

STUDIES IN ALKALOID CHEMISTRY

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PART I. MINOR ALKALOIDS OF LYCOPodium FLABELLIFORME

PART II. MODEL STUDIES LEADING TO A SYNTHESIS OF CULARINE

By

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A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

May 1964

DOCTOR OF PHILOSOPHY (1964)
(Chemistry)

McMASTER UNIVERSITY
Hamilton, Ontario

TITLE: Studies in Alkaloid Chemistry
Part I. Minor alkaloids of Lycopodium flabelliforme
Part II. Model studies leading to a synthesis of cularine

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NUMBER OF PAGES: viii, 141

SCOPE AND CONTENTS:

Part I.

The results of an investigation of the minor alkaloids of Lycopodium flabelliforme using elution chromatography and counter-current distribution are presented. Several alkaloids not previously reported to occur in this species were isolated and identified.

A new alkaloid, hydroxy-de-N-methyl- α -obscurine, has been isolated and a structure proposed for it on the basis of physical evidence.

A sufficient quantity of Lycopodium alkaloid L.5 which we have named flabellidine was isolated to permit the elucidation of its structure.

Part II.

A synthesis of 9-xanthenemethylamine and its rearrangement to the ring-expanded alcohol under the condition of the Demjanov reaction is described.

ACKNOWLEDGEMENTS

The author wishes to express his deepest gratitude to Professor D. B. MacLean for his patient direction and encouragement during the course of this investigation and in the writing of this thesis.

Financial assistance from the Canadian Commonwealth Scholarship Committee during the period 1960-1963 is gratefully acknowledged.

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PART I.

MINOR ALKALOIDS OF LYCOPODIUM FLABELLIFORME

GENERAL INTRODUCTION

The presence of alkaloids in plants of the family Lycopodiaceae has been known since 1881, when Boedeker first isolated an alkaloid, which he called lycopodine, from Lycopodium complanatum L. It has only been within the last two decades, however, that an extensive investigation of the alkaloidal content of the plants of this genus has been performed. So far about eighty Lycopodium alkaloids have been isolated and characterized from over a dozen species. Lycopodine has been found to be the most widely distributed and is the major alkaloid of at least six species.

The first structural elucidation of a Lycopodium alkaloid was achieved by Wiesner and co-workers in 1957 with annotinine, the major alkaloid of Lycopodium annotinum Linn. In the following year its relative stereochemistry was established on the basis of chemical evidence and X-Ray analysis. Since then about thirty of the Lycopodium alkaloids have had their structures elucidated. All known alkaloids possessing a single nitrogen atom, have a bridged hexahydrojulolidine ring system, whereas all the dinitrogen alkaloids, with the exception of selagine and flabelline, possess a hydrogenated m-phenanthroline ring system.

The work reported in this thesis involves an investigation, using countercurrent distribution and elution chromatographic techniques, of the minor alkaloids of Lycopodium flabelliforme. The

alkaloids which have previously been isolated from this plant by Manske and Marion and MacLean and Young are the following: Lycopodine, dihydrolycopodine, acetyldihydrolycopodine, alkaloids L.3, L.4, L.5 (flabellidine), α - and β -obscurine, flabelliformine, flabelline and nicotine. This investigation has resulted in the isolation of the alkaloids, annotinine, clavolonine, lycodine, de-N-methyl- α -obscurine and hydroxy-de-N-methyl- α -obscurine, in addition to α - and β -obscurine and L.5 already known to occur in this plant. Among these the alkaloid hydroxy-de-N-methyl- α -obscurine is new. After the isolation of de-N-methyl- α -obscurine in these laboratories, Ayer et al. reported its isolation from L. clavatum Lin. The alkaloids, lycodine, clavolonine and annotinine, although known, have not been previously isolated from L. flabelliforme.

A structure for hydroxy-de-N-methyl- α -obscurine has been proposed on the basis of evidence derived from infrared and ultraviolet spectroscopy, nuclear magnetic resonance spectroscopy and mass spectrometry.

The structure and stereochemistry of the alkaloid L.5, which we have named flabellidine, has been established on the basis of physical evidence and by a synthesis of its N-acetyl derivative from lycodine.

HISTORICAL INTRODUCTION

The family Lycopodiaceae includes the genera Lycopodium with some 180 species widely spread over most of the earth, and Phylloglossum found only in Australia.

In 1881, Boedeker (1) reported the first isolation of an alkaloid from a Lycopodium species, to which he assigned the formula $C_{32}H_{52}O_3N_2$ and the name lycopodine.

Somewhat later, Arata and Canzoneri (2) isolated from L. saururus. Lam., a base, $C_{15}H_{20}ON_2$, which they called pillijanine. Fifty years later, Deulofeu and Delange (3) while reinvestigating the same plant, failed to locate pillijanine, but they were able to isolate two new alkaloids, saurorine ($C_{10}H_{19}N$) and sauroxine ($C_{17}H_{26}ON_2$).

In 1934, Orechoff (4) reported the relatively high concentration of alkaloids in Lycopodium annotinum Linn., and in the following year, Muszynski (5) reported that alkaloids were present in three additional species. In 1938, Achmatowicz and Uzieblo (6) isolated three alkaloids from Lycopodium clavatum L., the most abundant of which was lycopodine, for which the correct formula $C_{16}H_{25}ON$ was established. Two other minor alkaloids which they isolated were named clavatine and clavatoxine.

The first comprehensive investigation of species of the genus Lycopodium for their alkaloidal content was carried out by

Manske and Marion (7-16) on ten distinct species. The first species to be examined by them was L. flabelliforme Fern (7) from which they isolated six new alkaloids in addition to lycopodine and nicotine. These are: dihydrolycopodine (L 1), acetyldihydrolycopodine (L 2), alkaloid L.3, alkaloid L.4 which may be anhydrodihydrolycopodine, alkaloid L.5 (flabellidine) and obscurine. A subsequent examination of obscurine by Moore and Marion (17) showed it to be a mixture of two alkaloids, α -obscurine and β -obscurine. The other species examined by Manske and Marion are: L. annotinum L., L. tristachyum Pursch., L. obscurum L., L. clavatum L., L. lucidulum Michx., L. sabinaefolium Willd., L. annotinum Var. acrifolium Fern., L. cernuum L., and L. densum Labill. As a result of their investigation, Manske and Marion were able to isolate about thirty-five new alkaloids in addition to the known lycopodine and nicotine. Lycopodine was found to be the most abundant and was detected in all species except L. cernuum L. Only five of the alkaloids which they isolated were given trivial names, the remainder being designated by the letter L and a number e.g., L.5. Some of the alkaloids which they designated by number only have since had their structures elucidated and have been assigned a trivial name.

Manske and Marion separated their alkaloids by fractional crystallization and distillation of the free bases or their salts and it is a tribute to their experimental skill that they were able to isolate as many bases as they did in a state of high purity. In the intervening years chromatographic methods and countercurrent distribution have been developed and used with remarkable success in

the separation of components of complex natural product mixtures. It is not surprising, therefore, that a number of Lycopodium species have been re-examined for their alkaloidal content making use of these new techniques. The plants which have been subjected to reinvestigation are the following: L. obscurum L. (18), L. flabelliforme (19, 20), L. clavatum (21, 22, 23, 24), L. annotinum (25, 26, 27, 28, 29) and L. lucidulum (30). In addition, two new Lycopodium species, L. fawcettii (31, 32, 33) and L. selago (34, 35) have also been examined. As a result of the application of the new techniques many new alkaloids have been found in species initially examined by Manske and Marion in addition to those which they originally separated.

To date about eighty Lycopodium alkaloids have been characterized of which lycopodine is the most widely distributed. It is the major alkaloid of at least six species and has been detected in all the species so far investigated except L. cernuum L., L. fawcettii and L. saururus Lam.

Table I summarizes the Lycopodium species which have been examined and the alkaloids contained therein with the references to their isolation. Table II indexes the Lycopodium alkaloids and gives formulas, melting points and references to degradation and structure.

TABLE I

Plants of the Lycopodium species investigated
and their contained alkaloids

Species	Alkaloid	Reference to isolation	
<u>L. annotinum</u> L. (Canadian)	lycopodine	8, 25, 29	
	O-acetylacrifoline (L.12)	8, 25, 29	
	acrifoline (L.27)	25, 29	
	annofoline	28	
	annotine (L.11)	8, 25, 29	
	annotinine (L.7)	8, 25, 29	
	fawcettiine	28, 29	
	lofoline	28, 29	
	lycodine	27, 29	
	lycodoline (L.8)	8, 25, 29	
	lycofoline	28	
	α -obscurine	8, 25, 29	
	β -obscurine	8, 25, 29	
	L.9a, L.9b, L.9c, L.9d	8	
	L.10	8	
	annotoxine	26, 25, 29	
	annopodine	29	
	alkaloid 2-A	29	
	<u>L. annotinum</u> L. (European)	lycopodine	26, 34
		acrifoline (L.27)	26, 34

TABLE I (continued)

Species	Alkaloid	Reference to isolation
	annotine (L.11)	26, 34
	annotinine (L.7)	26, 34
	isolycopodine	34
	lycodoline (L.8)	34
	nicotine	36
	α -obscurine	34, 36
	β -obscurine	36
	L.28	36
	L.29	36
	L.31	36
	U.1	36
	U.2	36
	U.3	36
	U.4	36
	U.5	36
	U.6	36
	U.7	36
	U.8	36
<u>L. annotinum</u> Var.	lycopodine	14
acrifolium Fern	acrifoline	14
(Canadian)	annotinine	14
	lycodoline	14
	L.28	14

TABLE I (continued)

Species	Alkaloid	Reference to isolation
	L.29	14
	L.31	14
<u>L. cernuum</u> L.	cernuine (L.32)	15
(Hawaiian and	L.33	15
Trinidadian)	nicotine	15
<u>L. clavatum</u> L.	lycopodine	11
(Canadian)	de-N-methyl- α -obscurine	23
	α -obscurine	23
	nicotine	11
	alkaloid L.13	11
	alkaloid L.18	11
	alkaloid L.19	11
<u>L. clavatum</u> L.	lycopodine	6
(Polish)	clavatine	6
	clavatoxine	6
<u>L. clavatum</u> L.	lycopodine	21, 22
(Jamaican)	dihydrolycopodine	22
	acetyldihydrolycopodine	22
	clavolonine	22
	fawcettiine	22
	fawcettimine	22
	deacetylfawcettiine	22

TABLE I (continued)

Species	Alkaloid	Reference to isolation
<u>L. clavatum</u> var. megastachyon Fern and Bissel (Canadian)	lycopodine	24
	clavolonine	24
	lycoclavine	24
	O-acetyllycoclavine	24
	base $C_{32}H_{52}O_3N$	24
	base $C_{16}H_{25-27}O_2N$	24
<u>L. complanum</u> L.	lycopodine	1
	N-methyl lycodine	23
<u>L. flabelliforme</u> var. ambiguum (Canadian)	lycopodine	20
	annotinine	T*
	clavolonine	T
	dihydrolycopodine	20
	de-N-methyl- α -obscurine	T
	flabelliformine	19, 20
	flabelline	20
	flabellidine (L.5)	T
	hydroxy-de-N-methyl- α -obscurine	T
	lycodine	T
	α -obscurine	T, 20
β -obscurine	T	

* T refers to this thesis.

TABLE I (continued)

Species	Alkaloid	Reference to isolation
<u>L. flabelliforme</u> Fern	lycopodine	7
(Canadian)	acetyldihydro-lycopodine (L.2)	7
	dihydrolycopodine	7
	nicotine	7
	α -obscurine	7
	β -obscurine	7
	L.3	7
	L.4 (L.14)	7
	L.5 (flabellidine)	7
<u>L. densum</u> Labill.	lycopodine	16
(New Zealand)	L.34	16
	L.35	16
<u>L. fawcettii</u> Lloyd & Underhill	acetylfawcettiine	21
(Jamaican)	deacetylfawcettiine	31
	diacetyllycofoline (base N)	21
	fawcettidine	31
	fawcettiine	21, 31
	fawcettimine	21, 31
	lycodoline	31
	lycofoline	21
	base E	31
	base G	31

TABLE I (continued)

Species	Alkaloid	Reference to isolation
	base I	21
	monoacetylyllycofoline (base M)	21
	base O	21
	base L	33
	base R	33
	lycodine	33
	de-N-methyl- α -obscurine	33
<u>L. lucidulum</u> Michx. (Canadian)	lycopodine	12
	nicotine	12
	L.13	12
	L.20	12, 30
	L.21	12
	L.22	12
	L.23	12
	L.24	12
	L.25	12
<u>L. obscurum</u> L. var. dendroideum (Michx.) D. C. Eaton (Canadian)	lycopodine	10, 37
	lycodine	23, 37
	α -obscurine	10
	β -obscurine	10
	L.13	10
	L.16	10
	L.17	10

TABLE I (continued)

Species	Alkaloid	Reference to isolation
<u>L. sabinaefolium</u> Willd.	lycopodine	13
(Canadian)	nicotine	13
	L.13	13
	L.26	13
<u>L. saururus</u> Lam.	pillijanine	2
(Argentinian)	saururine	3
	sauroxine	3
<u>L. selago</u> L.	lycopodine	34
(Polish)	acrifoline	34
	lycodoline	34
	pseudoselagine	34
<u>L. selago</u>	selagine	35
(N. American)		
<u>L. tristachyum</u> Pursh.	lycopodine	9
(Canadian)	anhydrodihydro- lycopodine (L.14)	9
	nicotine	9
	L.13	9
	L.15	9

TABLE II

Index of the Lycopodium alkaloids, with formula, melting point and related references

Alkaloid	Formula	Melting Point	References	
			Degradation	Structure
O-acetylacrifoline (L.12)	$C_{18}H_{25}O_3N$	119-120°	25	71, 73
acetyldihydrolycopodine (L.2)	$C_{18}H_{29}O_2N$	95-96°		61, 70
acetylfawcettiine	$C_{20}H_{31}O_4N$	117°		61, 70
acrifoline (L.27)	$C_{16}H_{23}O_2N$	97-104°	25, 26, 71, 72	71, 72, 73
anhydrodihydro- lycopodine (L.14)	$C_{16}H_{25}N$		60	61, 69
annofoline	$C_{16}H_{25}O_2N$	150-151° 156-157°	69, 74	69, 70, 74
annotine (L.11)	$C_{16}H_{21}O_3N$	174°	25, 26, 88	
annotinine (L.7)	$C_{16}H_{21}O_3N$	232°	14, 38-40, 41-47 48-57	51, 54, 55
annotoxine	$C_{32}H_{44}O_5N_2$	196-197°C		
annopodine	$C_{17}H_{25}O_3N$	212°C	29	
cernuine (L.32)	$C_{16}H_{26}ON_2$	106°		
clavatine	$C_{16}H_{25}O_2N$	212-214°	6	
clavatoxine	$C_{17}H_{27}O_2N$	185-186°	6	
clavolonine	$C_{16}H_{25}O_2N$	238°C	70, 75	70, 75

TABLE II (continued)

Alkaloid	Formula	Melting Point	References	
			Degradation	Structure
deacetylfawcettiine	$C_{16}H_{27}ON$	203-204, 207 ^o	70, 75	70, 75
de-N-methyl- α -obscurine	$C_{16}H_{24}ON_2$	266-268 ^o	23	23
diacetyllycofoline	$C_{20}H_{29}O_4N$	140 ^o	73	73
dihydrolycopodine	$C_{16}H_{27}ON$	168 ^o	60, 64	61, 70
fawcettidine (Base F)	$C_{16}H_{23}ON$		33	
fawcettiine	$C_{18}H_{29}O_3N$	166-167, 172-173 ^o		
fawcettinine	$C_{16}H_{25}O_2N$		33	
flabelliformine	$C_{16}H_{25}O_2N$	210-211 ^o	19, 20	19
flabelline	$C_{18}H_{30}ON_2$	186-188 ^o	20	20
hydroxy-de-N-methyl- α -obscurine	$C_{16}H_{24}O_2N_2$	300-305 ^o	T	T
isolycopodine	$C_{16}H_{25}ON$	136 ^o		
lofoline	$C_{18}H_{29}O_3N$	211-212 ^o	29, 70	29, 70
lycoclavine	$C_{18}H_{29}O_3N$		24	24
lycodine	$C_{16}H_{22}N_2$	118 ^o	18, 27, 37	18, 27, 37
lycodoline (L.8, L.30)	$C_{16}H_{25}O_2N$	180 ^o	25, 60, 89	89

TABLE II (continued)

Alkaloid	Formula	Melting Point	References	
			Degradation	Structure
lycofoline	$C_{16}H_{25}O_2N$	142°	28, 73, 90	73, 90
lycopodine	$C_{16}H_{25}ON$	116°	39, 60, 61-67	61, 62, 69, 70
N-methyllycodine	$C_{17}H_{24}N_2$	91-92°		18, 23
nicotine	$C_{10}H_{14}N_2$			
α-obscurine	$C_{17}H_{26}ON_2$	282-283°	17, 23, 39, 76	23, 76
β-obscurine	$C_{17}H_{24}ON_2$	317-318°	17, 23, 39, 76	23, 76
pillijanine	$C_{15}H_{24}ON_2$	64-65°		
pseudoselagine	$C_{16}H_{25}O_2N$	163-163.5°		
sauroxine	$C_{17}H_{26}ON_2$	198°		
saurorine	$C_{10}H_{19}N$			
selagine	$C_{15}H_{18}ON_2$	224-226°	35, 77	35, 77
L.3	$C_{18}H_{31}O_2N$			
L.4 (L.14)	$C_{16}H_{27}N$		60	61, 69
L.5 (flabellidine)	$C_{18}H_{28}ON_2$		T	T
L.9a	$C_{16}H_{23}ON$			
L.9b	$C_{16}H_{25}O_2N$			

TABLE II (continued)

Alkaloid	Formula	Melting Point	References	
			Degradation	Structure
L.9c	$C_{19}H_{31}O_3N$			
L.9d	$C_{20}H_{31}O_4N$	98°		
L.10	$C_{16}H_{27}ON$			
L.13	$C_{16}H_{25}ON$	130°		
L.15	$C_{20}H_{31}O_4N$			
L.16	$C_{16}H_{25}ON$			
L.17	$C_{18}H_{27}O_3N$			
L.18	$C_{11}H_{19}ON$			
L.19		231°		
L.20	$C_{16}H_{25}O_2N$	259°	24	24
L.21	$C_{13}H_{21}ON$			
L.22	$C_{16}H_{27}ON$	108°		
L.23	$C_{16}H_{25}O_2N$	161-162°		
L.24	$C_{16}H_{25}ON$			
L.25	$C_{16}H_{25}O_2N$			
L.26	$C_{15}H_{25}ON$	171°		

TABLE II (continued)

Alkaloid	Formula	Melting Point	References	
			Degradation	Structure
L.28	$C_{17}H_{27}O_2N$			
L.29	$C_{16}H_{23}O_2N$			
L.31	$C_{20}H_{29}O_4N$			
L.33		218°		
L.34	$C_{16}H_{25}O_2N$	236°		
L.35	$C_{14}H_{21}O_2N$	133°		
Base E	$C_{17}H_{25}O_2N$			
Base G	$C_{18}H_{27}O_3N$			
Base I		300°d		
Base M (Monoacetyllycofoline)	$C_{18}H_{27}O_3N$		73	73
Base O	$C_{20}H_{31}O_5N$	181-182°	21	
Base L	$C_{18}H_{31}NO_4$		33	
Base R	$C_{16}H_{24}N_2O_2$	129-130°	33	
U.1	$C_{10}H_{19}(21)ON$			
U.2	$C_{16}H_{25}ON$			
U.3	$C_{16}H_{21}O_3N$			

TABLE II (continued)

Alkaloid	Formula	Melting Point	References	
			Degradation	Structure
U.4	$C_{16}H_{23}ON$			
U.5	$C_{17}H_{25}O_2N$			
U.6	$C_{17}H_{25}O_3N$			
U.7	$C_{18}H_{25}O_3N$			
U.8	$C_{18}H_{25}O_4N$			
Alkaloid 2-A	$C_{17}H_{25}O_3N$	123°		

Structures and Stereochemistry of Lycopodium Alkaloids

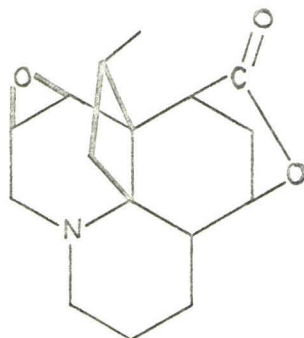
An examination of the Lycopodium alkaloids of known structure reveals that there are at present four different structural types. By far the largest number of alkaloids have the skeletal structure of that of lycopodine, the major alkaloid of many of the Lycopodium species. The next most abundant group of alkaloids has the skeletal structure of lycodine. The other two structural types are those of annotinine and selagine, but to date they are the sole representatives of their groups.

In the following section the alkaloids of known structure and stereochemistry are listed with a brief reference to the work leading to them.

Annotinine (I)

The preliminary investigation of the structure of annotinine was begun by Manske and Marion (14). The degradative studies leading to the complete structure were carried out by three groups of workers led by Marion (38-40) at the National Research Council in Ottawa, by MacLean (41-47) in these laboratories and by Wiesner (48-57) at the University of New Brunswick. The correct structure, shown on the next page, was first proposed in 1957 by Wiesner et al. (51) on the basis of chemical evidence. This structure was later confirmed by Przybylska and Marion (58, 59) by subjecting annotinine bromohydrin

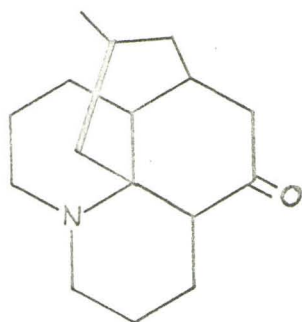
to an X-Ray crystallographic study. Their work established the configuration of the C-methyl group and verified the configuration previously assigned to other asymmetric centres in the molecule.



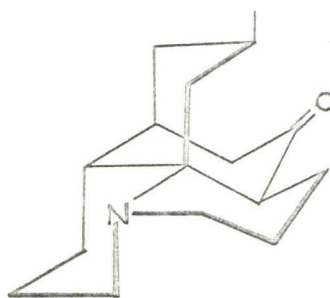
I

Lycopodine (IIa, IIb)

Two years after the elucidation of the structure of annotinine Harrison and MacLean (61, 62) proposed the total structure of lycopodine. The initial work was done by Manske, Marion and co-workers (60, 63, 64) while the remaining structural investigation was carried out by MacLean and co-workers (65-68) in these laboratories. The relative stereochemistry was worked out by Anet (69).



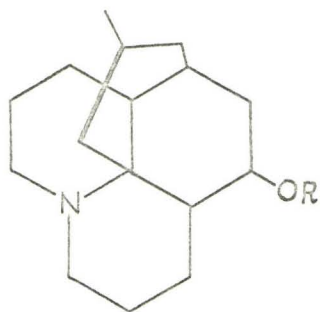
IIa



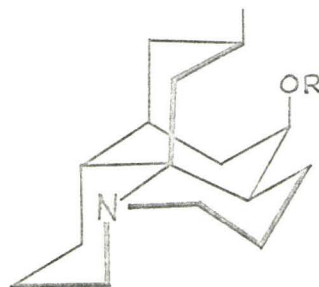
IIb

Acetyldihydrolycopodine (L.2) (IIIa, IIIb)

This alkaloid was shown to be an acetyl derivative of dihydrolycopodine by Douglas, Lewis and Marion (60). The stereochemistry was deduced by Burnell and Taylor (70).

IIIa R=COCH₃

IVa R=H



IIIb

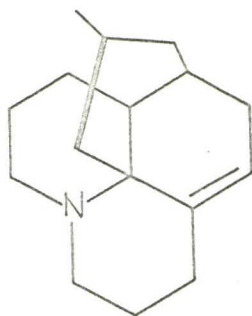
IVb

Dihydrolycopodine (L.1) (IVa, IVb)

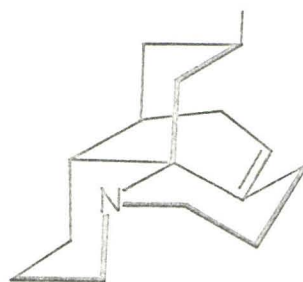
Marion *et al.* (60, 64) established its relationship with lycopodine. The stereochemistry has been established by Burnell and Taylor (70).

Anhydrodihydrolycopodine (L.14) (Va, Vb)

Its relationship to lycopodine was also established by Douglas, Lewis and Marion (60) while its stereochemistry has been discussed by Anet (69).



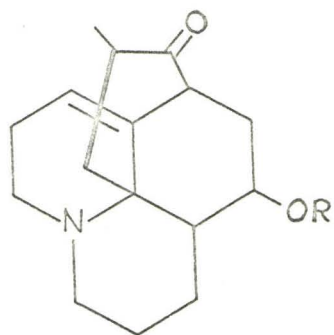
Va



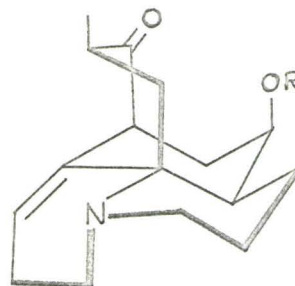
Vb

Acrifoline (VIa, VIb)

French and MacLean (68, 69) elucidated the structure of this alkaloid. The stereochemistry was established by Anet (69) and Burnell and Taylor (73).



VIa R=H

VIIa R=COCH₃

VIb

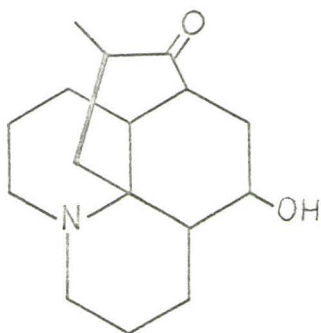
VIIb

O-Acetylacrifoline (L.12) (VIIa, VIIb)

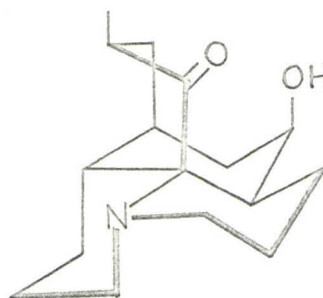
Perry and MacLean (25) were the first to show this alkaloid to be a derivative of acrifoline. The stereochemistry of this alkaloid follows from its derivation from acrifoline.

Annofoline (VIIIa, VIIIb)

The structure of this alkaloid was proposed by Anet and Khan (74). Anet also elucidated its stereochemistry and showed it to be epimeric at C₄ with dihydroacrifoline reported by Perry and MacLean (25). This assignment was confirmed by subsequent work of Burnell and Taylor (70).



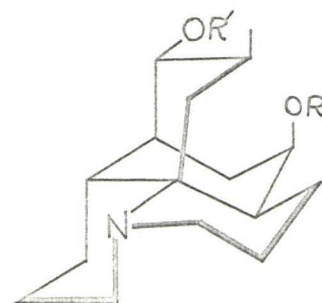
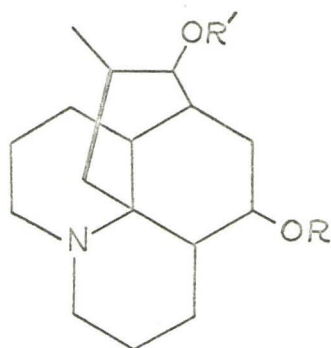
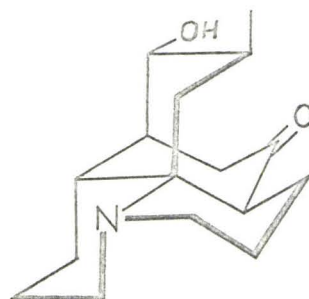
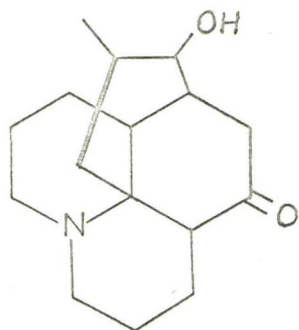
VIIIa



VIIIb

Clavolonine (IXa, IXb) and Fawcettiine (Xa, Xb)

The structure and stereochemistry of these two alkaloids were established by Burnell and Taylor (70, 75).



Xa R=COCH₃; R'=H Xb

XIa R=R'=COCH₃ XIIb

XIIa R=R'=H XIIb

Acetylfawcettiine (Base K) (XIa, XIIb)

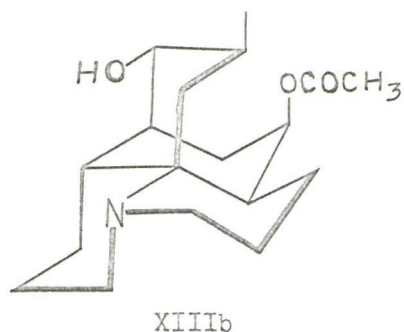
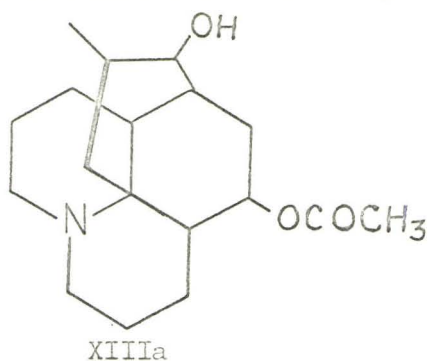
This alkaloid was shown to be an acetyl derivative of fawcettiine by Burnell and co-workers (21).

Deacetylfawcettiine (XIIa, XIIb)

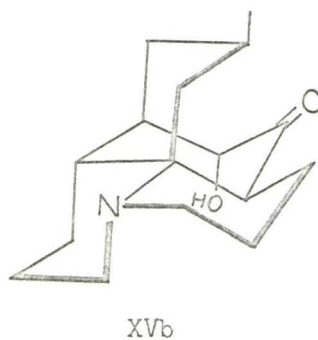
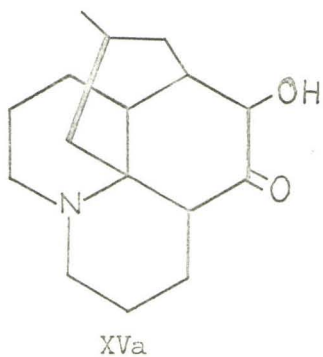
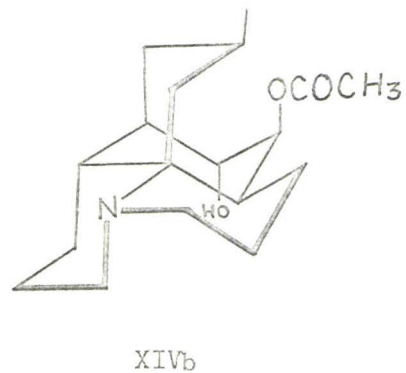
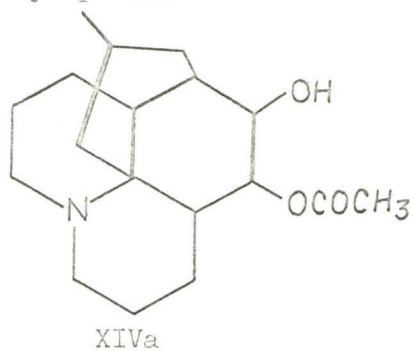
Burnell and Taylor (70, 75) were able to relate this alkaloid to fawcettiine. The structure of this alkaloid is represented by XIIa and its stereochemistry by XIIb.

Lofoline (XIIIa, XIIIb)

Initial structural elucidation was carried out by Anet and Khan (74). Burnell and Taylor (70) showed this alkaloid to be an epimer of fawcettiine and the structure and stereochemistry are represented by XIIIa and XIIIb respectively.

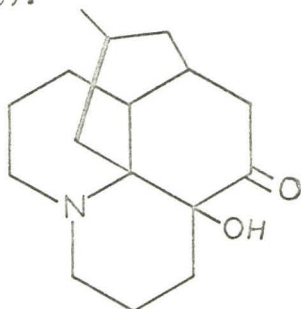
Lycoclavine (XIVa, XIVb) and L.20 (XVa, XVb)

Ayer *et al.* (24, 30) have recently elucidated the structure and stereochemistry of these two alkaloids by relating them to each other and to lycopodine.

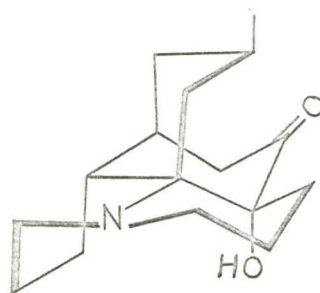


Flabelliformine (XVIa, XVIb)

The structure and stereochemistry of this minor alkaloid of L. flabelliforme have been elucidated by Curcumelli-Rodostamo and MacLean (19).



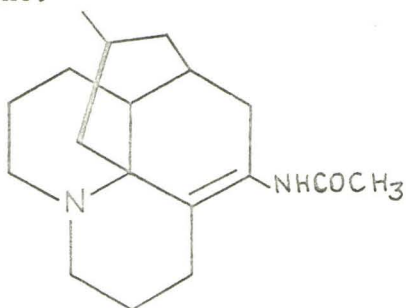
XVIa



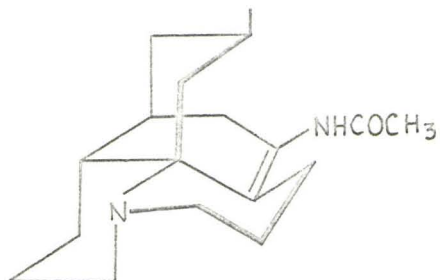
XVIb

Flabelline (XVIIa, XVIIb)

MacLean and Young (20) have recently reported the elucidation of the structure and stereochemistry of this alkaloid by relating it to lycopodine.



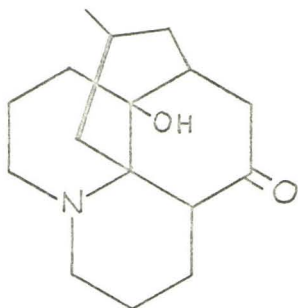
XVIIa



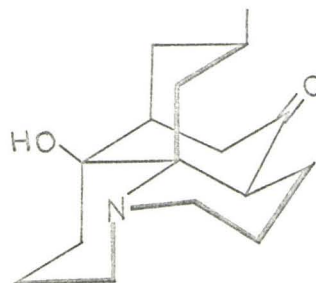
XVIIb

Lycodoline (L.8), L.30 (XVIIIa, XVIIIb)

Preliminary investigation on this alkaloid was carried out by Perry and MacLean (25). The complete structure was elucidated by Ayer and Iverach (89), who also established its stereochemistry.



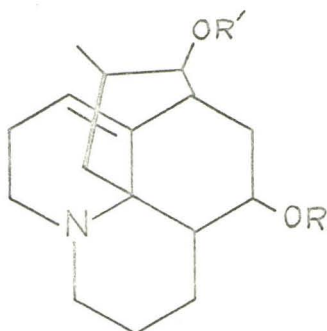
XVIIIa



XVIIIb

Lycofoline (XIXa, XIXb)

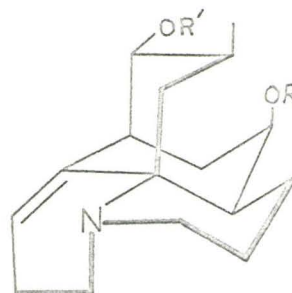
Anet and Khan (28) carried out the preliminary investigations which established the nature of the functional groups. The structure as well as its stereochemistry were established independently by Anet et al. (75) and by Burnell and Taylor (73).



XIXa

R=R'=H

XIXb

XXa R=COCH₃, R'=H XXbXXIa R=R'=COCH₃ XXIb

Monoacetyllycofoline (base M, XXa, XXb) and

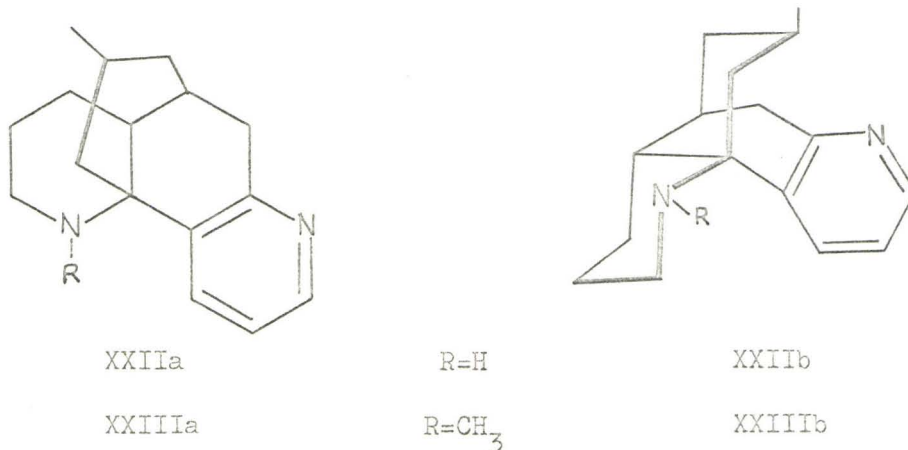
Diacetyllycofoline (base N, XXIa, XXIb)

Burnell and Taylor (73), related these two bases to lycofoline and their structures became apparent when the structure of lycofoline

was elucidated. The monoacetate and diacetate have the structures XXa and XXIa and stereochemistry XXb and XXIb respectively.

Lycodine (XXIIa, XXIIb)

The preliminary investigation was carried out by Anet and Eves (27) while the total structure XXIIa for lycodine was proposed by Ayer and Iverach (18). A rigorous proof of this structure was provided by the work of Anet and Rao (37) who related lycodine to lycopodine. This work also completely defined the stereochemistry of this alkaloid.

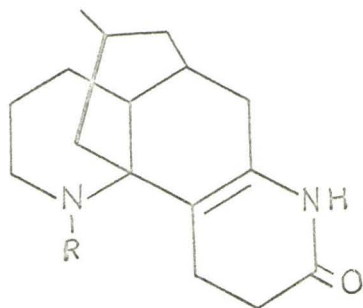


N-methyllycodine (XXIIIa, XXIIIb)

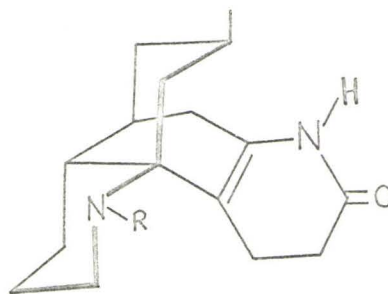
The structure and stereochemistry of this alkaloid follows from that of lycodine (23, 18).

α -obscurine (XXIVa, XXIVb) and β -obscurine (XXVIa, XXVIb)

Moore and Marion (17) showed that obscureine, which was first reported by Manske and Marion (2), was a mixture of two compounds, α - and β -obscurine. Ayer et al. (23, 76) deduced the structures of these alkaloids by relating them to one another and to lycodine and lycopodine.



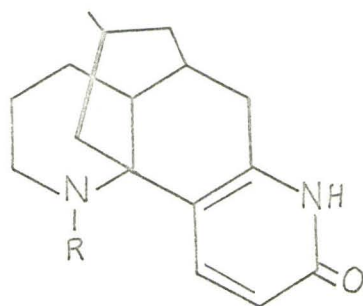
XXIVa

R=CH₃

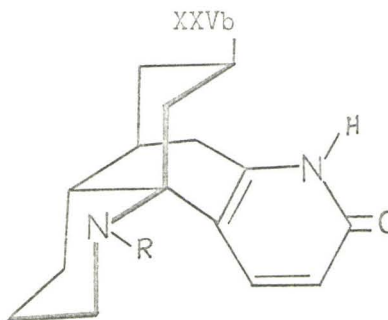
XXIVb

XXVa

R=H



XXVIa



XXVb

XXVIb

De-N-methyl- α -obscurine (XXVa, XXVb)

This alkaloid is derived from α -obscurine by demethylation and has the structure XXVa.

Hydroxy-de-N-methyl- α -obscurine

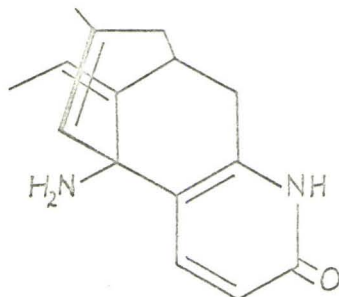
The elucidation of the structure of this alkaloid is described in a subsequent section of this thesis.

Flabellidine (L.5)

The structure and stereochemistry of this alkaloid has been established by relating it to lycodine. This work is also described in the discussion section of this thesis.

Selagine (XXVII)

The structure of selagine was deduced by Wiesner et al. (35, 77) and is represented by the structure below.



XXVII

Absolute stereochemistry of the Lycopodium alkaloids

The absolute stereochemistry of the Lycopodium alkaloids has been established by Wiesner et al. (57). From a measurement of the rotation dispersion curve of an annotinine derivative and application of Djerassi's Octant Rule (78) the absolute configuration of annotinine given in formula I was deduced. This configuration was further confirmed by Wiesner et al. (57) by two other unrelated methods.

Wiesner et al. (57) also elucidated the absolute configuration of lycopodine shown in formula (IIb) from a measurement of its optical rotatory dispersion and application of Djerassi's Octant Rule (78).

This configuration for the Lycopodium alkaloids was confirmed by the independent work of Burnell and Mootoo (70).

Ayer et al. (30) have recently provided further evidence for the absolute stereochemistry of lycopodine, deduced by Wiesner, from an optical rotatory dispersion measurement of 6 α -bromolycopodine and application of axial haloketone rule (78).

Mass spectra of Lycopodium alkaloids

Although mass spectrometry has been used by chemists for a long time in the analysis of hydrocarbon mixtures its use in the determination of organic structures is relatively recent. Despite this fact mass spectrometry has found wide application in solving organic structural problems and has been found particularly well suited to structural studies on substances available in small amounts. A number of reference books reviewing the application of mass spectrometry to organic structural problems have been published in recent years. Among these the books by Biemann (79), Beynon (80), Djerassi et al.(81) and McLafferty (82) are worthy of mention.

MacLean (83) has recently examined the mass spectra of some representative Lycopodium alkaloids and has proposed a fragmentation mechanism for these alkaloids. A brief review of this work is introduced here since mass spectrometry has figured prominently in the study of the structure of two alkaloids discussed in this thesis.

The spectra of lycopodine, dihydrolycopodine, anhydrodihydrolycopodine and acetyldihydrolycopodine are reproduced in Fig. 1, with the author's permission. A common feature of these spectra is the presence of a peak at $M-57$, which corresponds to the loss of the bridge plus a hydrogen atom. This peak is the most intense except in the spectrum of acetyldihydrolycopodine. Another peak shown in all the spectra appears at $M-43$, which may arise by loss of three bridge carbon atoms. With the exception of lycopodine all the spectra show

a peak at m/e 146. A fragmentation mechanism compatible with these findings has been proposed by MacLean (83) and is illustrated in the case of dihydrolycopodine shown in scheme I.

In the ion of m/e 174 it is unlikely that the double bonds are fixed, but instead may assume any of the positions shown in formula A, B and C. The loss of ethylene to yield the ion of m/e 146 may then proceed by either of the pathways shown by arrows.

Lycopodine carries a carbonyl group at the C_7 position and therefore cannot lose water with the subsequent formation of a double bond at the 7,8-position. Hence the peak due to the ion of m/e 146 is absent in its spectrum. The peaks at M-85 and M-113, which correspond to consecutive losses of 28 mass units are most likely due to loss of carbon monoxide and of ethylene.

It was proposed (83) that in the formation of the M-57 peak common to all the spectra the bridge and the hydrogen atom are lost in a concerted rather than in a stepwise process. Metastable peaks*

*

Metastable peaks are observed in a spectrum when ions of mass m_1 decompose in the accelerating region near the exit slit to ions of mass m_2 . The approximate value of m/e , m^* , at which such ions are recorded in the spectrum may be calculated by applying the relation $m^* = (m_2)^2/m_1$ (79). In practice, in the type of instrument used in this study, the observed values are higher, but usually by not more than one mass unit, than those calculated by the relation above. The metastable peaks are observed as broad peaks of low intensity and it is the intensity maximum which is reported. The presence of a metastable peak is evidence that the transformation m_1 to m_2 is a concerted rather than a stepwise process. In the text following, the symbolism $m_1 \longrightarrow m_2$ will be used to denote such a transformation.

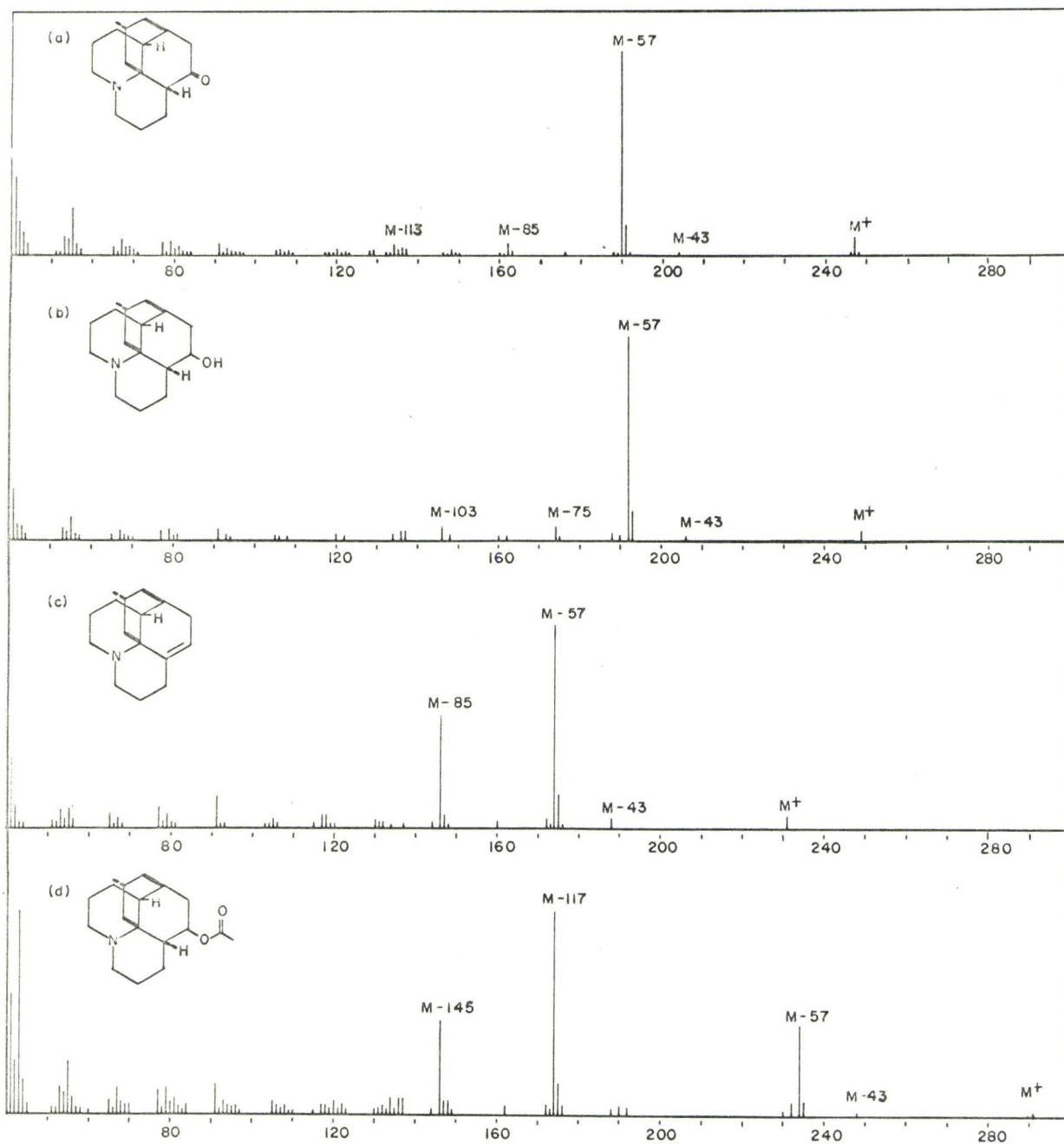
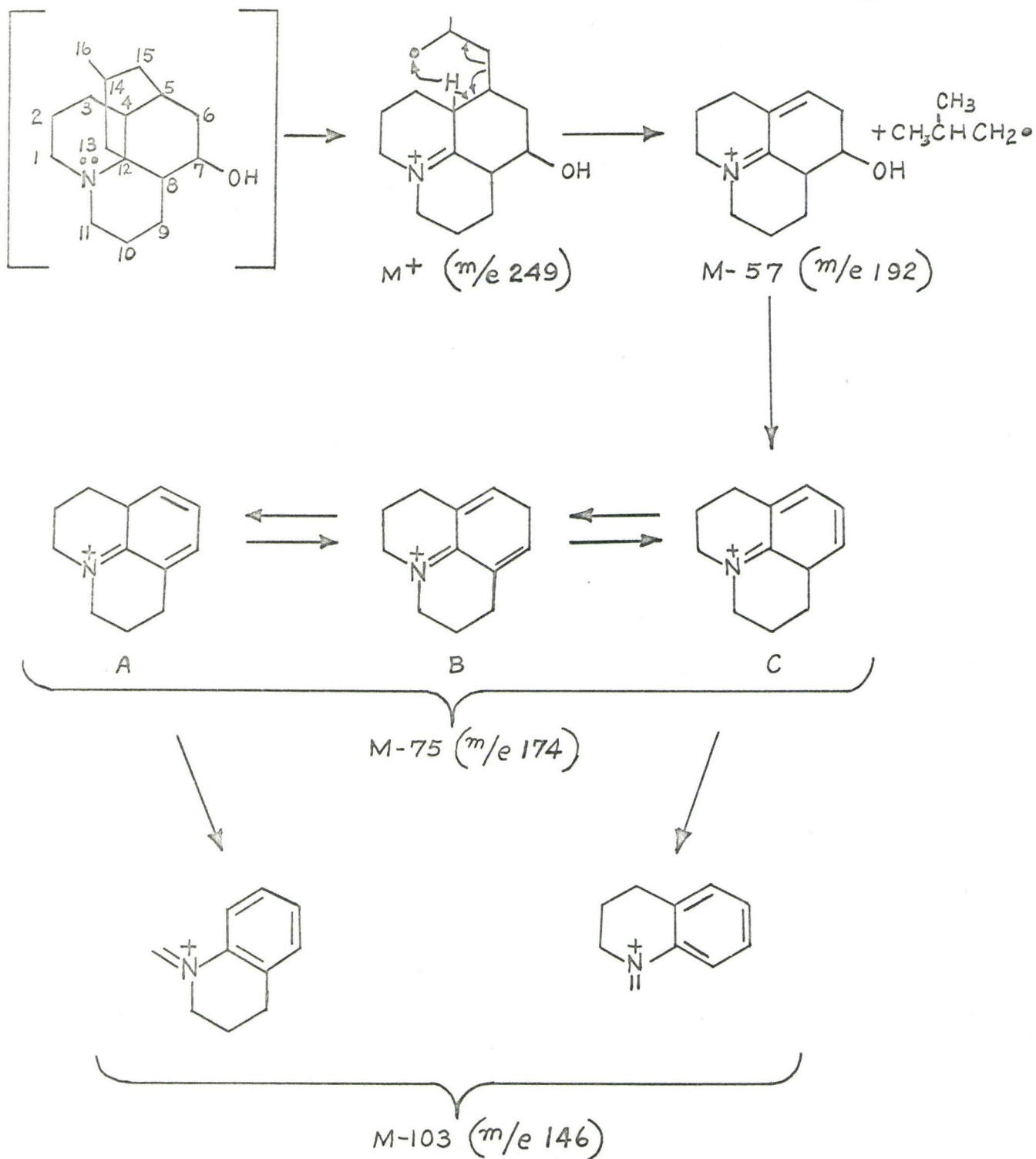


Fig. 1. Mass spectra of (a) Lycopodine, (b) dihydrolycopodine, (c) anhydrodihydrolycopodine, (d) acetyldihydrolycopodine. (83)



Scheme I. Proposed fragmentation mechanism for dihydrolycopodine.

found in the four spectra are in agreement with this proposition. It was also proposed that the hydrogen which is lost with the bridge carbon is derived from the hydrogen attached to carbon atom four.* No direct evidence is at hand in support of this postulate but it has been shown that alkaloids substituted at C_4 exhibit a different fragmentation pattern. Furthermore an examination of the mass spectrum of lycopodine- d_3 in which the hydrogens at C_6 and C_8 were exchanged for deuterium showed its major peak at m/e 193 corresponding to a loss of 57 mass from the molecular ion at m/e 250. Thus the hydrogens at carbon-6 are not involved in this fragmentation.

The spectra of three alkaloids - lofoline (XIII), clavolonine (IX), and annofoline (VIII) - each bearing a substituent on the bridge as well as at C_7 , were also examined and their spectra may be interpreted along the same lines as those outlined in scheme I. The major fragmentation pathway in each spectrum is still due to the loss of the bridge plus a hydrogen atom. The most significant feature of the three spectra is the fact that the substituents on the bridge do not alter appreciably the fragmentation pattern exhibited by lycopodine and its derivatives. Examination of the mass spectrum of an alkaloid, therefore, reveals the presence and nature of the substituents on the bridge.

Two alkaloids acrifoline (VI) and lycodoline (XVIII) which lack a hydrogen atom at C_4 were examined by MacLean (83) and their

*

Numbering as shown in scheme I for dihydrolycopodine.

spectra differed significantly from the spectra previously discussed. In the case of acrifoline which has a $\Delta^{3,4}$ -double bond it was found, for instance, that the peak at M-70 (loss of bridge) was more than twice as intense as that at M-71 (loss of bridge + hydrogen). Other features of the spectrum were in marked contrast to the spectra previously examined indicating that the absence of hydrogen at C_4 forces the fragmentation to follow a different pathway.

The spectrum of lycodoline which differs in structure from lycopodine in that the hydrogen at C_4 is replaced by an OH function was also very different from those of Fig. 1. The molecular ion peak was now the most intense peak in the spectrum and peaks of high intensity were observed at M-17, M-43, M-71 and M-73. In contrast the M-57 peak was less intense than any of the others listed and only twice as intense as the peak at M-56. The spectrum of lycodoline is reproduced in Fig. 6 and will be discussed in more detail in a later section.

Alkaloids with hydroxyl substitution on the bridge clavolonine (IX), at C_6 lycoclavine (XIV) and at C_8 flabelliformine (XVI) were all examined by MacLean (83) but only in the case of lycodoline (C_4 hydroxyl, XVIII) was a marked departure from the normal fragmentation mode observed. The presence of a substituent at C_4 is thus strikingly obvious in its effect upon the fragmentation pattern.

The second most abundant group of alkaloids are those related in structure to lycodine. In this category are found lycodine (XXII), its N-methyl derivative (XXIII), α - and β -obscurine (XXIV, XXVI),

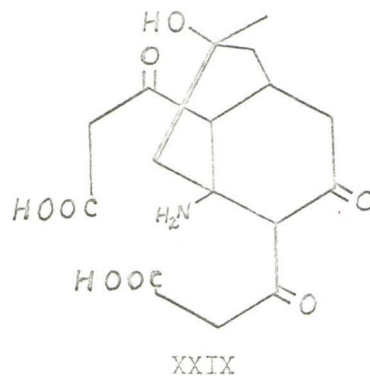
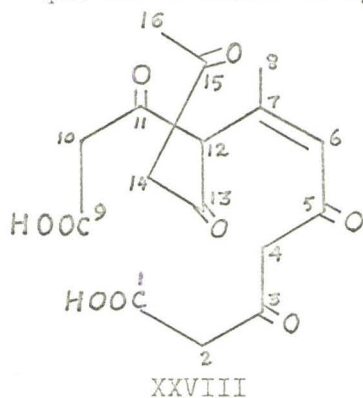
de-N-methyl- α -obscurine (XXV) and hydroxy-de-N-methyl- α -obscurine (XXXIV). The mass spectra of all these alkaloids, except the last one, which will be discussed in a later section, are very similar, the most noteworthy feature being their remarkable resemblance to those of the lycopodine group. However, alkaloids with two nitrogen atoms (a situation which pertains in the lycodine group) can be easily differentiated because of their even-numbered molecular weight. Confusion may arise only when the lycopodine skeleton carries a nitrogenous substituent group. The only known alkaloid of this type is flabelline, the mass spectrum of which will be discussed elsewhere in this thesis. It may be noted here, however, that it is possible to differentiate the two types by mass spectrometry.

The mass spectra of annotinine and selagine, which are the sole representatives of their groups, have also been examined. Although the spectra are complex, it is worthwhile to note that the expulsion of the bridge is still the dominant feature in the fragmentation pattern of these alkaloids. In the mass spectrum of annotinine, the loss of the bridge corresponds to a peak at M-42, which is therefore a characteristic peak for this structure and would differentiate an annotinine-like alkaloid from the other structural types. In the selagine spectrum again the peak at M-55 arises from the ion formed by loss of the bridge plus hydrogen.

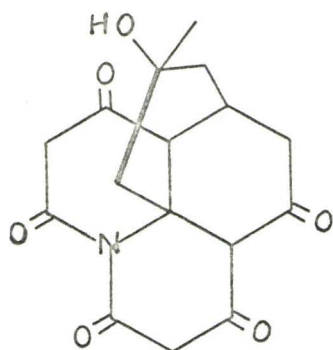
Biogenesis of Lycopodium alkaloids

Soon after the structure of lycopodine was elucidated, a biogenetic scheme for the Lycopodium alkaloids was proposed by Conroy (84) involving the condensation of two 3,5,7-triketo octanoic acid chains. Although there is no experimental evidence for this scheme, it has adequately served as a useful guide in arriving at probable structures of new Lycopodium alkaloids which could then be tested by chemical methods.

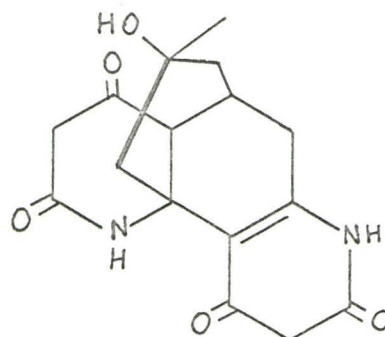
The first step in Conroy's scheme involves the condensation of two molecules of 3,5,7-triketo octanoic acid or its biological equivalent followed by dehydration to give the intermediate XXVIII



A second aldol condensation between C-8 and C-15, followed by reduction of the double bond and a Mannich condensation with ammonia linking C-4 and C-13 would give the intermediate XXIX. Two lactamizations would give the immediate precursor XXX of the lycopodine type alkaloids.



XXX

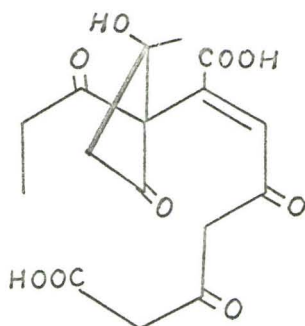


XXXI

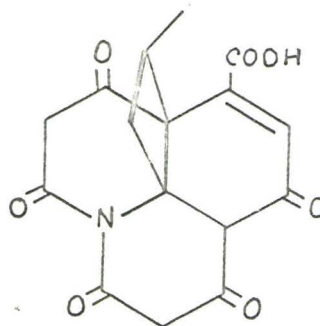
The obscurine type alkaloids may be accommodated in Conroy's scheme by addition of ammonia to XXIX at C₅, followed by dehydration and appropriate lactam closure to the immediate precursor XXXI of the obscurines.

Decarboxylation at C₉ prior to lactamization of the second amine affords a precursor of selagine.

Annotinine may be obtained by oxidation of the C-8 methyl group to carboxyl in XXVIII followed by closure between C-12 and C-15 leading to an intermediate (XXXII) containing the cyclobutane ring. Mannich reaction with ammonia and lactamization gives the carbon skeleton of annotinine (XXXIII).



XXXII



XXXIII

Conroy's scheme was proposed when lycopodine was the sole representative of that structural type. Since then the structures

of a number of Lycopodium alkaloids have been elucidated which incorporate the entire lycopodine molecule but display an additional site of oxygenation. The following are specific examples:

- (1) flabelliformine (8-hydroxylycopodine, XVI)
- (2) lycoclavine (6-hydroxy-O-acetyldihydrolycopodine, XIV)
- (3) annofoline (15-keto dihydrolycopodine, VIII)
- (4) lycodoline (4-hydroxylycopodine, XVIII)

The only known alkaloid of the lycodine group with an additional site of oxygenation is 4-hydroxy-de-N-methyl- α -obscurine, (this thesis).

All these structures may be obtained from XXX or XXXI by a series of selective reductions, dehydrations, hydrations, and oxidations.

It may be noted here that Iverach (29) has recently proposed a biogenetic scheme for the Lycopodium alkaloids utilizing lysine the isopentenyl pyrophosphate. This scheme provides for the observed additional sites of oxygenation in the alkaloids more directly than Conroy's scheme.

Results and Discussion

In the introductory section it was pointed out that Manske and Marion were the first to study the alkaloidal content of L. flabelliforme and were able to isolate from this species lycopodine, the major alkaloid, and six other alkaloids which they designated L.1, L.2, L.3, L.4, L.5 and L.6. Subsequent studies have shown that L.1 and L.2 are dihydro and acetyldihydro lycopodine respectively, and that L.6 which they called obscurine is a mixture of two bases α - and β -obscurine.

A few years ago we undertook a reinvestigation of the minor alkaloids of this species. The crude alkaloid mixture was separated into various fractions using chromatographic and countercurrent methods. Some of these fractions, described later, were examined by Young who isolated two new alkaloids, flabelliformine and flabelline, the structures of which have been elucidated in these laboratories (19, 20). The investigation of the remaining fractions constitutes the subject of the first part of this thesis.

In undertaking a thorough investigation of this species, we had in mind that new alkaloids might be found which would shed light on the biogenetic schemes which have been proposed for the formation of the Lycopodium alkaloids. The presence of alkaloids with bicyclic and tricyclic ring systems and of alkaloids with fewer than the sixteen carbon atoms normally found in the ring systems of these

alkaloids was considered likely. Although this objective was not realized a new alkaloid of the lycodine group was found and a sufficient quantity of alkaloid L.5 was isolated to allow its structural elucidation. In addition a number of alkaloids of known structure but not previously reported in L. flabelliforme were detected.

All the alkaloids isolated during this investigation were minor alkaloids, being present only in minute quantities. Consequently, it was necessary to rely heavily on instrumental methods such as ultraviolet, infrared and N.M.R. spectroscopy and mass spectrometry in the identification and particularly in the structural studies of the two alkaloids, hydroxy-de-N-methyl- α -obscurine and flabellidine, described in later sections.

Isolation of alkaloids

L. flabelliforme had been used as a source of lycopodine by other workers in these laboratories. The mother liquors, after removal of lycopodine, which had accumulated from a number of extractions were combined and constituted the starting material for the investigation of the minor alkaloids of this species.

The crude residue was first extracted with acid and filtered through celite to remove insoluble resinous material. Non-basic water-soluble material was then removed by ether extraction. Basification and chloroform extraction of the acid solution gave a mixture of crude alkaloids which was chromatographed on an alumina column using chloroform as the first eluant. This treatment removed the bulk of the crude bases. The more strongly adsorbed bases were removed

by eluting with 2% and finally 5% methanol in chloroform.

The chloroform fraction was investigated by Young (20) who isolated from it by crystallization a new alkaloid which was named flabelliformine. The mother liquor obtained from the separation of crude flabelliformine, was partitioned between citrate-phosphate buffer (pH 6.0) and chloroform in a twelve-separatory-funnel distribution. All the buffer fractions and chloroform fractions, except the last three, were examined by Young (20), who isolated, in addition to the known dihydrolycopodine and α -obscurine, another new alkaloid which was named flabelline ($C_{18}H_{28}ON_2$). The melting point of flabelline perchlorate ($280^{\circ} - 282^{\circ}$) was identical with that of alkaloid L.5 perchlorate, isolated by Manske and Marion (7). A comparison of the two showed, however, that they were not identical. The melting point of a mixture of the two was depressed and there were significant differences in their ultraviolet and infrared spectra.

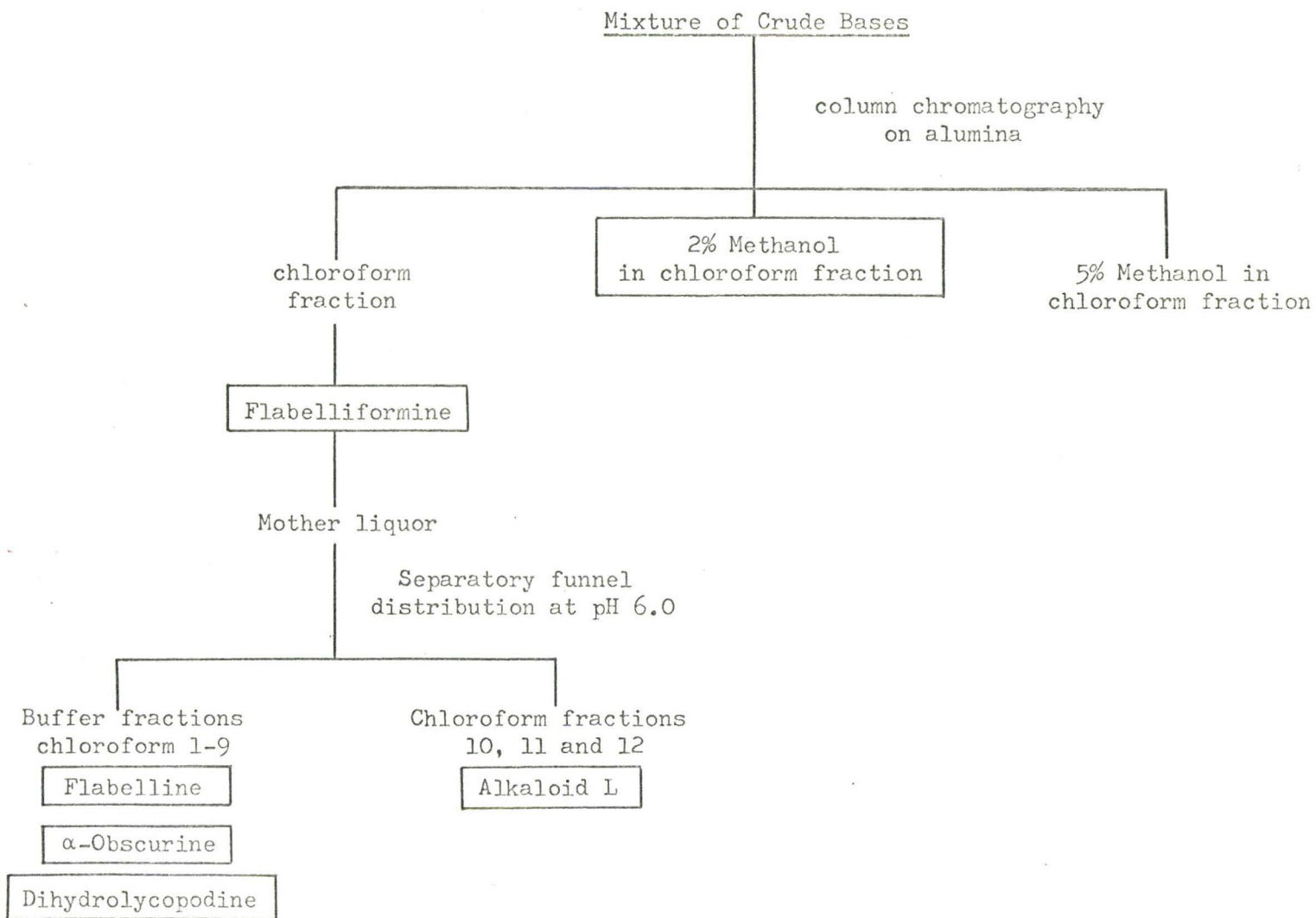
The structure of flabelline was subsequently elucidated by MacLean and Young (20). Flabelline is the first Lycopodium alkaloid shown to have an acetamido group and also the first alkaloid containing two nitrogen atoms shown to possess the hexahydrojulolidine ring system.

In this investigation the fractions which were not examined by Young were subjected to scrutiny. They included the fraction eluted by 2% methanol in chloroform from the original chromatography and the final three chloroform fraction (designated alkaloid L) from the distribution experiment. The alkaloid L fraction constituted approximately 50% of the total base subjected to the distribution experiment

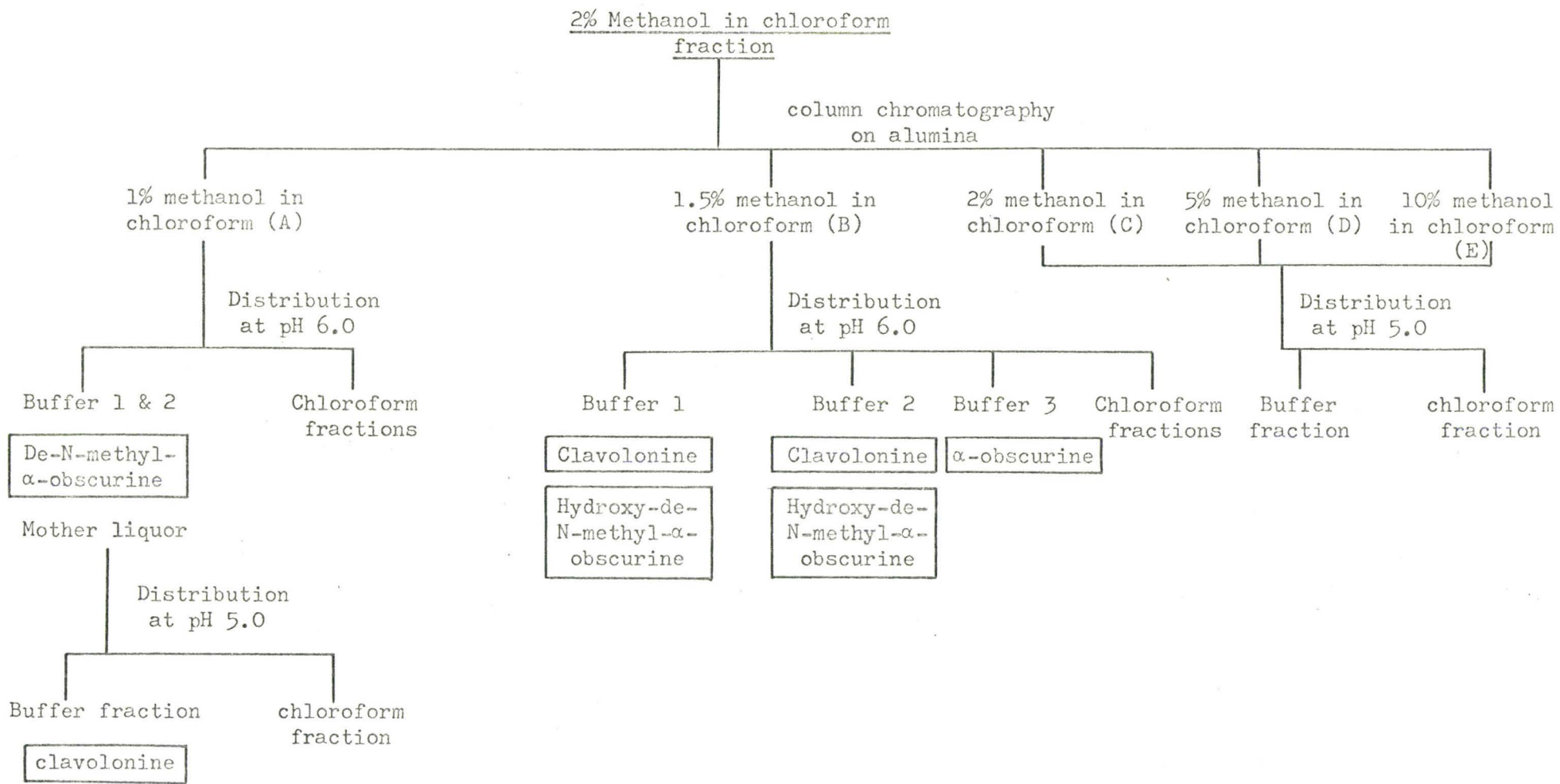
by Young. Extensive use of infrared and ultraviolet spectroscopy was made in following the course of the separation of these fractions into their components. Flow Sheet 1 (page 43a) depicts the general separation scheme followed by Young and indicates the fractions which were examined in the course of this work. In Flow Sheet 2 there is depicted the work which has been carried out on the 2% methanol in chloroform fraction and in Flow Sheet 3 the outcome of the analysis of the alkaloid L fraction is given.

The 2% methanol in chloroform fraction was subjected to further careful chromatography and separated in this way into five fractions, A, B, C, D, E.

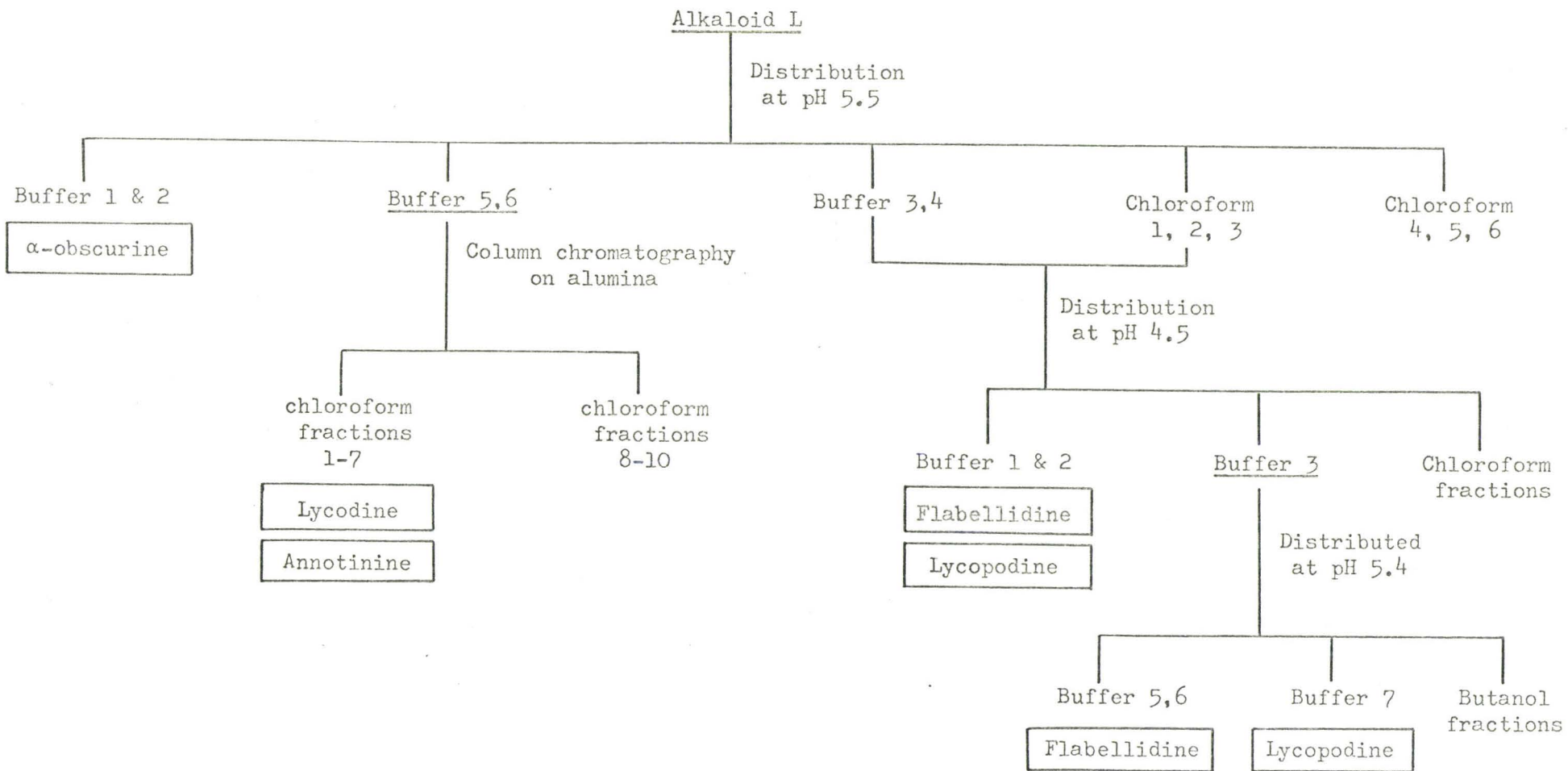
Fraction A was subjected to a six-funnel distribution between chloroform and buffer of pH 6.0. A new base was isolated from the first and second buffer fractions, analysis of which fitted the formula $C_{16}H_{24}ON_2$. Analysis for an N-methyl group was negative. The ultraviolet spectrum had an absorption maximum at $\lambda_{max} 255 m\mu$ indicating the presence of the same chromophoric group as in α -obscurine (17, 76). The infrared spectrum was very similar to that of α -obscurine, differing only in the fingerprint region. It appeared likely that the base was de-N-methyl- α -obscurine. A year after the isolation of this alkaloid, Ayer et al. (23) reported the isolation of de-N-methyl- α -obscurine from L. clavatum Lin. The melting points of our base and that of Ayer were found to be the same. An infrared spectrum of de-N-methyl- α -obscurine obtained from Dr. W. A. Ayer was found to be identical with that of the base isolated in these laboratories.



Flow Sheet diagram 1. General separation scheme followed by Young.



Flow Sheet diagram 2. Experiments performed on 2% methanol in chloroform fraction.



43c

Flow Sheet diagram 3. Analysis of 'Alkaloid L' fraction.

The mother liquor (after the separation of de-N-methyl- α -obscurine) was further subjected to a six-funnel distribution between chloroform and buffer of pH 5.0. Another alkaloid was isolated from the sixth buffer fraction, the analysis of which fitted the formula $C_{16}H_{25}O_2N$ and which melted at 237° - 238° . This base was characterized as clavolonine by comparing it with an authentic sample obtained from Dr. R. H. Burnell. There was very little material present in the buffer fractions 1-5 and all attempts to induce crystallization were unsuccessful. No further work was done on these fractions.

The 1.5% methanol in chloroform fraction (fraction B in Flow Sheet 2) was also subjected to a six-funnel distribution between chloroform and pH 6.0 buffer. A new base which we have designated hydroxy-de-N-methyl- α -obscurine, and an additional amount of clavolonine were isolated from the first and second buffer fractions. The infrared spectrum of hydroxy-de-N-methyl- α -obscurine showed characteristic absorption bands for a hydroxyl group, an NH group and a dihydropyridone ring system as in α -obscurine. The ultraviolet spectrum ($\lambda_{\max} = 255 \text{ m}\mu$, $\log \epsilon = 3.78$) was similar to that of α -obscurine ($\lambda_{\max} = 255 \text{ m}\mu$, $\log \epsilon = 3.75$). The structure of this alkaloid is discussed in a later section.

Following the isolation of hydroxy-de-N-methyl- α -obscurine in these laboratories, Burnell et al. (21) reported the isolation of Base I from *L. fawcettii*, the infrared spectrum of which showed peaks at 3480 cm^{-1} , 3200 cm^{-1} and 1662 cm^{-1} , respectively, almost the same as that of hydroxy-de-N-methyl- α -obscurine. Furthermore, the ultraviolet spectrum of base I showed absorption at $\lambda_{\max} 252 \text{ m}\mu$ ($\log \epsilon = 3.77$).

Finally, the melting point reported for base I (300° C) is about the same as that of hydroxy-de-N-methyl- α -obscurine ($300-305^{\circ}$). It is therefore likely that the two alkaloids are identical, but a direct comparison has not been made.

The third buffer fraction gave α -obscurine. The buffer fractions 4-6 did not crystallize and since there was not much material in these fractions, their investigation was not pursued further.

Fractions C, D and E (flow sheet 2) were combined because of their similar infrared spectra but further countercurrent extraction did not lead to any crystalline material.

In a second experiment* the 2% methanol in chloroform fraction (from the original chromatography, flow sheet 1) was partitioned between chloroform and buffer of pH 6.0. The first and second buffer fractions were individually chromatographed on an alumina column and different fractions were collected. In this experiment, in addition to the alkaloids already isolated in the first experiment, a small amount of β -obscurine was isolated. Buffer fractions 3-6 failed to give any crystalline material.

Many of the buffer fractions and all the chloroform fractions from the separatory funnel distribution experiments yielded dark resinous material. The residues which could not be induced to crystallize were not examined further. In the case of the buffer

* In the first experiment, the 2% methanol in chloroform fraction was first carefully rechromatographed and then subjected to countercurrent distribution.

fractions which yielded crystalline material, it was found that at best only 75% of the original amount of each buffer fraction could be isolated. The remaining amount that apparently was lost during the isolation of the alkaloids must have been comprised of solvent and decomposition products.

The separatory funnel distribution performed in this study were carried out with relatively large amounts of material. In view of the fact that the mixtures distributed were very complex and the number of transfers small, one could not hope to get a separation other than the weak from the strong bases. It is not surprising, therefore, that the weight analysis pattern of some of the distribution experiments did not show any clear cut separation of the components of the alkaloidal mixtures.

Alkaloid L (flow sheet 3)

The chloroform fractions 10, 11 and 12 on combination and redistribution between chloroform and citrate-phosphate buffer (pH 5.5) gave more α -obscurine from the first and second buffer fractions. The residues from the fifth and sixth buffer yielded crystals of lycodine and annotinine. Although these two alkaloids are known, this is the first recorded isolation of these alkaloids from L. flabelliforme and the first recorded isolation of annotinine from a Lycopodium species other than L. annotinum.

The bases from the third and fourth buffer fractions and first, second and third chloroform fractions from the above distribution experiment had similar infrared spectra and all showed absorption

in the ultraviolet at λ_{\max} 237 $m\mu$. Paper chromatography of these fractions revealed two spots, the Rf value of each spot being the same in all the fractions. These fractions were, therefore, combined and subjected to a further six funnel distribution using pH 4.5 buffer.

The first three buffer fractions had similar infrared and ultraviolet spectra. A base was isolated from the first buffer fraction as the perchlorate salt. It melted at 281° - 282° and its analysis fitted the formula $C_{18}H_{28}ON_2 \cdot HClO_4$. The melting point of this perchlorate is the same as that of L.5 perchlorate reported by Manske and Marion (7). The ultraviolet and infrared spectra were identical with those of L.5 perchlorate*. The melting point of a mixture of the two was undepressed. The isolated base was thus identified as alkaloid L.5. We have chosen the name flabellidine for this alkaloid. The elucidation of its structure is described in a later section.

Another base was isolated from the second buffer fraction as the methiodide salt which melted at 332° - 334° . The analysis of the methiodide fitted the formula $C_{16}H_{25}ON \cdot CH_3I$. This methiodide was identified as lycopodine methiodide by comparison with an authentic sample.

Paper chromatography showed that the third buffer fraction was a mixture of at least three bases. Vapour phase chromatography

* A sample of L.5 perchlorate was kindly supplied by Dr. R. H. F. Manske.

showed that the two major components of the mixture were lycopodine and flabellidine (L.5). The minor components were not identified.

The buffer fractions 4-6 contained very little material. This could not be induced to crystallize and was not examined further.

Alkaloid L.2, L.3 and L.4 isolated by Manske and Marion (7) have not been detected in this investigation. Our failure to find alkaloid L.2 (acetyldihydrolycopodine) may be a consequence of the isolation methods used in this study, which involved many acid extractions, repeated adsorption chromatography on alumina and countercurrent distributions involving acidic buffer solutions. The acetyl group might have been hydrolyzed during any or all of these treatments leading to the formation of dihydrolycopodine which was isolated by Young.

Structure of hydroxy-de-N-methyl- α -obscurine

The presence of a hydroxyl group in hydroxy-de-N-methyl- α -obscurine is deduced from an examination of its infrared spectrum in nujol and in chloroform solution. In nujol a band at 3510 cm^{-1} indicates the presence of a hydroxyl group. In chloroform solution the spectrum also shows a band at 3510 cm^{-1} which is independent of the concentration of the solution. We have assigned this band to an intramolecularly hydrogen-bonded hydroxyl group (85a).

The infrared spectrum also suggests the presence of a secondary amide group in the molecule. It shows a split band at 3220 cm^{-1} and 3150 cm^{-1} , the peaks of which are attributed to intermolecularly bonded NH stretching vibrations and a band at 1665 cm^{-1} attributed to an amide carbonyl group. Along with the carbonyl absorption at 1665 cm^{-1} , there is a medium intensity band at 1690 cm^{-1} , which may be attributed to >C=C< stretching vibrations (85b). In chloroform solution the band at 3510 cm^{-1} remains unaffected but another concentration-independent new peak appears at 3400 cm^{-1} . This band is attributed to non-hydrogen bonded amide NH stretching. The infrared spectrum of de-N-methyl- α -obscurine, which was isolated along with the hydroxy-de-N-methyl- α -obscurine from the L. flabelliforme, shows the same bands except for that at 3510 cm^{-1} attributed to the hydroxyl group. The two alkaloids also differ in the fingerprint region. The infrared spectra of α -obscurine, de-N-methyl- α -obscurine and hydroxy-de-N-methyl- α -obscurine, in the region $3600\text{-}1500\text{ cm}^{-1}$ are

reproduced in Fig. 2. The striking similarity in the carbonyl region is obvious. In the region $3100-3400\text{ cm}^{-1}$ the spectra are also very similar. Since α -obscurine does not have an amino NH, the peaks which are observed in this region must be due to the amide NH which is common to all three compounds.

The ultraviolet spectrum of the hydroxy compound (Fig. 3a) shows absorption at $\lambda_{\text{max}} = 255\text{ m}\mu$ ($\log \epsilon = 3.78$) indicating that the amide carbonyl is a part of a conjugated system. A comparison of this spectrum with those of α -obscurine (Fig. 3b, $\lambda_{\text{max}} 255\text{ m}\mu$, $\log \epsilon = 3.75$) and de-N-methyl- α -obscurine (Fig. 3c, $\lambda_{\text{max}} 255\text{ m}\mu$, $\log \epsilon = 3.81$) again shows their remarkable similarity. In the case of the latter two alkaloids, the observed spectral properties have been attributed by Ayer and Iverach (23, 29) to the presence of a 5,6 disubstituted 3,4-dihydropyridone ring system. Ayer and Iverach (23) were able to show that the de-N-methyl- α -obscurine differs from α -obscurine only by the absence of the methyl group on the amino nitrogen atom. It is most likely, therefore, that the hydroxy compound has the same chromophoric system.

The mass spectrum of the hydroxy compound shows a molecular weight of 276 which corresponds to the molecular formula $\text{C}_{16}\text{H}_{24}\text{O}_2\text{N}_2$. This formula differs in composition from de-N-methyl- α -obscurine ($\text{C}_{16}\text{H}_{24}\text{ON}_2$) by one oxygen atom.

The nuclear magnetic resonance (N.M.R.) spectrum (Fig. 4) of the hydroxy compound shows a three proton doublet at $\tau = 9.18$ corresponding to the presence of a >CH-CH_3 group. In the low field region the spectrum shows a signal at $\tau = 2.7$ which may be attributed

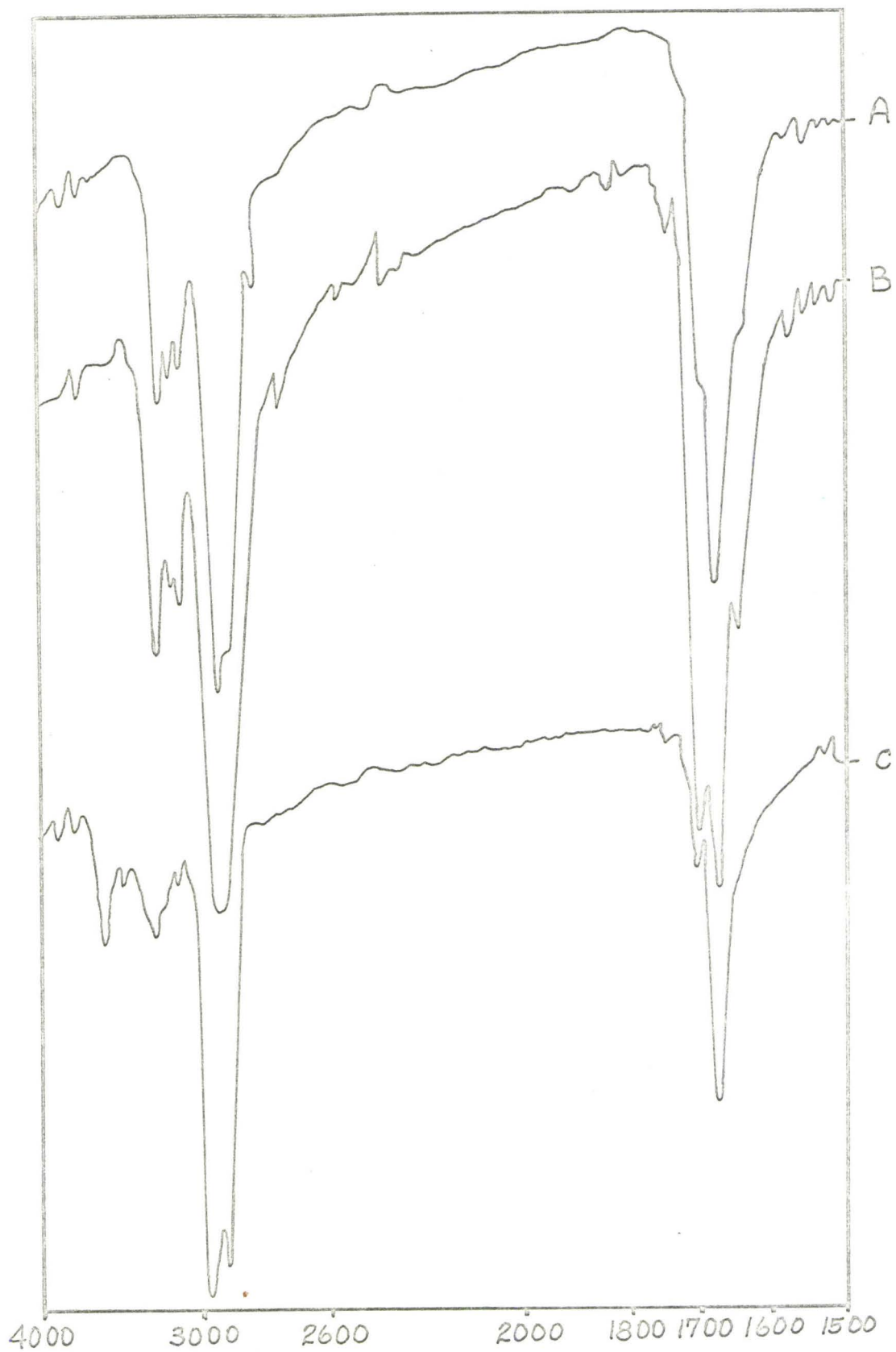


Fig. 2. Infrared spectra of (a) α -obscurine, (b) de-N-methyl- α -obscurine, (c) hydroxy-de-N-methyl- α -obscurine.

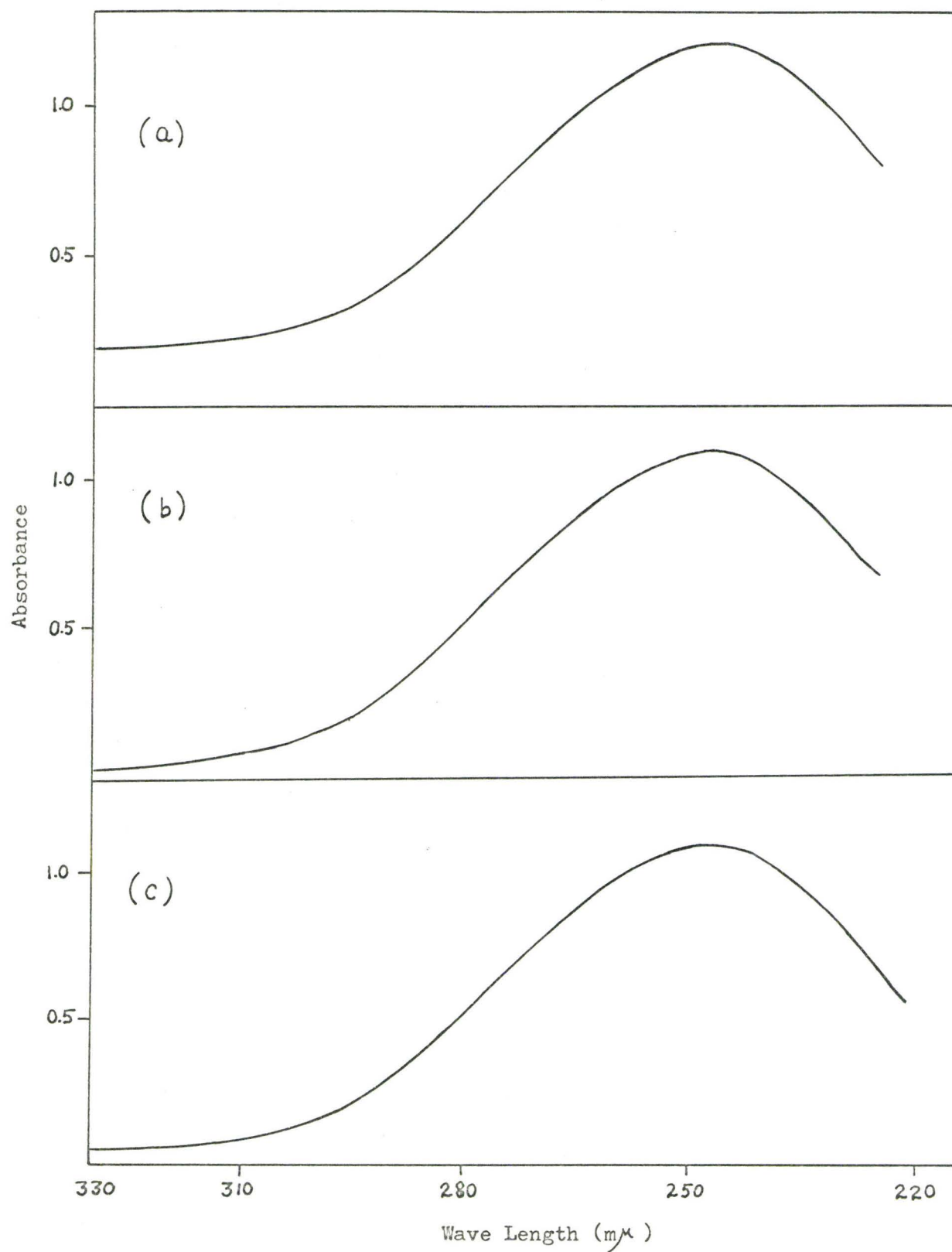


Fig. 3. Ultraviolet spectra of (a) Hydroxy-de-N-methyl- α -obscurine, (b) α -obscurine, and (c) de-N-methyl- α -obscurine.

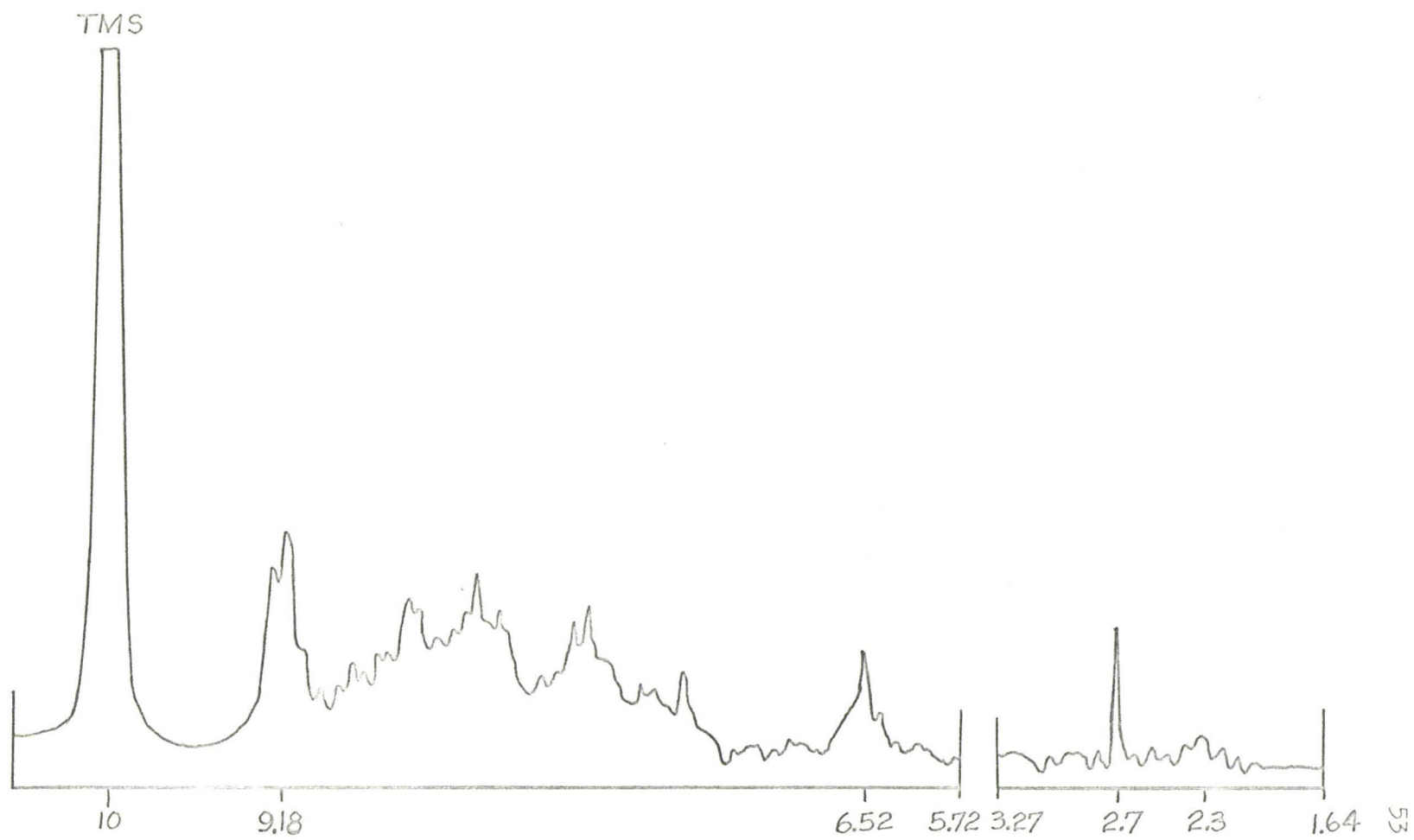
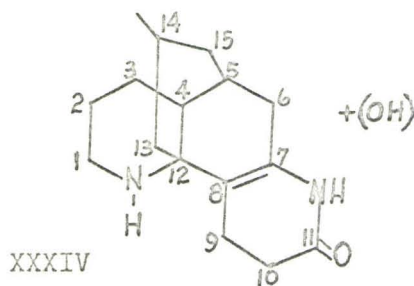


Fig. 4. N.M.R. spectrum of hydroxy-de-N-methyl- α -obscurine (CDCl_3).

to the hydrogen on the amide nitrogen atom (86a) and a weak broad signal at $\tau = 2.3$ which may be due to secondary amino proton. No signal corresponding to an $>N-CH_3$ group is found in the spectrum indicating that if the alkaloid conforms to the obscurine structure, the second nitrogen atom is secondary in nature. Since there is no other signal in the low field region below $\tau = 6.5$, it may be concluded that the double bond is tetrasubstituted.

The apparent similarity between the infrared and ultraviolet spectra of de-N-methyl- α -obscurine, α -obscurine and the hydroxy compound has been mentioned before. The fact that this base occurs with the other two and that it contains a secondary C-methyl group and may contain a secondary nitrogen atom suggests that this base may be hydroxy-de-N-methyl- α -obscurine. Thus it seems reasonable to assign structure XXXIV assuming that the two molecules (hydroxy compound and de-N-methyl- α -obscurine) have the same ring system.



The nature and the site of the hydroxyl function will now be discussed. The absence of any signal in the N.M.R. spectrum below $\tau = 6.5$ (other than at $\tau = 2.7$ and 2.3) indicates that the hydroxyl group may be attached to a tertiary carbon atom. This observation coupled with the fact that the hydroxyl group is intramolecularly hydrogen bonded makes it possible to reduce the number of possible

sites of attachment of the hydroxyl group to formula XXXIV. Attachment at C₁₄ may be excluded on the basis of the N.M.R. spectrum which shows a split peak at $\tau = 9.18$ corresponding to a $>\text{CH}-\text{CH}_3$ group. Also C₅ may be ruled out because intermolecular hydrogen bonding is forbidden on steric grounds. Thus it appears that the most likely position for the hydroxyl group is at C₄. The hydrogen bonding will then involve the lone pair electron of the amino nitrogen. A similar kind of hydrogen bonding involving the nitrogen lone pair has been reported for the alkaloid lycodoline (89) which has the hydroxyl group in the same relative position. The location of the hydroxyl group at C₄ is further borne out by an examination of the fragmentation pattern of hydroxy-de-N-methyl- α -obscurine upon electron impact.

In Fig. 5 the mass spectra of de-N-methyl- α -obscurine and hydroxy-de-N-methyl- α -obscurine are shown. For convenience of discussion, the mass spectra of lycopodine and lycodoline are shown in Fig. 6.

A comparison of the spectra of de-N-methyl- α -obscurine and lycopodine shows their remarkable resemblance. The mass spectrum of de-N-methyl- α -obscurine is relatively simple and the major peaks appear at M-43, M-57 and M-85, as in the case of lycopodine. A fragmentation mechanism compatible with the observed peaks in the mass spectrum of de-N-methyl- α -obscurine is formulated in scheme II. In both the spectra the loss of the bridge is the dominant feature. Other modes of fragmentation are of minor importance. The spectra of other Lycopodium alkaloids of the obscure type examined by MacLean (83) also showed the same pattern.

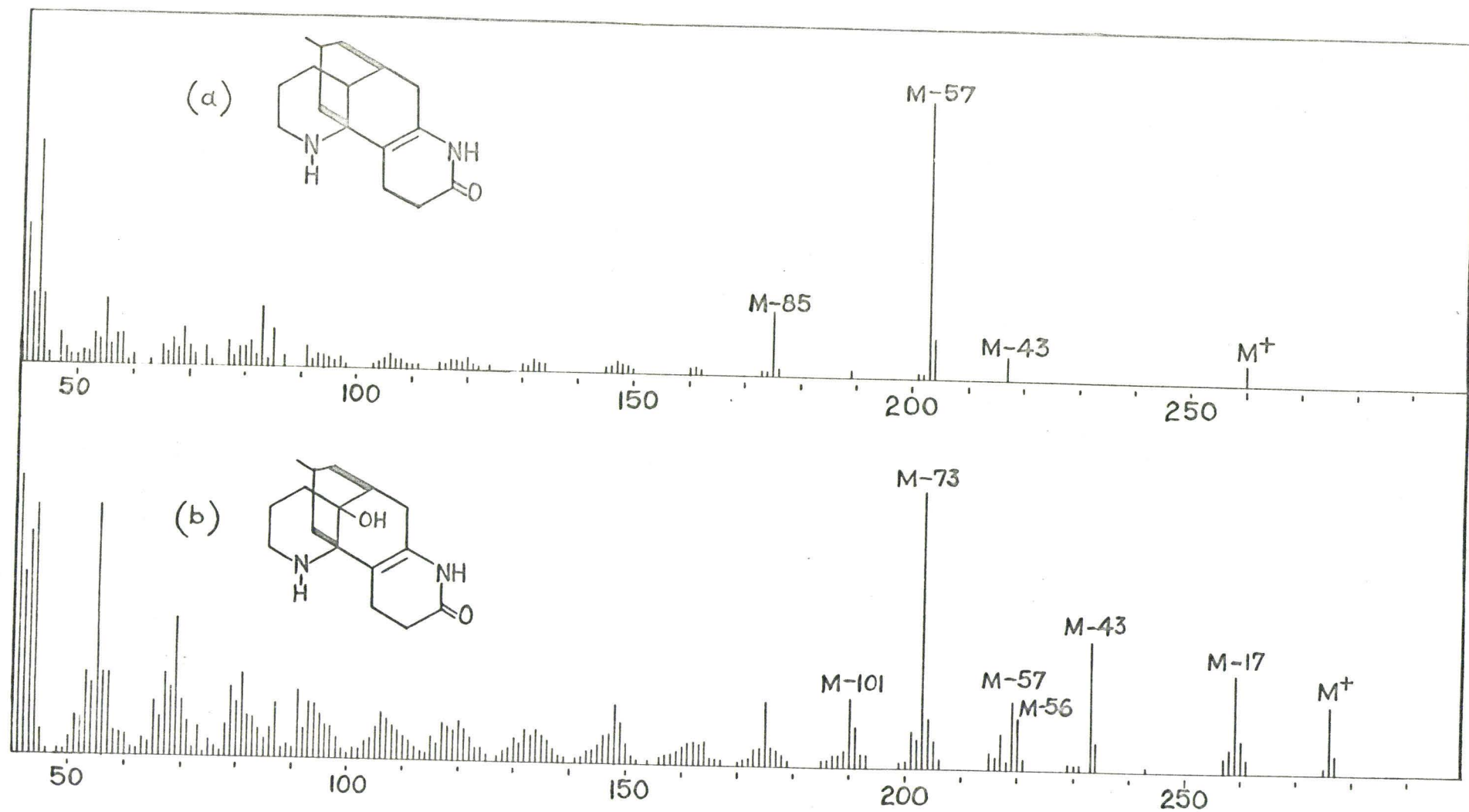


Fig. 5. Mass spectra of (a) de-N-methyl- α -obscurine, (b) hydroxy-de-N-methyl- α -obscurine.

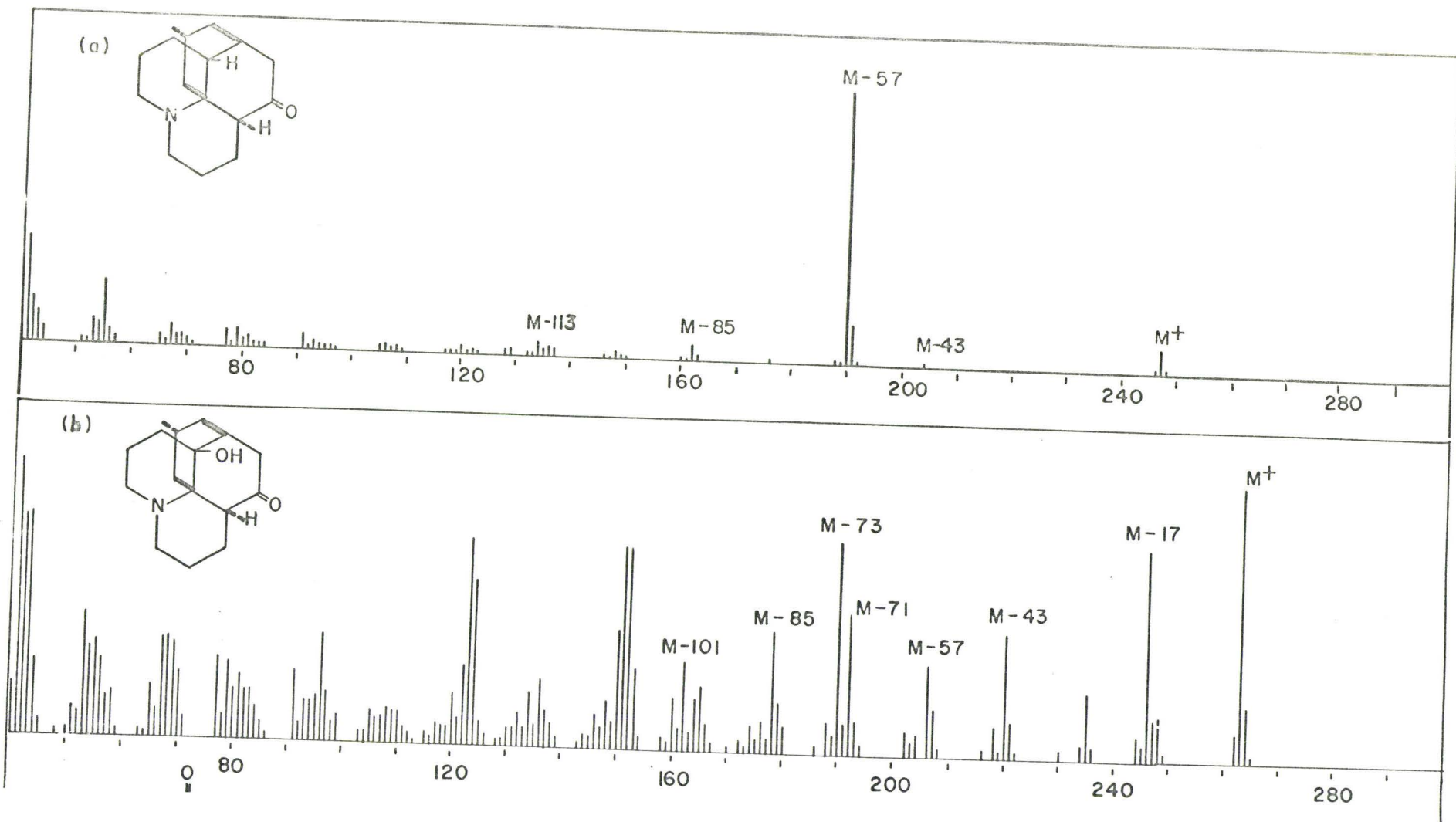
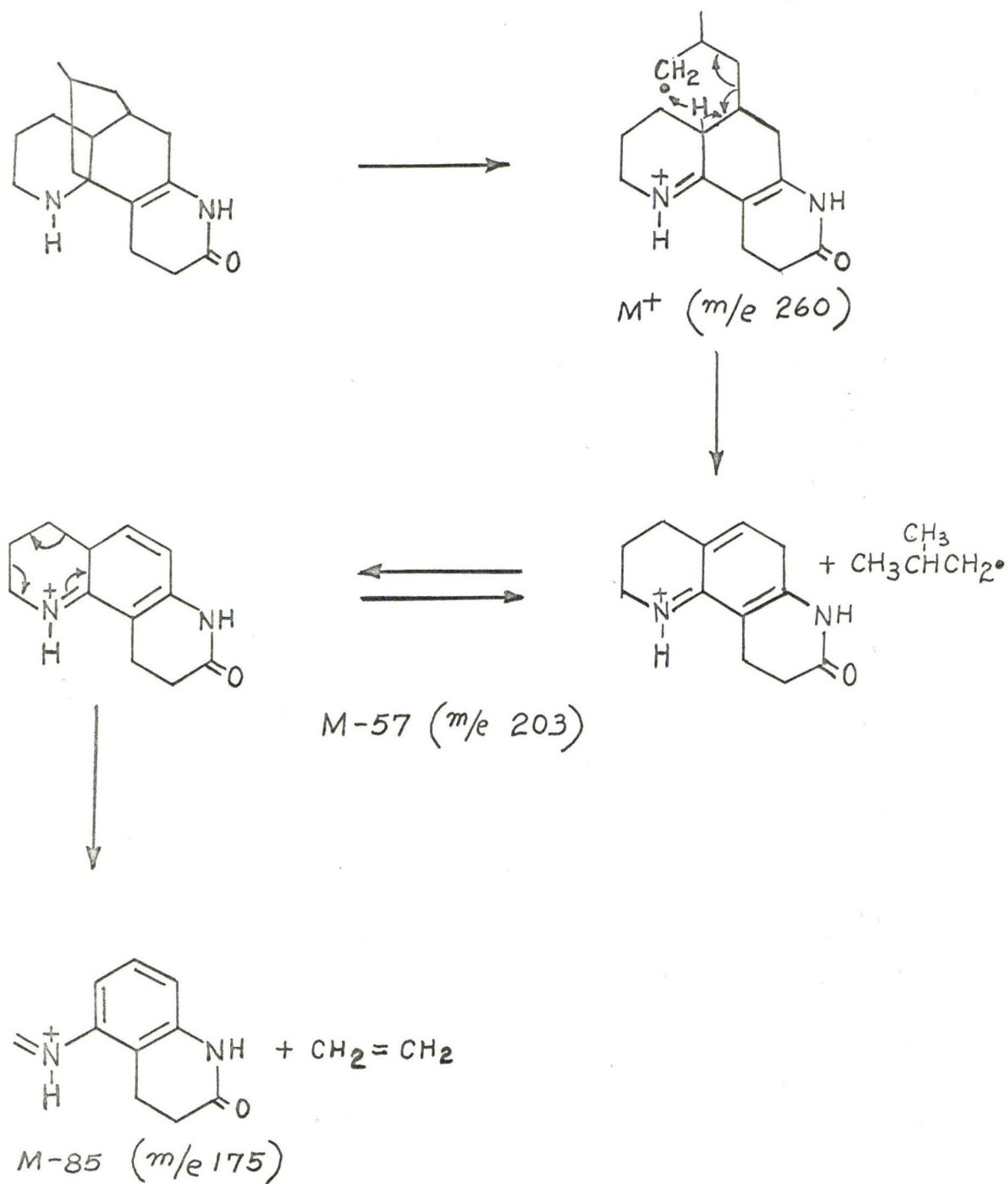


Fig. 6. Mass spectra of (a) lycopodine, (b) lycodoline. (83)



Scheme II. Proposed fragmentation for de-N-methyl- α -obscurine.

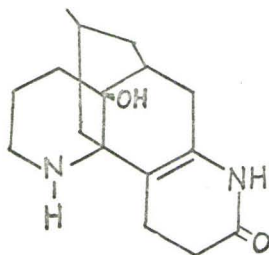
An examination of the mass spectrum of hydroxy-de-N-methyl- α -obscurine shows a more complex fragmentation pattern. The M-73 peak (m/e 203) is now the most intense peak in the spectrum while the M-57 peak (m/e 219) which is the most intense peak in the spectrum of de-N-methyl- α -obscurine, is now less intense than several other peaks in the spectrum. In addition the spectrum has major peaks at M-17 (m/e 259), M-43 (m/e 233) and M-101 (m/e 175). The M-73 peak undoubtedly arises by loss of the bridge plus the hydroxyl group. A mechanism consistent with the observed fragmentation pattern is formulated in scheme III.

The M-43 peak which arises most probably by loss of three of the bridge carbon atoms (83), although present in the spectra of lycopodine and de-N-methyl- α -obscurine, is more intense in the spectrum of hydroxy-de-N-methyl- α -obscurine than the peak at M-57 (loss of bridge plus hydrogen), a reflection of the lack of hydrogen at C₄. Another noteworthy feature is that the M-56 peak which is a normal isotope peak of the M-57 peak in the spectrum of de-N-methyl- α -obscurine, is now about 75% as intense as the M-57 peak. The intensity of the molecular ion peak is also greatly increased.

The spectrum of lycodoline differs from that of lycopodine in the same way as the spectrum of hydroxy-de-N-methyl- α -obscurine differs from that of de-N-methyl- α -obscurine. The spectrum of lycodoline has been discussed in detail elsewhere (83) and it is only necessary to indicate here the points of similarity with the spectrum of hydroxy-de-N-methyl- α -obscurine. We find for example an intense M-17 peak and an intense M-73 peak. The M⁺ peak is also more intense than in

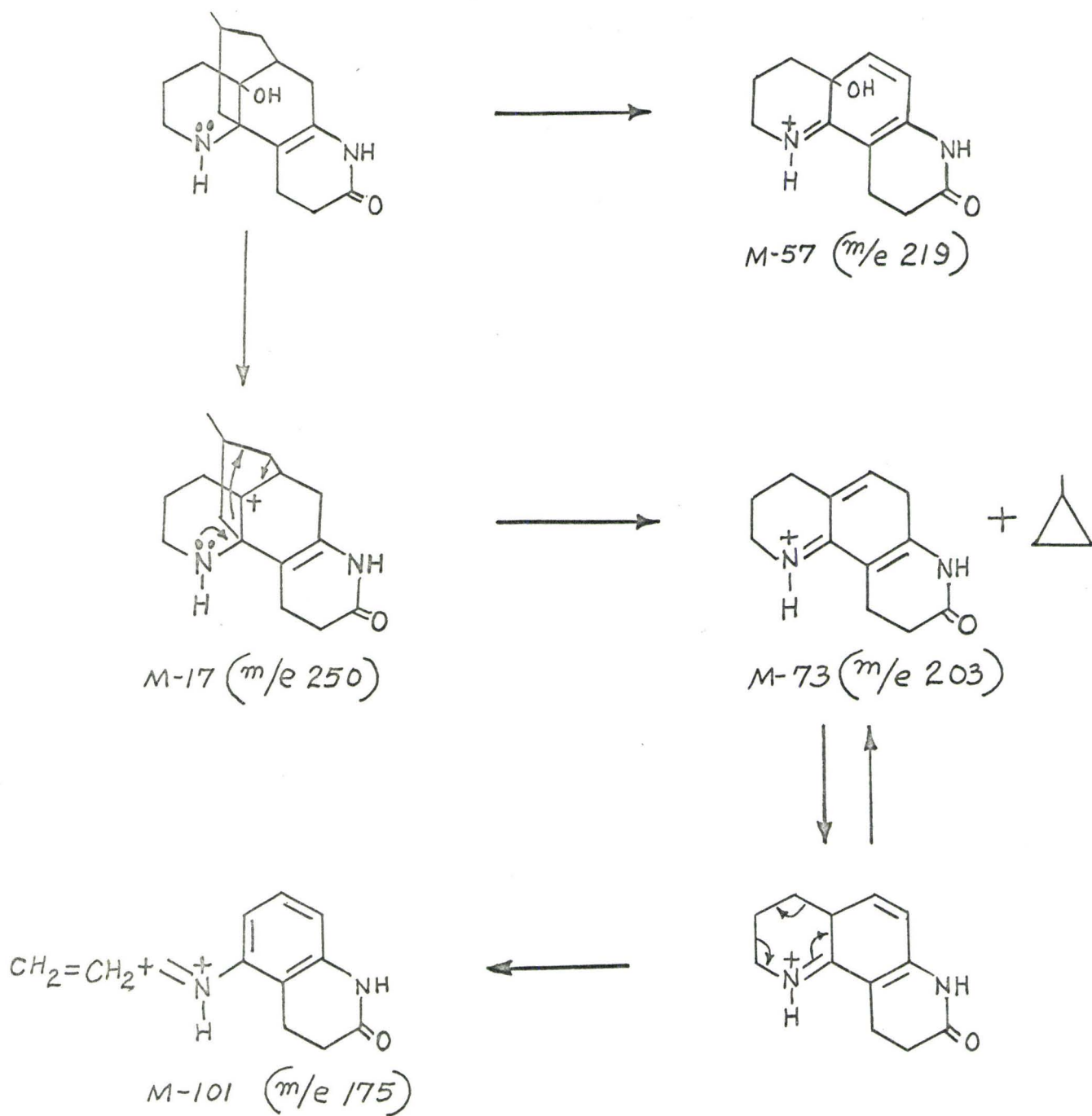
lycopodine indicating that the molecule is less prone to fragment. The peak at M-57 is no longer the most intense peak in the spectrum and the M-56 peak is much stronger than a normal isotope peak. The peaks at M-28 and M-71 in lycodoline are characteristic of this compound and are associated with the presence of the carbonyl group. The M-28 peak may be attributed to loss of carbon monoxide from the molecular ion and the M-71 peak to loss of 43 mass units from the M-28 peak.

The similarity in the spectra of hydroxy-de-N-methyl- α -obscurine and of lycodoline suggests that the hydroxyl group is situated in the same relative position in the two molecules. It was pointed out earlier in the introductory section that hydroxyl substitution at other sites in the lycopodine nucleus does not have such a profound effect on the fragmentation pattern. It is proposed, therefore, on the basis of the infrared, ultraviolet, N.M.R. and mass spectrometric examination that hydroxy-de-N-methyl- α -obscurine has the structure shown below.



XXXV

The evidence on which structure XXXV for hydroxy-de-N-methyl- α -obscurine is proposed does not provide any firm evidence about the stereochemistry of the molecule. The relative and absolute stereochemistry of the alkaloids of the lycodine group has been established by Ayer et al. (23, 76) by inter-relating these alkaloids to one



Scheme III. Proposed fragmentation of hydroxy-de-N-methyl- α -obscurine.

another and by relating de-N-methyl- α -obscurine to lycopodine, the relative and absolute stereochemistry of which is known (57). Since all these alkaloids have been found to have the same stereochemistry as shown in structure XXIVb for α -obscurine, it is unlikely that the hydroxy-de-N-methyl- α -obscurine will deviate from this pattern.

Although in structure XXXV we have placed the methyl group at C₁₄, the evidence at hand does not preclude the possibility of its attachment to any one of the three bridge carbon atoms. All the alkaloids of known structure of the lycopodine and lycodine group have, however, this methyl group attached to the C₁₄ position. It is, therefore, reasonable to assume that in hydroxy-de-N-methyl- α -obscurine, the methyl group is present in the same relative position. This assignment is also compatible with the biogenetic scheme proposed by Conroy (84) and by Iverach (29).

Structure of flabellidine

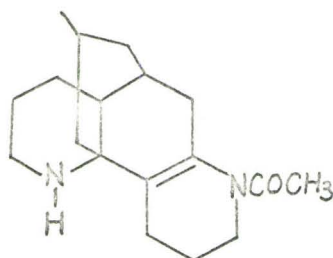
The alkaloid, flabellidine, was isolated as its perchlorate salt. The analysis indicated that the alkaloid has the molecular formula $C_{18}H_{28}ON_2$. The melting point of flabellidine perchlorate (280° - 282°) is the same as that of alkaloid L.5 perchlorate (280° - 282°) and a comparison of flabellidine perchlorate with an authentic sample of L.5 perchlorate obtained from Dr. R. H. F. Manske showed that the two samples were identical. Although Manske and Marion (7) assigned the formula $C_{18}H_{28}O_2N_2$ to alkaloid L.5, the elemental composition reported by them can be better accommodated by the formula $C_{18}H_{28}ON_2$. The latter formula is consistent with the molecular weight of 288 found by mass spectrometry.

The presence of a secondary amino group in flabellidine is inferred from its infrared spectrum which shows an absorption band at 3280 cm^{-1} (nujol), which may be assigned to a hydrogen bonded N-H stretching absorption (85c). In the infrared spectrum of flabellidine perchlorate, this band is very weak and in addition a series of bands between 2700 cm^{-1} - 2625 cm^{-1} appear. This weakening of the normal N-H stretching band on salt formation and appearance of a complex series of absorption bands in the region 2700 - 2600 cm^{-1} ($\overset{+}{N}H_2$ stretching vibrations) has been shown (85d) to be characteristic of the secondary amino group. The formation of an acetyl derivative of flabellidine, described later, substantiates the secondary nature of the basic nitrogen.

The infrared spectrum also shows bands at 1640 cm^{-1} (s) and 1680 cm^{-1} (m) which may be attributed to an amide carbonyl group and a carbon-carbon double bond respectively (85b). The absence of amide II and III bands suggests that the amide function may be tertiary. This is further supported by the fact that the perchlorate salt of flabellidine does not show any amide N-H stretching absorption. It has been found (20) in the case of flabelline, which contains both a basic nitrogen and a secondary amide group, that the perchlorate salt has a medium intensity band in the N-H stretching region in the infrared spectrum.

The N.M.R. spectrum of flabellidine (Fig. 7) shows a split peak ($\tau = 5$ c.p.s.) at $\tau = 9.12$ (3 proton) indicating the presence of a >CHCH_3 group, and a singlet at $\tau = 7.9$ (3 proton) which may be assigned to an >NCOCH_3 group (86b). In the low field region, the spectrum has only a very weak broad signal at $\tau = 2.84$ which may be due to the proton on the secondary nitrogen atom. Since there is no signal below $\tau = 6.10$ in the low field region, other than that at $\tau = 2.84$, the double bond must be tetrasubstituted. The molecular weight 288 and the molecular formula $\text{C}_{18}\text{H}_{28}\text{ON}_2$, combined with the spectral evidence, indicate that flabellidine is tetracyclic.

These structural features deduced from the infrared and N.M.R. spectra of flabellidine may be accommodated within the framework of the known ring systems of the Lycopodium alkaloids in structure XXXVI for flabellidine. This structure is also compatible with the ultra-violet spectrum and the mass spectrum discussed below.



XXXVI

The ultraviolet spectrum of flabellidine shows an absorption at λ_{\max} 237 $m\mu$ (perchlorate, λ_{\max} 237, $\log \epsilon = 3.84$). Flabelline (XVIIa) which is isomeric with flabellidine, in contrast, shows no absorption above 220 $m\mu$ while α -obscurine shows a band at λ_{\max} 255 $m\mu$ ($\log \epsilon = 3.75$). All these alkaloids have the chromophoric system >C=C-NCO- . In flabelline only the double bond is incorporated in a ring system, in flabellidine both the double bond and the nitrogen atom are incorporated in the system, while in α -obscurine, the double bond, nitrogen and carbonyl are all present in a six-membered ring. One may explain the shift in the absorption maximum by the greater degree of planarity enforced upon the chromophoric system as one moves from flabelline to α -obscurine.

Confirmation of structure (XXXVI) for flabellidine was obtained by the conversion of lycodine into N-acetyl-flabellidine.

Lycodine contains all the ring carbon atoms of the structure proposed for flabellidine. We considered that high pressure reduction of lycodine in the presence of acetic anhydride would lead to the formation of N-acetyl flabellidine for the following reasons. A

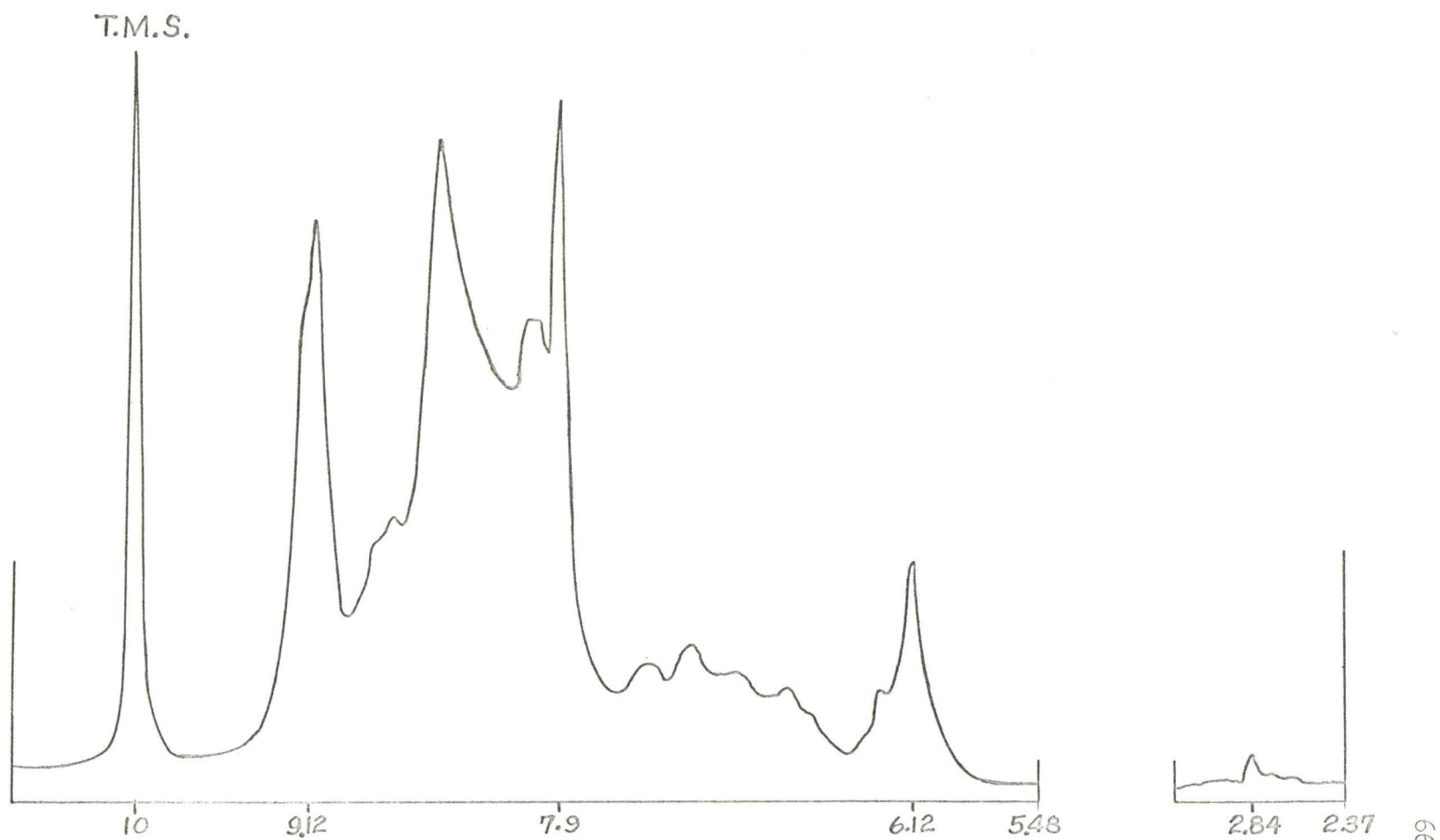


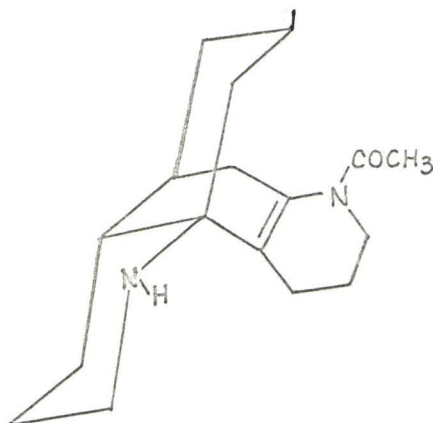
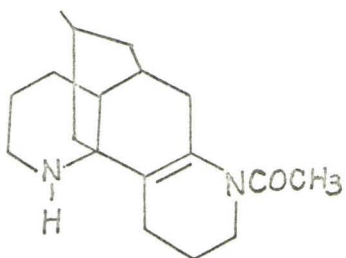
Fig. 7. N.M.R. spectrum of flabellidine (CDCl₃).

7,8 double bond in the lycopodine system has been shown to be highly resistant to saturation. Thus anhydrodihydrolycopodine is not affected by hydrogen at 1050 p.s.i.g. and 110° C. (92). Similarly it is known (20) that flabelline is formed upon high pressure reduction of lycopodine oxime in the presence of acetic anhydride. In the obscurine series it is found (18) that β -obscurine is converted to the α -obscurine with lithium in ammonia, thereby showing that the 7,8 double bond is resistant to reduction. Thus it seemed likely that the reduction of lycodine in the presence of acetic anhydride, hydrogen and Raney nickel would stop at the tetrahydro stage and that the two nitrogen atoms would undergo acylation under the conditions employed.

Accordingly lycodine was treated with acetic anhydride over Raney nickel and hydrogen at high pressure (1160 p.s.i.g.) and temperature (110°). The acidic reaction mixture, after decomposing with water, was extracted with chloroform, leaving behind any basic material. The chloroform extract yielded a neutral crystalline compound melting at 150°-152°.

The acetyl derivative of flabellidine was prepared by heating flabellidine with acetic anhydride and purifying the product by column chromatography. The crystalline compound thus obtained melted at 150°-152°. The melting point of a mixture of this compound with the synthetic acetyl flabellidine was undepressed. The infrared and ultraviolet spectra of the two, as well as the mass spectra, which are discussed in the next section, were identical.

Since the structure and the stereochemistry of lycodine is known (37) the following structure and stereochemistry can be assigned to flabellidine.



Mass spectra of flabelline, flabellidine and acetylflabellidine

The fragmentation pattern observed upon electronic impact is consistent with the structure XXXVI for flabellidine.

An examination of the mass spectrum of flabellidine (Fig. 8a) shows peaks in the high mass region at M-43, M-57, M-85, M-99 and M-127. The M-57 peak is by far the most intense peak in the spectrum. The origin of this peak is undoubtedly due to the loss of bridge plus hydrogen, a process common to Lycopodium alkaloids of the lycodine and lycopodine group. The M-85 peak, which may arise by the loss of 28 mass units from the M-57 fragment ion, and the M-43 peak are also common to alkaloids of both ring systems. The peaks characteristic of this alkaloid are at M-99 and M-127 which arise because of the presence of the N-acetyl group. It has been shown by Djerassi *et al.* (87) that amides readily lose ketene ($\text{CH}_2=\text{C}=\text{O}$) upon electronic impact and the ions of M-99 and M-127 may arise by loss of ketene from the ions of M-57 and M-85 respectively. A mechanism consistent with the formation of these fragment ions is shown in scheme IV.

The mass spectrum of acetyl flabellidine (Fig. 8b) and the mass spectrum of the acetylated product obtained from reduction of lycodine are identical. The fragmentation pattern is similar to that of flabellidine. Additional peaks are present, however, because of the presence of the second acetyl group. The peaks observed are at M-43, M-57, M-71, M-85, M-99, M-112, M-127, M-141, and M-169 mass units. The most intense peak in the spectrum is again the M-57

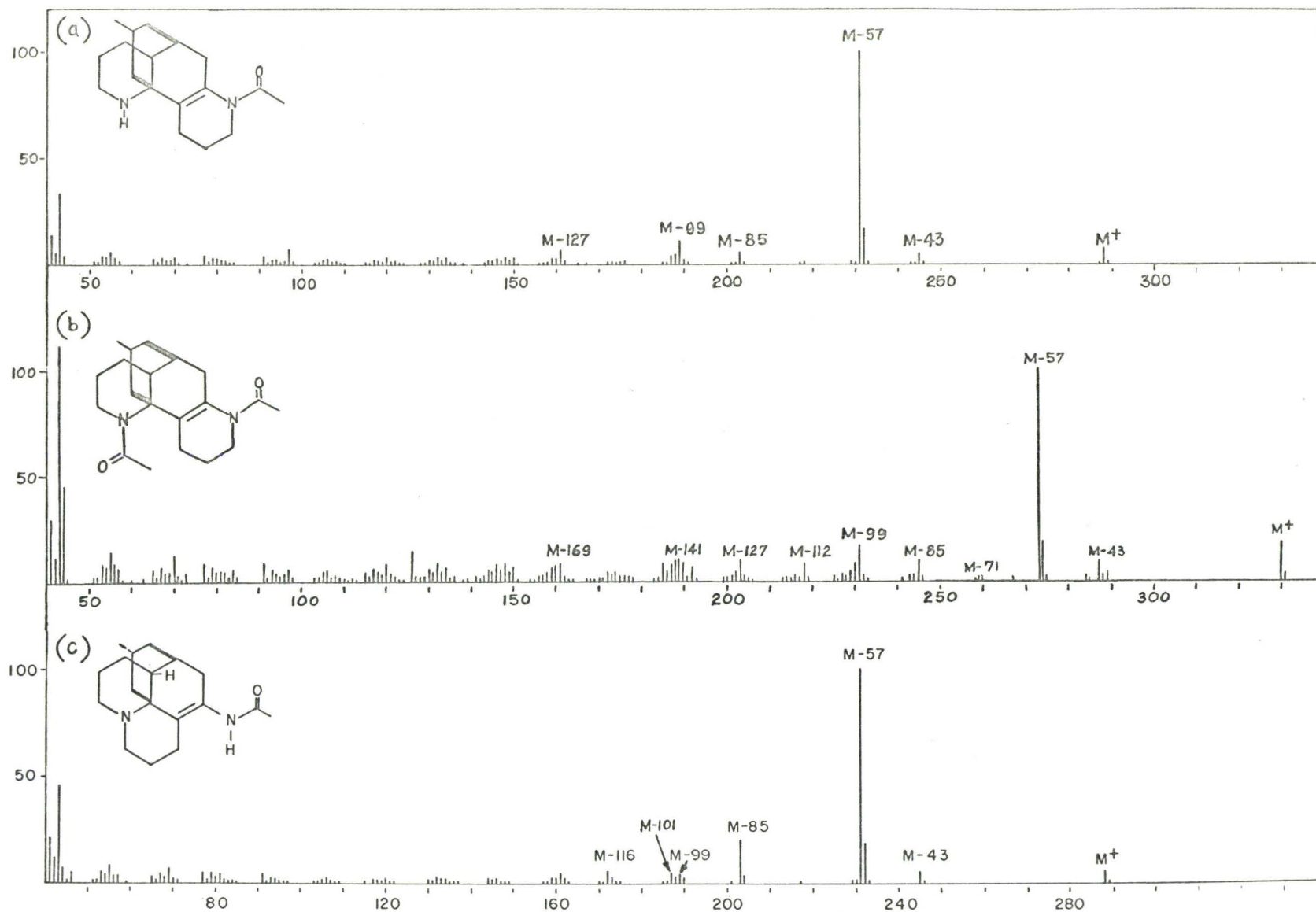
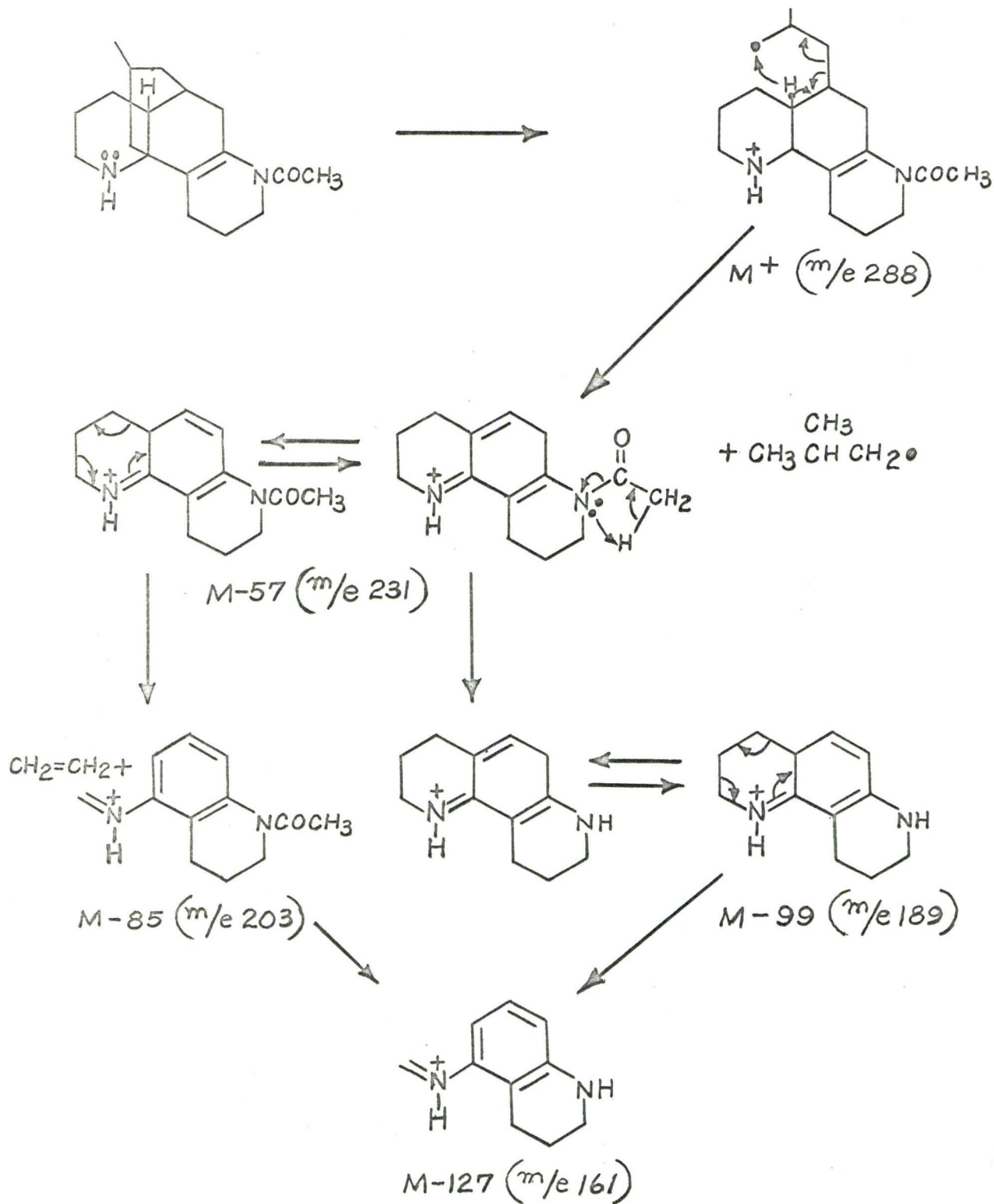


Fig. 8. Mass spectra of (a) flabellidine, (b) acetylflabellidine, (c) flabelline.



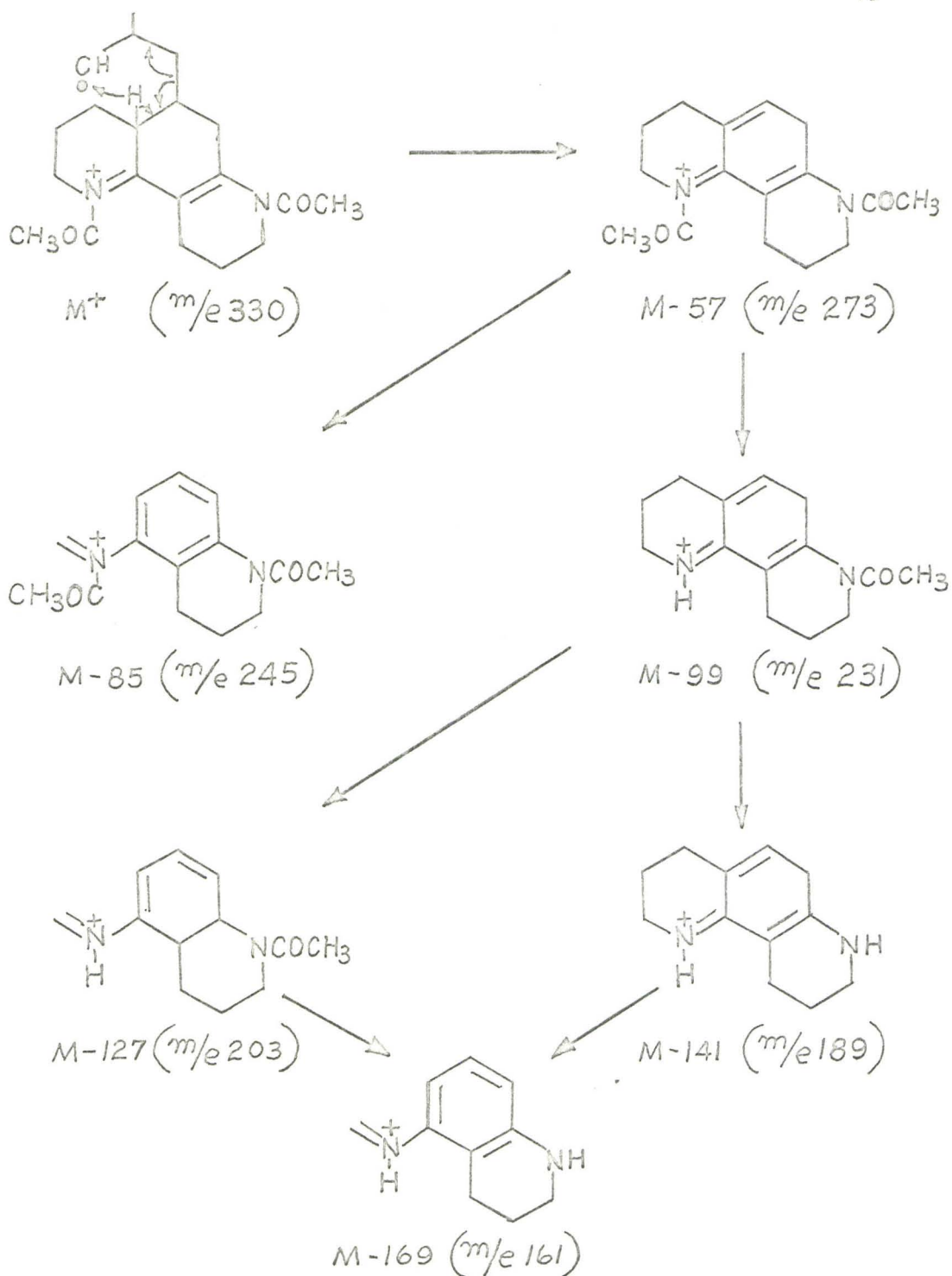
Scheme IV. Proposed fragmentation mechanism for flabellidine.

peak. Loss of 28 mass units from the fragment ion m/e 273 will give the peak at M-85 (m/e 245) mass units. On the other hand loss of 42 mass units ($\text{CH}_2=\text{C}=\text{O}$) from the fragment ion m/e 273 will give the M-99 peak (m/e 231). Loss of another ketene molecule from m/e 231 ion will result in the formation of the fragment ion, m/e 189 (M-141), which then may lose another 28 mass units ($\text{CH}_2=\text{CH}_2$) forming the fragment ion m/e 161 (M-169). The peak at M-127 (m/e 203) may arise by the loss of 28 mass units from the fragment ion m/e 231 (M-99), which may lose 42 mass units giving rise to the M-169 peak (m/e 161). For clarity of understanding a possible fragmentation mechanism is shown in scheme V.

The M-43 peak found in the Lycopodium alkaloids of the lycopodine and lycodine group has been shown (83) to have its origin through the loss of three of the bridge carbon atoms. The M-71 fragment ion present in acetyl flabellidine may arise by the loss of 28 mass units from the M-43 fragment ion. A plausible mechanism for the formation of the M-43 peak is postulated in scheme VI.

The fragmentation pattern of flabellidine and acetyl flabellidine upon electron impact are in agreement with the proposed structure (XXXVI) and with the fragmentation patterns of other Lycopodium alkaloids.

Although the mass spectra of flabelline and flabellidine are similar, a comparison shows that the presence of a secondary amide substituent in flabelline results in additional characteristic peaks in its spectrum. The spectrum (Fig. 8c) has peaks in the high mass region at M-43, M-57, M-85, M-99, M-101, M-116 and M-127 mass



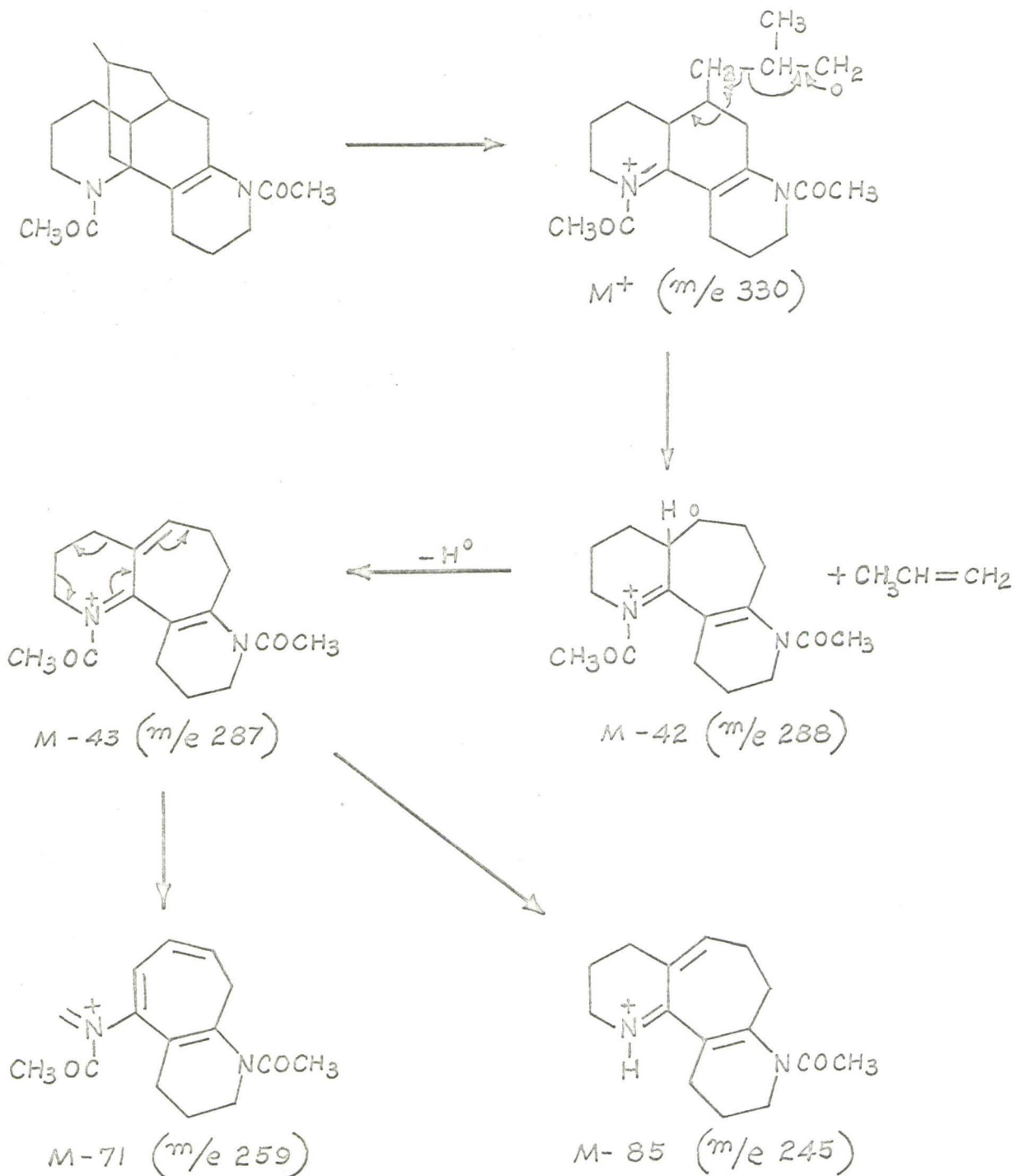
Scheme V. Proposed fragmentation for acetyl flabellidine.

units. The M-43, M-57 and M-85 peaks are again common to all Lycopodium alkaloids of the lycopodine and lycodine groups. The peaks at M-99 and M-127 are present in the spectrum of both flabelline and flabellidine confirming the assumption that they arise because of the presence of an N-acetyl group. The peak at M-116 however is absent in the spectrum of flabellidine and hence is characteristic of flabelline, although of relatively low intensity. A fragmentation mechanism accounting for the formation of these ions is postulated in scheme VII.

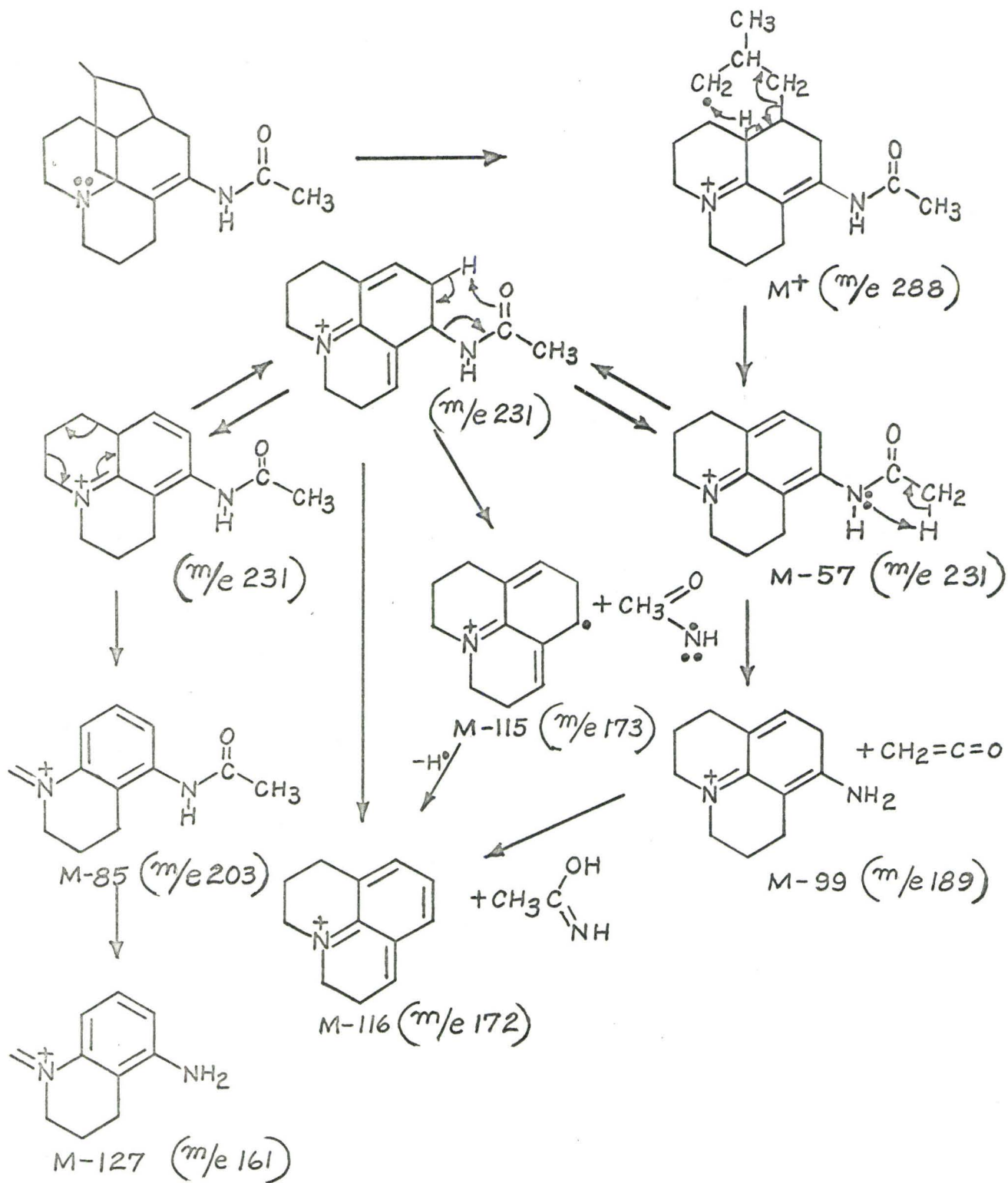
The peak at m/e 172 (M-116) corresponds to the loss of acetamide from the fragment of m/e 231 (M-57), and it appears that acetamide may be lost in more than one manner. In one stepwise process it is proposed that ketene is first eliminated to yield the ion of m/e 189 (M-99), which then loses ammonia. In support of this process there is a strong metastable peak in the spectrum at m/e 157.8, corresponding to loss of 17 mass units from the fragment of m/e 189 [$m^*(189 \longrightarrow 172) = 156.8$]. Another stepwise process, the loss of $(\text{CH}_3\text{C} \begin{smallmatrix} \text{=O} \\ \text{<NH} \end{smallmatrix})$ followed by the loss of $\text{H}\cdot$, is suggested by the presence of metastable peaks at m/e 129.8 and 171.5, corresponding respectively to the transformations m/e 231 \longrightarrow 173 (m^* calc. = 129.5) and m/e 173 \longrightarrow 172 (m^* calc. = 171). A final possibility, the concerted elimination of acetamide, cannot be ruled out from the evidence at hand. In order to accommodate the formation of the fragments of m/e 203, 189 and 172, it is necessary to postulate that the double bonds and the hydrogen atoms in the fragment ions are very mobile.

In the fragmentation of flabelline, the loss of one nitrogen atom in the transformation m/e 189 \longrightarrow 172 is observed. The loss of one

nitrogen atom as an ammonia molecule or as an amine fragment, is less likely from a heterocyclic system and indeed is not a discernible transformation in the spectra of lycodine or α -obscurine reported by MacLean (83) or that of flabellidine discussed above. Thus it appears that the mass spectrometric method will differentiate between alkaloids with the lycopodine skeletal structure carrying a nitrogenous substituent and alkaloids with a lycodine skeleton, where both nitrogens are incorporated into the ring system.



Scheme VI. Proposed mechanism for the formation of M-43 peak.



Scheme VII. Proposed fragmentation mechanism for flabelline.

Experimental

Apparatus, Methods, Materials

Melting points were recorded on a Kofler Micro hot stage melting point apparatus, and are uncorrected.

The infrared spectra were recorded on a Beckman Model IR-5 recording spectrophotometer in nujol mull unless otherwise stated.

Ultraviolet spectra were measured in methanol on a Perkin-Elmer Model 4000 spectracord.

The paper chromatograms were run on Whatman No. 1 paper buffered as described in the text, and developed with butanol saturated with water. The absolute Rf values of the alkaloids varied appreciably, but the relative values were constant. The alkaloidal spots were developed by spraying with Dragendorff's reagent (91).

The analyses were performed by E. Thommen, Basel, Switzerland; Galbraith Laboratory, Inc., Knoxville, Tennessee; and C. Daessle, Montreal, P. Q.

The mass spectra were determined on a C.E.C.-103 mass spectrometer at an ionizing potential of 70 eV and an ionizing current of 50 μ a. Samples were introduced through a heated inlet system maintained at 140° C. The samples were run through the courtesy of Professor K. Biemann, Massachusetts Institute of Technology, Cambridge, Massachusetts. All mass spectra are plotted in terms of relative abundance, with the most intense peak ("base peak") being taken as 100%. In one mass

spectrum (Fig. 8b) the most intense peak in the high mass region was taken as 100%.

The N.M.R. spectra were measured in deuteriochloroform solutions with tetramethylsilane as an internal standard on a Varian V-4300 B high-resolution spectrometer at a frequency of 60 Mc/sec.

The vapour phase chromatography was done on an RSCO-"600 series" vapour phase chromatograph. The column packing consisted of silicone rubber gum S.E.30 supported on celite (B.D.H. 80-120 mesh size).

Source of crude *L. flabelliforme* extracts

The crude extracts used in these experiments were isolated in these laboratories over a period of several years from *Lycopodium flabelliforme* collected in the Wentworth Valley of the province of Nova Scotia. The dry, ground plant material was extracted by the procedure of Manske and Marion (8). Lycopodine was isolated by column chromatography as described by Barclay and MacLean (65). The rest of the material was eluted from the column and combined to give the mixture of crude alkaloids used in these experiments.

Preliminary purification of crude alkaloids

Several litres of water and several hundred grams of tartaric acid were added to the mixture of the crude alkaloids obtained after removal of lycopodine. The resultant mixture was steam-distilled to remove organic solvents used in the original purification. More water was added to the distillation residue, and the aqueous solution was shaken with about 60 grams of powdered celite and then suction-filtered through a pad of celite. After extracting the filtrate twice

with ether to remove any fatty, non-basic material, the aqueous solution was basified with ammonia and extracted with chloroform. The chloroform extract was washed with water and evaporated to dryness, when about 200 grams of a black tar-like residue was obtained.

Chromatography of crude alkaloid

The crude alkaloid extract (75 grams) was dissolved in the minimum volume of chloroform and was chromatographed through a 5 x 54 cm column of alumina. The column was first eluted with chloroform until the eluate was almost colourless. The eluates were combined and evaporated to dryness, yielding 50 grams of dark resinous material. The column was then eluted with 2% methanol in chloroform and 5% methanol in chloroform. These fractions on removal of solvent yielded dark resinous material weighing 14 grams and 3 grams respectively. The residue obtained from the chloroform fraction was further subjected to a twelve separatory funnel countercurrent distribution between citrate-phosphate buffer of pH 6.0 and chloroform by Young (20). The chloroform fractions 10, 11 and 12, obtained from this distribution were combined and used in the experiments described later in this section. This combined fraction was labelled alkaloid L.

Rechromatography of 2% methanol in chloroform fraction

The material obtained from this fraction was dissolved in the minimum volume of chloroform and adsorbed on an alumina column. The column was eluted successively with chloroform, 1% methanol in chloroform (fraction A), 1.5% methanol in chloroform (fraction B), 2% methanol in chloroform (fraction C), 5% methanol in chloroform

(fraction D) and 10% methanol in chloroform (fraction E). No alkaloid was present in the chloroform fraction. The residues obtained from the other fractions did not crystallize and attempts to prepare perchlorate salts were also unsuccessful. The weight of the residues obtained from different fractions are given in Table III.

Table III

Column chromatography of 2% methanol in chloroform fraction.

Fraction	Chloroform	1% Methanol in Chloroform	1.5% Methanol in Chloroform	2% Methanol in Chloroform	5% Methanol in Chloroform	10% Methanol in Chloroform
Weight in grams	None	2.20	8.75	0.10	1.51	0.43

Separatory funnel distribution of fraction A with the system chloroform-citrate phosphate buffer (pH 6.0)

Fraction A (2.2 g) dissolved in chloroform (200 ml) was placed in a separatory funnel and partitioned between chloroform and buffer of pH 6.0 (200 ml), in a six-funnel distribution, the chloroform being the moving layer. The buffer layers were separated from the chloroform layers, basified with ammonium hydroxide solution, extracted with chloroform and the extract dried over anhydrous sodium sulphate. The residue, after evaporation of the solvent, was weighed to give the distribution shown in Fig. 9a. The samples obtained from the weight analysis were submitted to paper chromatography and their infrared and ultraviolet spectra measured to obtain some indication about the

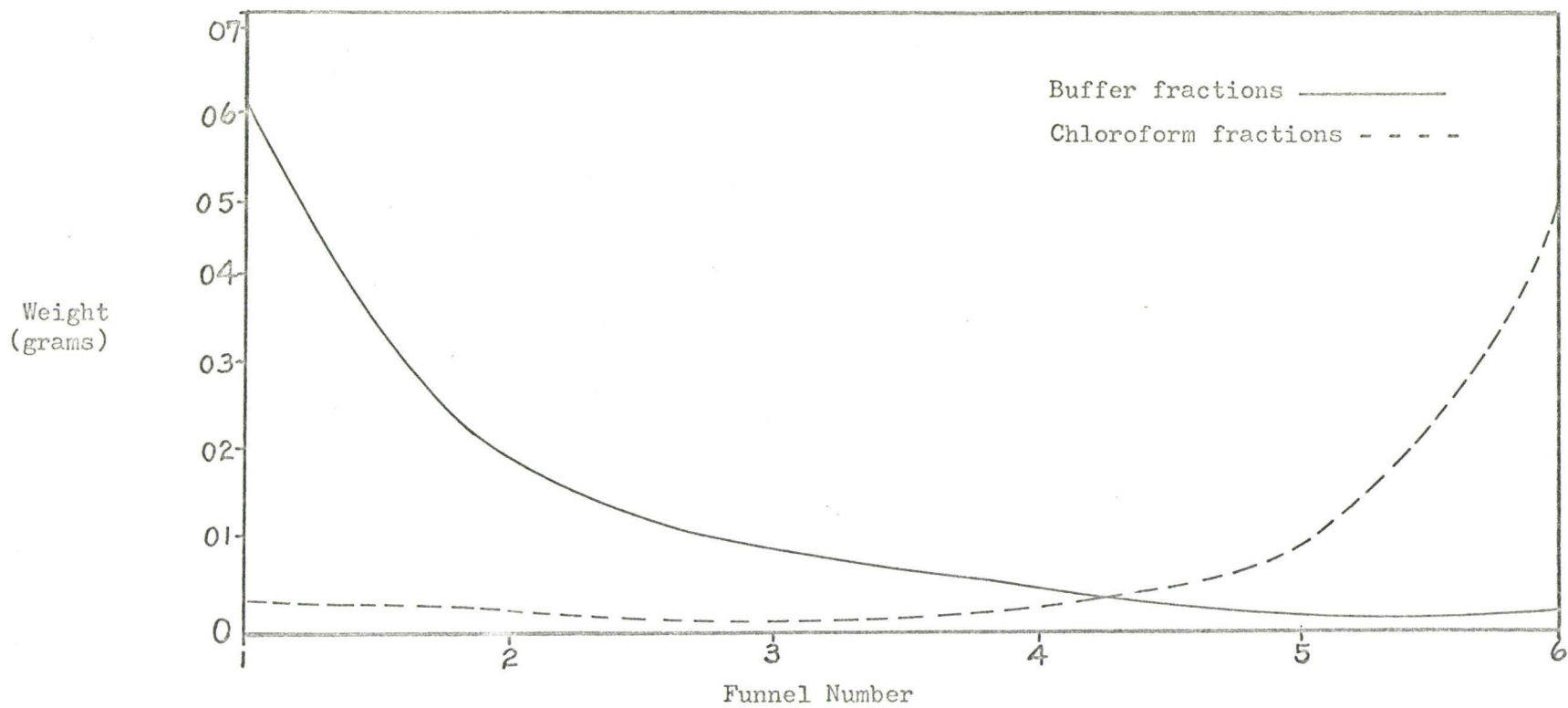


Fig. 9a. Separatory funnel distribution of fraction A.

composition of the material. Most of the material was present in the first two buffer fractions. The residue from the buffer fractions 3-6 could not be induced to crystallize and was not examined further.

Isolation of de-N-methyl- α -obscurine

The residue from the buffer fractions 1 and 2 crystallized in needles from a chloroform-acetone mixture and on recrystallization from the same solvent system melted at 268° - 270° C. The compound was very soluble in chloroform but only moderately soluble in acetone. Calculated for $C_{16}H_{24}ON_2$: C, 73.80%; H, 9.30%

Found: C, 73.98%; H, 9.51%; No N-methyl or O-methyl groups were present.

The ultraviolet spectrum showed a single maximum at $250\text{ m}\mu$ ($\log \epsilon = 3.81$) similar to that of α -obscurine.

The infrared spectrum showed a strong band at 1665 cm^{-1} (amide $\text{>C}=\text{O}$) and a medium intensity band at 1690 cm^{-1} ($\text{>C}=\text{C}<$). It also showed peaks at 3232 cm^{-1} and 3150 cm^{-1} indicating the presence of a secondary amide group. A well-defined peak at 1420 cm^{-1} suggested that the carbonyl group is adjacent to a methylene group. The infrared spectrum of this compound was very similar to that of α -obscurine differing only in the finger print region. The melting point, the composition and the spectral properties are identical with those reported for de-N-methyl- α -obscurine (23).

An infrared spectrum of de-N-methyl- α -obscurine obtained from Dr. W. A. Ayer was identical with the above spectrum.

Concentration of the mother liquor yielded a second crop of crystals which were shown to be a mixture of two compounds by paper chromatography (paper buffered at pH 5.0). Accordingly the crystalline

material and the residue obtained by evaporation of the mother liquor were combined and subjected to another six-funnel countercurrent distribution at a lower pH.

Separatory funnel distribution of the residue obtained after the separation of de-N-methyl- α -obscurine

Isolation of clavolonine

The second distribution was performed exactly as above except that a citrate-phosphate buffer of pH 5.0 was used. The fractions were worked up as described previously. The weight analysis gave a distribution pattern as shown in Fig. 9b.*

Insufficient material was present in buffer fractions 1-5 to warrant investigation. The 6th buffer fraction however yielded crystals from a methanol-acetone mixture which on recrystallization from the same solvent yielded a compound melting at 237° - 238° C.

The infrared spectrum showed absorption peaks at 1690 cm^{-1} and 3210 cm^{-1} indicating the presence of carbonyl and hydroxyl groups. A well-defined peak present at 1412 cm^{-1} is indicative of the grouping $-\text{CH}_2-\text{CO}-$.

Calculated for $\text{C}_{16}\text{H}_{25}\text{O}_2\text{N}$: C, 72.96%; H, 9.57%; N, 5.32%

Found: C, 72.61%; H, 9.72%; N, 5.60%

The composition and the spectral properties are identical with those reported for clavolonine. An authentic sample of clavolonine was obtained from Dr. R. H. Burnell for comparison and the two were found to be identical.

* The observed distribution pattern does not correspond to the theoretical curve. The deviation may be the result of incomplete equilibration.

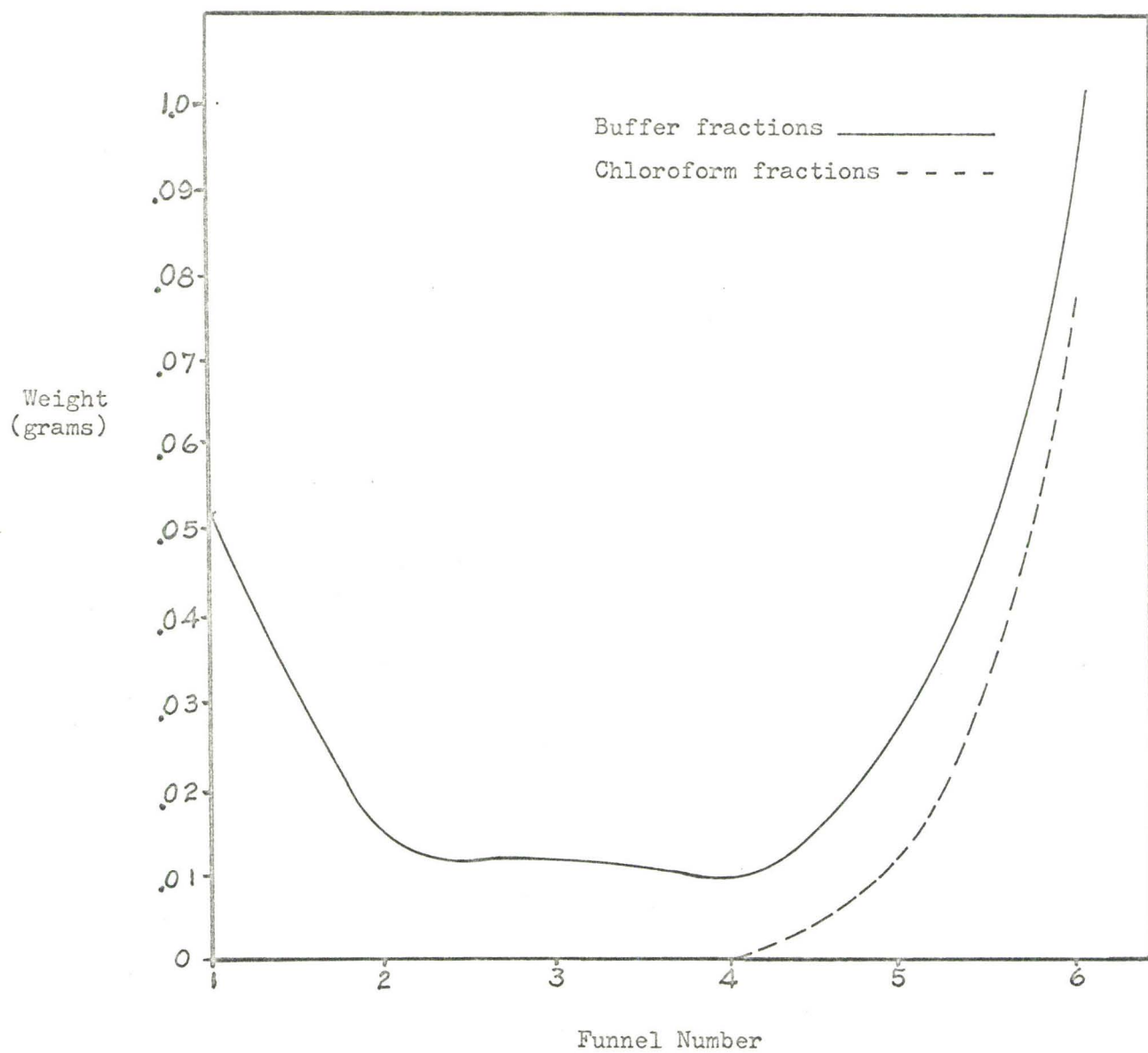


Fig. 9b. Separatory funnel distribution of mother liquor after isolation of de-N-methyl- α -obscurine.

Fraction B (See flow sheet diagram 2)

Isolation of hydroxy-de-N-methyl- α -obscurine

The material obtained from this fraction was also subjected to a six-separatory funnel distribution between chloroform and buffer of pH 6.0 and was worked up as described above. The weight analysis of this distribution is shown in Fig. 9c. Crystalline material could be obtained only from the first three buffer fractions. The rest of the buffer fractions were not examined further.

The material obtained from the first two buffer fractions yielded crystals from a methanol-acetone mixture which were identified as clavolonine.

After the separation of clavolonine, the mother liquor, on concentration, yielded a crop of crystals (0.035 g) from a chloroform-acetone mixture. The compound was not very soluble in cold chloroform or acetone and only moderately soluble in cold methanol. This material was recrystallized from a methanol-acetone mixture and melted at 300-305° C with decomposition.

The ultraviolet spectrum showed an absorption maximum at $\lambda_{\max} 255 \text{ m}\mu$ ($\log \epsilon = 3.78$) like that of α -obscurine. The infrared spectrum had absorption peak at 3510 cm^{-1} (OH), a split band at 3220 cm^{-1} and 3150 cm^{-1} ($>\text{N-H}$), and peaks at 1665 cm^{-1} (secondary amide $>\text{C=O}$), 1690 cm^{-1} ($>\text{C=C}<$) and 1410 cm^{-1} ($-\text{CH}_2-\text{CO}$).

The mass spectrum showed a molecular weight of 276 for this alkaloid.

The N.M.R. spectrum (Fig. 4) had a three proton split signal at $\tau = 9.18$ (CH-CH_3). The other signals in the spectrum were at

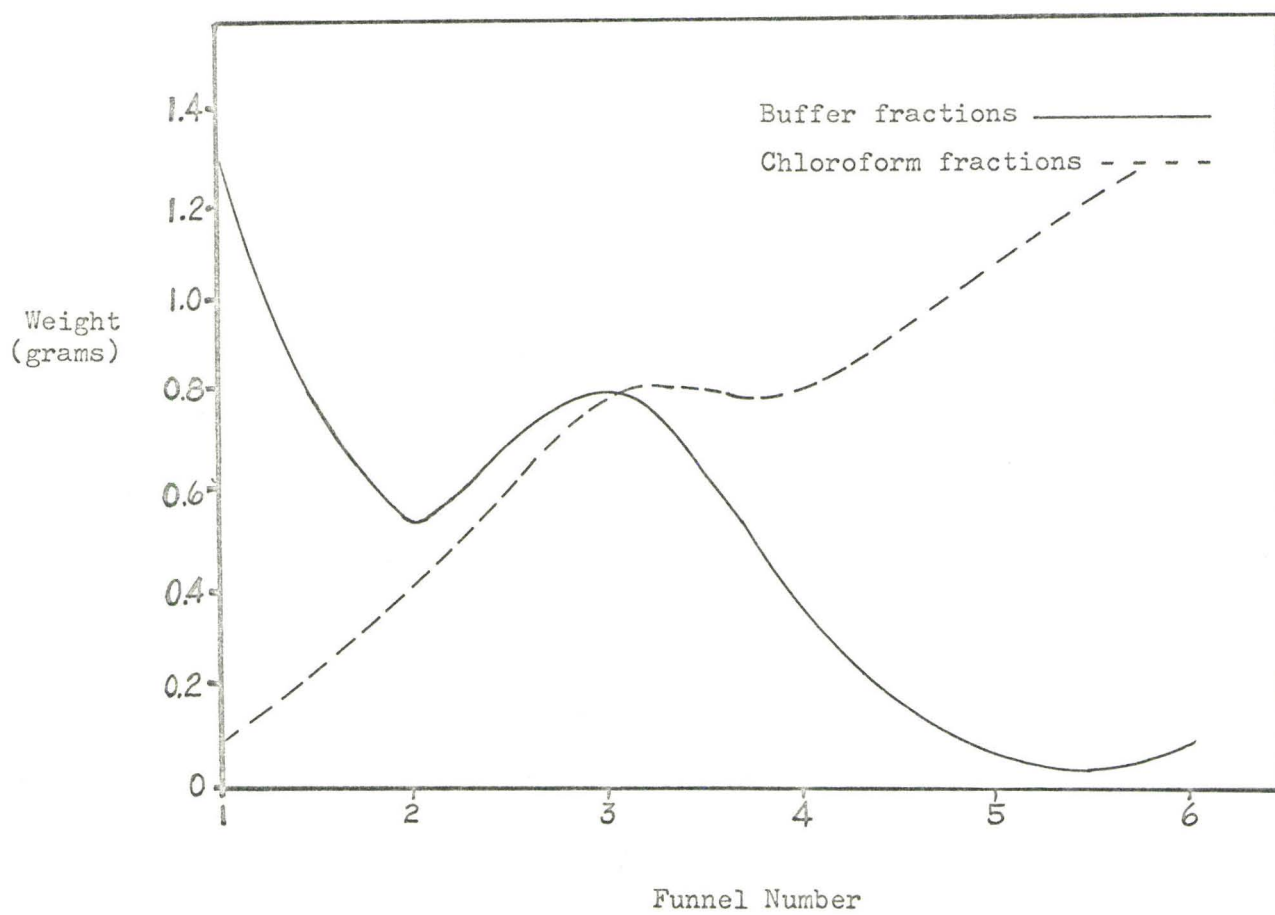


Fig. 9c. Separatory funnel distribution of fraction B.

$\tau = 2.7$ (N-H proton) $\tau = 2.3$ and $\tau = 6.52$.

Isolation of α -obscurine

The third buffer fraction after working up in the same way as described previously yielded crystals of α -obscurine (m.p. 280° - 282° C), which was identified by comparison with an authentic sample available in these laboratories.

Separatory funnel distribution of fraction C, D and E (See flow sheet diagram 2)

The infrared spectra of fractions C, D and E were alike and accordingly these fractions were combined and subjected to a six-separatory funnel distribution between citrate-phosphate buffer of pH 5.0 and butanol. After working up, the weight analysis gave a distribution pattern as shown in Fig. 9d. No bases could be induced to crystallize from any of these fractions.

Isolation of β -obscurine

A second batch of 2% methanol in chloroform fraction (4 g) was distributed between chloroform and citrate-phosphate buffer (pH 6.0) in an eight funnel distribution. Most of the substance was found in the first two buffer fractions and the last two chloroform fractions. Since the 3-6 buffer fractions contained very little material and could not be induced to crystallize, they were not examined further.

The residue from the first and second buffer fractions was adsorbed on an alumina column and the column was eluted successively

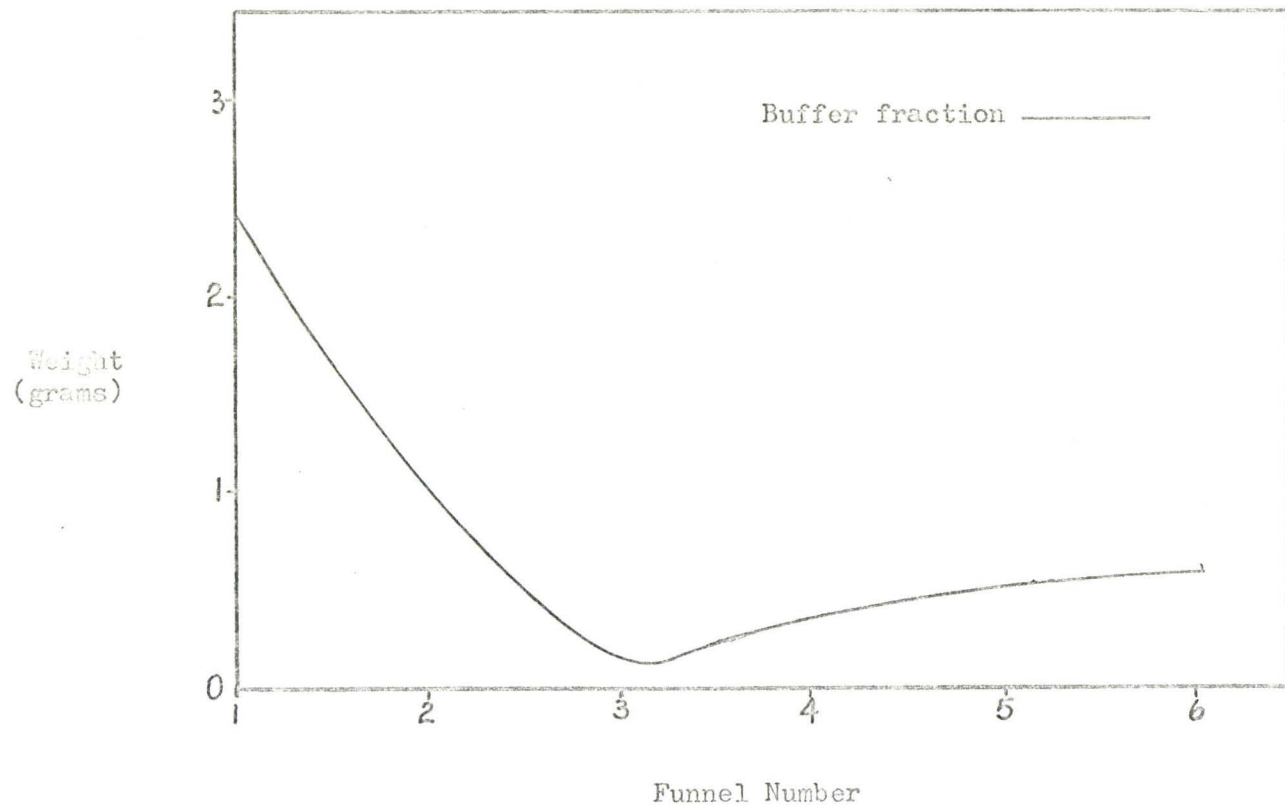


Fig. 9d. Separatory funnel distribution of fractions C, D, E.

with benzene, chloroform (two fractions of 250 ml each) and 1% methanol in chloroform. The first chloroform fraction yielded clavolonine and de-N-methyl- α -obscurine whereas the second chloroform fraction yielded de-N-methyl-hydroxy- α -obscurine. The residue from the 1% methanol in chloroform fraction crystallized from methanol and after recrystallization from the same solvent it melted at $325^{\circ} - 328^{\circ}$. The ultraviolet and infrared spectra of this compound were identical with those of β -obscurine and a melting point of a mixture of the two was undepressed.

Alkaloid L

The alkaloid L fraction (12 g), the origin of which was described previously was distributed between citrate-phosphate buffer (pH 5.5) and chloroform in a six funnel distribution. The weight analysis distribution pattern, is shown in Fig. 9e. The infrared spectrum of the residue from each fraction was examined and compared and as a result of this comparison buffer fraction 1 and 2, and 5 and 6 were combined before proceeding with further purification. Also buffer fraction 3 and 4, and chloroform fractions 1, 2 and 3 were combined.

Examination of buffer fraction 1 and 2

Isolation of α -obscurine

The residues from the first and second buffer fractions yielded crystals from acetone-ether which melted at $281-282^{\circ} \text{C}$. The ultraviolet spectrum showed an absorption maximum at $\lambda_{\text{max}} 255 \text{ m}\mu$ ($\log \epsilon = 3.73$) similar to that of α -obscurine. A mixed melting point with an authentic sample of α -obscurine was undepressed and the

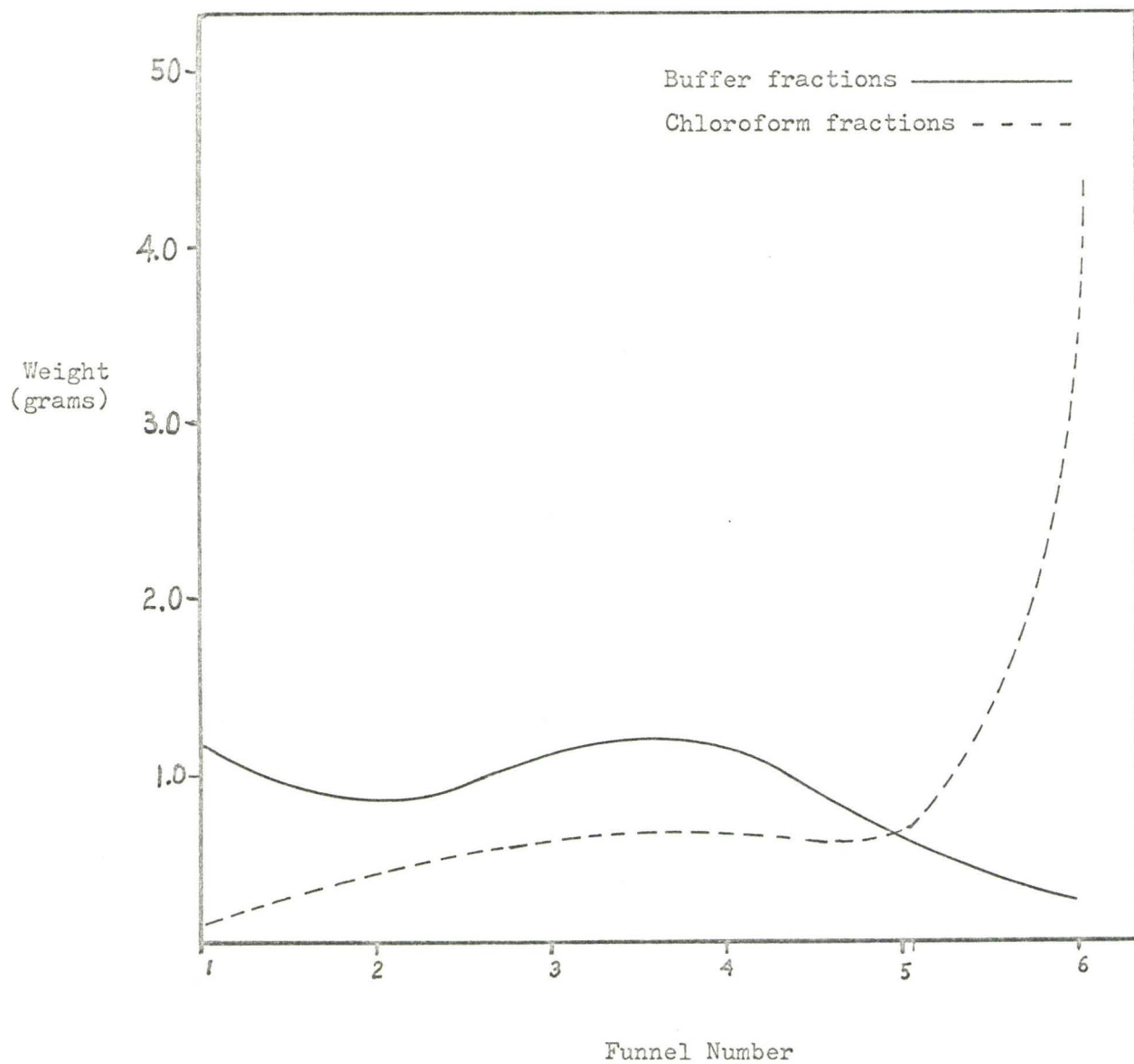


Fig. 9e. Separatory funnel distribution of alkaloid L.

infrared spectra of both samples were superimposable.

Examination of buffer fractions 5 and 6

Isolation of lycodine and annotinine

The residue from the fifth and sixth buffer fractions from the above distribution were chromatographed on an alumina column. The column was eluted with (1:1) benzene-n-hexane, benzene, and lastly with chloroform. The material on the column was eluted only with chloroform and was collected in different fractions of 25 ml each. Chloroform fractions 1-7 contained the majority of the material from the column. The residue from these fractions after evaporation of chloroform gave crystals from ether which proved to be a mixture. The mixture was separated into two components by taking account of their differential solubilities in ether. The more soluble compound was separated by trituration with ether and upon crystallization from this solvent gave a substance melting at 117° - 118° .

The ultraviolet spectrum showed two peaks at λ_{\max} 269 $m\mu$ ($\log \epsilon = 3.72$) and 277 $m\mu$ ($\log \epsilon = 3.62$). The infrared spectra had a peak at 3260 cm^{-1} (N-H group) and the presence of a pyridine group was indicated by peaks at 1570 cm^{-1} , 1470 cm^{-1} , 800 cm^{-1} and 725 cm^{-1} . The ultraviolet and infrared spectra proved to be identical with the spectra of an authentic sample of lycodine available in these laboratories. Also the melting point was undepressed when mixed with this authentic sample of lycodine.

The ether insoluble component was recrystallized from methanol and gave a compound melting at 227° - 228° . Recrystallization from methanol raised the melting point to 230° - 232° . The infrared

spectrum showed a strong single peak in the carbonyl region at 1776 cm^{-1} indicating a γ -lactone group. The spectrum was identical with that of an authentic sample of annotinine. The melting point of a mixture of the two was undepressed. Subsequent fractions eluted from the column could not be induced to crystallize and were not worked further.

Examination of buffer fractions 3 and 4 and chloroform fractions 1, 2 and 3

All five fractions were found to exhibit an absorption band in the ultraviolet at $\lambda_{\text{max}}\ 237\text{ m}\mu$ indicative of a new alkaloid since absorption at this wave length had not been previously reported for a Lycopodium alkaloid. Paper chromatography (paper buffered at pH 4.5) showed the sample to be a mixture of at least two compounds. Attempts to isolate the compound responsible for the absorption at $237\text{ m}\mu$ by crystallization of the bases or of their salts or by adsorption chromatography met with failure. It was decided, therefore, to attempt still another countercurrent distribution which was carried out in this case with citrate-phosphate buffer of pH 4.5.

Distribution between buffer (pH 4.5) and chloroform

Accordingly the fractions were combined and partitioned between pH 4.5 buffer (50 ml) and chloroform (50 ml) in a six-funnel system. The buffer fractions were worked up as in previous experiments. The first, second and third buffer fractions had similar infrared spectra. Absorption peaks were present at 3300 cm^{-1} , 1735 cm^{-1} , 1690 cm^{-1} , 1650 cm^{-1} and 1635 cm^{-1} . The ultraviolet spectrum of each of these fractions showed a peak at $\lambda_{\text{max}}\ 237\text{ m}\mu$.

The weight distribution pattern is shown in Fig. 9f. The residue from each buffer fraction was separately tried for crystallization using various solvent systems without success.

Paper chromatography (paper buffered at pH 5.4) showed that all the buffer fractions were mixtures of at least two compounds. Vapour phase chromatography showed, however, that one of the compounds, designated base M_1 was concentrated in the first buffer fraction whereas the other designated base M_2 was concentrated in the second buffer fraction.

Preparation of perchlorate salt of base M_1 (L.5)

The residue from the first buffer fraction was dissolved in ether and was carefully neutralized with perchloric acid (60%). An oily layer separated which, on scratching, solidified. The solid was collected, washed with ether repeatedly and crystallized from acetone. The white needles melted at $275^\circ - 277^\circ$. After repeated recrystallization from acetone, the melting point was raised to $281^\circ - 282^\circ$.

Calculated for $C_{18}H_{28}ON_2 \cdot HClO_4$: C, 55.52%, H, 7.50%; N, 7.20%

Found: C, 55.49%, H, 7.65%; N, 7.37%

The infrared spectrum showed a broad peak in the N-H region, a strong peak at 1628 cm^{-1} ($-\text{NH}-\overset{\text{O}}{\parallel}{\text{C}}-$), a peak at 1650 cm^{-1} ($>\text{C}=\text{C}<$) and a strong broad peak at 1100 cm^{-1} (perchlorate ion). The ultra-violet spectrum had an absorption peak $\lambda_{\text{max}} 237 \text{ m}\mu$ ($\log \epsilon = 3.85$). The free base regenerated from the perchlorate salt had similar absorption. The N.M.R. spectrum (Fig. 7) of the free base showed

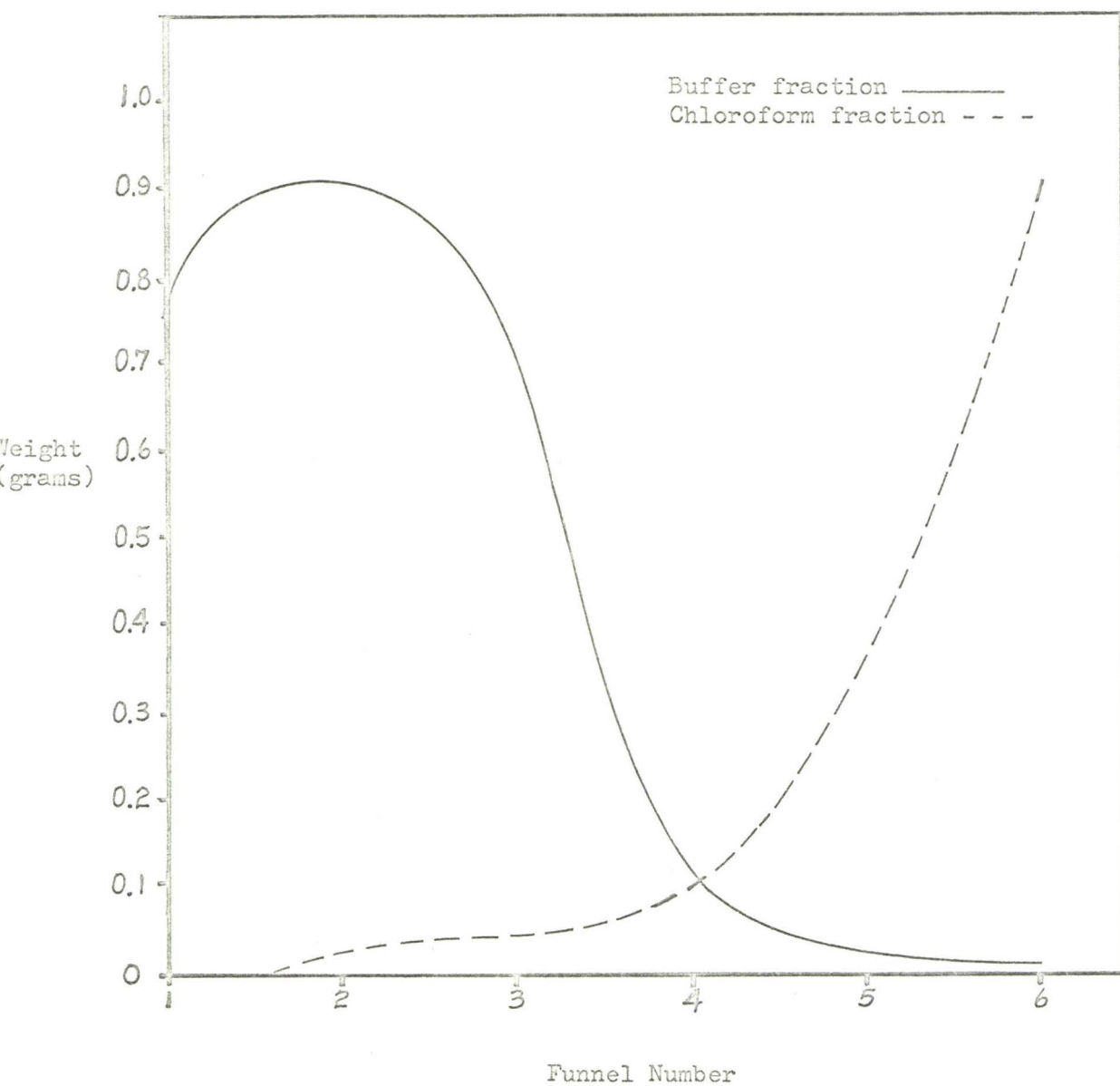


Fig. 9f. Separatory funnel distribution of fractions showing ultra-violet absorption at λ_{\max} 237 m μ .

a three proton doublet at $\tau = 9.12$ ($J = 5$ c.p.s.), a signal at $\tau = 7.9$ (singlet, three proton) and a weak and broad signal at $\tau = 2.84$ which may be due to an N-H proton. The mass spectrum of the free base (Fig. 8a) showed a molecular weight of 288.

The perchlorate proved to be identical with L.5 perchlorate by comparison with an authentic sample kindly supplied by Dr. R. H. F. Manske. The ultraviolet and infrared spectra of the two were identical and the melting point of a mixture of the two was undepressed.

Preparation of the methiodide of base M₂ (lycopodine)

The free base (0.1 g) regenerated from the perchlorate salt obtained from the second buffer fraction was heated under reflux for one hour with acetone (2 ml) and methyl iodide (1.5 ml). On slow evaporation of the solvent a crystalline material was obtained (0.08 g), which on recrystallization from a methanol-acetone mixture melted at $340^{\circ} - 341^{\circ}$ (d).

Calculated for $C_{16}H_{25}ON \cdot CH_3I$: C, 52.49%; H, 7.20%; N, 3.59%

Found: C, 52.21%; H, 7.34%; N, 3.18%

The infrared spectrum had a strong sharp peak at 1700 cm^{-1} ($>C=O$) and a well-defined peak at 1412 cm^{-1} ($-CH_2CO-$) and a broad absorption peak in 2700 cm^{-1} region. These crystals were identified as lycopodine by comparison with an authentic sample prepared in these laboratories.

The melting point of a mixture of the two was undepressed and the infrared spectra were identical.

Preparation of acetyl Flabellidine (Acetyl L.5)

Flabellidine (37 mg) regenerated from the perchlorate salt was heated under reflux for one hour and fifteen minutes with acetic anhydride (15 ml). The reaction mixture was cooled and poured on crushed ice (50 g) and the aqueous mixture was extracted with chloroform. The chloroform extract was washed with 10% aqueous sodium carbonate solution and water and dried over anhydrous sodium sulphate. Removal of the solvent yielded a gummy substance which did not show any peak corresponding to flabellidine upon vapour phase chromatography but instead showed a new peak with higher retention time. This material was adsorbed on a non-basic alumina (Woelm) column and was eluted successively with petroleum ether, benzene and chloroform. Most of the material was eluted with benzene. The residue from the benzene fraction after removal of the solvent crystallized from ether. After recrystallization from acetone-ether it melted at 150° - 152° . The ultraviolet spectrum of this compound showed an absorption at λ_{\max} 237 $m\mu$. The infrared spectrum in chloroform solution showed an absorption peak at 1620 cm^{-1} , but no absorption in the N-H region. The mass spectrum showed a molecular weight of 330 for this compound. Calculated for $C_{20}H_{30}ON_2$: C, 72.70%; H, 9.10%; N, 8.48%
Found: C, 72.25%; H, 9.11%; N, 8.06%

High Pressure hydrogenation and acetylation of lycodine

Lycodine (0.095 g) was dissolved in freshly distilled acetic anhydride (30 ml) and Raney Nickel (200 - 300 mg) added. The mixture was shaken with hydrogen (at 1160 p.s.i.g.) at 110° C for 16 hours. After removal of most of the acetic anhydride under reduced pressure,

the reaction mixture was poured into crushed ice and the aqueous mixture extracted with chloroform. The chloroform extract was washed with water, dried over anhydrous sodium sulphate and the solvent removed. The residue crystallized from acetone-ether, and on recrystallization from the same solvent melted at 150-152^o. A mixed melting point with acetyl flabellidine was undepressed. The ultraviolet spectrum and the infrared spectrum were superimposable on those of acetylflabellidine. The mass spectrum (Fig. 8b) showed a molecular weight of 330 and was identical with that of acetyl flabellidine.

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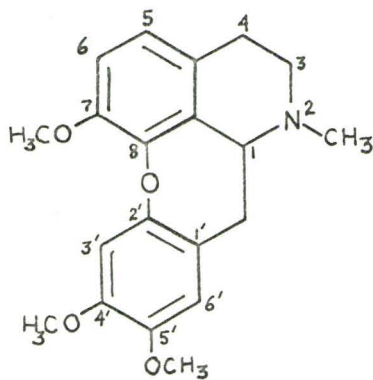
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PART II.

MODEL STUDIES LEADING TO A SYNTHESIS OF CULARINE

GENERAL INTRODUCTION

Cularine is a benzyl isoquinoline alkaloid with the unusual feature that position 8 of the isoquinoline ring is joined through an ether linkage to position 2' of the benzyl group. At the time this



work was initiated cularine had not been synthesised although several attempts had been reported. These attempts apparently failed because of the inability of earlier investigators to obtain in good yield a suitably substituted dibenz[b,f]oxepin from which the complete structure might be elaborated.

For the construction of the dibenz[b,f]oxepin system ring expansion of a xanثone or xanثone derivative appeared to be a promising approach. In model studies in this direction an investigation of the ring expansion of 9-xanthenemethylamine was undertaken.

The present work describes a new synthesis of the amine and its rearrangement under the condition of the Demjanov reaction to the ring-expanded alcohol.

INTRODUCTION

The alkaloid, cularine, $C_{20}H_{30}O_4N$, was isolated by Manske (1) from Corydalis claviculata and from Dicenta cucullaria, D. eximia, D. formosa and D. oregana. The structure of cularine (I) was elucidated by Manske (2) who showed it to be a benzyl isoquinoline alkaloid. An unusual feature of this structure is that the benzyl ring is joined to the 8-position of the isoquinoline ring by an ether linkage. The key products in his degradation experiments are shown on page 107 (II - IVa).

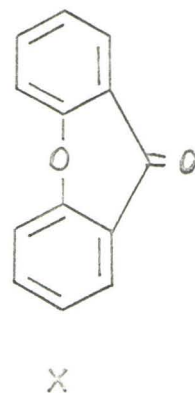
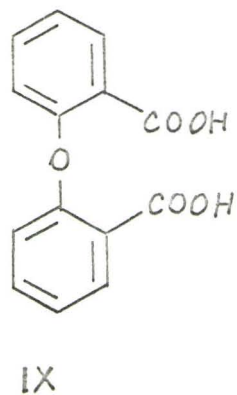
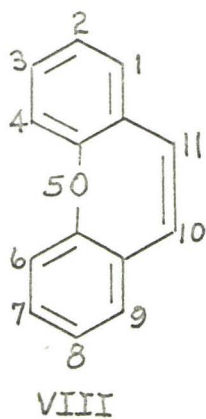
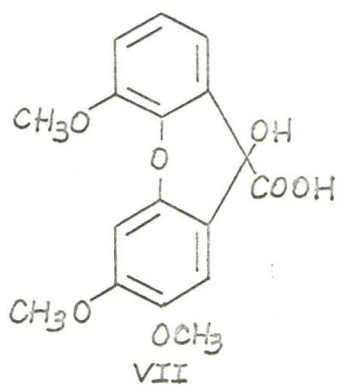
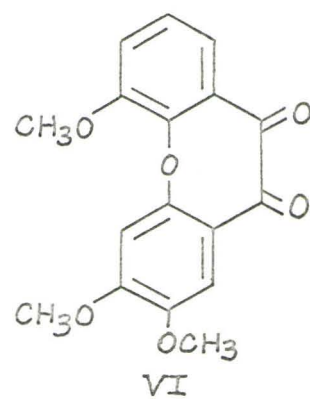
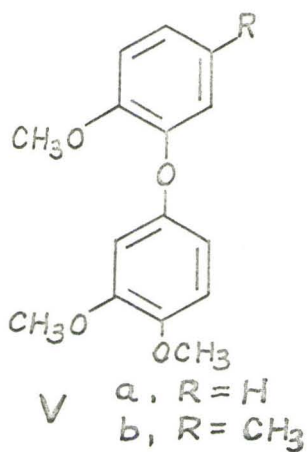
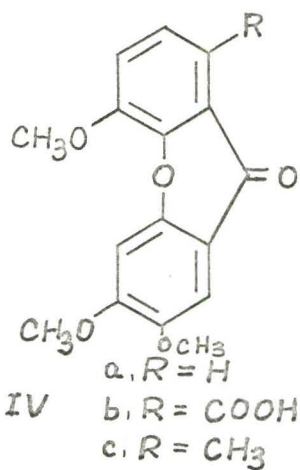
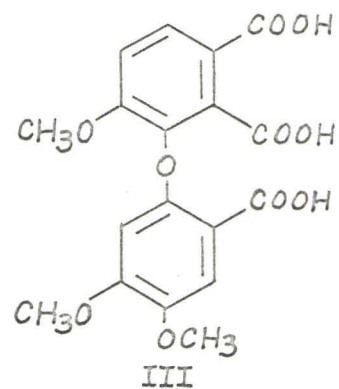
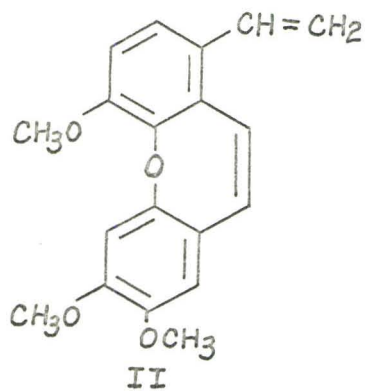
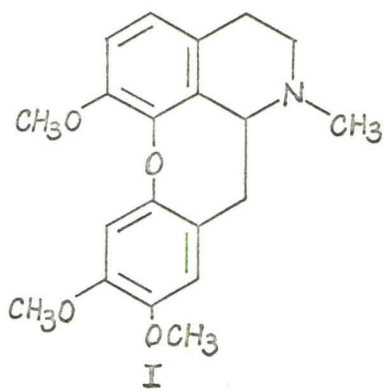
A two stage Hofmann degradation of cularine gave II which was then oxidized with potassium permanganate to yield a mixture of III and IVb. The formation of the xanthone (IVb) was visualized to proceed through the formation of an intermediate diketone (VI), a benzilic acid rearrangement of the diketone (VI) to the hydroxy acid (VII), and decarboxylation and oxidation of (VII) to the xanthone (IVb). The inference that the xanthone (IVb) arises from (II) was confirmed by Manske (3) by subjecting a synthetic specimen of dibenz[b,f]oxepin (VIII) to the same oxidation conditions, when, in addition to the expected diphenyl ether -2,2'-dicarboxylic acid (IX), a 6-7% yield of 9-xanthenone (X) was obtained. The structure of the xanthone (IVb) was verified by L'Ecuyer and Constantin (4) by synthesis of this compound in an unambiguous manner. The synthetic compound was found to be identical with the degradation product obtained by Manske.

The demonstration of the presence of a dihydrodibenz [b,f] - oxepin ring-system in the alkaloid cularine stimulated interest in synthetic routes to this ring-system and to the alkaloid itself. Until then this ring-system was known only in by-products arising from application of Pschorr's (5) phenanthrene synthesis.

Manske and Ledingham (3) synthesised the dibenz [b,f] oxepin (VIII) in the following way. An Ullmann condensation of iodobenzene and the copper salt of salicylaldehyde gave o-phenoxybenzaldehyde which was converted to the corresponding arylacetic acid via the azlactone and the aryl pyruvic acid. The chloride of the o-phenoxyphenylacetic acid was converted by the Friedel-Crafts reaction to dibenzoxepinone which on reduction and dehydration gave dibenz [b,f] - oxepin (VIII). Manske and Kulka (6) also synthesised a number of derivatives modelled on the substitution pattern of the alkaloid but yields in all cases were low thereby preventing elaboration of the synthesis to the ring system of cularine itself.

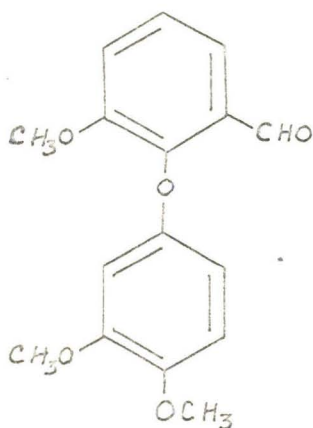
Following methods similar to that of Manske, Kimoto et al. (7), in 1954, reported the synthesis of derivatives of dibenz [b,f] - oxepin but did not state the yield.

For a practicable synthesis of the type attempted by Manske et al. two stages are critical, one being the formation of the diphenyl ether linkage and the other the closure of the oxepin ring. The Ullmann condensation (8-11) is of limited value for the synthesis of hindered diphenyl ethers. A more powerful method is the well-known reaction of a phenol with an activated aryl halide in the presence of a base (12-14). Utilizing this method, Loudon and Summers (15)

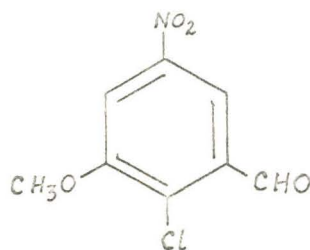


were able to synthesise 2-aryloxy-5-nitrobenzaldehydes in good yield from condensation of 2-chloro-5-nitrobenzaldehyde with phenol and methoxy phenols in aqueous alkali. The 2-aryloxy-5-nitrobenzaldehydes were converted to the corresponding pyruvic acids and acetic acids which were cyclized to derivatives of 2-nitrodibenz[*b,f*]oxepin in nearly 40% yield. They did not, however, report the synthesis of dibenzoxepinone or its derivatives with methoxy substitution modelled on the alkaloid cularine.

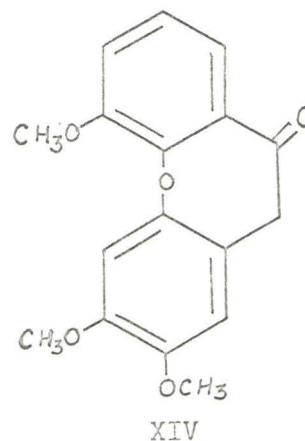
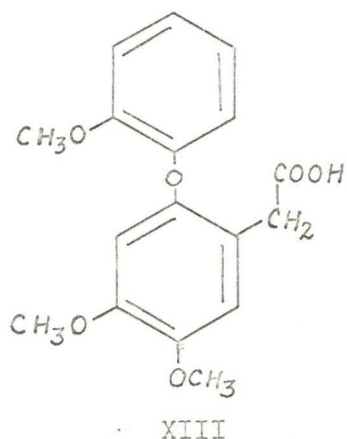
Another method (11, 16, 17) for the synthesis of hindered diphenyl ethers, in which the key step is the reaction of a phenol with an activated N-phenyl pyridinium salt, has the advantage that less activation is required. L'Ecuyer and Constantin (18) utilized this method for synthesising several substituted diphenyl ethers in good yield but could not synthesise XI required for the synthesis of cularine (according to their scheme), as they failed to obtain the required starting material, 2-chloro-3-methoxy-5-nitrobenzaldehyde (XII).



XI

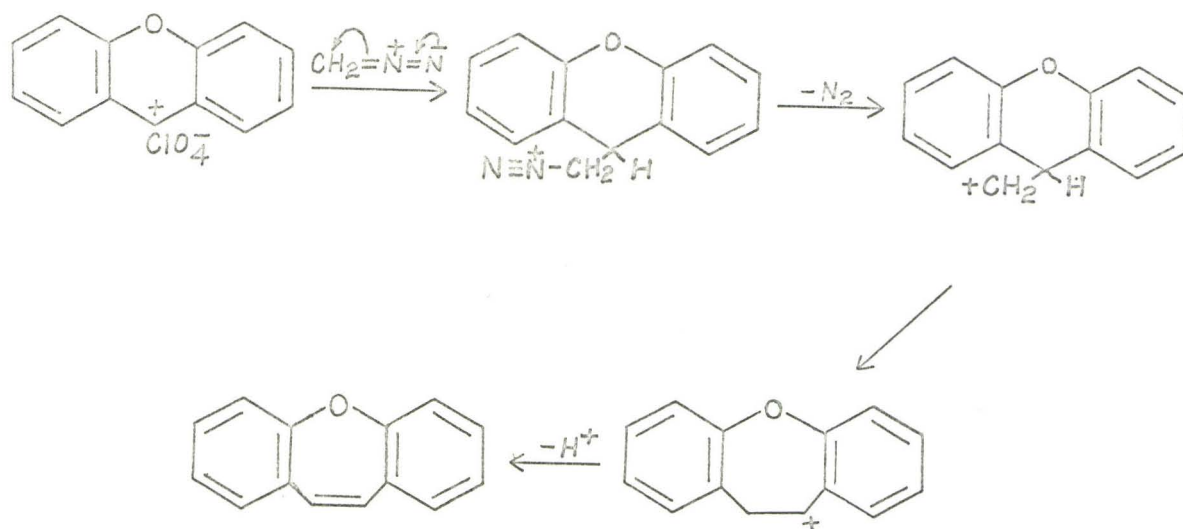


XII



The difficulties encountered by others in the synthesis of the biphenyl ether XIII and its subsequent cyclodehydration to the oxepinone derivative XIV led us to consider other methods for the synthesis of this oxepinone derivative. One method that seemed promising was the ring expansion of the xanthenone XIV, a compound which had already been synthesized in good yield by L'Ecuyer and Constantin (4).

The ring expansion of xanthenone derivatives to dibenz[*b,f*]-oxepin is already known. Thus Anet and Bavin (19) have reported the synthesis of dibenz[*b,f*]oxepin and 10-methyldibenz[*b,f*]oxepin by Wagner-Meerwein rearrangement of 9-xanthenemethanol and 9-hydroxy-methyl-9-methylxanthenone. In the latter case the yield was reported to be 68.5% whereas in the former case the yield was not mentioned. More recently Whitlock (20) has reported the formation of dibenz[*b,f*]-oxepin in 61% yield when xanthylium perchlorate is treated with ethereal diazomethane. Presumably diazomethane attacks xanthylium perchlorate with the formation of a primary stable carbonium ion which then undergoes a Wagner-Meerwein rearrangement as shown on page 110:



These ring expansions led directly to the dibenzoxepin whereas for the synthesis of cularine the dibenzoxepinone or its corresponding alcohol with the functional group properly orientated with respect to the methoxyl groups seemed more desirable so that one might proceed with construction of the isoquinoline ring system of structure (I).

Accordingly we were led to consider other methods for ring expansion of the xanthone (IV) to the dibenz [b, f] oxepin system and these are discussed in the subsequent section.

While this work was in progress, Kametani and Fukumato (21) reported the total synthesis of cularine. By using Manske's method but varying the reaction conditions, they were able to increase the yield of the oxepinone XIV (22). The oxepinone was converted into the isoquinoline derivative by the Pomeranz-Fritsch reaction with amino acetal. The resulting isoquinoline, after converting it to its methiodide, was reduced with sodium borohydride yielding (\pm) cularine.

RESULTS AND DISCUSSION

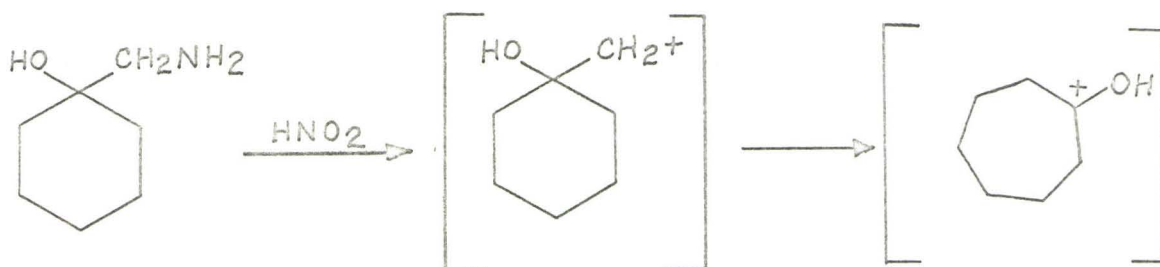
Cularine had not been synthesised when this work was begun and the major barrier to a successful synthesis appeared to be an efficient preparation of oxepinone XIV or a derivative suitable for elaboration of the isoquinoline ring of cularine. The methods reported for synthesis of oxepinones were all based on cyclization of o-phenoxyphenylacetic acids. In looking for an alternative method we were led to consider xanthenes as possible precursors, in ring expansion reactions, of the oxepinones or the corresponding alcohols. In surveying possible ring expansion processes we confined this study to the readily available 9-xanthenone.

The reaction in which ketones are treated with diazomethane to form the homologous ring expanded ketone was investigated first. It has been reported (23) that 9-fluorenone reacts with diazomethane to give the ring expanded compound in 30% yield. This suggested that 9-xanthenone might also undergo a similar reaction to give 10,11-dihydrodibenz [b,f]oxepin 10 (11, H)-one. However, when a solution of 9-xanthenone in chloroform-methanol mixture, containing a small amount of sodium carbonate which has been reported (24) to act as catalyst, was treated with ethereal diazomethane, only unreacted 9-xanthenone was recovered.

We next turned our attention to the Demjanov reaction and its companion the Tiffeneau-Demjanov reaction. In the Demjanov reaction (25, 26) aminomethylcycloalkanes are treated with nitrous acid to produce cycloalkanols in which the ring is larger by one

carbon atom. The reaction is most useful in the preparation of five, six and seven-membered rings. In the Tiffeneau-Demanjov reaction (27) a 1-aminomethylcycloalkanol is treated with nitrous acid to yield a ring-enlarged ketone. Both the Demjanov and Tiffeneau-Demjanov ring enlargements may be regarded as special cases of the rearrangement of a carbonium ion (28-30). The rearrangement is always competitive, therefore, with a substitution reaction which precedes rearrangement and both rearranged and initial ions may combine with any base present in the solution. Furthermore hydride migration with the formation of a tertiary carbonium ion has also been observed. Consequently the rearranged alcohol is only one of several products that may form in a Demjanov reaction.

Although the mechanisms of the Demjanov and Tiffeneau-Demjanov reaction are fundamentally the same, two important factors are operative in the latter which favour ring expansion. First, the absence of hydrogen on the β -carbon atom eliminates the competitive hydride migration and second, in the rearranged carbonium ion, the positive charge is located on a protonated carbonyl group as shown below, a species generally of much lower energy than a simple carbonium ion.



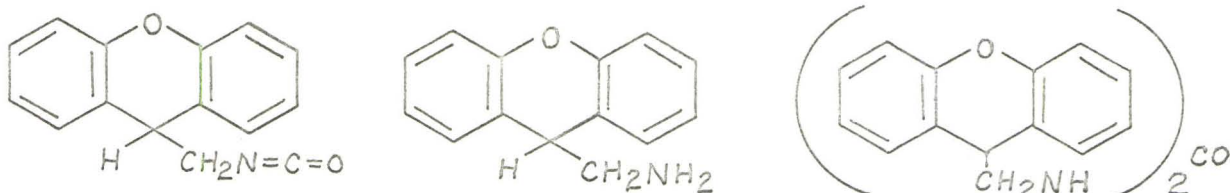
The literature on the Demjanov and Tiffeneau-Demjanov reaction up to the end of the year 1957 has been surveyed by P. A. S. Smith and D. R. Baer (31). In a careful search through this review and in a survey of literature we were unable to find that either of these reactions had been applied to xanthene derivatives. Our intention to study the Tiffeneau rearrangement was frustrated by our inability to prepare the requisite starting material 9-aminomethyl-9-xanthenol. Accordingly we turned our attention to the Demjanov rearrangement and set about to prepare 9-xanthenemethylamine.

Synthesis of 9-xanthenemethylamine

The preparation of 9-xanthenemethylamine has been reported in the literature. Thus 9-xanthenenitromethane has been prepared by condensation of nitromethane with 9-xanthenol followed by reduction with zinc-dust and acetic acid to the amine (32). This method, however, was considered unsuitable because of the poor yield of the 9-xanthenenitromethane (10%). In another method 9-cyanoxanthene has been converted to the amine by reduction with lithium aluminum hydride (33). Our inability to obtain the nitrile led us to discard this approach even though the yields in this process were reported to be high.

Goldberg and Wragg (34) reported that 9-xantheacetic acid can be prepared from 9-xanthenol and malonic acid in 68% yield. Conversion of 9-xantheacetic acid to 9-xantheacetyl azide and a Curtius rearrangement (35) on the azide would be expected to lead to 9-xanthenemethylamine. Since 9-xanthenol is readily available, it was decided to apply this method to the synthesis of the amine.

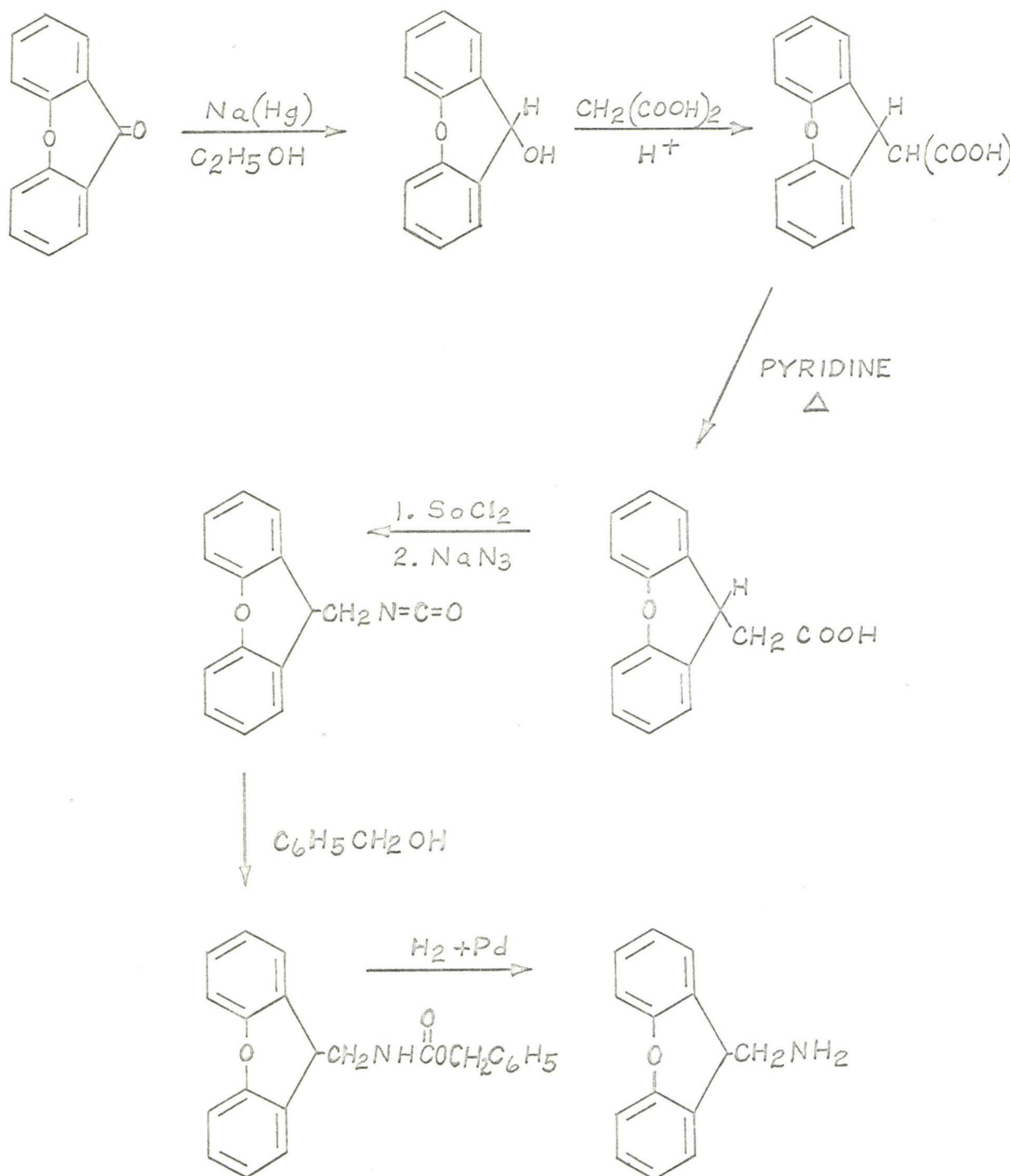
9-Xantheneacetic acid was prepared in 63% yield by heating 9-xanthanol and malonic acid in pyridine (36). A better yield was achieved when 9-xanthenemalonic acid was prepared first and then decomposed in a separate step to 9-xantheneacetic acid by decarboxylation in pyridine solution. The acid chloride of 9-xantheneacetic acid was prepared in 82% yield by reaction with thionyl chloride. It in turn was converted to the azide (92% yield) which underwent the Curtius rearrangement, on heating in benzene, to the isocyanate in quantitative yield. Treatment of the isocyanate with alkali, however, gave N,N'-di(9-xanthenemethyl)urea instead of the expected amine.



The formation of the urea was avoided by conversion of the crude isocyanate to the ethylurethane followed by hydrolysis of the urethane to 9-xanthenemethylamine, isolated as the hydrochloride salt, in a yield of 41.5% based on the 9-xantheneacetic acid. The yield was improved to 52.5% by treatment of the crude isocyanate with benzyl alcohol followed by reduction of the benzylurethane to the amine. The reactions leading to the amine are summarized in Scheme I.

Demjanov rearrangement of 9-xanthenemethylamine

9-xanthenemethylamine was treated with nitrous acid in acetate buffer using experimental conditions normally employed in the Demjanov rearrangement. The infrared spectrum of the crude oily

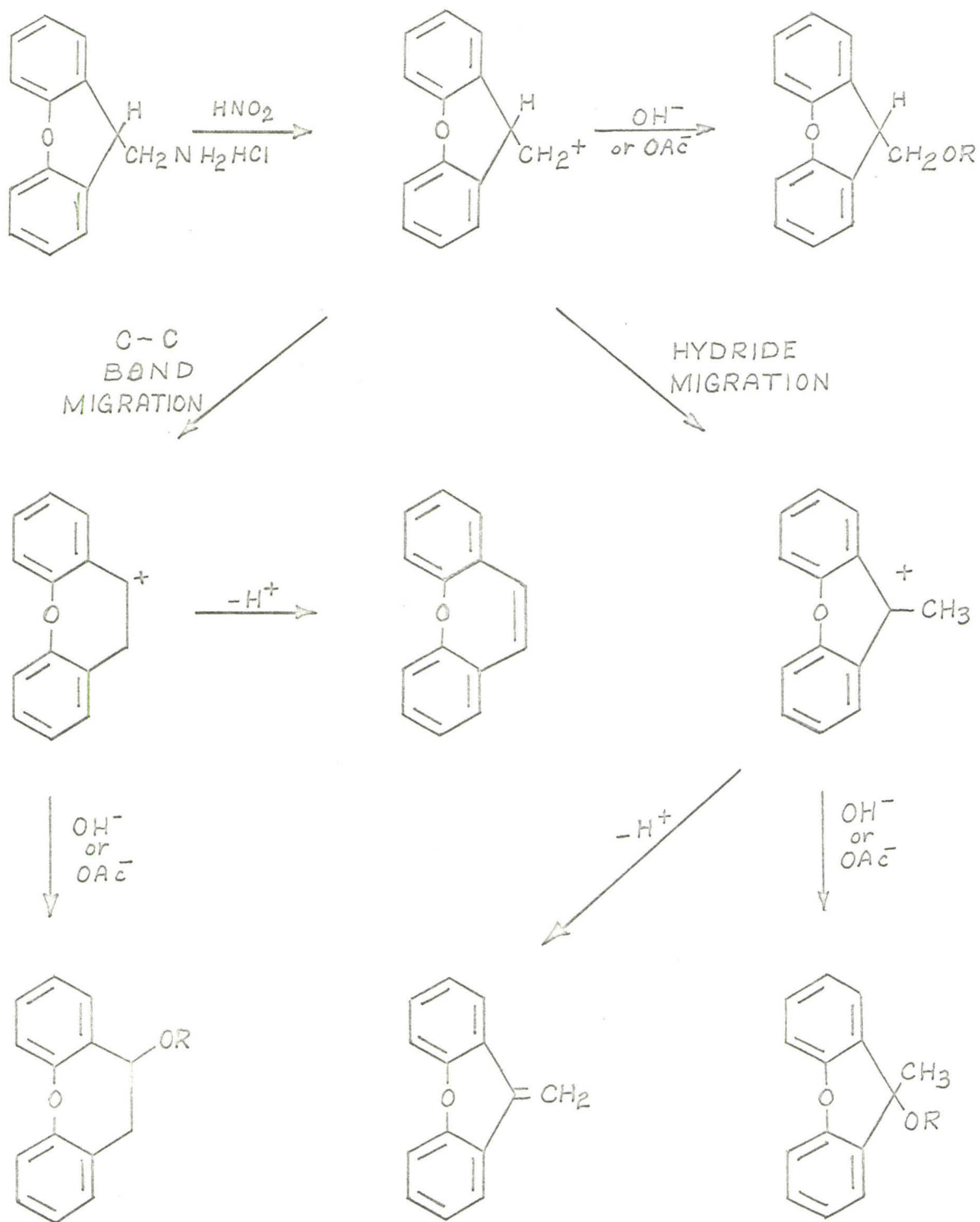


Scheme I. Synthesis of 9-xanthenemethylamine.

reaction product showed a strong peak at 3400 cm^{-1} (OH), a weak peak at 1720 cm^{-1} indicative of the presence of an acetyl group and two peaks at 1600 and 1570 cm^{-1} . The peaks at 1600 and 1570 cm^{-1} are characteristic of aromatic absorption associated with xanthene and all its derivatives. These two peaks are present in the spectra of the derivatives of dibenz[b,f]oxepin that were examined as well as in that of the parent compound itself but in the latter their intensity is diminished. The products that might be expected from this reaction are shown in Scheme II. They include the three isomeric alcohols, their acetate esters and two olefins. The tertiary alcohol might dehydrate, however, under the reaction conditions employed.

In order to reduce the complexity of the product the crude oil was treated with methanolic potassium hydroxide to hydrolyze the esters to the corresponding alcohols. The product isolated after this treatment no longer had absorption at 1720 cm^{-1} in its infrared spectrum but showed the other absorption bands noted above.

A preliminary analysis of the hydrolysis product was carried out using chromatographic techniques. Thin-layer chromatography showed it to be a mixture of at least three components. Vapour phase chromatography revealed three peaks, the area under the highest peak, that of longest retention time, being 96% of the total area of the three combined peaks. Column chromatography on an alumina column effected a separation of the alcoholic components from the olefinic components and showed that the alcoholic fraction accounted for 96% of the total eluate. In order to ascertain the exact composition of



Scheme II. Possible products from Demjanov reaction of 9-xanthenemethylamine. $\text{R} = \text{H}$ or CH_3CO .

the alcohol fraction it seemed necessary to synthesise each of three alcohols which might form in the reaction and investigate their properties.

9-Xanthene methanol was prepared by the method of Anet and Bavin (37) by the reduction of 9-xanthenecarboxylic acid. The infrared spectrum (chloroform) showed peaks at 3600 and 3450 cm^{-1} (OH), two peaks at 1600 and 1570 cm^{-1} (aromatic), two peaks at 1120 and 1098 cm^{-1} and one peak at 895 cm^{-1} . The last three peaks were characteristic of this alcohol and allow its differentiation from its isomers.

The N.M.R. spectrum of 9-xanthenemethanol (Fig. 10) shows a one proton signal at $\tau = 7.72$ (OH proton), a doublet centred at $\tau = 6.52$ (two protons) and a triplet centred at $\tau = 6.12$ (one proton). We have assigned the triplet to the tertiary >C-H proton and the doublet to the methylene protons (38a). The coupling constant is found to be 6.6 c.p.s. The line positions and intensities are typical of an AB_2 spectrum with equal AB coupling constants (38b) which is expected for such a system.

10,11-dihydrodibenz[b,f]oxepin-10(11,H)-ol was prepared by borohydride reduction of the corresponding oxepinone which was synthesised by the method of Manske (3). The infrared spectrum (in chloroform solution) showed peaks at 3600 and 3450 cm^{-1} (OH) and two peaks at 1600 and 1570 cm^{-1} . This spectrum differed from that of 9-xanthenemethanol by the absence of the 895 cm^{-1} peak and by the presence of a peak at 1112 cm^{-1} replacing the two peaks at 1120 and 1098 cm^{-1} in the spectrum of 9-xanthenemethanol.

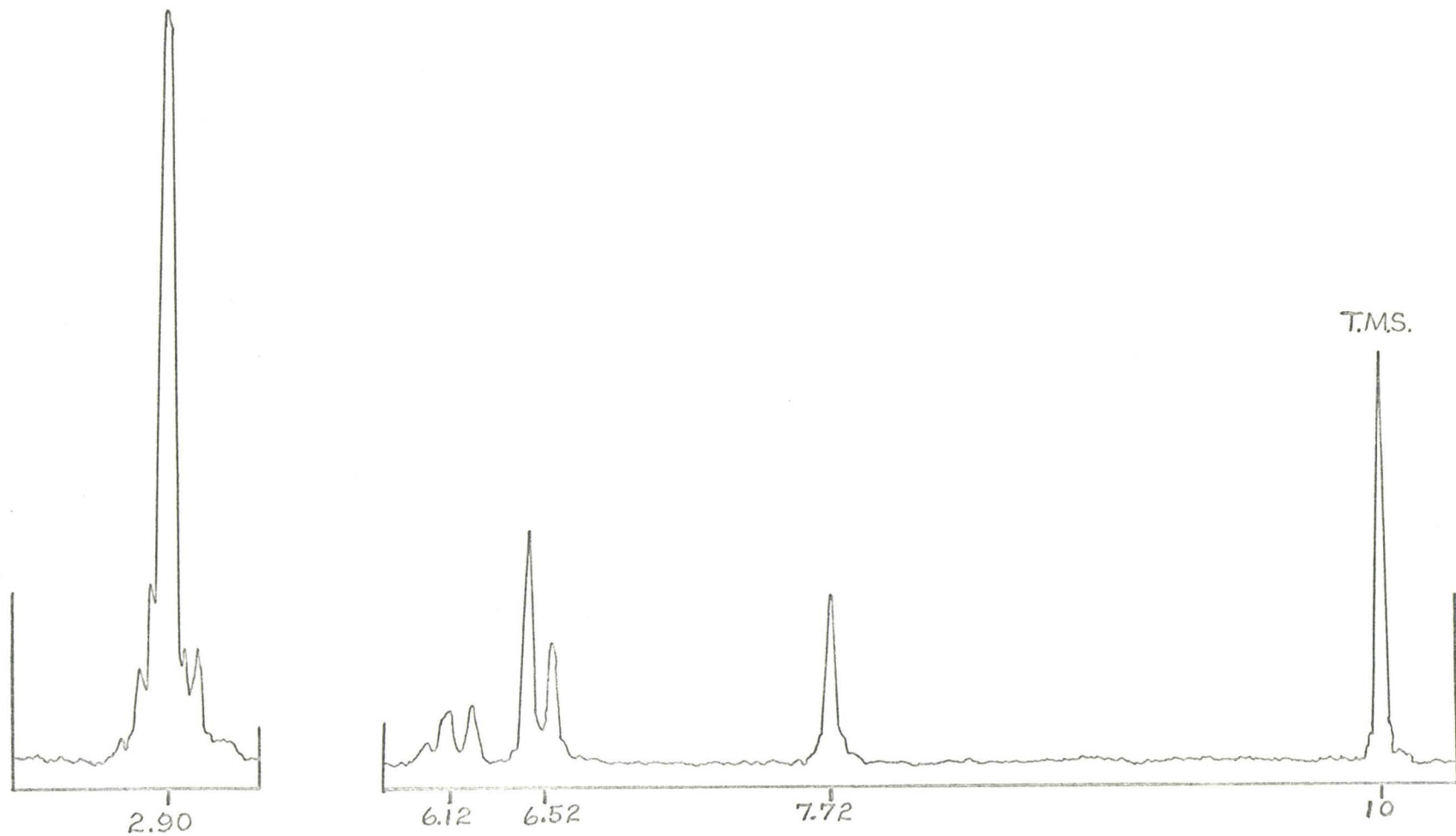


Fig. 10. N.M.R. spectrum of 9-xanthenemethanol (CDCl_3).

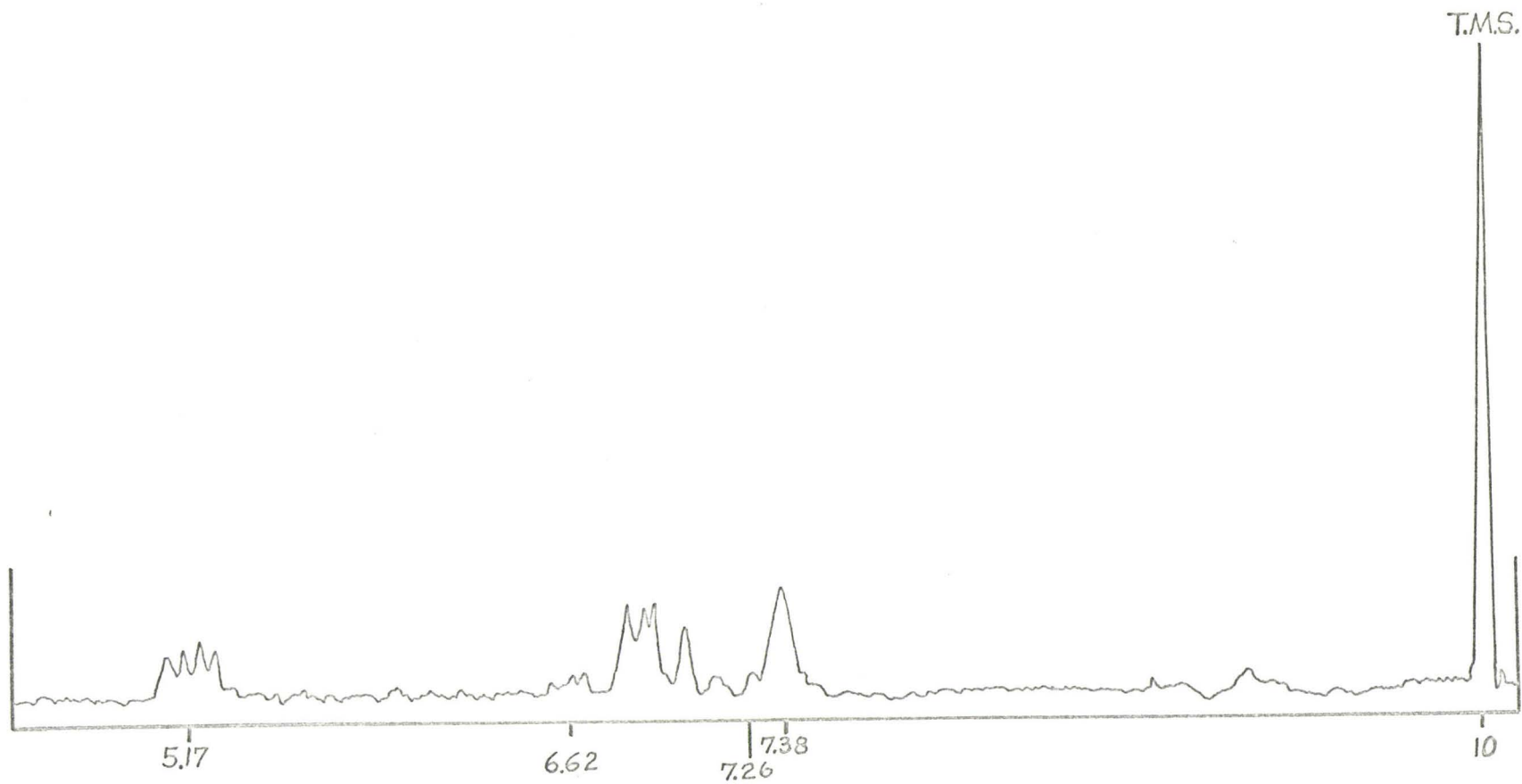


Fig. 11. N.M.R. spectrum of 10,11-dihydrodibenz [b, f] oxepin-10(11,H)-ol. (CDCl_3).

The N.M.R. spectrum of 10,11-dihydrodibenz[*b,f*]oxepin-10, (11,H)-ol (Fig. 11) shows a symmetrical quartet at $\tau = 5.7$ (one proton), a one proton signal at $\tau = 7.38$ which may be assigned to the hydroxyl proton, and a multiplet of eight signals between τ values, 7.26 and 6.62 (two protons). This spectrum is reminiscent of an ABX type (38c). Because of the rigidity enforced upon the system by the ether linkage, it can be seen from a Dreiding model of this molecule that the electronic environment around the two methylene protons is not identical. Furthermore, the chemical shift of the proton on the tertiary carbon atom will be different from that of the methylene protons because of the attached hydroxyl group. Consequently the expected spectrum would be of the ABX type. Thus the quartet may be assigned to the $\text{-}\underline{\text{C}}\text{HOH}$ proton and the multiplet to the methylene protons.

The tertiary alcohol, 9-methyl-9-xanthenol, was prepared by the method described by Decker (39) except that the Grignard complex was decomposed by cold 3% ammonium chloride instead of hydrochloric acid in order to inhibit dehydration to olefin. The infrared spectrum of the tertiary alcohol showed peaks at 3300 cm^{-1} , 1600 cm^{-1} , 1570 cm^{-1} and 930 cm^{-1} . The peak at 930 cm^{-1} was found to be characteristic of this alcohol.

Solutions of the tertiary alcohol rapidly discoloured and even the solid itself decomposed on standing. Vapour phase chromatography of the freshly prepared alcohol exhibited a single peak corresponding in retention time to dibenz[*b,f*]oxepin. The tertiary alcohol must dehydrate, therefore, in the vapourizer of the chromatograph to 9-methylenexanthene which apparently has the same retention time, on the

column employed, as dibenz [b,f] oxepin. When the tertiary alcohol was placed on an alumina column and the column eluted with chloroform, the product recovered was not the alcohol. The eluted product showed no hydroxyl absorption in the infrared spectrum but otherwise was not investigated further. The instability of the alcohol, apparently due to its tendency to dehydrate readily, precludes its presence in the alcohol fraction derived from the Demjanov rearrangement.

An examination of the infrared spectrum of the alcohol fraction obtained from the Demjanov reaction indicated that the mixture consisted of the primary and the secondary alcohol. To ascertain the composition of this mixture we turned first to vapour phase chromatography but found it unsuitable. The primary and the secondary alcohol exhibited identical retention times on a column packed with 2.5% silicone rubber gum, SE 30, adsorbed on silica-gel and a mixture of the two appeared as a single peak. We also tested a second column, 5% carbowax, 1540, on chromosorb P, with a similar result. Instead of searching for a suitable column we turned to N.M.R. spectroscopy as a means of estimating the composition.

An examination of the N.M.R. spectrum of the two alcohols shows that two peaks, that of the CHOH peak of the secondary alcohol at $\tau = 5.16$ and that of the $\text{CH-CH}_2\text{OH}$ peak at $\tau = 6.12$ of the primary alcohol are distinct from other peaks in each of the two alcohols. An examination of synthetic mixtures of the two showed that the composition could be estimated to $\pm 5\%$ by measurement of the integrated areas underlying these two peaks. In the case of the alcohol mixture derived from the Demjanov rearrangement, the integration of the area

under the two peaks, at $\tau = 5.13$ and 6.12 , shows that 80% (± 5) of the alcohol is the ring expanded alcohol. Thus it appears that ring expansion has taken place in the Demjanov reaction in about 65% yield, based on 9-xanthenemethylamine.

Although no separation was obtained on an alumina column, chromatography on a silica-gel column containing 12% water (W/W) using chloroform as eluant separated the ring-expanded alcohol from the primary alcohol. The composition of the alcohol mixture based on this chromatographic analysis was in agreement with that reached by N.M.R. analysis.

The foregoing results show that the Demjanov rearrangement may be successfully applied to the synthesis of 10,11-dihydrodibenz $[b, f]$ -oxepin-10(11,H)-ol and dibenz $[b, f]$ oxepin itself. Manske and Ledingham have shown (3) and we have confirmed that the alcohol may be converted quantitatively to the olefin.

In the course of this work it was necessary to prepare the dibenzoxepinone and dibenzoxepin. The N.M.R. spectrum of both of these compounds was recorded and since they have not been reported elsewhere they are reported here as a matter of interest.

The spectrum of dibenz $[b, f]$ oxepin is composed of two signals as expected. There is a two proton signal at $\tau = 3.25$ (singlet) due to the olefinic protons and an eight proton multiplet centred at $\tau = 2.75$ due to the aromatic protons. The N.M.R. spectrum of dibenzoxepinone is more interesting showing a two proton singlet at $\tau = 6.02$ (methylene protons) a multiplet centred at $\tau = 2.8$

(aromatic protons) and a doublet (one proton) at $\tau = 2.1$ and 1.95. These two peaks may be assigned to the aromatic proton adjacent to the carbonyl group. This signal is shifted downfield owing to diamagnetic anisotropy of the adjacent carbonyl group (38d). The splitting into a doublet may be attributed to spin-spin coupling with the adjacent aromatic proton. The other half of the AB system is probably embedded in the general aromatic resonance multiplet. This observation is of importance in that in substituted oxepinones N.M.R. spectroscopy might provide a method of locating the position of the carbonyl group with respect to substitution in the aromatic nucleus.

Although Kametani and Fukumato (21) have been able to synthesise cularine, they encountered difficulties in the construction of the isoquinoline ring from the dibenzoxepin derivatives. In only one of nine experiments were they able to get even an 8.35% yield of the isoquinoline derivative. Previous workers (6, 15) who attempted this synthesis of the isoquinoline ring from dibenzoxepinone derivatives were even less successful.

While undertaking the investigation of Demjanov reaction as a possible route to dibenzoxepin derivatives we had considered several methods for elaboration of this system to synthesis of the isoquinoline ring system of cularine among which was that applied, with limited success, by Kametani and Fukumato.

In the light of their experience a more attractive scheme would appear to be a synthesis based upon the xanthone IVb. By making use of the carboxyl group at the 1-position a nitrogen-containing

side chain might be constructed before or after ring expansion of the xanthone to an oxepin derivative. Cyclization to the tetrahydroisoquinoline would then be expected to proceed without difficulty.

EXPERIMENTAL

Apparatus, Methods and Materials

The apparatus and methods used in these experiments are the same as those described previously in the first part of this thesis.

The chemicals used were analytical reagent grade unless otherwise specified.

Preparation of 9-xanthenol

9-xanthenol was prepared by the method described in Organic Synthesis (40) by the reduction of 9-xanthenone. The sodium amalgam used in the reduction was prepared by the method of Bachmann (41). The yield of 9-xanthenol was comparable to that described in Organic Synthesis.

Preparation of 9-xantheneacetic acid

Method A. This method is due to Matsuno and Okabayashi (36). Freshly prepared 9-xanthenol (10 g) and malonic acid (6.5 g) were heated in pyridine (20 ml) solution for 3 hours at 70° and then for 2 hours at 100°. The mixture was cooled and poured into 5% aqueous hydrochloric acid (170 ml). The precipitate which separated was filtered, redissolved in 10% aqueous sodium carbonate solution, filtered and the filtrate acidified with dilute hydrochloric acid to reprecipitate the acid. The crude product was recrystallized from aqueous methanol to give 9-xantheneacetic acid (7.6 g, 63%) which melted at 154 - 155°. The reported melting point is 155 - 156° (36).

Method B. The method of Goldberg and Wragg (34) was found to give a better yield of xantheneacetic acid. A solution of malonic acid (5 g) in glacial acetic acid (50 ml) was added to a solution of 9-xanthanol (10 g) in glacial acetic acid (25 ml). The mixture was kept at room temperature overnight and then poured with stirring into ice water (300 ml). The precipitate was filtered, dissolved in 10% sodium carbonate solution, filtered again and reprecipitated by acidification of the filtrate with dilute hydrochloric acid. The crude product was filtered, washed with water and dried to give crude 9-xanthene-malonic acid (12 g). A portion of the crude acid was crystallized from aqueous methanol to give pale green needles which melted at 150 - 151°. The reported melting point is 150 - 152° (34). The crude 9-xanthene-malonic acid was boiled under reflux with pyridine (31 ml) for half an hour, cooled and poured into stirred 5M aqueous hydrochloric acid solution (156 ml). The white precipitate was filtered, washed with cold water, dissolved in aqueous ammonia and filtered again. The filtrate on acidification gave 9-xantheneacetic acid as white crystals which on recrystallization from methanol melted at 154 - 156° (8.2 g, 68%). The reported melting point is 154 - 156° (34).

Preparation of the methyl ester of 9-xantheneacetic acid

9-xantheneacetic acid (1 g) dissolved in a mixture of benzene and ether was treated with an ethereal solution of diazomethane. The mixture was let stand overnight after which the solvent was removed on a rotary evaporator. The residue was dissolved in chloroform and the chloroform solution was dried over anhydrous sodium sulphate. Removal

of chloroform on a rotary evaporator gave a solid which crystallized from methanol and melted at 50 - 52°. The mass spectrum of this compound showed a molecular ion peak at m/e 254.

Preparation of 9-xantheneacetyl chloride

Purified thionyl chloride (4 ml) was cooled in an ice bath and 9-xantheneacetic acid (4 g) was added slowly with mechanical stirring when a semisolid green mass formed. Additional thionyl chloride (4 ml) was added and the mixture boiled gently under reflux for 25 minutes. The excess thionyl chloride was then removed under reduced pressure and the residual green solid crystallized from benzene to give 9-xantheneacetyl chloride (3.5 g, 82%), m.p. 95 - 96°. The reported melting point for the acid chloride is 98° C (34).

Preparation of 9-xantheneacetic azide

A solution of sodium azide (1.0 g) in water (2 ml) was added dropwise to a stirred ice-cooled solution of 9-xantheneacetyl chloride (1.0 g) in acetone (15 ml) and the mixture allowed to stand for 15 minutes. The resulting oily mixture was diluted with water (30 ml) and the crystalline azide filtered and dried at room temperature under reduced pressure. The azide was used in subsequent experiments without further purification. The yield was 0.92 g (90.2%). The infrared spectrum of the azide showed a strong peak at 2120 cm⁻¹ (azide) and 1730 cm⁻¹ (>C=O).

Preparation of N,N'-di(9-xanthenemethyl)urea

The azide prepared above was dissolved in dry benzene (5 ml) and heated under reflux for 2 hours (or until nitrogen evolution

ceased). Removal of solvent on a rotary evaporator gave the isocyanate as a brownish solid. The crude isocyanate was dissolved in benzene (10 ml), treated with 10 ml of 50% potassium hydroxide solution and heated on a steam bath for one hour. Removal of benzene on a rotary evaporator followed by cooling of the aqueous layer gave a crystalline solid which was separated and washed with water. Crystallization from methanol gave N,N'-di(9-xanthenemethyl)urea which melted at 222 - 223°. The infrared spectrum showed an absorption peak at 3330 cm^{-1} (-NH absorption) and a peak at 1627 cm^{-1} attributed to the carbonyl group of urea linkage.

Calculated for $\text{C}_{29}\text{H}_{24}\text{O}_3\text{N}_2$: C, 77.90%; H, 5.36%; N, 6.27%

Found: C, 78.31%; H, 5.18%; N, 6.34%

Preparation of 9-xanthenemethyl isocyanate

9-xanthenecetyl azide (0.9 g) was dissolved in dry benzene (5 ml) and heated under reflux for 2.5 hours when the evolution of nitrogen had ceased. Upon evaporation of benzene a brownish solid (0.8 g) was obtained. The infrared spectrum showed that the azide and the carbonyl peak had disappeared and a new peak at 2265 cm^{-1} (-N=C=O) appeared. The yield of the isocyanate was quantitative (0.8 g).

Preparation of 9-xanthenemethylamine

Method A. The crude isocyanate (0.6 g) was dissolved in absolute ethanol (20 ml) containing potassium hydroxide (4 g) and boiled under reflux for 15 hours during which time a small amount of a reddish precipitate formed. Additional absolute ethanol (15 ml) was added

to dissolve the precipitate and the mixture was boiled under reflux for a further 30 minutes. The mixture was filtered, the filtrate acidified with diluted hydrochloric acid and filtered free of potassium chloride. The filtrate was then refluxed for one hour, cooled, basified with ammonia and most of the ethanol removed on a rotary evaporator. Water was added to the residue and the mixture extracted with ether. The ether extract, dried over anhydrous sodium sulphate, was saturated with dry hydrogen chloride gas whereupon white crystals separated which after drying weighed 0.26 g (41.5% based on the acid chloride) and melted at 225 - 230°. Recrystallization from ethanol (95%) gave 9-xanthenemethylamine hydrochloride, which melted at 235 - 236°. The melting point reported for this compound is 235 - 236° (33).

Method B. The crude azide (2 g) prepared by the method described previously was heated under reflux with benzyl alcohol (5 ml) and dry toluene (5 ml) for three hours. The mixture was poured into a porcelain evaporating dish and toluene was allowed to evaporate slowly. The crystalline residue was transferred to a Buchner funnel, washed with a little absolute ethanol and dissolved in a mixture of methanol (15 ml) and glacial acetic acid (5 ml). This solution was hydrogenated at 50 p.s.i.g. in the presence of palladium black catalyst (0.5 g) for 6 hours, filtered, concentrated under reduced pressure and poured into cold water (200 ml). The aqueous solution was treated with 10% hydrochloric acid solution (50 ml) and the mixture was extracted twice with ether to remove non-basic impurities. Basification with ice cold 10% sodium hydroxide solution gave a white

residue which was extracted with ether. The ether extract, dried over anhydrous sodium sulphate, was saturated with dry hydrogen chloride gas when 1.0 g (53.8% based on acid chloride) of crystalline 9-xanthenemethylamine hydrochloride was obtained which melted at 230 - 231°. A sample recrystallized from ethanol (95%) melted at 235 - 237°. The infrared spectrum showed a continuous series of bands between 3030 cm^{-1} and 2500 cm^{-1} characteristic of NH_3^+ absorption and absorption peaks at 1600 and 1570 cm^{-1} characteristic of aromatic absorption of xanthene derivatives, but no absorption in the carbonyl region.

Calculated for $\text{C}_{14}\text{H}_{13}\text{ON}\cdot\text{HCl}$: C, 68.00%; H, 5.65%

Found: C, 67.88%; H, 5.70%

The mass spectrum of this compound showed a molecular ion peak at m/e 211.

Demjanov rearrangement of the 9-xanthenemethylamine

9-xanthenemethylamine hydrochloride (1.0 g, 0.004 mole) dissolved in acetic acid-sodium acetate buffer (pH 5.8, 20 ml) was cooled to 0° C in an ice bath and the stirred solution was treated with a solution of sodium nitrite (0.4 g) in water (2 ml). When the addition was complete the reaction mixture was allowed to warm up slowly to room temperature and was kept there for six hours. The mixture was then heated on a steambath until nitrogen evolution had ceased (approximately 45 minutes). The cooled reaction mixture was then extracted with ether, the ether extract washed with water and dried over anhydrous sodium sulphate. Evaporation of the ether gave

a yellow oil (0.8 g). This oil was heated under reflux for 1 hour with methanolic potassium hydroxide solution (5%, 50 ml) in order to hydrolyse acetates to the corresponding alcohols. The reaction mixture was cooled, methanol removed under reduced pressure, more water added and the reaction mixture extracted with ether. The ether extract was washed with water and dried over anhydrous sodium sulphate. On removal of ether a yellow oil (0.74 g) was obtained which could not be induced to crystallize.

Vapour phase chromatography on a 2.5% silicone gum rubber on silica gel column showed that, approximately, 96% of this material was a mixture of isomeric alcohols, the rest being a mixture of isomeric olefins. However, no separation of the alcohol mixture was achieved by vapour phase chromatography. The peak assigned to the alcohol mixture was established by comparing with the peak of a mixture of synthetic alcohols. A mixture of the synthetic primary and secondary alcohol showed a single peak with the same retention time as the alcohol fraction from Demjanov reaction.

This oil was adsorbed on an alumina column (non-basic alumina, Woelm, Germany) and eluted successively with petroleum ether (b.p. 30 - 60°), ether and chloroform. The ether fraction on evaporation gave yellow crystals (0.02 g) which melted over a wide range. Recrystallization from ether did not yield a pure compound. This fraction was not further investigated.

The chloroform fraction on removal of solvent gave a yellow oil (0.71 g) which could not be induced to crystallize. The infrared spectrum showed an absorption of medium intensity at 1645 cm^{-1} and

1610 cm^{-1} , a strong peak at 1570 cm^{-1} , and a strong peak in the OH region at 3400 cm^{-1} . The ultraviolet spectrum showed absorption at λ_{max} 337, 270 and 260 $\text{m}\mu$ and continuous absorption beyond 241 $\text{m}\mu$. Vapour phase chromatography on the column described above showed only one peak with trace of another peak corresponding to dibenz[b,f]oxepin. Thin layer chromatography, however, indicated that the mixture was composed of at least three components. The composition of the mixture was estimated from its nuclear magnetic resonance spectrum by comparison with that of a synthetic mixture of the 9-xanthenemethanol and 10,11-dihydrodibenz[b,f]oxepin-10,(11,H)-ol. It was found that the ratio of the rearranged alcohol to the unrearranged primary alcohol was approximately 4:1. Thus it may be calculated that the rearranged product is obtained in 65% yield based on the weight of the free amine.

The alcohol fraction (0.5 g) obtained from alumina chromatography was chromatographed on a silica gel column (12% water) (W/W) eluting the column with ether, benzene-chloroform mixture (1:1) and finally with chloroform. There was no material in the ether fraction. The (1:1) benzene fraction on evaporation yielded a viscous oil (0.09 g) which would not crystallize. The chloroform fraction gave a viscous oil (0.4 g), the infrared spectrum of which was identical with that of synthetic 10,11-dihydrodibenz[b,f]oxepin 10,(11,H)-ol.

Preparation of o-phenoxy-benzaldehyde

This compound was prepared according to the procedure of Manske and Ledingham (3). Copper salicylaldehyde (86 g) was heated under reflux with iodobenzene (170 g) in an atmosphere of nitrogen for 12 hours. The separated copper salt was removed by filtration and the excess reagent removed by distillation under reduced pressure.

The residue was dissolved in benzene, washed with dilute hydrochloric acid, sodium hydroxide, and then water. Removal of benzene gave an oily residue which on distillation yielded 18 grams of pale yellow oil, collected at a temperature of $140 - 160^{\circ}$ and a pressure of 0.8 mm of mercury. The infrared spectrum showed absorption peaks at 1700 cm^{-1} (CHO) and 1600 cm^{-1} (aromatic).

Preparation of o-phenoxy-benzaldehyde azlactone

A mixture of the above aldehyde (18 g), hippuric acid (18 g), anhydrous sodium acetate (7.5 g) and acetic anhydride (65 ml) was heated on a steam bath for 2.5 hours. Orange yellow crystals separated soon after the commencement of the heating. When the reaction was complete the crystals were separated, washed with ether and water to remove inorganic impurities and dried in a desiccator. The yield of azlactone was 18 grams. A portion of the azlactone, on recrystallization from acetic acid, melted at $184 - 186^{\circ}$. The reported melting point for this compound is 186° (3). The infrared spectrum of the azlactone showed absorption peaks at 1770 cm^{-1} (lactone carbonyl) and at 1640, 1590 and 1550 cm^{-1} .

o-phenoxy-phenylacetic acid

The azlactone prepared above was gently boiled with potassium hydroxide (26.3 g) in water (90 ml) until the evolution of ammonia had ceased (1.5 hours). The cooled solution, after dilution with water (275 ml), was saturated with sulfur dioxide. The mixture was warmed to dissolve the bisulfite addition product and filtered from benzoic acid. The aqueous solution was acidified with hydrochloric

acid and heated under reduced pressure to expel sulphur dioxide. The oily keto acid which separated crystallized readily on cooling. After filtering, washing and drying, the keto acid weighed 13.5 grams. A portion recrystallised from benzene melted at $145 - 148^{\circ}$. The reported melting point is $146 - 150^{\circ}$ (3). The infrared spectrum of the keto acid in nujol showed peaks at 2660 and 2540 cm^{-1} assigned to the hydroxy absorption of the carboxyl group and carbonyl absorption at 1680 cm^{-1} with a shoulder at 1645 cm^{-1} .

The crude keto acid dissolved in 5% sodium hydroxide solution (450 ml) was cooled to 0° and maintained at this temperature while 30% hydrogen peroxide (50 ml) was added. The mixture was allowed to come to room temperature and let stand for 24 hours. The solution was filtered and the filtrate acidified with hydrochloric acid. The crystals which formed upon acidification were washed with water and dried in a desiccator (10 g). Recrystallization from benzene-hexane gave crystals melting at $90 - 92^{\circ}$. The reported melting point is 91° (3). The infrared spectrum in nujol showed two peaks at 2700 and 2650 cm^{-1} and a single peak in the carbonyl region at 1710 cm^{-1} .

Preparation of 10,11-dihydrodibenz[b,f]oxepin-10(11,H)-one

A solution of the above *o*-phenoxy-phenylacetic acid in chloroform (15 ml) and thionyl chloride (20 g) was heated under reflux for 3 hours. The residual acid chloride obtained after removal of excess thionyl chloride under reduced pressure was dissolved in nitrobenzene and gradually added to a suspension of aluminum chloride (8 g) in nitrobenzene (15 ml) maintained at a temperature below 20° .

The mixture, after remaining at room temperature for 24 hours, was decomposed with ice and steam-distilled to remove the nitrobenzene. The aqueous layer was extracted with ether. The extract was washed with dilute hydrochloric acid, aqueous sodium bicarbonate and water and the ether removed. The residue on distillation yielded a pale yellow oil (7 g) collected at 160 - 165° at 2 mm which solidified on cooling. Recrystallization from hexane gave the cyclic ketone which melted at 55 - 56°. The melting point reported by Manske and Ledingham is 56° (3). The infrared spectrum showed a carbonyl absorption at 1675 cm^{-1} and two peaks at 1600 and 1570 cm^{-1} . The mass spectrum showed a molecular weight of 210.

Preparation of 10,11-dihydrodibenz[b,f]oxepin, 10(11,H)-ol

The oxepinone (1 g) was taken up in absolute ethanol (15 ml) and sodium borohydride (2 g) was added to it in portions. The mixture was let stand overnight, and the excess borohydride destroyed by adding acetone in portions. The clear solution was then acidified with diluted hydrochloric acid and filtered. The solution was extracted with chloroform, the extract washed with water and dried over anhydrous sodium sulphate. The removal of solvent gave a viscous oil (0.8 g) which could not be induced to crystallize. This oil was chromatographed on an alumina column (Woelm, non-basic), eluting with ether and chloroform. A small amount of gummy material was obtained from the ether fraction, the infrared and ultraviolet spectra of which indicated it to be dibenz[b,f]oxepin. The chloroform fraction, on removal of solvent, gave a viscous oil which could not be induced

to crystallize. Thin layer chromatography showed only a single spot. The infrared spectrum showed a peak at 3400 cm^{-1} (OH) and peaks at 1605 cm^{-1} and 1575 cm^{-1} (aromatic ring). The mass spectrum of this alcohol showed a molecular weight of 212. The ultraviolet spectrum showed absorption maxima at λ_{max} 300, 270 and $264\text{ m}\mu$ with a shoulder at $275\text{ m}\mu$ and intense absorption beyond $228\text{ m}\mu$.

Preparation of dibenz[b,f]oxepin

A portion of the alcohol (0.4 g) in toluene (10 ml) was heated under reflux for fifteen minutes with p-toluenesulfonic acid (0.5 g). The reaction mixture was poured on crushed ice and the toluene layer separated and washed with water. Evaporation of the toluene under reduced pressure yielded dark coloured crystals (0.35 g). The crystals were purified by eluting with ether through an alumina column. The eluted material crystallized from methanol and melted at $110 - 111^{\circ}$. The melting point reported in the literature is 111° (3). The infrared spectrum showed two peaks of medium intensity at 1600 and 1575 cm^{-1} and a series of strong peaks in the low-frequency region. The mass spectrum showed a molecular weight of 194. The ultraviolet spectrum was identical with that reported by Anet and Bavin (37) for dibenz[b,f]oxepin.

Preparation of 9-xanthenemethanol

Xanthene-9-carboxylic acid (1 g) was dissolved in ether (50 ml) and reduced with an excess of ethereal lithium aluminum hydride. The mixture was allowed to stand overnight and the excess reagent decomposed with wet ether and enough water added to coagulate the precipitate.

The ether layer was washed with aqueous sodium carbonate and dried over anhydrous sodium sulphate. The residue obtained after removal of ether crystallized from an ether-pentane mixture (0.69 g). The crystals melted at $67 - 68^{\circ}$. The reported melting point for 9-xanthenemethanol is 68° (37). The infrared spectrum showed peaks at 3600, 3450, 1600, 1570, 1120, 1098 and 895 cm^{-1} . The N.M.R. spectrum showed a one proton signal at $\tau = 7.72$ (OH proton), a doublet centred at $\tau = 6.52$ (two protons) and a triplet centred at $\tau = 6.12$ (one proton).

The mass spectrum showed a molecular weight of 212.

Preparation of 9-methyl-9-xanthenol

9-Xanthenone (5 g) was treated with methylmagnesium iodide (2.16 g Mg + 11.84 g methyl iodide) in dry ether in a Soxhlet apparatus. A yellow solid separated during the course of the reaction. The whole was boiled under reflux for 1 hour after which the solid was filtered, washed with ether and decomposed with cold 3% ammonium chloride solution. The mixture was extracted with ether and the ether extract dried over anhydrous sodium sulphate. Removal of ether gave a crystalline product (3.9 g, 71%) which melted over a wide range. Repeated recrystallization from petroleum-ether and ether mixture raised the melting point to $95 - 99^{\circ}$ (capillary). The reported melting point for 9-methyl-9-xanthenol is $96 - 99^{\circ}$ (39). The infrared spectrum of 9-methyl-9-xanthenol showed absorption peaks at 3300, 1600, 1570 and 930 cm^{-1} . The mass spectrum did not show the molecular ion peak instead the strongest peak in the spectrum was at m/e 194 (M-18) which corresponds to loss of water from the molecular ion.

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