 3.36 and 3.44). As well its ^{13}C-NMR spectrum showed 24 signals for 24 carbon atoms. From the composition of E and the ^{13}C-NMR data of the saponification product, a molecular formula $C_{24}H_{39}NO_7$ was inferred for the latter. Both the ^{1}H-NMR and ^{13}C-NMR spectral data were consistent with those reported for delectinine (30)[32,37,47] (see table 6 for ^{13}C-NMR data), i.e. establishing this to be the parent carbinolamine which is esterified with methylsuccinimidoanthranilic acid to give E.

Further support for (29) as the structure of E was provided by acetylation with acetic anhydride in pyridine: an acetylation which, in the case of lycoctonine-type alkaloids, leaves the tertiary OH's at C(7)

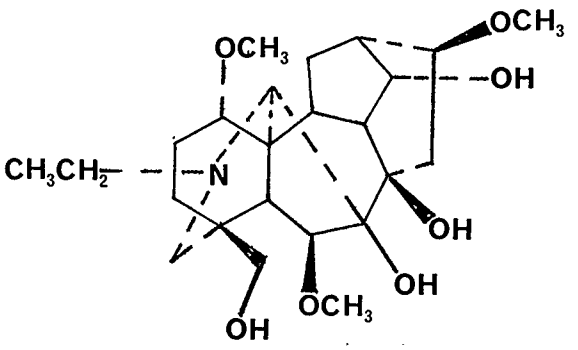
TABLE 6

Carbon-13 Chemical Shifts and Assignments for Saponified 29 and Delectinine (30)[37]

C-atom	Compound[a] Saponified 29	30
C-1	85.2	85.1
2	25.4	25.3
3	31.6	31.6
4	38.9	38.8
5	45.1	45.1
6	90.1	90.1
7	89.9	89.0
8	76.3	76.3
9	49.4	49.5
10	36.3	36.5
11	48.1	48.2
12	27.5	27.5
13	46.1	46.1
14	75.3	75.3
15	33.0	33.1
16	81.7	81.8
17	65.4	65.4
18	67.7	67.6
19	52.6	52.8
NCH_2	51.3	51.3
CH_3	14.3	14.2
CH_3O-1	56.0	56.0
6	58.0	58.1
16	56.5	56.5

[a] In $CDCl_3$

and C(8) unesterified. The product was found to be nudicauline (28)[48] by ^{1}H-NMR spectroscopy and comparison with an authentic specimen of that alkaloid. Accordingly alkaloid E was named deacetylnudicauline. The isolated yield accounted for about 7% of A1.

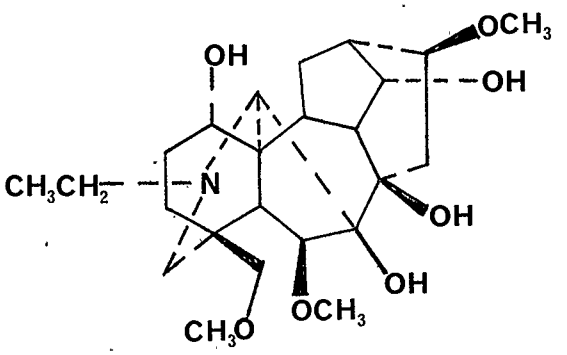


30 Delectinine

Alkaloid F

This alkaloid crystallized from hexane-acetone as colourless crystals (mp 204-207°C). The ^{1}H-NMR spectrum revealed the presence of an N-ethyl group (3H, t, J=7.2Hz, centred at δ 1.10) and three methoxyl groups (each 3H, s, δ 3.33, 3.36 and 3.37). The mass spectrum exhibited a molecular ion peak at m/z 453, with high mass fragment ions at 438 (M-15), 436 (M-17), 435 (M-18), 422 (M-31), 420 (M-33), 418 (M-35). The ^{13}C-NMR showed 24 signals corresponding to 24 carbon atoms. Off-resonance decoupling gave the multiplicity of each signal. On the basis of this data, a C_{19}-lycoctonine type alkaloid of formula $C_{24}H_{39}NO_7$ was once again inferred. Since 14-acetyldelcosine had earlier been isolated and identified (cf. alkaloid A), it was suspected that delcosine might be present in the alkaloid mixture A1. Of the known C_{19}-diterpenoid alkaloids with three methoxyls and a molecular formula $C_{24}H_{39}NO_7$, delcosine was therefore the first candidate for comparison. Indeed the ^{13}C-NMR

spectrum showed a perfect correspondence with that reported for delcosine (31) (Table 7).[32,34] The identity was further established by comparison with an authentic specimen of delcosine. They co-chromatographed on TLC and their IR spectra were identical. The isolated yield of this alkaloid accounted for 13.5% of A1.



31 Delcosine

Alkaloid G

This alkaloid gave a MS which contained an apparent molecular ion of composition $C_{23}H_{37}NO_7$ (found 439.2569, Calcd. 439.2570), with fragment ions at 424 (M-15), 422 (M-17), 408 (M-31), 406 (M-33), 390 (M-49), 250 (M-189). The ^{1}H-NMR spectrum revealed the alkaloid to contain an N-ethyl group (3H, t, J=7.2Hz, centred at δ 1.09) and two methoxyl groups (each 3H, s, δ 3.33, 3.39). On the basis of its spectral characteristics, the alkaloid was also suspected to have a C_{19}-lycoctonine type skeleton. The ^{13}C-NMR spectrum showed 22 signals, in which the absorption at δ 45.1 was assigned to two carbon atoms as it corresponded to about twice the intensity of other proton-bearing carbon atoms. Off-resonance decoupling gave the multiplicity of each signal. As no tertiary C-methyl group was observed in the ^{1}H-NMR or ^{13}C-NMR spectrum,

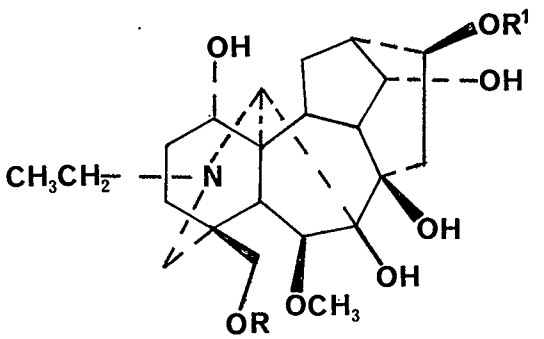
TABLE 7

Carbon-13 Chemical Shifts and Assignments for Alkaloid F and Delcosine (31)[32,34]

	Compound[a]	
C-atom	F	31
C-1	72.6 d	72.7
2	27.4 t	27.5
3	29.3 t	29.4
4	37.5 s	37.6
5	43.9 d	44.0
6	90.0 d	90.1
7	87.8 s	87.9
8	78.0 s	78.1
9	45.2 d	45.3
10	39.3 d	39.4
11	48.8 s	48.9
12	29.2 t	29.4
13	45.1 d	45.3
14	75.7 d	75.8
15	34.4 t	34.5
16	81.9 d	82.0
17	66.3 d	66.3
18	77.3 t	77.4
19	57.1 t	57.1
NCH_2	50.4 t	50.4
CH_3	13.7 q	13.7
CH_3O-1	57.3 q	57.4
16	56.3 q	56.4
18	59.0 q	59.1

[a] In $CDCl_3$

C(18) bears an oxygen function. An absorption at 77.2 ppm in the ^{13}C-NMR spectrum was consistent with a C(18) bearing a methoxyl group. As noted previously, in the case of C(18)-hydroxyl, the signal appears about 66.5-68.5 ppm. The signals at 37.6 and 48.5 ppm due to non- oxygenated quaternary carbon atoms were assigned to C(4) and C(11) respectively. The low-field signals at 78.4 and 87.8 ppm due to carbons bearing OH/OMe groups were assigned to C(7) and C(8) respectively, each bearing a hydroxyl. A methoxyl group at C(1) was unlikely because of the up-field signals at 27.5 and 29.3 ppm assigned to C(2) and C(3) respectively which are characteristic of a C(1)-α-hydroxylated system. A C(16) bearing a β-methoxyl group absorbs around 79.5-84.5 ppm.[32] Since no such a signal was observed in the spectrum of G, it appeared that C(16) bears a hydroxyl group. Similarly the chemical shift of C(14) bearing an α-methoxyl group appears at about 83.5-85.0 ppm,[32] and since no signal was observed in this region, C(14) appeared most likely to be α-hydroxylated, and the signal at 76.1 ppm was ascribed to this carbon atom. The signal at 89.9 ppm was attributed to C(6) bearing a β-methoxyl group. This evidence led to the structure (33) for alkaloid G, which apparently had not been described before. It was designated



32 R=H, $R^1=CH_3$; Takaosamine

33 $R=CH_3$, R^1=H; Alkaloid G

macrocentridine. Thus macrocentridine has the basic structure of delcosine (31), but with the C(16)-β-methoxyl replaced by a β-hydroxyl. Correlation of the fragmentation pattern in the mass spectrum with that found for delcosine,[23,33,49] showed consistency with the proposed structure. Both spectra had an ion at M-15 as the most intense high mass fragment. As well both spectra showed intense M-17, M-31 and M-33 fragment ions, and whereas delcosine showed an ion at m/z 264 (M-189), which has been ascribed to a fragment in which the C/D ring system is retained,[49] alkaloid G showed a similar peak at 250 (M-189), consistent with the latter lacking a methoxyl at C(16). Although the ^{1}H-NMR spectrum of macrocentridine closely resembled that reported for takaosamine (33),[32,48,51] this possibility was ruled out by the non-identity of their ^{13}C-NMR spectra (Table 8). Macrocentridine is the second example a C_{19}-lycoctonine type alkaloid bearing a hydroxyl rather than a methoxyl at C(16). The first one to be reported being delbiterine (24), for which unfortunately no ^{13}C-NMR data was available. The isolated yield of macrocentridine accounted for ca. 3.5% of A1.

2.3 Alkaloids from the pH 7 (A2) fraction

By employing conventional, gravity-flow column chromatography over neutral alumina, fraction A2, comprising the moderately basic alkaloids that extracted into chloroform at pH 7, was further fractionated. The results of this fractionation were as given in Table 13 (see experimental section, p.). Fractions of similar composition (TLC) were pooled, and further separation done using PTLC. This resulted in the isolation of four alkaloids (H-L), which were characterized by spectroscopic

TABLE 8

Carbon-13 Chemical Shifts and Assignments for Alkaloid G (33) and Takaosamine (32)[48]

C-atom	Compound[a] 33	32
C-1	72.6 d	72.6 d
2	27.5 t	26.9 t
3	29.3 t	29.3 t
4	37.6 s	38.2 s
5	43.9 d	44.8 d
6	89.9 d	90.1 d
7	87.8 s	87.8 s
8	78.4 s	78.0 s
9	45.1 d	43.9 d
10	43.1 d	45.2 d
11	48.5 s	48.8 s
12	28.7 t	26.9 t
13	45.1 d	39.3 d
14	76.1 d	75.7 d
15	38.7 t	34.4 t
16	72.3 d	81.9 d
17	66.1 d	66.3 d
18	77.2 t	66.8 t
19	57.0 t	57.0 t
NCH_2	50.4 t	50.4 t
CH_3	13.7 q	13.7 q
CH_3O-1	57.4 q	57.8 q
16	-	56.3 q
18	59.1 q	-

[a] In $CDCl_3$

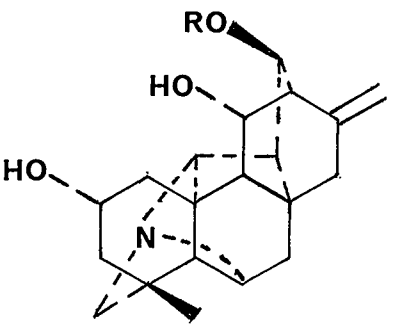
methods (^{1}H-NMR, ^{13}C-NMR, MS, IR).

Alkaloid H

This alkaloid was found to be identical with alkaloid D, which had been identified earlier from the pH 5 (A1) fraction as browniine (27) (see page 25). The isolated yield of the alkaloid comprised 6% of A2.

Alkaloid J

The ^{1}H-NMR revealed the presence of a tertiary C-methyl group (3H, s, δ 0.96), an exocyclic methylene function (1H, s, each at δ 4.79 and 4.98), and a benzoyl group (3H, m, δ 7.43 and 2H, dd, J=10Hz, 2Hz, δ 8.17). The mass spectrum showed a molecular ion peak at m/z 433 with intense fragment ions at m/z 312 (M-121), 282 (M-151), 105 (M-328) and 77 (M-356). The IR spectrum revealed characteristic absorptions at ν_{max}3359 (OH), 1728, 1600, 1588, 1279, and 735 (benzoate), 1650 and 916 ($>C=CH_2$) 1122 and 1064 (C-O). These findings led to the assumption that this alkaloid possessed a C_{20}-carbon skeleton, which was considered to be most probably the atisine skeleton or its derivative. The intense (base peak) fragment ion at m/z 105 (M-328) in the MS led to the suspicion that alkaloid J was a hetisine benzoate of molecular formula $C_{27}H_{31}NO_4$, since hetisine (34) has the molecular mass 329. Comparison of the ^{1}H-NMR spectrum of J with that reported for 13-O-acetylhetisine (35),[52] showed a close correspondence, especially the chemical shifts attributed to the C-methyl, the exomethylene functionality, C(2)-β-H, C(11)-β-H and C(13)-α-H. The alkaloid was therefore tentatively identified as hetisine-13-O-benzoate (36). Unfortunately due to lack of sufficient material, it was not possible to completely establish its identity. The amount isolated accounted for about 4% of A2.



34 R=H; Hetisine

35 R=Ac; 13-O-Acetylhetisine

36 R=Bz; Hetisine-13-O-benzoate

Alkaloid K

Alkaloid K crystallized from chloroform as colourless needles (mp 275-279°C). It was found to be identical with alkaloid N, first isolated from pH 9 (A3) and pH 11 (A4) fractions. Its identification is described later (page 44). The amount of Alkaloid K isolated accounted for ca. 5% of A2.

Alkaloid L

This alkaloid was found to be the same as alkaloid M (38), first isolated from the pH 9 (A3) and pH 11 (A4) fractions, and whose identification as macrocentrine is described later (page 40). The amount isolated comprised about 4% of A2.

2.4 Alkaloids from the pH 9 (A3) and pH 11 (A4) Fractions

Fractions A3 and A4 were similar and further fractionation was achieved by exploiting differences in their solubility in acetone. The

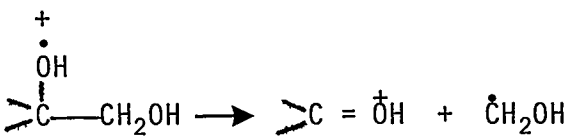
fractions A3 and A4 were each divided into acetone-insoluble and soluble portions. The insoluble portion yielded alkaloid M while the soluble part gave alkaloids N and P.

Alkaloid M

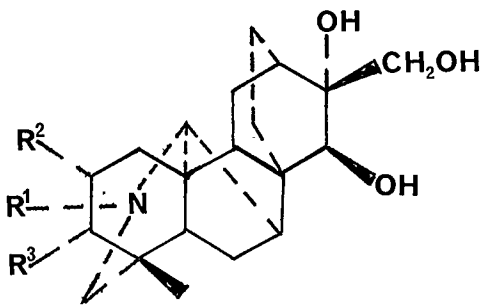
The alkaloid was isolated as a solid, homogeneous on TLC analysis. It crystallized from EtOH-H_2O in colourless tablets (mp 207-209°C). It gave a MS which contained as the base peak an apparent molecular ion of composition $C_{22}H_{35}NO_5$ (found m/z 393.2511, Calcd. 393.2516) with high mass fragment ions at m/z 376 (M-17), and 362 (M-31). The IR spectrum had ν_{max} 3400 cm^{-1} (brs, OH), but was devoid of absorptions attributable to carbonyl or olefinic functionalities. The ^{1}H-NMR spectrum revealed the presence of a tertiary C-methyl (3H, s, δ 0.81) and an N-ethyl group (3H, t, J=7Hz, δ 1.11), and absence of methoxyl or methylenedioxy groups.

The C_{22} formulation of alkaloid M taken together with the absence of methoxyl or methylenedioxy groups excluded a structure based on lycoctonine and attention was therefore turned to a consideration of hexacyclic derivaties of the C_{20} skeleton carrying an N-ethyl function.

The ^{13}C-NMR spectrum of the alkaloid in pyridine-d_5 and CD_3OD-$CDCl_3$, respectively, revealed resonances for 22 carbon atoms, in accord with the molecular composition deduced from the MS evidence; and together with the IR spectrum excluded olefinic and carbonyl functions. It was hypothesized that the formation of the m/z 362 fragment ion corresponded to the loss of a CH_2OH unit, which might occur readily from an exocyclic methylene group were it to be reduced and hydroxylated and particularly so had it been converted to a diol i.e.



Looking for models for such a system dictyzine (37)[53] was encountered. The very close correspondence of the ^{13}C-NMR resonances (Table 9) attributed to the C/D ring system, and pendant hydroxymethyl group, of dictyzine[54] with those found for alkaloid M, was striking. It was thus



37 R^1=CH_3, R^2=R^3=H; Dictyzine

38 R^1=Et, R^2=R^3=OH, Alkaloid M

deduced that the alkaloid was a dihydroxy N-ethyl homologue of dictyzine.

Placement of the two additional hydroxyl groups was more problematical. Sites on rings C and D were excluded in order to preserve the correspondence of the ^{13}C-NMR data, and so attachments to rings A and B were considered. Returning to the ^{1}H-MR spectrum of alkaloid M, it was observed that there were signals for 5 protons in the region 3.5-5 ppm: an AB pair, δ 4.00 and 3.52 (each J=11.5Hz); a singlet at δ 3.90; a rather broad multiplet at δ 3.76 ($w_{1/2}$ = ca. 10Hz); and a doublet at

TABLE 9

Carbon-13 Chemical Shifts and Assignments for Alkaloid M (38) and Dictyzine (37)[54]

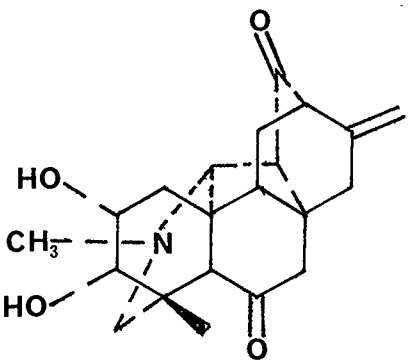
	Compound		
C-atom	38[a]	38[b]	37[c]
C-1	31.9 t	33.0 t	40.2 t
2	69.0 d	70.1 d	20.8 t
3	67.5 d	68.5 d	27.7 t
4	38.7 s	39.3 s	34.4 s
5	39.6 d	41.0 d	44.2 d
6	27.5 t	28.7 t	26.6 t
7	35.7 d	36.1 d	36.2 d
8	41.9 s	42.4 s	42.0 s
9	51.5 d	52.5 d	52.8 d
10	45.4 s	46.0 s	45.6 s
11	21.5 t	22.8 t	21.9 t
12	42.7 d	43.6 d	42.8 d
13	23.3 t	24.4 t	23.1 t
14	22.2 t	22.6 t	26.6 t
15	86.0 d	86.4 d	86.7 d
16	79.2 s	80.4 s	79.8 s
17	67.3 t	67.3 t	59.8 t
18	21.7 q	22.8 q	23.6 q
19	48.7 t	50.1 t	67.8 t
20	75.9 d	76.8 d	73.5 d
NCH_2	49.7 t	49.4 t	-
CH_3	12.2 q	12.7 q	-

a In CD_3OD

b In Py-d_5

c In $CDCl_3$

δ 3.19 (J=4.5Hz). These were attributed to the diastereotopic hydroxymethyl function, the isolated C(15)-H, and the 'extra' diol group respectively. Selective decouplings revealed that irradiation at δ 3.80 collapsed the doublet a δ 3.19 to a singlet and also simplified a multiplet at δ 1.88, while irradiation at δ 1.88 converted the multiplet at δ 3.76 to a clean doublet (J=4.3Hz). It was concluded that the two 'extra' hydroxyl groups formed a vic-diol unit of the type $CH_nCH(OH)CH(OH)-C<$. This then had to be accommodated in ring A, and it was thought that the relatively low abundance of an (M-17) ion in the MS of alkaloid M excluded a hydroxyl at C(1),[33] thus leading to a 2,3-diol. The magnitude of the observed coupling between the carbinyl protons indicated that these could not be in an axial-axial orientation. As 2α-hydroxylation of C_{20}-diterpenoids is relatively common,[21,22] this stereochemical assignment was made and, guided by the precedent provided by hetidine (39),[55] a cis-diol with 3α-OH was constructed.



39 Hetidine

Structure (38) was thus arrived at for alkaloid M, but without definite proof for the geometry of the diol system. The alkaloid (M) was designated as macrocentrine.

An X-ray crystallographic analysis (Fig. 7) of the alkaloid,[56] revealed macrocentrine to indeed have the structure and relative stereochemistry depicted in (38). The amount of macrocentrine isolated comprised 25% of A3 and 50% of A4.

Alkaloid N

This alkaloid crystallized from chloroform as colourless needles (mp 275-279°C). The ^{1}H-NMR spectrum revealed the alkaloid to possess a tertiary C-methyl (3H, s, δ 1.37) and an exomethylene function (each 1H, s, δ 4.56 and 4.71). The IR spectrum showed characteristic absorptions at ν_{max} 3400 (OH), 1708 (>C=O), 1650 and 885 (>C=CH_2), 1073, 1041 (C-O). The mass spectrum showed a molecular ion peak at m/z 327 with fragment ions at 299 (M-28), 271 (M-56), 242 (M-85), 176 (M-151), 105 (M-222).

The absence of absorptions in the ^{1}H-NMR spectrum of alkaloid N which could be attributed to N-ethyl or N-methyl substituents together with evidence for an exocyclic >C=CH_2 unit, suggested that it belonged to the C_{20}-diterpenoids. The molecular mass 327 for alkaloid N was found to be identical with that of the known C_{20}-diterpene alkaloid hetisinone (40), of molecular formula $C_{20}H_{25}NO_3$. The presence of a strong carbonyl absorption at 1708 cm^{-1} in the IR spectrum led to the suspicion that the alkaloid was in fact hetisinone. However, direct comparison of the IR and ^{1}H-NMR spectra of N with those of an authentic specimen of hetisinone revealed that they were non-identical. But the very close resemblance of the spectra of N and hetisinone suggested that alkaloid N was probably an isomer of hetisinone bearing the keto group on either C(11) or C(13).[57-58] Due to insufficient material, the

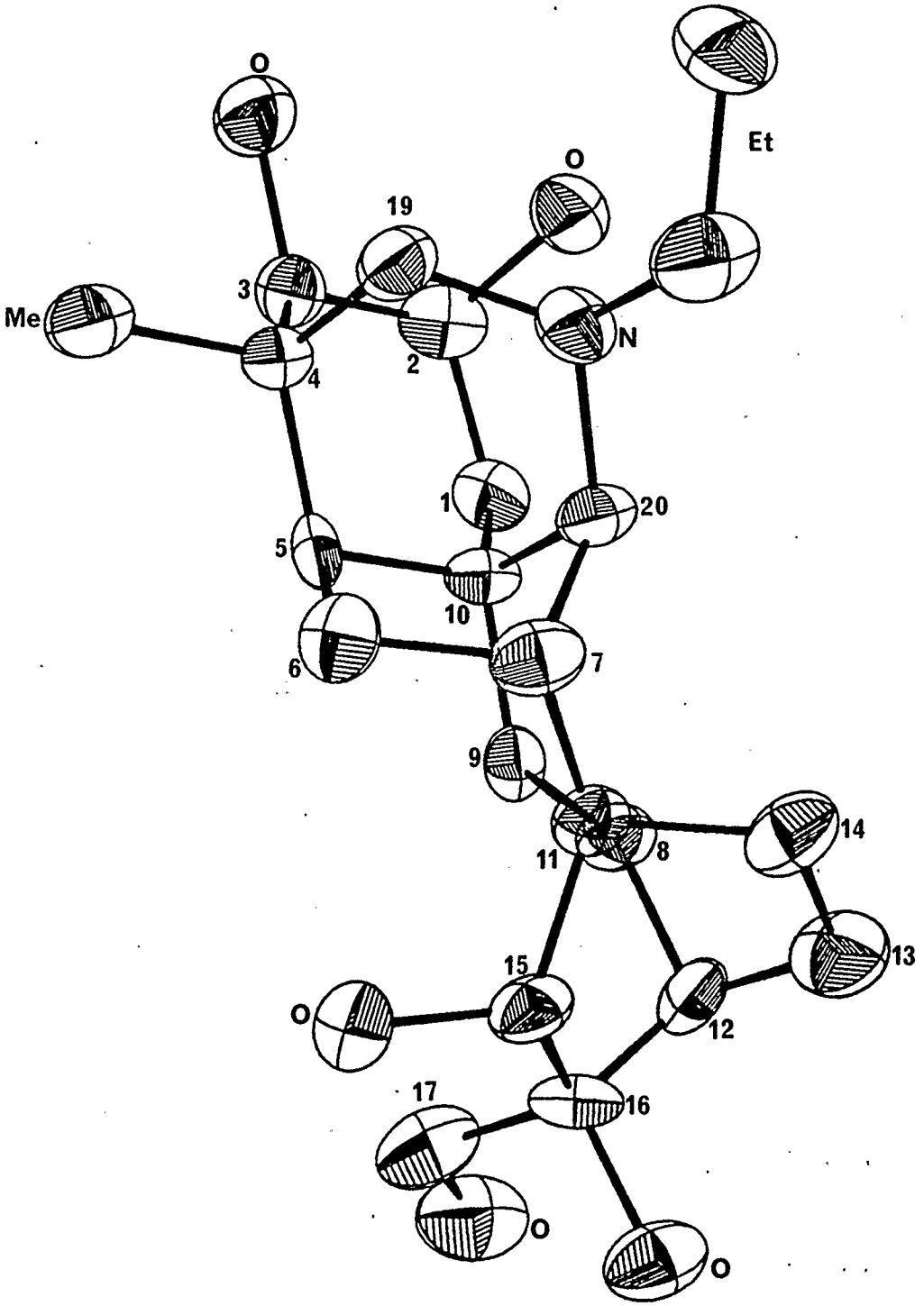
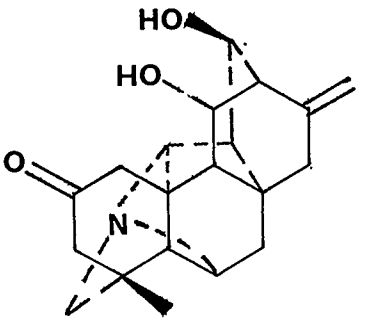


Figure 7: ORTEP-diagram of X-ray Crystallographic Structure of Macrocentrine (38).

identity could not be established beyond doubt.

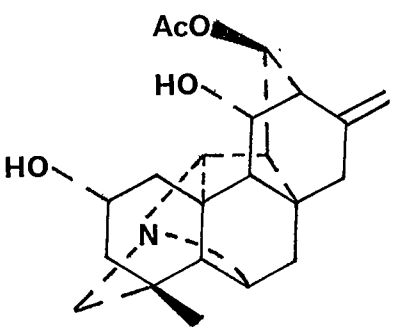


40 Hetisinone

The isolated yield of this alkaloid accounted for about 2% of A3 and 5% of A4.

Alkaloid P

The ^{1}H-NMR spectrum showed the presence of a tertiary C-methyl (3H, s, δ 0.97), an acetate (3H, s, δ 2.18) and an exomethylene group (1H, s, each at δ 4.73 and 4.90). The ^{13}C-NMR spectrum revealed 22 signals apparently corresponding to 22 carbon atoms. From these spectral characteristics, it was concluded that the alkaloid belonged to the C_{20}-diterpenoids. The alkaloid was suspected to be 13-O-acetylhetisine (35), because of the C_{22} formulation taken together with the presence of an acetate group. It was tentatively asssigned the formula $C_{22}H_{29}NO_4$.



35 13-O-acetylhetisine

Indeed its ^{13}C-NMR spectrum was in excellent agreement (see table 10) with that reported for 13-O-acetylhetisine.[52] The reported features of the ^{1}H-NMR spectrum[52] were also in accord with those found for alkaloid P. The isolated yield of Alkaloid P accounted for about 6% of A3 and A4.

2.5 Conclusions

Delphinium macrocentrum, like delphiniums elsewhere in the world, yielded C_{19}- and C_{20}-diterpenoid alkaloids, six of which were identified as the known compounds 14-acetyldelcosine, deltatsine, methyllycaconitine, browniine, delcosine, and 13-O-acetylhetisine. As well it gave some novel alkaloids which were named as deacetylnudicauline, macrocentridine, macrocentrine, hetisine-13-O-benzoate, and a dehydrohetisine. Delcosine, macrocentrine and methyllycaconitine were found to be the major components.

The presence of methyllycaconitine and delcosine as major components of D. macrocentrum is an indication that the plants should be toxic to mammals and anthropods. Both delcosine and methyllycaconitine have been shown[7] to have toxic properties, while delcosine is known[59] to be insecticidal. It would be of interest to study the toxicological and pharmacological properties of D. macrocentrum.

Two rather unusual alkaloids, macrocentridine and macrocentrine are biosynthetically noteworthy. Macrocentridine is the second example, after delbiterine, of a C_{19}-diterpenoid alkaloid of the lycoctonine-type lacking a C(16)-β-methoxyl and suggests that 16-O-methylation is not necessarily earlier than at other sites e.g. C(1). Macrocentrine on the

TABLE 10

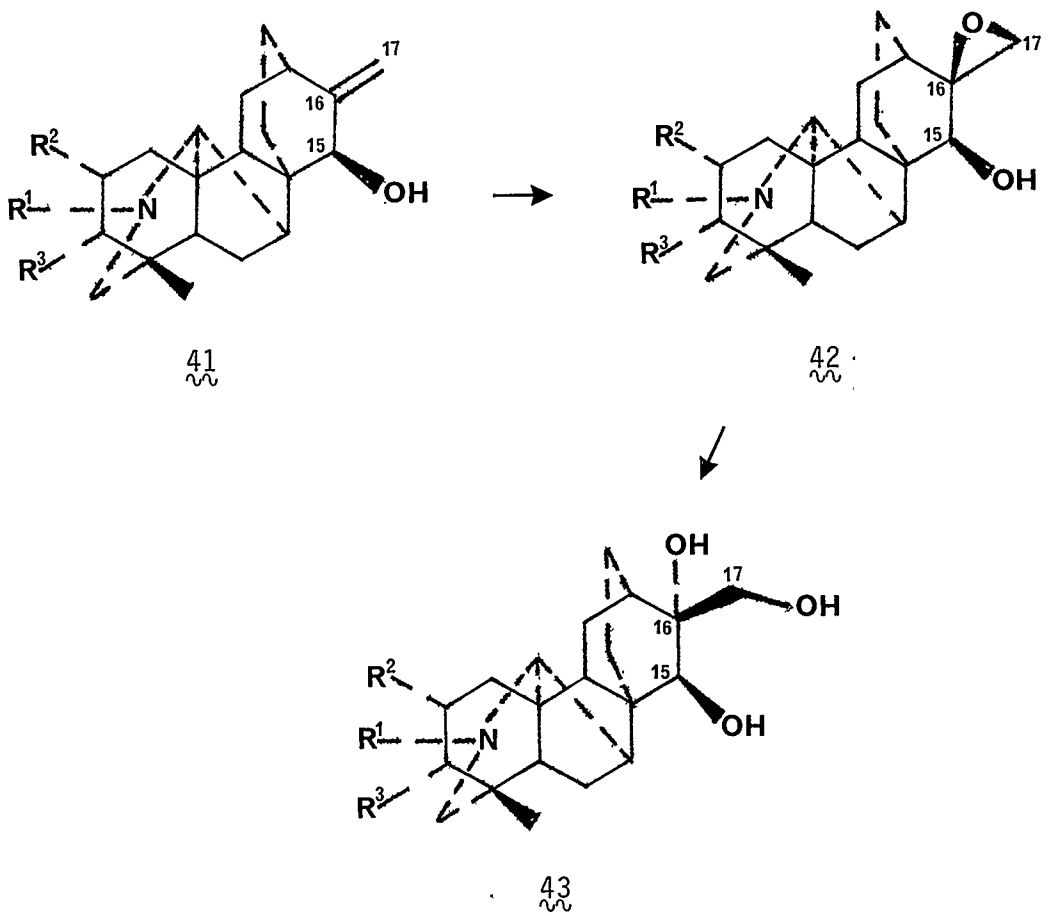
Carbon-13 Chemical Shifts and Assignments for Alkaloid P and 13-O-Acetylhetisine (35)[52]

C-atom	Compound[a] P	35
C-1 *	33.6 t	33.7 t
2 *	74.6 d	74.5 d
3 *	36.2 t	36.2 t
4	36.7 s	36.7 s
5	61.6 d	61.6 d
6	64.3 d	64.4 d
7 *	33.9 t	34.0 t
8	43.7 s	43.7 s
9	55.3 d	55.4 d
10	50.6 s	50.7 s
11 *	68.8 d	68.8 d
12 *	48.6 d	48.6 d
13 *	75.8 d	75.8 d
14 *	50.3 d	50.4 d
15	40.5 t	40.5 t
16	144.8 s	144.9 s
17	108.8 t	108.7 t
18	29.8 q	29.8 q
19	63.6 t	63.7 t
20	67.0 d	67.0 d
C = O	170.1 s	170.1 s
CH_3	21.3 q	21.3 q

* Tentative assignments

[a] In $CDCl_3$

other hand, is the second example, after dictyzine of a C_{20}-diterpenoid alkaloid in which the unit usually present as an exocyclic methylene group has been converted to a vic-diol. This suggests a biosynthetic generation via epoxidation and subsequent ring-opening of the oxirane (Fig. 8), and prompts the thought that these alkaloids may be clues to the construction of their C_{19}-relatives, i.e. that this hydroxylation paves the way for detachment of C(17) from the C_{20} system while, as originally suggested,[26,27] a C(15)-β-OH may provide the site for a leaving group which results in a rearrangement to the C_{19}-aconitine/ lycoctonine ring system (see pp.9 and 12).



41 42 43

Figure 8: A hypothetical Biosynthesis of the Dictyzine-Macrocentrine Triol-System

3. EXPERIMENTAL

3.1 General Methods

Melting Points

Melting points (mp) were determined on a Leitz microscope hotstage melting point apparatus, and are uncorrected.

Infrared (IR) Spectra

Infrared spectra were recorded on a Perkin-Elmer 467 grating infrared spectrometer and on a Nicolet 5DX FT-IR spectrometer. Spectra were measured on samples dispersed in KBr and pressed into pellets. Occasionally samples were examined as solutions in chloroform. Absorption maxima (ν_{max}) are in cm^{-1}.

Proton Nuclear Magnetic Resonance (^{1}H-NMR) Spectra.

^{1}H-NMR spectra were obtained at 200MHz with a Varian XL-200 spectrometer. The spectra were recorded in deuterochloroform ($CDCl_3$), unless otherwise specified. Tetramethylsilane (TMS) (δ=0 ppm) or residual chloroform (δ 7.27 ppm) was used as an internal standard. Chemical shifts (δ) are reported in parts per million (ppm) relative to TMS, and multiplicities are indicated by s=singlet, d=doublet, t=triplet, q=quartet, and m=multiplet, spin-spin coupling constants (J) are given in Hz.

Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR) Spectra

^{13}C-NMR data were collected on a Varian XL-200 spectrometer at 50.3 MHz, in $CDCl_3$, unless otherwise specified. TMS was used as an internal standard. Broadband and Off-resonance decoupled spectra, were used to assign the different carbon atoms, whose chemical shifts (δ) are given in ppm with the multiplicity in parentheses.

Mass Spectra (MS)

Mass spectra were recorded with a Kratos MS-80 spectrometer at an ionization potential of 70eV, and are given as mass to charge ratios (m/z) in atomic mass units, with relative ion intensities in parentheses. The samples were introduced via a direct probe.

Optical Rotations

Specific optical rotations, $[\alpha]_D$ were measured at the sodium D-line (589.5 nm) with a Rudolf Autopol III Polarimeter using a 1cm light path. The solvents, and temperatures were as specified.

Thin Layer Chromatography (TLC)

Samples were spotted on precoated silica gel TLC plates (Merck, 60F-254, 2.5cm x 7.5cm, or 5cm x 20cm, 0.25mm layer thickness) and after elution with a suitable solvent system, examined under UV light (254 nm), and developed in an iodine chamber. Chloroform:methanol 5:1 v/v was used as the eluting solvent, unless specified otherwise. Here and elsewhere in the text, all solvent mixtures were prepared on a volume/volume basis.

Preparative Thin Layer Chromatography (PTLC)

A concentrated solution of the sample was applied to a precoated silica gel plate (Merck, 60F-254, 20cm x 20cm, 1mm thickness). After development, the zone corresponding to the compound of interest was scraped off and extracted repeatedly with a suitable solvent. The zones of interest were located by dusting the plate margins with SiO_2-I_2 powder, after examination under UV light.

Alkaloid Spot Test Reagent

The presence of alkaloids in the aqueous extracts was detected with

Mayer's reagent: an aqueous solution of a mixture of mercuric chloride (1.36g) and potassium iodide (5.00g) made up to 100mL with water in a volumetric flask. A white precipitate is formed if alkaloids are present.

3.2 Plant Material

The aerial parts (stems and leaves) of Delphinium macrocentrum Oliv. were collected just before the flowering period on Sirmon Track (Approximately 9,000-10,000 ft altitude) of Mt. Kenya. The plants were identified by Mr. S.G. Mathenge of the Herbarium, Department of Botany, University of Nairobi. A voucher specimen (fig.1) is deposited in the Herbarium of the University of Calgary.

3.3 Extraction and Separation Procedures

The air dried and ground plant material (665g) was extracted with 95% ethanol (5x4L) in a Waring blendor until the extracts were almost clear. The extracts were combined, filtered and concentrated under reduced pressure (cyclone evaporator) to yield a dark greenish syrup (approximately 100mL). This was then partitioned between chloroform (500mL) and dilute aqueous sulphuric acid (0.5M, 500mL). The resulting two layers were separated and the chloroform layer further extracted with portions of dilute sulphuric acid (5x300mL) until the aqueous acid extracts showed only a very faint positive result when tested with Mayer's reagent.

The first half of the aqueous acid extracts (1L) was basified, after adding some crushed ice, with saturated aqueous sodium carbonate to pH 7 and extracted with chloroform (3x200mL). The aqueous acid

solution was basified further to pH 11 and extracted with chloroform (2x 200mL). The chloroform extracts at pH 7 and pH 11 were combined and dried with anhydrous magnesium sulphate. The solvent was then removed under reduced pressure (Rotovap, bath temperature ca. 50°C) to leave a brownish residue (I, 2.8g). The second portion of the aqueous acid extracts (1L) was similarly treated and gave a brownish residue (II, 0.7g). The crude alkaloid mixtures I and II were each dissolved in chloroform and then combined (ca. 50ml). This was followed by extraction with dilute (0.5M) sulphuric acid (3x150mL) until the aqueous extract showed only a very faint positive result when tested with Mayer's reagent. The combined acid extracts (1.5L) were basified to pH 5 (indicator paper) using saturated aqueous sodium carbonate and extracted with chloroform (3x100mL). The aqueous acid solution was then successively basified to pH 7, pH 9 and pH 11 and extracted with chloroform (3x100mL) at each of these pH points. The extracts at pH 5, pH 7, pH 9 and pH 11 were individually dried over anhdydrous magnesium sulphate, followed by removal of the solvent under reduced pressure to yield the purified alkaloid mixtures A1 (1.15g), A2 (0.36g), A3 (0.43g) and A4 (0.02g) respectively. The total alkaloid content, on a dry weight basis, was therefore found to be ca. 0.4%.

TLC analysis (see figure 6 page 14) revealed A1 to contain four major components and three minor ones. Under the same conditions A2 showed two major and two minor components. Fractions A3 and A4 were similar and each appeared to be comprised of two major components.

3.4 Isolation of the Components of Fraction A1

Separation of the components of the alkaloid mixture A1 was carried out using conventional column chromatography (CC) and Vacuum Liquid Chromatography (VLC).

3.4.1 Vacuum Liquid Chromatography (VLC)

Part of the alkaloid mixture A1 (0.53g) was subjected to short-column Vacuum Liquid Chromatography. A sintered glass Buchner filter funnel (60 mL) fitted with a fritted disk (10-15 M) and packed with alumina (Merck, 60HF-254, basic type E, 50g) was used as the chromatographic column. The funnel was filled with the alumina and tapped on the bench, followed by vacuum suction (water aspirator). A small flat bottomed Erlenmeyer flask was used to tamp down the alumina until a very hard smooth upper surface was produced. The vacuum was released, toluene poured quickly onto the surface, and then the vacuum reapplied. The solvent was sucked through the column and the vacuum again released. The alkaloid mixture dissolved in just enough toluene to cover the top surface of the column was then added, and the vacuum was reapplied gently, to draw the sample into the packing. The column was eluted first with toluene, then toluene with increasing amounts of chloroform, chloroform, then chloroform with increasing amounts of methanol, ethyl acetate with increasing amounts of methanol, and finally with methanol. Each portion of solvent (50mL) was sucked through and collected, the column being sucked dry between each fraction. After each fraction had been collected, fresh eluent was added to the top of the column without vacuum. The vacuum was then reapplied to draw the solvent through. Fifty-six fractions were collected. The individual fractions were

TABLE 11

Vacuum Liquid Chromatography Data for the Fractionation of A1 (0.53g)

Fraction No.	Eluent	Weight (mg)[a]
1	Toluene	-
2	"	-
3	Toluene:$CHCl_3$ (9:2)	-
4	" "	-
5	Toluene:$CHCl_3$ (7:3)	-
6	" "	-
7	" "	-
8	" "	-
9	" "	-
10	Toluene:$CHCl_3$ (3:2)	-
11	" "	< 10
12	" "	< 10
13	" "	< 10
14	" "	18
15	Toluene:$CHCl_3$ (1:1)	
16	" "	
17	" "	10
18	" "	
19	Toluene:$CHCl_3$ (2:3)	
20	" "	
21	" "	28
22	" "	
23	Toluene:$CHCl_3$ (1:3)	
24	" "	
25	" "	
26	" "	85
27	$CHCl_3$	
28	"	10
29	"	20
30	"	20

TABLE 11 (continued)

Fraction No.	Eluent	Weight (mg)[a]
31	"	23
32	"	16
33	"	< 10
34	$CHCl_3$:MeOH (98:2)	30
35	" "	51
36	$CHCl_3$:MeOH (97:3)	25
37	" "	20
38	" "	< 10
39	" "	< 10
40	" "	< 10
41	$CHCl_3$:MeOH (93:7)	< 10
42	" "	< 10
43	" "	
44	" "	10
45	EtOAc:MeOH (85:15)	
46	" "	
47	" "	10
48	" "	
49	EtOAc:MeOH (50:50)	
50	" "	
51	" "	< 10
52	" "	
53	Methanol	
54	"	12
55	"	
56	"	

[a] ± 10mg

All the fractions were 50mL each.

monitored by TLC. See Table 11 for the chromatographic details.

Fractions 14-15 each yielded a colourless gum upon concentration in vacuo. TLC showed the two fractions to be similar. Each revealed three components (R_f 0.8, 0.7 and 0.6). The spot at R_f 0.8 appeared to be predominant. The two fractions were combined, and purified by Preparative Thin Layer Chromatography (PTLC) to yield the main component as a solid, A (18mg) homogeneous on TLC (R_f 0.8), $[\alpha]_D^{23}$ +30(±3)° (c 1.0, EtOH). It was identified as 14- acetyldelcosine (20) on the basis of the following properties:

^{1}H-NMR: δ 1.09 (3H, t, J=7Hz, N-CH_2CH_3), 2.06 (3H, s, -$OCOCH_3$), 3.32, 3.33, 3.34 (each 3H, s, 3 x -OCH_3), 4.80 (1H, t, J=5Hz, C(14)-H). For the literature values see refs. 32, 35.

MS: m/z 495 (6), 480 (38), 478 (32), 464 (23), 463 (7), 462 (24), 306 (3), 111 (10), 108 (9), 98 (8), 91 (10), 85 (9), 71 (18), 58 (56), 45 (39), 43 (100), 41 (17).

See Table 1 for the ^{13}C-NMR spectrum.

Fractions 18-25, on concentration in vacuo, each yielded a colourless gum. TLC showed them to be similar, each showing three spots (R_f 0.8, 0.7 and 0.6),, with the middle spot appearing as the major component. The combined fractions 18-25 (28mg) were purified by further VLC as described above, but with a smaller sintred glass Buchner filter funnel (15mL) packed with alumina (7.5g). Elution was done with 40% chloroform in toluene → 70% chloroform in toluene. A total of 37 fractions (each ca. 10mL) were collected, and analysed by TLC. Fractions 9'-12' were combined and yielded a slightly impure gum, B (15mg), $[\alpha]_D^{23}$ +30(±3)° (c 0.6, EtOH). TLC revealed one main spot (R_f 0.7) with

traces of impurities showing at R_f 0.6 and 0.8. It was identified as deltatsine (25) on the basis of the following spectral characteristics.

^{1}H-NMR: δ 1.09 (3H, t, J=7.3Hz, $NCH_2\underline{CH_3}$), 3.37, 3.39, 3.40, 3.47 (each 3H, s, 4 x OCH_3), 3.65 (1H, Brs, C(1)-β-H), 3.83 (1H, s, C(6)-α-H), 4.01 (1H, t, J=5Hz, C(14)-β-H). For the literature values see ref. 43.

MS: m/z 467 (5), 452 (29), 436 (88), 420 (100), 404 (10), 390 14), 388 (10), 376 (11), 374 (11), 264 (43), 178 (7), 114 (19), 108 (20), 91 (24), 71 (35), 58 (80), 45 (83).

See Table 2 for the ^{13}C-NMR spectrum.

IR ($CHCl_3$), ν_{max} 3527 (OH), 2941 (C-H), 1091 (ether C-O).

On concentration in vacuo, fraction 26-27 each yielded a colourless gum. TLC revealed a single spot (R_f 0.6) in each of the fractions. This material, C(85mg), was identified as methyllycaconitine (26) by its spectral characteristics.

^{1}H-NMR: δ 1.04 (3H, t, J=7Hz, $NCH_2\underline{CH_3}$), 3.26, 3.41 (each 3H, s, 2 x OCH_3), 3.34 (6H, s, 2 x OCH_3), (1H, dd, J=7.5Hz, 2Hz, δ 7.34; 1H, dt, J=7.5Hz, 2Hz, δ 7.60; 1H, dt, J=7.5Hz, 2Hz, δ 7.74; 1H, dd, J=7.5Hz, 2Hz, δ 8.10; aromatic protons). For the literature values see refs. 32, 45.

For the ^{13}C-NMR spectrum see Table 3.

HI Salt of Alkaloid C

A crystalline salt of alkaloid C was obtained by forming its HI derivative. The alkaloid was dissolved in EtOH and ethanolic HI slowly added until the mixture was just acidic (Congo Red indicator). Precipitation was done using diethyl ether, followed by recrystallization from

EtOH. This yielded yellowish crystals, $[\alpha]_D^{23}$ +27(±3)° (c 0.6, MeOH), whose IR showed the following features:

IR: ν_{max} 3473 (OH), 1716 (Benzoyl $\geq$C=O), 1773, 1695 (Imide $\geq$C=O), 1396 (C-CH_3), 1256 (Ester C-O), 1085 (Ether C-O). This spectrum was identical to that of authentic methyllycaconitine HI salt.

Fraction 29 on concentration in vacuo resulted in the isolation of a light yellowish gum. TLC analysis revealed two spots (R_f 0.6 and 0.5), with the lower spot being predominant. It was purified by PTLC to yield the main component, D (20mg, R_f 0.5), $[\alpha]_D^{23}$ +39(±2)° (c 1.5, EtOH). This was identified as browniine (21) on the basis of the following spectral data.

^{1}H-NMR δ 1.04 (3H, t, J=7Hz, $NCH_2\underline{CH_3}$), 3.25, 3.30, 3.36, 3.41 (each 3H, s, 4 x OCH_3). For the literature values see refs. 32, 35.

MS: m/z 467(2), 452(22), 437(22), 436(74), 435(7), 417(14), 406(13), 108(22), 91(18), 71(50), 58(58), 43(100).

For the ^{13}C-NMR spectrum see Table 4.

Alkaloid D co-chromatographed on TLC with an authentic specimen of browniine.

Fractions 31-32 were concentrated in vacuo and each afforded a colourless gum. TLC showed them to be similar, each revealing three spots (R_f 0.8, 0.7 and 0.6), with the middle spot appearing predominant. The two fractions were combined and purified by PTLC to yield an apparently unknown alkaloid E (39mg, R_f 0.7), $[\alpha]_D^{23}$ +30(c 1.6, EtOH), as the main component. The alkaloid was assigned structure (29) on the basis of the following spectral properties:

^{1}H-NMR: δ 1.06 (3H, t, J=7.2Hz, $NCH_2\underline{CH_3}$), 3.25 (3H, s, OCH_3),

3.36 (6H, s, 2 x OCH_3), 4.09 (1H, s, C(6)-α-H), 4.35 (1H, d, C(14)-β-H), (1H, dd, J=7.5Hz, 2Hz, δ 7.30; 1H, dt, J=7.5Hz, 2Hz, δ 7.57; 1H, dt, J=7.5Hz, 2Hz, δ 7.72; 1H, dd, J=7.5Hz, 2Hz, δ 8.06; armoatic protons).

MS: m/z 668 (2), 651 (14), 638 (13), 637 (26), 635 (13), 583 (15), 436 (15), 216 (22), 120 (20), 71 (26), 43 (100).

For the ^{13}C-NMR spectrum see Table 5.

Saponification of Alkaloid E

The unknown alkaloid E (46mg) was dissolved in methanol (ca. 5mL) and 10% aqueous sodium hydroxide (1mL) added. The mixture was then kept at room temperature for 24h. The solvent was removed in vacuo, and the residue partitioned between water (ca. 2mL) and chloroform (3x2mL). The combined organic layers were dried ($MgSO_4$). Removal of the solvent then afforded the hydrolysed product (30mg). Spectral analysis revealed the saponification product to have properties in accord with those reported for delectinine (30).

^1H-NMR: δ 1.05 (3H, t, J=7.1Hz, NCH_2CH_3), 3.26, 3.36, 3.44 (each 3H, s, 3 x OCH_3), 3.82 (1H, s, C(6)-α-H), 3.99 (1H, brs, C(14)-β-H). For the literature values see ref. 47.

For the ^{13}C-NMR spectrum see Table 6.

Acetylation of Alkaloid E

A mixture of E (20mg) and acetic anhydride (0.5mL) plus pyridine (0.5mL) was left at room temperature for 48h. After working up, an acetylated derivative of alkaloid E was obtained. The spectral data (^1H-NMR), and co-chromatography on TLC with an authentic specimen of nudicauline (28) showed the acetylation product of E to be identical with this alkaloid.

^{1}H-NMR, δ 1.04 (3H, t, J=7Hz, $NCH_2\underline{CH_3}$), 3.25, 3.32, 3.34 (each 3H, s, 3 x OCH_3), 2.05 (3H, s, $COCH_3$), 4.08 (1H, s, C(6)-α-$\underline{H}$), 4.75 (1H, t, J=5Hz, C(14)-β-$\underline{H}$), (1H, dd, J=7.5Hz, 2Hz, δ 7.30; 1H, dt, J=7.5Hz, 2Hz, δ 7.57; 1H, dt, J=7.5Hz, 2Hz, δ 7.70; 1H, dd, J=7.5Hz, 2Hz, δ 8.06; aromatic Protons). For the literature values see ref. 48.

Concentration of fractions 35-36 under reduced pressure resulted in the isolation of yellowish gums. TLC revealed a single spot (R_f 0.6) in each of the fractions. Both fractions crystallized from hexane-acetone to yield colourless crystals of alkaloid F (75mg) (mp 204-207°C), $[\alpha]_D^{23}$ +58(±5)° (c 0.6, EtOH), which was identified as delcosine (31), on the basis of the following properties:

^{1}H-NMR: δ 1.10 (3H, t, J=7.2Hz, $NCH_2\underline{CH_3}$), 3.33, 3.36, 3.37 (each 3H, s, 3 x OCH_3), 4.02 (1H, s, C(6)-α-$\underline{H}$), 4.11 (1H, t, J=5Hz, C(14)-β-$\underline{H}$). For the literature values see refs. 32, 35, 51.

MS: m/z 453 (20), 438 (93), 436 (67), 435 (29), 422 (75), 421 (38), 420 (87), 418 (27), 403 (28), 264 (48), 71 (44), 58 (88), 43 (100).

For the ^{13}C-NMR spectrum see Table 7.

IR: ν_{max} 3518, 3473, 3357, 3352 (OH), 2949, 2930, 2865 (CH), 1467, 1452 (ether CH_3), 1399, 1388 (OH), 1110, 1082 (ether C-O). This spectrum exactly matched that of an authentic specimen of delcosine. The alkaloid (F) also co-chromatographed on TLC with the authentic sample of delcosine.

Removal of solvent under reduced pressure from fractions 43-49 afforded yellowish gums. TLC showed them to be similar, by revealing one major spot (R_f 0.4) with some minor impurities, in each of the

fractions. They were combined and purified by PTLC to yield another apparently unknown alkaloid, G (20mg, R_f 0.4), $[\alpha]_D^{23}$ +27(±3)° (c 1.0, EtOH), as the main component. This alkaloid was identified as (33) on the basis of the following spectral properties:

^{1}H-NMR, δ 1.09 (3H, t, J=7.2Hz, $NCH_2\underline{CH_3}$), 3.33, 3.39 (each 3H, s, 2 x OCH_3), 4.04 (1H, s, C(1)-β-H), 4.10 (1H, s, C(6)-α-H), 4.28 (1H, t, J=5Hz, C(14)-β-H).

MS: m/z 439 (10), 424 (80), 422 (64), 408 (53), 406 (61), 390 (49), 250 (20), 114 (25), 108 (26), 91 (38), 71 (32), 58 (100), 45 (83).

For the ^{13}C-NMR spectrum see Table 8.

IR ($CHCl_3$): ν_{max} 3433 (OH), 2913 (CH), 1097 (ether C-O).

3.4.2 Column Chromatography (CC)

The remainder of the alkaloid mixture A1 (0.62g) was chromatographed on a column of alumina (Woelm, neutral, activity III, 1.5cm x 30cm). The column was packed by introducing dry alumina into the glass column filled with toluene. The sample was then dissolved in little toluene, loaded onto the column, and eluted with toluene, toluene with increasing amounts of chloroform, chloroform, then chloroform with increasing amounts of methanol, methanol, then methanol-aqueous ammonia 20:1, and finally the column was washed with water. Fifty-five fractions (each about 50mL) were collected (see Table 12). The fractions were monitored by TLC.

Fractions 13-14, each gave a colourless gum on concentration in vacuo. TLC showed the two fractions to be similar by revealing three spots (R_f 0.8, 0.6 and 0.5) with the top spot being predominant in each of the fractions. The two fractions were combined and purified by PTLC,

TABLE 12

Column Chromatography Data for the Fractionation of A1 (0.62g)

Fraction No.	Eluent	Volume (mL)	Weight (mg)[a]
1	Toluene	50	-
2	"	50	-
3	"	50	-
4	"	50	-
5	Toluene:$CHCl_3$ (9:1)	50	-
6	" "	50	-
7	" "	50	-
8	" "	50	-
9	Toluene:$CHCl_3$ (3:2)	50	-
10	" "	50	-
11	" "	50	< 10
12	" "	50	< 10
13	Toluene:$CHCl_3$ (2:3)	50	33
14	" "	50	
15	" "	50	20
16	" "	50	10
17	" "	50	10
18	" "	50	20
19	" "	50	10
20	" "	50	10
21	Toluene:$CHCl_3$ (1:4)	50	10
22	" "	50	30
23	" "	50	20
24	" "	50	10
25	$CHCl_3$	50	20
26	"	50	20
27	"	50	10
28	"	50	10
29	"	50	< 10
30	"	50	< 10

TABLE 12 (continued)

Fraction No.	Eluent	Volume (mL)	Weight (mg)[a]	
31	"	50	< 10	
32	"	50	< 10	
33	$CHCl_3$:MeOH (9:1)	40	40	
34	" "	20	40	
35	" "	20	30	
36	" "	20	10	
37	" "	20	20	
38	" "	20	< 10	
39	" "	50	10	
40	" "	50	10	
41	" "	50	< 10	
42	" "	50	< 10	33
43	" "	50	< 10	
44	$CHCl_3$:MeOH (3:2)	50	10	
45	" "	50	< 10	
46	" "	50	10	
47	" "	50	< 10	
48	MeOH	50	< 10	
49	"	50	< 10	
50	"	50	10	
51	"	50	< 10	
52	"	50	10	
53	"	150	10	
54	MeOH:NH_4OH (20:1)	210	60	
55	H_2O	250	100	

[a] ± 10mg

to yield as the main component a homogeneous material (30mg, R_f 0.8). This compound was identical in all aspects to alkaloid A, earlier on identified as 14-acetyldelcosine (20), from its spectral data (see page 57).

On concentration under reduced pressure, fractions 15-16 gave colourless gums. TLC analysis revealed three spots (R_f 0.8, 0.7 and 0.5) in each of the two fractions, with the middle spot appearing as the main component. The fractions were combined and purified by PTLC. This resulted in the isolation of a substance (25mg), homogeneous on TLC (single spot R_f 0.7). Its spectral characteristics showed it to be identical with alkaloid B, earlier identified as deltatsine (25) (see page 57).

Fractions 19-21, on concentration under reduced pressure gave yellowish gums. TLC analysis revealed three spots (R_f 0.8, 0.7 and 0.5), in each of the fractions, with the bottom spot appearing predominant. The three fractions were combined and subjected to PTLC, which resulted in the isolation of the major component (30mg) (R_f 0.5). This compound was found to be the same in all respects with alkaloid D, earlier identified as browniine (21) (see page 59).

Removal of solvent from each of the fractions 22-24 gave a colourless gum. TLC examination of fraction 22 showed three spots (R_f 0.7, 0.6 and 0.5) with the middle spot predominating. Fractions 23 and 24, each showed two spots (R_f 0.7 and 0.6), the lower spot appearing as the major component. The three fractions were combined and subjected to PTLC. This yielded, as the main component, a substance (60mg),

homogeneous on TLC (R_f 0.6), and identified from its spectral characteristics as alkaloid C earlier on found to be methyllycaconitine (26) (see page 58).

Concentration in vacuo of fractions 25-26 afforded colourless gums. TLC analysis revealed two spots (R_f 0.7 and 0.6) in each of the two fractions, with the upper spot appearing predominant. The two fractions were combined and purified by PTLC, to yield as the main component an apparently unknown compound (40mg, R_f 0.7). This alkaloid was identical in all respects to alkaloid E (29), earlier isolated and designated as deacetylnudicauline (see page 59).

Fractions 33-34, on concentration under reduced pressure gave yellowish gums (80mg). TLC analysis revealed a single spot (R_f 0.6) in each of the two fractions. Crystallization from hexane-acetone furnished colourless crystals, (mp 204-207°C), which were identical to those obtained earlier as alkaloid F and identified as delcosine (31) (see page 61).

Fractions 38-47, on concentration in vacuo resulted in the isolation of yellowish gums. TLC analysis revealed a major spot (R_f 0.4) in each of the fractions, with some minor impurities. These fractions were combined and purified by PTLC, to afford as the major component an apparently unknown substance (20mg, R_f 0.4). Spectral characterization showed it to be identical to alkaloid G, isolated earlier, assigned structure (33) and designated macrocentridine (see page 61).

Although fractions 54 and 55 would appear as major fractions from the CC, analytical TLC showed them to be complicated mixtures. PTLC resulted in the isolation of only traces (<10mg) of alkaloidal materials which could not be characterized further.

3.5 Isolation of the Components of Fraction A2

Fraction A2 (0.36g) was chromatographed on a column of alumina (Woelm, neutral, activity III, 1cm x 27cm), as described for A1 (Section 3.4.2). Starting with hexane:chloroform (3:2), the column was eluted with hexane containing increasing amounts of chloroform, chloroform, then chloroform with increasing amounts of methanol, methanol, and fi-finally with methanol:water (5:1). A total of 44 fractions (each about 30mL) were collected (see Table 13) and monitored by TLC ($CHCl_3$-MeOH 4:1).

Fractions 5-11 on concentration under reduced pressure yielded light yellowish gums. TLC analysis revealed two main spots (R_f 0.7 and 0.06), with the upper spot appearing predominant. They were separated by PTLC to yield two components H(20mg, R_f 0.7) and J(15mg, R_f 0.06). Alkaloid H was found to be identical to alkaloid D, earlier isolated from A1 and identified as browniine (21). See page 59. Alkaloid J, $[\alpha]_D^{23}$ -10(±1)° (c 0.5, EtOH), had the following spectral properties.

^{1}H-NMR: δ 0.96 (3H, s, C-CH_3), 4.06 (1H, br s, >C<u>H</u>OH), 4.42 (1H, d, >C<u>H</u>OH), 4.79 and 4.98 (each 1H, s, >C=CH_2), 5.40 (1H, dt, J=10Hz, 2Hz, >C<u>H</u>OBz), 7.43 and 8.17 (3H, m and 2H, dd, J=10Hz and 2Hz, benzoate).

MS: m/z 433 (30), 416 (9), 313 (10), 312 (43), 283 (16), 282 (23), 144 (9), 106 (12), 105 (100), 77 (59).

IR ($CHCl_3$): ν_{max} 3359 (OH), 1728, 1600, 1588, 1279 and 735 (benzoate), 1650, 916 (>C=CH_2), 1122, 1064 (C-O).

On the basis of the above properties alkaloid J was assigned structure (36).

TABLE 13

Column Chromatography Data for the Fractionation of A2 (0.36g)

Fraction No.	Eluent	Volume (mL)	Weight (mg)[a]
1	$CHCl_3$:Hexane (3:2)	30	-
2	" "	30	< 10
3	" "	30	< 10
4	" "	30	< 10
5	" "	30	< 10
6	" "	30	30
7	" "	30	12
8	" "	30	11 } 68
9	$CHCl_3$:Hexane (4:1)	30	13
10	" "	30	< 10
11	" "	30	10
12	" "	30	< 10
13	" "	30	< 10
14	" "	30	< 10
15	" "	30	< 10
16	$CHCl_3$	30	< 10
17	"	30	< 10
18	"	30	< 10
19	"	30	10
20	"	30	< 10
21	"	30	< 10
22	"	30	< 10
23	"	30	10
24	"	30	< 10
25	"	30	< 10
26	$CHCl_3$:MeOH (5:1)	20	73
27	" "	20	52
28	" "	30	17
29	" "	30	10
30	" "	30	< 10
31	" "	30	< 10

TABLE 13 (continued)

Fraction No.	Eluent	Volume (mL)	Weight (mg)[a]	
32	" "	30	< 10	
33	" "	30	< 10	
34	" "	30	< 10	
35	$CHCl_3$:MeOH (2:1)	30	< 10	
36	" "	30	< 10	12
37	" "	30	< 10	
38	" "	30	< 10	
39	" "	30	< 10	10
40	MeOH	30	< 10	
41	"	30	< 10	
42	"	30	< 10	
43	"	50	< 10	
44	MeOH:H_2O (5:1)	125	50	

[a] ± 10mg

Fraction 26 on concentration in vacuo gave a yellowish gum. TLc showed it to be a mixture of at least 3 components. PTLC on this fraction resulted in the isolation of one pure component, K (15mg, R_f 0.4), which crystallized from $CHCl_3$ as colourless needles (mp 275-279°C). This alkaloid was found to be identical with alkaloid N, first encountered in fractions A3 and A4 and thought to be isomeric with hetisinone (40). Its characterization is described later (see p. 71).

Concentration of fractions 35-40 under reduced pressure resulted in the isolation of colourless gums. TLC showed them to be similar by revealing a single main spot (R_f 0.3). The combined fractions crystallized from EtOH-H_2O as colourless tablets (mp 207-209°C). These crystals (alkaloid L (15mg)) were found to be identical to those of alkaloid M, documented later as isolated from fractions A3 and A4, designated as macrocentrine and assigned structure (38). See below.

3.6 Isolation of the Components of Fractions A3 and A4

The residues A3 (0.43g) and A4 (0.02g) obtained from the chloroform extracts at pH 9 and pH 11, respectively were quite similar. Trituration in acetone of each of the fractions, resulted in their further fractionation into acetone-insoluble and soluble portions.

The acetone insoluble fractions crystallized from EtOH-H_2O as colourless tablets (mp 207-209°C), $[\alpha]_D^{23}$ -43(±4)° (c 0.7, EtOH). This alkaloid M (115mg), was apparently new. It was assigned structure (38) and designated macrocentrine on the basis of the following spectral data.

^{1}H-NMR (CD_3OD): δ 0.81 (3H, s, C-CH_3), 1.11 (3H, t, J=7Hz, $NCH_2\underline{CH_3}$), 3.52 and 4.00 (AB pair each 1H, d, J=11.5Hz, -CH_AH_BOH), 3.19 (1H, d, J=4.5Hz, $CH_2CH(OH)\underline{CH}(OH)C\lessgtr$) and 3.76 (1H, m, $CH_2\underline{CH}(OH)CH(OH)C\lessgtr$), and 3.90 (1H, s, C(15)-H).

MS: m/z 393 (99), 376 (36), 362 (51), 318 (37), 186 (45), 91 (34), 58 (100).

IR: ν_{max} 3400 (OH), 2927, 2879 (C-H), 1205, 1124, 1060 (C-O).

For the ^{13}C-NMR spectrum see Table 9.

The acetone-soluble fractions, on removal of solvent <u>in vacuo</u> followed by crystallization from $CHCl_3$ resulted in the isolation of alkaloid N (7mg) as colourless needles (mp 275-279°C). This alkaloid was thought to be isomeric with hetisinone (<u>40</u>) on the basis of the following spectral characteristics.

^{1}H-NMR (CD_3OD): δ 1.37 (3H, s, C-CH_3), 3.12 and 3.88 (each 1H, d, J=12Hz, $\gtrless\underline{CH}OH$), 4.56, 4.71 (each 1H, s, $\gtrless C=CH_2$).

MS: m/z 327 (70), 299 (24), 270 (21), 242 (22), 176 (26), 105 (36), 91 (68), 85 (72), 83 (100), 55 (81).

IR: ν_{max} 3400 (OH), 2938, 2916 (C-H), 1708 ($\gtrless C=O$), 1650 and 885 ($\gtrless C=CH_2$), 1073, 1041 (C-O).

The mother liquors remaining after the crystallization of alkaloid N were combined. TLC revealed the presence of several components. By subjecting them to PTLC, one of the components, P (27mg), $[\alpha]_D^{23}$ - 8(±1)° (c 1.8, EtOH), homogeneous (R_f 0.05) on TLC ($CHCl_3$:MeOH 4:1) was isolated. This alkaloid was identified as hetisine-13-O-acetate, (<u>35</u>) on the basis of the following data:

^{1}H-NMR: δ 0.97 (3H, s, C-CH_3), 2.18 (3H, s, $COCH_3$), 4.20 (2H, m,

$W^{1/2}$ ca. 10Hz, CHOH), 4.73 and 4.90 (each 1H, s, $>C=CH_2$), 5.18 (1H, dt, J=10Hz, 2Hz, CHOAc). For the literature values see ref. 52.

For the ^{13}C-NMR spectrum see Table 10.

REFERENCES

1. W.B. Turril and E. Milne-Redhead (Eds.), "Flora of Tropical East Africa, Ranunculaceae", No. 154, Crown Agents, London, 1952, pp.19-22.
2. A.D.Q. Agnew, "Upland Kenya Wild Flowers: A Flora of the Ferns and Herbaceous Flowering Plants of Upland Kenya", Oxford University Press, Nairobi, 1974, pp.78, 80 and 81.
3. J.M. Kingsbury, "Poisonous Plants of The United States and Canada", Prentice Hall, Inc., New York, 1964, p.131.
4. F. Fyles, "Principal Poisonous Plants of Canada", Agriculture Bulletin No. 39, Ottawa, 1920, pp.39-41.
5. M. Stuart (Ed.), "The Encyclopedia of Herbs and Herbalism", Orbis Publishing, London, 1979, pp.103, 183.
6. R.N. Chopra, P.L. Badhawar, and S. Ghosh, "Poisonous Plants of India", Vol. 1, Government of India Press, Delhi, 1949, pp.95 and 125.
7. M.H. Benn, and J.M. Jacyno, "The Toxicology and Pharmacology of Diterpenoid Alkaloids" in "Alkaloids: Chemical and Biological Perspectives" ed. S.W. Pelletier, John Wiley and Sons, New York, 1983, p.153 and references therein.
8. C.L. Porter, "Taxonomy of Flowering Plants", W.H. Freeman and Co., San Francisco, 1967, pp.252, 258.
9. B. Evarard and B.D. Morley, "Wild Flowers of the World", Rainbird Reference Books Ltd., London, 1970, pp.27, 52 and 91.
10. T.H. Everett, "The New York Botanical Garden Illustrated: Encyclopedia of Horticulture", Vol. 3, Garland Publishing Inc. New York, 1980, p.1029.

11. R. Le Strange, "A History of Herbal Plants", Angus and Robertson Publishers, London, 1977, pp.101-103.

12. T. Sollmann, "A Manual of Pharmacology, and its Applications to Therapeutics and Toxicology", W.B. Saunders Co. Philadelphia, 1977, p.676.

13. M. Grieve, "A Modern Herbal", Vol. 2, Hafner, New York, 1961, pp.464,770.

14. M. Moore, "Medicinal Plants in the Mountain West", Museum of New Mexico Press, Santa Fe, 1979, p.95.

15. W.A. Emboden, "Narcotic Plants", McMillan Publishing Co., Inc., New York, 1979, pp.18, 127.

16. J.D. Olsen, J. Range Manage., 1983, 36, 550.

17. E.H. Cronin, D.B. Nielsen, N. Madsen, J. Range Manage., 1976, 29, 364.

18. E.H. Cronin and D.B. Nielsen, in "Effects of Poisonous Plants on Livestock", (R.F. Keeler, K.R. Van Kampen and L.F. James, Eds.), Academic Press, New York, 1977, pp.521-534. See also the following chapter on "Larkspur Toxicosis: A Review of Current Research", by J.D. Olsen, pp.535-543.

19. P.N. Nation, M.H. Benn, S.H. Roth and J.L. Wilkens, Can. Vet. J., 1982, 23, 264.

20. V.N. Aiyar, M.H. Benn, Y.Y. Huang, J.M. Jacyno and A.J. Jones, Phytochemistry, 1978, 17, 1453.

21. S.W. Pelletier and S.W. Page, Nat. Prod. Reports, 1986, 3, 451; and references therein.

22. S.W. Pelletier and N.V. Mody, in "The Alkaloids: Chemistry and Physiology", Vol. 18 eds. R.H.F. Manske and R.G.A. Rodrigo, Academic Press, New York, 1981, p.99.

23. S.W. Pelletier and N.V. Mody, in "The Alkaloids: Chemistry and Physiology", Vol. 17 eds. R.H.F. Manske and R.G.A. Rodrigo, Academic Press, New York, 1973, p.1 and references therein.

24. C.A. West, in "Biosynthesis of Isoprenoid Compounds", (J.W. Porter, and S.L. Spurgeon, Eds.) Vol. 1, John Wiley and Sons, New York, 1981, p.375.

25. D.R. Dalton, "The Alkaloids: The Fundamental Chemistry, A Biogenetic Approach", Mercel Dekker, Inc., New York 1979, pp.630-659.

26. Z. Valenta and K. Wiesner, Chem. and Ind., 1956, 354.

27. R.C. Cookson and M.E. Trevett, J. Chem. Soc., 1956, 3121.

28. E.J. Herbert and G.W. Kirby, Tetrahedron Letters, 1963, 1505.

29. M.H. Benn and J. May, Experientia, 1964, 20, 252.

30. G.M. Frost, R.L. Hale, G.R. Waller, L.H. Zalkow, and N.N. Girotra, Chem. and Ind.. 1967, 320.

31. S.W. Pelletier, B.S. Joshi and H.K. Desai, "Techniques for isolation of alkaloids" in "Advances in Medicinal Plant Research" Ed. A.J. Vlietinck and R.A. Dommisse, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 1985, pp.153-195.

32. S.W. Pelletier, N.V. Mody, B.S. Joshi and L.C. Schramm in "Alkaloids: Chemical and Biological Perspectives", vol. 2, ed. S.W. Pelletier, Wiley-Interscience, New York, 1984, p.205. This includes an extremely useful review and catalogue of the ^{13}C-NMR shift assignments of diterpenoid alkaloids with C_{19}-skeletons.

33. M.S. Yunusov, Ya. V. Rashkes, V.A. Tel'nov, and S. Yu Yunusov, Khim. Prir. Soedin, 1969, 515.

34. S.W. Pelletier, N.V. Mody, R.S. Sawhney, and J. Battacharyya, Heterocycles, 1977, 1, 327.

35. S.W. Pelletier, R.S. Sawhney, H.K. Desai and N.V. Mody, J. Nat. Prod., 1980, 4, 395.

36. A.J. Jones and M.H. Benn, Can. J. Chem. 1973, 51, 486.

37. S.W. Pelletier and R.S. Sawhney, Heterocycles, 1978, 9, 463.

38. S.W. Pelletier, N.V. Mody and R.S. Sawhney, Can. J. Chem., 1979, 57, 1652.

39. S.W. Pelletier, N.V. Mody, A.P. Venkov and N.M. Mollov, Tetrahedron Letters, 1978, 5045.

40. S.W. Pelletier, N.V. Mody and R.C. Desai, Heterocycles, 1981, 16, 747.

41. M.N. Sultankhodzaev, L.V. Beshitaishvili, M.S. Yunusov, M.R. Yagudov and S. Yu Yunusov, Khim. Prir. Soedin., 1980, 665.

42. H. Hikino, Y. Kuroiwa and C. Konno, J. Nat. Prod., 1983, 46, 178.

43. B.S. Joshi, J.A. Glinski, H.P. Chokshi, S. Chen, S.K. Srivastava and S.W. Pelletier, Heterocycles, 1984, 22, 2037.

44. B.T. Salimov, M.S. Yunusov and S. Yu. Yunusov, Khim. Priv. Soedin., 1978, 106.

45. S.W. Pelletier, O.D. Dailey, Jr. and N.V. Mody, J. Org. Chem., 1981, 46, 3284.

46. S.W. Pelletier, N.V. Mody and O.D. Dailey Jr., Can. J. Chem., 1980, 58, 1875.

47. B.T. Salimov, N.D. Abdullaev, M.S. Yunusov, and S. Yu. Yunusov, Khim. Prir. Soedin., 1978, 235.

48. P. Kulanthaivel and M.H. Benn, Heterocycles, 1985, 23, 2515.

49. G.R. Waller (Ed.), "Biochemical applications of mass spectrometry", Wiley-Interscience, New York, 1972, p.662.

50. S. Sakai, N. Shinma, S. Hasegawa and T. Okamoto, J. Pharm. Soc. Japan, 1978, 98, 1376.

51. S. Sakai, H. Takayama and T. Okamoto, J. Pharm. Soc. Japan, 1979, 99, 647.

52. M.H. Benn, J.F. Richardson and W. Majak, Heterocycles, 1986, 24, 1605.

53. B.T. Salimov, B. Tashkhodzaev and M.S. Yunusov, Khim. Prir. Soedin., 1981, 86.

54. B.S. Joshi, J.K. Wunderlich and S.W. Pelletier, Can. J. Chem., 1987, 65, 99.

55. S.W. Pelletier, K.N. Iyer, V.K. Bhalla, M.G. Newton and R. Aneja, J. Chem. Soc. Chem. Commun., 1970, 393.

56. Details of this structural determination have been submitted to Acta. Cryst. Sec. C.

57. R.T. Aplin, M.H. Benn, S.W. Pelletier, J. Solo, S.A. Telang and H. Wright, Can. J. Chem., 1968, 46, 2635.

58. S.W. Pelletier, R. Aneja and K.W. Gopinath, Phytochemistry, 1968, 7, 625.

59. W.M. Davidson, J. Econ. Entomol., 1929, 22, 226.