MINOR ALKALOIDS OF LYCOPODIUM FLABELLIFORME

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By

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SCOPE AND CONTENTS:

An examination of the alkaloids of L. flabelliforme var. ambiguum was undertaken. By using chromatographic and counter-current distribution methods, the known alkaloids lycopodine, dihydro-lycopodine and α-obscurine have been isolated. In addition two new minor alkaloids, flabelliformine ($C_{16}H_{25}O_2N$) and flabelline ($C_{18}H_{30}ON_2$) have been isolated. The structure of flabelline has been established by infrared and nuclear magnetic resonance data, and chemical examination.

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TABLE OF CONTENTS

	Page
Descriptive Note	ii
Acknowledgements	iii
GENERAL INTRODUCTION	1
HISTORICAL INTRODUCTION	2
Isolation of _Lycopodium_ Alkaloids	2
Methods of Isolation	34
Methods of Separation and Purification	35
Fractional crystallization	36
Formation of salts	36
Fractional distillation	36
Adsorption chromatography	37
Counter-current distribution	38
Paper chromatography	43
Other techniques with limited application	44
Pharmacological Action	45
Structure and Stereochemistry of _Lycopodium_ Alkaloids	45
Biogenesis of _Lycopodium_ Alkaloids	55
DISCUSSION OF RESULTS	59
Isolation of Alkaloids	59
Structure of Flabelline	64

Stereochemistry of Flabelline 71

Biogenesis of Flabelline 72

EXPERIMENTAL 73

Apparatus, Methods, and Materials 73

Source of Crude _L._ _flabelliforme_ Residues 73

Preliminary Purification of Crude Alkaloids 74

First Chromatography of Crude Alkaloids 74

Isolation of Flabelliformine 75

Preparation of Flabelliformine Methiodide 76

First Separatory Funnel Distribution of Crude Bases (SD1) 76

Resolution of SD1.1B 79

Resolution of SD1.2B 79

Resolution of SD1.3B 79

Resolution of SD1.4B 80

Counter-current Distribution of SD1.3B Residues and SD1.4B 80

Resolution of SD1.5B 83

Second Chromatography of Crude Alkaloid Extracts 84

Second Separatory Funnel Distribution of Crude Bases (SD2) 85

Resolution of SD2.1B 85

Resolution of SD2.2B 85

Resolution of SD2.3B 88

Resolution of SD2.4B 88

Resolution of SD2.5B 89

Resolution of Fractions SD2.6B to SD2.9B 89

Resolution of SD2.10B 90

Resolution of SD2.11B 90

Resolution of SD2.12B 90

Third Separatory Funnel Distribution of Crude Bases (SD3) 90

Preparation of Flabelline Methiodide 91

Preparation of Flabelline Perchlorate 91

Attempted Hydrogenation of Flabelline 92

Attempted Hydrolysis of Flabelline 92

Preparation of Lycopodine Oxime 92

Attempted Platinum Oxide Reduction of Lycopodine Oxime 93

Lithium Aluminum Hydride Reduction of Lycopodine Oxime 93

Acetylation of Lycopodine Oxime Reduction Product with Acetyl Chloride-Pyridine Complex 94

High Pressure Reduction and Acetylation of Lycopodine Oxime with Raney Nickel and Acetic Anhydride 95

REFERENCES 97

LIST OF FIGURES

		Page
Figure 1.	Counter-current Distribution	40
Figure 2.	Counter-current Distribution Curves	40
Figure 3.	Inter-relationship of *Lycopodium* Alkaloids	56
Figure 4.	Infrared Spectrum of Flabelline in Nujol	65
Figure 5.	Infrared Spectrum of Flabelline in Chloroform	67
Figure 6.	NMR Spectrum of Flabelline	69
Figure 7.	Separatory Funnel Distribution SD1	78
Figure 8.	Counter-current Distribution CD1	82
Figure 9.	Separatory Funnel Distribution SD2	87

LIST OF TABLES

		Page
Table I	Plants of the *Lycopodium* Species and Their Contained Alkaloids	9
Table II	Index of the *Lycopodium* Alkaloids, With Formulas, Melting Points and Related References	15
Table III	Formula Index of the *Lycopodium* Alkaloids	22
Table IV	Melting Point Index of the *Lycopodium* Alkaloids	24
Table V	Melting Point Index of Hydrobromide Salts	26
Table VI	Melting Point Index of Hydrochloride Salts	26
Table VII	Melting Point Index of Hydriodide Salts	27
Table VIII	Melting Point Index of Methiodide Salts	27
Table IX	Melting Point Index of Methochloride Salts	29
Table X	Melting Point Index of Methoperchlorate Salts	30
Table XI	Melting Point Index of Methopicrate Salts	30
Table XII	Melting Point Index of Nitrate Salts	31
Table XIII	Melting Point Index of Picrate Salts	31
Table XIV	Melting Point Index of Perchlorate Salts	32
Table XV	Distribution of Alkaloids in SD1	77
Table XVI	Distribution of Alkaloids in CD1	81
Table XVII	Distribution of Alkaloids in SD2	86

GENERAL INTRODUCTION

The presence of alkaloids in plants of the genus Lycopodiaceae has been known since 1881, but only during the past two decades has an extensive investigation of these alkaloids been performed. At the present time about 75 *Lycopodium* alkaloids have been isolated and characterized from over a dozen species. The structures of twenty-seven of these alkaloids have been elucidated and many have had their stereochemistry established. All alkaloids of known structure possessing a single nitrogen atom have a hexahydrojulolidine ring system, whereas all the di-nitrogen alkaloids, with the exception of selagine and flabelline, possess a hydrogenated m-phenanthroline ring system.

The work reported in this thesis involves an investigation, by use of counter-current distribution and chromatographic techniques, of the minor alkaloids of *L. flabelliforme* var. *ambiguum*. In addition to lycopodine, dihydrolycopodine, and α-obscurine, isolated from this plant by earlier workers, there have been isolated two new alkaloids: flabelliformine and flabelline. The nature of the functional group of flabelline was established by infrared and nuclear magnetic resonance spectroscopy, while the structure has been determined by synthesis from lycopodine. Flabelline is the first di-nitrogen *Lycopodium* alkaloid found to have the hexahydrojulolidine ring system and an amide group.

HISTORICAL INTRODUCTION

Isolation of *Lycopodium* Alkaloids

Spermatophyta and Pteridophyta are the two major divisions into which the vascular plants are divided. The plants in the Spermatophyta (flowering plants) have been examined more fully for their chemical content than those in the Pteridophyta, since the latter are of only secondary economic importance and frequently are inconspicuous in appearance. The family Lycopodiaceae, a member of the Pteriodophyta, includes the genera *Lycopodium*, with some 180 species widely spread over most of the earth, and *Phylloglossum* found only in Australia.

The Lycopodiaceae of central European origin which have been studied for their alkaloidal content are: *L. annotinum* L., *L. clavatum* L., *L. complanatum* L., and *L. selago* L. Because of their yard-long, evergreen stems creeping over the ground, *L. annotinum* L. and *L. clavatum* L. are often called snake moss. From Canadian sources, the species *L. annotinum* L., *L. annotinum* var. *acrifolium* Fernald., *L. clavatum* L., *L. complanatum* L., *L. flabelliforme* Fernald., *L. lucidulum* Michx., *L. obscurum* L. var. *dendroideum* (Michx.) D. C. Eaton, *L. sabinaefolium* Willd., *L. selago* L., and *L. tristachyum* Pursh. have been examined. The species *L. clavatum* L., and *L. fawcettii* Lloyd and Underhill from the Blue Mountain Range of Jamaica, *L. saururus*

Lam from Argentina, L. densum Labill. from New Zealand, and L. cernuum L. of Hawaiian and Jamaican origin have also been investigated for their alkaloidal content.

Bödeker (1) was the first to report the occurrence of an alkaloid in the Lycopodiaceae. In 1881, from L. complanatum L., he isolated a crystalline base, assigning to this alkaloid the formula $C_{22}H_{52}O_3N_2$ and the name lycopodine.

In 1892, Arata and Canzoneri (2) reported the isolation of pillijanine ($C_{15}H_{20}ON_2$) from the South American L. saururus Lam. Reinvestigating the same plant fifty years later, Deulofeu and DeLange (3) failed to locate any pillijanine. However, they isolated two new alkaloids: saururine ($C_{10}H_{19}N$) and sauroxine ($C_{17}H_{26}ON_2$).

In the mid-thirties, Orekhov (4) reported that L. annotinum L. is a source of alkaloids and Muszynski (5) followed with a preliminary account of work on the alkaloids of five European Lycopodium species.

It was not until 1938 that the correct formula for lycopodine was proposed by Achmatowicz and Uzieblo (6). In a paper on the alkaloids of L. clavatum L., they reported isolation of three bases. It was assumed that the major alkaloid was identical with Bödeker's lycopodine and was assigned the formula $C_{16}H_{25}ON$. Clavatine ($C_{16}H_{25}O_2N$) and clavatoxine ($C_{17}H_{27}O_2N$) were the two minor alkaloids isolated.

The first comprehensive investigation of the genus was begun by Manske and Marion (7-16) in 1942 on ten Lycopodium species. From these species they isolated about thirty-five new alkaloids, in addition to the known lycopodine and nicotine. Lycopodine was found to be the major alkaloid in most of the species investigated, and was detected in all species except L. cernuum L. Only five of the new

alkaloids were given trivial names, the remainder being designated by the letter L and a number, e.g., L.25. Another five have subsequently been given trivial names. The detection of nicotine in the Lycopodiaceae is the first recorded instance of an alkaloid being found in both the Pteridophyta and Spermatophyta.

In 1952, Bertho and Stoll (17) reported isolating and characterizing seven alkaloids from L. annotinum L. of German origin. Three of these, acrifoline, annotinine, and lycopodine were already known at that time. Annotine ($C_{16}H_{21}O_3N$), which was later shown by Achmatowicz and Rodewald (18) to be identical with L.11, was incorrectly reported as $C_{16}H_{23}O_4N$. Annotoxine ($C_{32}H_{44}O_5N_2$), incorrectly reported as $C_{31}H_{42}O_5N_2$, was also investigated by Achmatowicz and Rodewald (18) and found to be an equimolecular complex of acrifoline and annotine. Bertho and Stoll also isolated base U.1* ($C_{16}H_{25}ON$) isomeric with lycopodine, and another base U.2 not well characterized ($C_{10}H_{19(21)}ON$).

A more intensive investigation of L. annotinum L. of Polish origin has been reported by Achmatowicz and Rodewald (18,19). In these two publications, differences between the varieties of European and Canadian L. annotinum were shown not to be as great as was originally supposed by Bertho and Stoll (17). Isolation of alkaloids by Perry and MacLean (20) from undetermined varieties of L. annotinum growing in eastern Canada has further substantiated this view. The alkaloids

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* For sake of convenience, unnamed alkaloids have been designated with the letter U and a number.

found common to Canadian and European varieties are: acrifoline, annotine, annotinine, lycodoline, lycopodine, obscurine, L.28, L.29 and L.31. Achmatowicz and Rodewald (18) found that when the hydriodide and methiodide salts of annotoxine were fractionally crystallized, the corresponding salts of annotine and acrifoline were isolated separately, whereas attempted fractional crystallization of annotoxine itself afforded no separation. Manske and Marion (8) as well as Bertho and Stoll (in two of three plant extracts) separated their bases by fractional crystallization of salts, undoubtedly explaining why they failed to detect annotoxine. Achmatowicz and Rodewald (18) believe that Canadian L. annotinum L. should contain acrifoline as well as annotine, and that acrifoline was left in one of the L.9 fractions. New alkaloids reported by Achmatowicz and Rodewald include: isolycopodine ($C_{16}H_{25}ON$), which they hint may be identical with L.13 isolated from five different species by Manske and Marion; base U.4 ($C_{16}H_{23}ON$) believed to be identical with U.2 ($C_{16}H_{25}ON$) isolated by Bertho and Stoll; and five more which are undoubtedly new: U.3 ($C_{16}H_{21}O_3N$), U.5 ($C_{17}H_{25}O_2N$), U.6 ($C_{17}H_{25}O_3N$), U.7 ($C_{18}H_{25}O_3N$) and U.8 ($C_{18}H_{25}O_4N$). Isolation of nicotine was also reported.

L. selago L. of Polish origin has also been investigated by Achmatowicz and Rodewald (21). In 1956, they reported isolating acrifoline, lycodoline, lycopodine, and the base pseudoselagine ($C_{16}H_{25}O_2N$) which is either a new alkaloid, or identical with L.23, found in L. lucidulum Michx. by Manske and Marion (12).

In 1958, Anet and Eves (22) began another investigation of L. annotinum native to Canada and succeeded in isolating a new minor

alkaloid, lycodine ($C_{17}H_{24}N_2$). This formula was later changed to $C_{16}H_{22}N_2$, when the structure of lycodine became known (23,24). Reporting further on L. annotinum, Anet and Khan (25) in 1959 announced isolation of four more new alkaloids: annofoline ($C_{16}H_{25}O_2N$), lycofoline ($C_{16}H_{25}O_2N$), and α- and β-lofoline (both $C_{18}H_{29}O_3N$). The names α- and β-lofoline were later changed to lofoline and fawcettiine, respectively.

About the same time that Anet and co-workers were investigating L. annotinum, Burnell began studying plants of the Lycopodium species growing in the Blue Mountain Range of Jamaica. In 1959, Burnell (26) reported extraction of seven new alkaloids from L. fawcettii Lloyd and Underhill: base A, called fawcettimine ($C_{16}H_{27}O_2N$, later changed to $C_{16}H_{25}O_2N$); base B, called lycodoline and identical with L.8 and L.30 ($C_{16}H_{25}O_2N$); base C, called fawcettiine ($C_{18}H_{29}O_3N$); base D, deacetylfawcettiine ($C_{16}H_{27}O_2N$); base E ($C_{17}H_{25}O_2N$); base F, fawcettidine ($C_{16}H_{23}ON$); and base G ($C_{18}H_{27}O_3N$). Continuing the isolation of alkaloids from L. fawcettii, Burnell et al. (27) obtained lycofoline (base H) and a small amount of base I (a base resembling α-obscurine) by hydrolysis of crude bases from the plant. By using a milder extraction technique, they were able to isolate four more new bases: base K, which is acetylfawcettiine ($C_{20}H_{31}O_4N$); base M ($C_{17}H_{27}O_2N$ later changed to $C_{18}H_{27}O_3N$); base N, diacetyllycofoline ($C_{20}H_{29}O_4N$); and base O ($C_{20}H_{31}O_5N$). They were unable to locate any lycopodine in this plant.

Since extraction of alkaloids from L. clavatum L. collected in Poland by Achmatowicz and Uzieblo (6) and in Canada by Manske and Marion

(11) yielded different bases, Burnell and Mootoo (28) were prompted to investigate the alkaloidal content of this species growing in Jamaica. In 1961, they reported the isolation of seven alkaloids. Six of these, fawcettiine, deacetylfawcettiine, fawcettimine, lycopodine, dihydrolycopodine (L.1) and acetyldihydrolycopodine (L.2) were already known, while the seventh, clavolonine ($C_{16}H_{25}O_2N$) was new.

In 1960, Wiesner et al. (29) published the structure of selagine ($C_{15}H_{18}ON_2$), isolated from L. selago. They did not state whether other alkaloids were obtained from this plant.

While working on the structure of lycodine, Ayer and Iverach (23) found that this alkaloid is readily separable from the crude base extracts of L. obscurum L. Recently, Ayer et al. (30) noted the isolation of α-obscurine and des-N-methyl-α-obscurine from L. clavatum L., and N-methyllycodine from L. complanatum. The isolation of lycoclavine from L. clavatum L. has also been reported recently by Ayer et al. (31). A more complete report on the minor alkaloids of L. clavatum L. will be published by Ayer in the future.

In June of this year, Curcumelli-Rodastamo and MacLean (32) reported the isolation and structure of flabelliformine, a minor alkaloid of L. flabelliforme. Isolation of other new alkaloids from L. flabelliforme is mentioned elsewhere in this thesis.

To date, at least seventy-five Lycopodium alkaloids have been isolated, and one-third of these have had their structures elucidated. Lycopodine occurs most frequently throughout the species examined. It is the major alkaloid of at least six species, and has been detected in all species except L. cernuum L., L. fawcettii, and L. saururus Lam.

Table I summarizes the *Lycopodium* species which have been examined, and alkaloids contained therein. Table II indexes the *Lycopodium* alkaloids, and gives formulas, melting points and references to isolation, degradation and structure. Tables III - XIV index the formulas and melting points of the free bases and various salts.

TABLE I

Plants of the *Lycopodium* Species

and Their Contained Alkaloids

Species	Alkaloid	References
L. annotinum L. (Canadian)	lycopodine	8,20
	o-acetylacrifoline (L.12)	8,20
	acrifoline (L.27)	20
	annofoline	25
	annotine (L.11)	8,20
	annotinine (L.7)	8,20
	fawcettiine	25
	lofoline	25
	lycodine	22
	lycodoline (L.8)	8,20
	lycofoline	25
	α-obscurine	8,20
	β-obscurine	8,20
	L.9a,L.9b,L.9c,L.9d	8
	L.10	8
L. annotinum L. (European)	lycopodine	17,18
	acrifoline (L.27)	17,18
	annotine (L.11)	17,18
	annotinine (L.7)	17,18
	isolycopodine	18
	lycodoline (L.8)	18

TABLE I (Continued)

Species	Alkaloid	References
	nicotine	19
	α-obscurine	18,19
	β-obscurine	19
	L.28	19
	L.29	19
	L.31	19
	U.1,U.2	17
	U.3	19
	U.4	19
	U.5	19
	U.6	19
	U.7	19
	U.8	19
<u>L</u>. <u>annotinum</u> var. <u>acrifolium</u> Fern. (Canadian)	lycopodine	14
	acrifoline	14
	annotinine	14
	lycodoline	14
	L.28	14
	L.29	14
	L.31	14
<u>L</u>. <u>cernuum</u> L. (Hawaiian & Trinidadian)	cernuine (L.32)	15
	L.33	15
	nicotine	15

TABLE I (Continued)

Species	Alkaloid	References
L. clavatum L. (Canadian)	lycopodine	11
	des-N-methyl-α-obscurine	30
	lycoclavine	31
	nicotine	11
	α-obscurine	30
	L.13	11
	L.18	11
	L.19	11
L. clavatum L. (Polish)	lycopodine	6
	clavatine	6
	clavatoxine	6
L. clavatum L. (Jamaican)	lycopodine	27,28
	acetyldihydrolycopodine (L.14)	28
	clavolonine	28
	deacetylfawcettiine	28
	dihydrolycopodine (L.1)	28
	fawcettiine	27,28
	fawcettimine	28
L. complanatum L.	lycopodine	1
	N-methyllycodine	30
L. flabelliforme var. ambiguum (Canadian)	lycopodine	T*
	annotinine	33
	clavolonine	33
	des-N-methyl-α-obscurine	33

TABLE I (Continued)

Species	Alkaloid	References
	dihydrolycopodine	T
	flabelliformine	32,T
	flabelline	T
	lycodine	33
	α-obscurine	T
	hydroxy obscurine	33
L. flabelliforme Fern. (Canadian)	lycopodine	7
	acetyldihydrolycopodine (L.2)	7
	dihydrolycopodine	7
	nicotine	7
	α-obscurine	7
	β-obscurine	7
	L.3	7
	L.4 (L.14)	7
	L.5	7
L. densum Labill. (New Zealand)	Lycopodine	16
	L.34	16
	L.35	16
L. fawcettii Lloyd and Underhill (Jamaican)	acetylfawcettiine	27
	deacetylfawcettiine	26
	diacetyllycofoline	27
	fawcettidine	26
	fawcettiine	26,27
	fawcettimine	26,27
	lycodoline	26

TABLE I (Continued)

Species	Alkaloid	References
	lycofoline	27
	Base E	26
	Base G	26
	Base I	27
	Base M	27
	Base O	27
L. lucidulum Michx. (Canadian)	lycopodine	12
	nicotine	12
	L.13	12
	L.20	12
	L.21	12
	L.22	12
	L.23	12
	L.24	12
	L.25	12
L. obscurum L. var. *dendroideum* (Michx.) D. C. Eaton (Canadian)	lycopodine	10
	lycodine	23
	α-obscurine	10
	β-obscurine	10
	L.13	10
	L.16	10
	L.17	10

TABLE I (Continued)

Species	Alkaloids	References
L. sabinaefolium Willd. (Canadian)	lycopodine	13
	nicotine	13
	L.13	13
	L.26	13
L. saururus Lam. (Argentinian)	pillijanine	2
	saururine	3
	sauroxine	3
L. selago L. (Polish)	lycopodine	21
	acrifoline	21
	lycodoline	21
	pseudoselagine	21
L. selago (N. American)	selagine	29
L. tristachyum Pursh. (Canadian)	lycopodine	9
	anhydrodihydrolycopodine (L.14)	9
	nicotine	9
	L.13	9
	L.15	9

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* T refers to this thesis.

TABLE II

Index of the lycopodium Alkaloids, With Formulas,

Melting Points, and Related References

Alkaloid	Formula	Melting Point	References: Isolation	References: Degradation	References: Structure
O-acetylacrifoline (L.12)	$C_{18}H_{25}O_3N$	119-120°	8,20,28	20	52,55
acetyldihydrolycopodine (L.2)	$C_{18}H_{29}O_2N$	95-96°	7,28		57,85
acetylfawcettiine	$C_{20}H_{31}O_4N$	117°	27		57,81
acrifoline (L.27)	$C_{16}H_{23}O_2N$	97-104°	14,17,18 20,21	17,20,52 53	52,53,55
anhydrodihydro-lycopodine (L.14)	$C_{16}H_{25}N$		9,28	82	54,85
annofoline	$C_{16}H_{25}O_2N$	150-151° 156-157°	25	54,56	54,56,57
annotine (L.11)	$C_{16}H_{21}O_3N$	174°	8,17,18 20	17,20,34	
annotinine (L.7)	$C_{16}H_{21}O_3N$	232°	8,14,17 18,20,33	14 58-78	74-80
annotoxine	$C_{32}H_{44}O_5N_2$	196-197°	17,20		

TABLE II (Continued)

Alkaloid	Formula	Melting Point	References		
			Isolation	Degradation	Structure
cernuine (L.32)	$C_{16}H_{26}ON_2$	106°	15		
clavatine	$C_{16}H_{25}O_2N$	212-214°	6	6	
clavatoxine	$C_{17}H_{27}O_2N$	185-186°	6	6	
clavolonine	$C_{16}H_{25}O_2N$	238°	28,33	57,81	57,81
deacetylfawcettiine	$C_{16}H_{27}ON$	203-204° 207°	26,28	57,81	57,81
des-N-methyl-α-obscurine	$C_{16}H_{24}ON_2$	266-268°	30,33		30
diacetyllycofoline	$C_{20}H_{29}O_4N$	140°	27	55	55
dihydrolycopodine	$C_{16}H_{27}ON$	168°	7,28,T	82,88	57,85
fawcettidine	$C_{16}H_{23}ON$		26		
fawcettiine	$C_{18}H_{29}O_3N$	166-167° 172-173°	25-28	27,57,81	57,81
fawcettimine	$C_{16}H_{25}O_2N$		26-28		
flabelliformine	$C_{16}H_{25}O_2N$	210-211°	32,T	32	32
flabelline	$C_{18}H_{30}ON_2$	186-188°	T	T	T

TABLE II (Continued)

Alkaloid	Formula	Melting Point	References: Isolation	Degradation	Structure
hydroxyobscurine	$C_{17}H_{26}O_2N_2$	300°	33		
isolycopodine	$C_{16}H_{25}ON$	136°	18		
lofoline	$C_{18}H_{29}O_3N$	211-212°	25	57	57
lycoclavine	$C_{18}H_{29}O_3N$		31		31
lycodine	$C_{16}H_{22}N_2$	118°	22,23,33	22,23,24	23,24
lycodoline (L.8, L.30)	$C_{16}H_{25}O_2N$	180°	8,14,18 20,21,26	20,82,83	83
lycofoline	$C_{16}H_{25}O_2N$	142°	25,27	25,55,84	55,84
lycopodine	$C_{16}H_{25}ON$	116°	6-14 16-18 20,21,27 28,T	61,82 85-91	54,57,85 86
N-methyllycodine	$C_{17}H_{24}N_2$	91-92°	30		23,30
nicotine	$C_{10}H_{14}N_2$		7,9,11 12,13,15 19		
α-obscurine (L.6)	$C_{17}H_{26}ON_2$	282-283°	7,8,10 18,19,20 30,T	30,43,61 93	30,93

TABLE II (Continued)

Alkaloid	Formula	Melting Point	References		
			Isolation	Degradation	Structure
β-obscurine (L.6)	$C_{17}H_{24}ON_2$	317-318°	7,8,10 19,20	30,43,61 93	30,93
pillijanine	$C_{15}H_{24}ON_2$	64-65°	2		
pseudoselagine	$C_{16}H_{25}O_2N$	163-163.5°	21		
sauroxine	$C_{17}H_{26}ON_2$	198°	3		
saururine	$C_{10}H_{19}N$		3		
selagine	$C_{15}H_{18}ON_2$	224-226°	29	29,94	29,94
L.3	$C_{18}H_{31}O_2N$		7		
L.4 (L.14)	$C_{16}H_{27}N$		7	82	54,85
L.5	$C_{18}H_{28}O_2N_2$		7		
L.9a	$C_{16}H_{23}ON$		8		
L.9b	$C_{16}H_{25}O_2N$	122°	8		
L.9c	$C_{19}H_{31}O_3N$		8		
L.9d	$C_{20}H_{31}O_4N$	98°	8		
L.10	$C_{16}H_{27}ON$		8		

TABLE II (Continued)

Alkaloid	Formula	Melting Point	References		
			Isolation	Degradation	Structure
L.13	$C_{16}H_{25}ON$	130°	9-13		
L.15	$C_{20}H_{31}O_4N$		9		
L.16	$C_{16}H_{25}ON$		10		
L.17	$C_{18}H_{27}O_3N$		10		
L.18	$C_{11}H_{19}ON$		11		
L.19		231°	11		
L.20	$C_{16}H_{25}O_2N$	259°	12		31
L.21	$C_{13}H_{21}ON$		12		
L.22	$C_{16}H_{27}ON$	108°	12		
L.23	$C_{16}H_{25}O_2N$	161-162°	12		
L.24	$C_{16}H_{25}ON$		12		
L.25	$C_{16}H_{25}O_2N$		12		
L.26	$C_{15}H_{25}ON$	171°	13		
L.28	$C_{17}H_{27}O_2N$		14,19		
L.29	$C_{16}H_{23}O_2N$		14,19		

TABLE II (Continued)

			References		
Alkaloid	Formula	Melting Point	Isolation	Degradation	Structure
L.31	$C_{20}H_{29}O_4N$		14,19		
L.33		218°	15		
L.34	$C_{16}H_{25}O_2N$	236°	16		
L.35	$C_{14}H_{21}O_2N$	133°	16		
Base E	$C_{17}H_{25}O_2N$		26		
Base G	$C_{18}H_{27}O_3N$		26		
Base I		300°d	27		
Base M	$C_{18}H_{27}O_3N$		27	55	55
Base O	$C_{20}H_{31}O_5N$	181-182°	27	27	
U.1	$C_{10}H_{19(21)}ON$		17		
U.2	$C_{16}H_{25}ON$		17		
U.3	$C_{16}H_{21}O_3N$		19		

TABLE II (Continued)

Alkaloid	Formula	Melting Point	References		
			Isolation	Degradation	Structure
U.4	$C_{16}H_{23}ON$		19		
U.5	$C_{17}H_{25}O_2N$		19		
U.6	$C_{17}H_{25}O_3N$		19		
U.7	$C_{18}H_{25}O_3N$		19		
U.8	$C_{18}H_{25}O_4N$		19		

TABLE III

Formula Index of the *Lycopodium* Alkaloids

Formula	Alkaloids
$C_{10}H_{14}N_2$	nicotine
$C_{10}H_{19(21)}ON$	U.1
$C_{10}H_{19}N$	saururine
$C_{11}H_{19}ON$	L.18
$C_{13}H_{21}ON$	L.21
$C_{14}H_{21}O_2N$	L.26
$C_{15}H_{18}ON_2$	selagine
$C_{15}H_{24}ON_2$	pillijanine
$C_{16}H_{21}O_3N$	annotine, annotinine, U.3
$C_{16}H_{22}N_2$	lycodine
$C_{16}H_{23}ON$	fawcettidine, L.9a, U.4
$C_{16}H_{23}O_2N$	acrifoline, L.29
$C_{16}H_{24}ON_2$	des-N-methyl-α-obscurine
$C_{16}H_{25}ON$	isolycopodine, lycopodine, L.13, L.16, L.24, U.2
$C_{16}H_{25}O_2N$	annofoline, clavatine, clavolonine, flabelliformine, lycofoline, lycodoline, pseudoselagine, L.9b, L.20, L.23, L.25, L.34
$C_{16}H_{25}N$	anhydrodihydrolycopodine
$C_{16}H_{26}ON_2$	cernuine
$C_{16}H_{27}ON$	deacetylfawcettiine, dihydrolycopodine, L.10, L.22
$C_{16}H_{27}N$	L.4
$C_{17}H_{24}ON_2$	β-obscurine
$C_{17}H_{24}N_2$	N-methyllycodine

TABLE III (Continued)

Formula	Alkaloids
$C_{17}H_{25}O_2N$	Base E, U.5
$C_{17}H_{25}O_3N$	U.6
$C_{17}H_{26}ON_2$	α-obscurine, sauroxine
$C_{17}H_{26}O_2N_2$	hydroxyobscurine
$C_{17}H_{27}O_2N$	clavatoxine, L.28, Base M
$C_{18}H_{25}O_3N$	O-acetyl acrifoline, U.7
$C_{18}H_{25}O_4N$	U.8
$C_{18}H_{27}O_3N$	L.17, Base G
$C_{18}H_{28}O_2N_2$	L.5
$C_{18}H_{29}O_2N$	acetyldihydrolycopodine
$C_{18}H_{29}O_3N$	fawcettiine, lofoline, lycoclavine
$C_{18}H_{30}ON_2$	flabelline
$C_{18}H_{31}O_2N$	L.3
$C_{19}H_{31}O_2N$	L.9c
$C_{20}H_{29}O_4N$	diacetyllycofoline, L.9d, L.15
$C_{20}H_{31}O_5N$	Base O
$C_{32}H_{44}O_5N_2$	annotoxine

TABLE IV

Melting Point Index of the *Lycopodium* Alkaloids

Melting Point	Alkaloid
64-65°	pillijanine
91-92°	N-methyllycodine
95-96°	acetyldihydrolycopodine
97-104°	acrifoline
98°	L.9d
106°	cernuine
108°	L.22
116°	lycopodine
117°	acetylfawcettiine
118°	lycodine
119-120°	O-acetylacrifoline
122°	L.9b
130°	L.13
133°	L.35
136°	isolycopodine
140°	diacetyllycofoline
142°, 144-145°	lycofoline
150-151° 156-157°	annofoline
161-162°	L.23
163-163.5°	pseudoselagine
166-167° 172-173°	fawcettiine
168°	dihydrolycopodine

TABLE IV (Continued)

Melting Point	Alkaloid
171°	L.26
174°	annotine
178-179° 180°	lycodoline
181-182°	Base O
185-186°	clavatoxine
186-188°	flabelline
196-197°	annotoxine
198°	sauroxine
203-204° 207°	deacetylfawcettiine
210-211°	flabelliformine
211-212°	lofoline
212-213°	clavatine
218°	L.33
224-226°	selagine
231°	L.19
232°	annotinine
236°	L.34
238°	clavolonine
259°	L.20
266-268° 270°	des-N-methyl-α-obscurine
282-283°	α-obscurine
300°	hydroxyobscurine

TABLE IV (Continued)

Melting Point	Alkaloid
300°d	Base I
317-318° 322-323°	β-obscurine

TABLE V

Melting Point Index of Hydrobromide Salts

Melting Point	Alkaloid
274-275°	lycofoline
over 315°	acrifoline
over 337°d	flabelliformine
360°	lycopodine

TABLE VI

Melting Point Index of Hydrochloride Salts

Melting Point	Alkaloid
209.5-211°	annotinine
270-273°	isolycopodine
306-307°d	pseudoselagine
over 300°	annotine

TABLE VII

Melting Point Index of Hydriodide Salts

Melting Point	Alkaloid
237-239° 241.5-242°	annotinine
258-259°d	acrifoline
282-284°	O-acetylacrifoline
297°	annotine
304-305°	lycodoline
307-307.5°	α-obscurine
321°	isolycopodine

TABLE VIII

Melting Point Index of Methiodide Salts

Melting Point	Alkaloid	Melting Point	Alkaloid
184.8°	annotine	261°	U.2
216-217°	U.3	263-264°	lycofoline
236-237°d	annotine	265°	U.4
242-244°	saururine	266-267°	lofoline
249-250°d	acrifoline	267-268°	acrifoline
258°	sauroxine	272°	U.7

TABLE VIII (Continued)

Melting Point	Alkaloid	Melting Point	Alkaloid
279-280°	fawcettiine (hydrate)	304°	L.28
279.5°	pseudoselagine	308-309°	annofoline
280-281°	acrifoline	315°	U.6
281-282°	flabelline	317°	annotine
283°	U.8	317°d	lycodoline
289-290°	deacetylfawcettiine	317-318°	clavatine
ca. 290°	U.1	321°	isolycopodine
292°	L.31	324°	U.5
293-296° 296-297°	fawcettiine	325-328°d	clavolonine
294°	L.29	over 335°	flabelliformine
295.6°	lycopodine		

TABLE IX

Melting Point Index of Methochloride Salts

Melting Point	Alkaloid
210°	U.4
238°	acrifoline
238-240°	lycopodine
244-245°	annotine
250°	U.7
255°	U.3
258°	L.28
261°	L.31
263°	L.29
268°	pseudoselagine
269°	U.6
270°	U.8
270°	isolycopodine
273°d	lycopodine
274°	U.5
313°	lycodoline
316°	U.5

TABLE X

Melting Point Index of Methoperchlorate Salts

Melting Point	Alkaloid
234-236°	U.3
259-260°	U.8
267-268°	U.7
270°	U.4
287.5°	L.29
295°	L.31
321°	L.28
335°	U.6

TABLE XI

Melting Point Index of Methopicrate Salts

Melting Point	Alkaloid
78-80°	U.5
134-136°	U.3
151°	U.6
163°	L.29
219°	U.8
230-232°	U.4

TABLE XII

Melting Point Index of Nitrate Salts

Melting Point	Alkaloid
215-217°	annotoxine
222-223°	annotinine
236°	acrifoline
268-270°	lycopodine
over 380°	annotine

TABLE XIII

Melting Point Index of Picrate Salts

Melting Point	Alkaloid
107°	L.21
121°	U.1
133-134°	α-obscurine
195°	L.18
199-201°	isolycopodine
202°	saururine
205-207°	lycopodine
222-223°	fawcettidine
226°	nicotine
229-233°	lycodine
241-241.5°d	U.2
254°	β-obscurine

TABLE XIV

Melting Point Index of Perchlorate Salts

Melting Point	Alkaloid
110°	cernuine
198-200°	Base G
201°	L.21
209°	L.9c
211°	L.28
217°	L.31
221°	L.16
221-222°	fawcetimine
223°	L.10
223-224°	dihydrolycopodine
224-227°	deacetylfawcettiine
225°	L.4
227-228°	annotoxine
231°	L.15
238-239°	anhydrodihydro-lycopodine
239°	annotine
241-241.5°	U.2
244°	O-acetylacrifoline
246°	L.3
246-247°	acetyldihydro-lycopodine
254°	L.22

TABLE XIV (Continued)

Melting Point	Alkaloid
266°	acrifoline
267°	L.9c
267°	annotinine
267-269°	Base E
271°	L.20
272-275°d	fawcettiine
273.5°	L.9d
274°	L.13
274°	L.29
274°	isolycopodine
276°	L.9a
278°	L.24
280-282°	Base M
280-282°	flabelline
282°	L.5
283°	lycopodine
294-295°d	pseudoselagine
296°	L.17
297°	L.25
300°	L.23
314-315° 318°	lycodoline

Methods of Isolation

The presence of alkaloids in a plant may be established qualitatively by testing extracts from the plant with various alkaloidal reagents. Mayer's reagent (potassium mercuric iodide) is most commonly used as it is usually the most sensitive. About one gram of plant material is digested with 5-10 ml. of dilute HCl and the filtrate tested with a few drops of the reagent. A creamy precipitate or turbidity generally indicates the presence of alkaloids. Other common alkaloid-indicating reagents include Wagner's (iodine-potassium iodide), Dragendorff's (potassium bismuth iodide), Sonnenschein's (phosphomolybdic acid) and Scheibler's (phosphotungstic acid). Other have been listed by Cromwell (35).

Extraction of the total alkaloids from the plant material is generally accomplished either by extraction with acids, or with organic solvents. Solvent extraction is the more common procedure. To remove plant oils, the dried plant material is usually subjected to a preliminary extraction with light petroleum in which most alkaloids are insoluble. However, it is always advisable to extract the light petroleum with dilute acid, and to test the acid solution for the presence of alkaloids in case they are removed by this procedure. The plant material is then treated with alkali (calcium hydroxide, ammonium hydroxide or sodium carbonate) to liberate the bases from their salts and then extracted with a suitable organic solvent (chloroform, ether, benzene, methanol, ethanol or methylene chloride). The alkaloids are then extracted with dilute acid (sulfuric or hydrochloric), followed by basification of the

acid solution and extraction with chloroform or ether to give a crude mixture of bases.

Since most of the alkaloids and their salts are soluble in methanol or ethanol, these solvents can be used for the extraction of the plant material without previous treatment with alkali. After removal of the alcohol, the residue is treated with dilute aqueous acid and the solution filtered to remove resins and fatty material which separates out. The acid filtrate is then basified and extracted with chloroform or ether. Removal of solvent affords a crude mixture of alkaloids. This is the procedure followed by Manske and Marion (7-16) and others (18-26).

Another method is to extract the dried plant material with dilute acid, such as hydrochloric or tartaric. Where there is danger of decomposition of the plant base, the extraction is done in the cold. The aqueous solution is basified and either extracted with an organic solvent or steam-distilled to remove volatile components. Deulofeu and DeLanghe (3) followed this procedure using hydrochloric acid, while Achmatowicz and Uzieblo (6) as well as Burnell and co-workers (27,28) chose tartaric acid. All three sets of workers obtained the crude bases by solvent extraction of the basified aqueous solution.

Methods of Separation and Purification

The separation of individual alkaloids from a solution of crude bases is often necessary as most of the alkaloid-producing plants yield a mixture of closely related alkaloids. Until fairly recently, fractional crystallization, fractional distillation and preparation of

derivatives were the methods generally relied upon for separation of mixtures of alkaloids. Although these processes are often difficult and tedious, excellent separations have been achieved. However, some alkaloid mixtures are so complex that for a while, only the more abundant components were isolated. The development of modern techniques of separation have made possible the study of many minor components. The most commonly applied methods of separation are discussed below.

1. Fractional Crystallization - Some times a single solvent can be used to separate a mixture of two or more alkaloids, but often it is necessary to use a mixture of miscible solvents. Most alkaloids are readily soluble in chloroform and less so in the other organic solvents, the general order being chloroform $>$ acetone $>$ ethanol $>$ methanol $>$ ethylacetate $>$ ether $>$ benzene $>$ hexane. Should fractional crystallization of the free bases fail, fractional crystallization of the salts may effect a separation.

2. Formation of Salts - The salts most frequently used for separation or purification are the hydrochlorides, hydrobromides, hydriodides, methiodides, nitrates, perchlorates, oxalates, and picrates. The acids may be used in aqueous or methanolic solution. The salts can be precipitated from methanolic solution by addition of ether. Hydrochlorides can often be crystallized from hot acetone containing a small proportion of methanol. Addition of ethylacetate to acetone solutions of perchlorates, oxalates and picrates sometimes induces precipitation of these salts.

3. Fractional Distillation - Little need be said on the general principles of this method. Manske and Marion (7-16) used this technique

extensively in their isolation of *Lycopodium* alkaloids.

4. Adsorption Chromatography (36,37) - This technique involves passing a mixture in solution through a porous, insoluble, adsorptive medium. Owing to their selective adsorptions, the constituents of the mixture migrate through the adsorbent at different rates and separate from one another as a series of zones. The rate of migration of a given solute also depends upon the adsorption capacity of the adsorbent, the concentration of the solute, the rate of flow of the solution, and the solvent itself. Clearly, substances which are weakly adsorbed migrate rapidly, while strongly adsorbed substances migrate more slowly.

Activated alumina is the adsorbent most commonly used for separation of alkaloids. Generally speaking, adsorption takes place more readily from non-polar solvents such as petroleum ether and benzene, and less from highly polar solvents such as alcohols and pyridine. When choosing a solvent one must bear in mind that if the mixture is too strongly adsorbed, difficulties arise in development and in the final elution, whereas weak adsorption allows the mixture to run quickly through the column before any separation of constituents takes place. Benzene and chloroform have been found to be quite satisfactory solvents for chromatography of most *Lycopodium* alkaloids.

Ever since Moore and Marion reported the separation of α- and β-obscurine by column chromatography (43) this technique has been widely used in the separation and purification of the *Lycopodium* alkaloids.

5. Counter-current Distribution (38) - This is a liquid-liquid multiple extraction technique very useful for fractionating mixtures. It involves a series of discrete extractions, each being followed by a transfer of one immiscible liquid phase relative to the other.

Liquid-liquid extraction involves the distribution of one or more components between two liquid phases. The fraction of the total of a given solute found in a single phase at equilibrium is determined by a number of factors, such as the relative volumes of the phases, dissociation, association with the solvent, etc. Assuming the simplest case in which the molecules in each phase are in the same state of aggregation, the ratio of the concentrations in the two phases is a constant at a fixed temperature. Thus at equilibrium

$$\frac{C_1}{C_2} = K \qquad (1)$$

where C_1 and C_2 are the concentrations in the lighter and heavier phases, respectively, and the constant K is usually referred to as the distribution constant or partition coefficient. Since extration is primarily concerned with the fraction of total solute in a single phase, matters are simplified when C_1 and C_2 are expressed in weight per unit volume rather than in moles. Fortunately, for most extractions of interest to organic chemists, a certain amount of deviation from constant partition coefficients does not detract seriously from the use of this technique.

To simplify the discussion of fractionating by counter-current distribution, the following assumptions will be made: when two or more solutes are extracted in a two phase system, each behaves independently

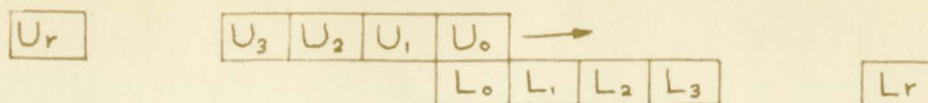
of the others; partition coefficients remain constant over the concentration range used; and the volumes of the two liquid phases are equal.

To further simplify the following discussion it is assumed that the solute has a partition coefficient of 1 in the solvent pair. The separate extraction tubes are numbered 0,1,2,3,...r and the transfers are numbered 0,1,2,3,...n. If unit quantity of the solute is dissolved in L_o and U_o moved over it, the process will be in its initial position. The two phases in tube 0 are equilibrated and, after they have separated, the upper phases can be shifted so that U_o will be over L_1 and U_1 will be over L_o. The first transfer has now been accomplished. Clearly, since the partition coefficient is 1 and the volumes of the two phases are equal, 50% of the solute has been transfered to tube 1 in the upper layer. For the second transfer, both tubes are equilibrated and the upper layers of both shifted so that U_o is over L_2, U_1 is over L_1, and U_2 is over L_o. This process can be continued indefinitely. The table of Figure 1 illustrates the distribution achieved for each transfer.

It can be shown that the fraction $T_{n,r}$ of solute present in the rth tube for n transfers is given by

$$T_{n,r} = \frac{n!}{r!(n-r)!} \left(\frac{1}{K+1}\right)^n K^r \qquad (2)$$

To graphically illustrate a counter-current distribution, one plots either the fraction of total solute or weight of solute as the ordinate against the tube number as abscissa. Figure 2 illustrates three distributions of eight transfers each of solutes with partition coefficients of 0.333, 1.0 and 3.0 corresponding to curves A, B and C respectively.



Tube Number

Transfer Number	0	1	2	3		r
0	1.0					
1	·50	·50				
2	·25	·50	25			
3	·125	375	375	·125		
4	.0625	25	375	25	.0625	
n						

Figure 1 Counter-current Distribution

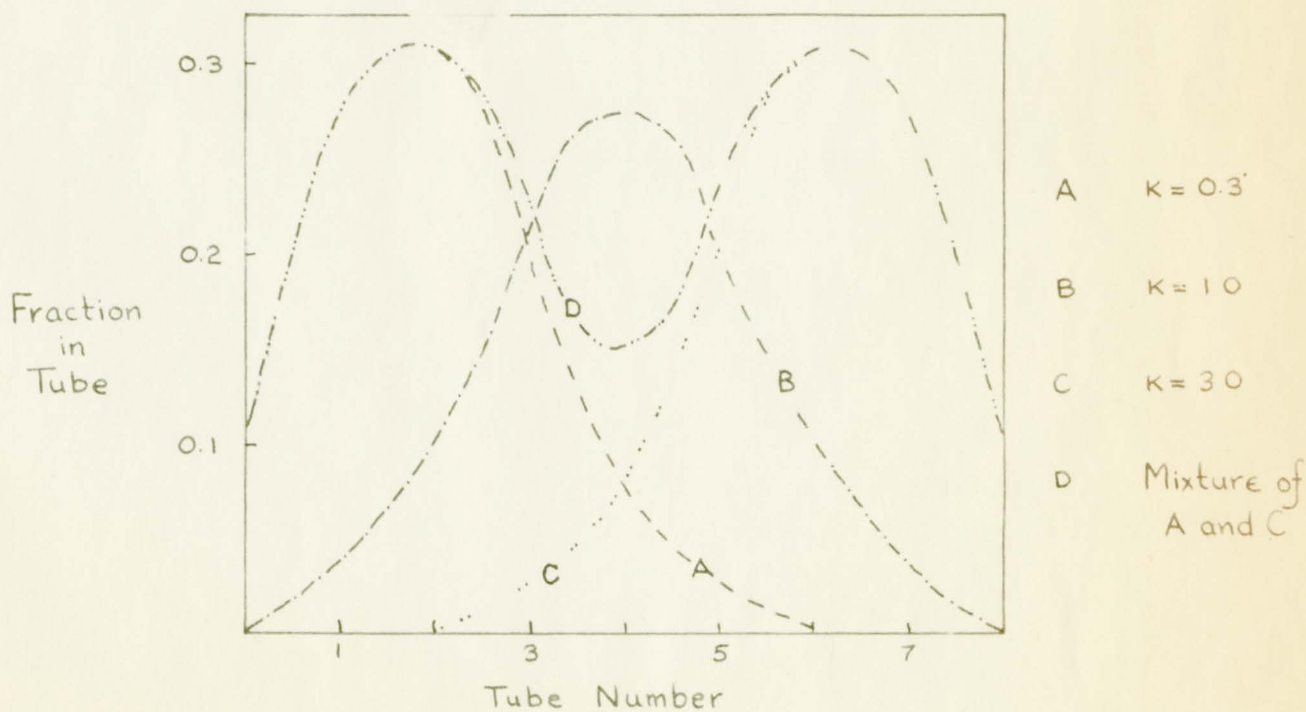


Figure 2. Counter-current Distribution Curves

The position of the maximum of a particular band for any given number of tubes after the band has cleared tube 0 is given by

$$N = \frac{nK}{K + 1} \qquad (3)$$

where n is the total number of transfers and N is the position of the maximum on the abscissa.

When two substances having different partition coefficients are present as solutes, two bands, overlapping or otherwise, will be obtained. For example, if two solutes present in equal amounts having partition coefficients of 1/3 and 3 are distributed with eight transfers, one obtains curve D in Figure 2. Rose and Rose (44) have given as a standard separation, a recovery of 80% of each component at a purity of 95% or better. The separation given in Figure 2 would just give this. However, for two substances having more closely related partition coefficients, such as in curves A and B, one would not get a standard separation. By increasing the number of transfers, from equation 3 it is clear that the distance between the maxima of the two bands will increase. As the number of transfers is increased, the number of tubes occupied by each solute also increases; however, the percentage of the total number of tubes occupied decreases. For example, a solute having K = 1 occupies 60% of the tubes for 25 transfers, 30% of the tubes for 100 transfers, and only 9.5% of the tubes for 1000 transfers.

When using more than 25 transfers, it is advantageous to be able to distribute larger quantities of solute. Unfortunately, if the amount of solute at the beginning is increased, difficulty is encountered, for generally, partition coefficients do not remain sufficiently constant

to permit the use of higher concentrations. By dividing the solute equally among several tubes at the start of the process, higher concentrations can be avoided. This would then give a distribution which is the sum of as many overlapping separate distributions as the number of tubes in which the solute was placed at the start. However, the effect is small if the solute is placed in not more than 5% of the total tubes used.

The number of individual extractions, E, for n transfers is given by

$$E = \frac{n(n+1)}{2} \qquad (4)$$

Thus for, say 25 transfers, 225 individual extractions would be required. From a practical stand point, this would involve too great an expenditure of time. A number of apparatuses have been designed to equilibrate all the tubes for a given transfer, so that in the above example, only 25 equilibrations would be required.

There are several procedures which can be used in counter-current distribution. The more common ones have been classified as:

(a) Fundamental - This is the procedure which has been treated thus far. Here nothing is withdrawn from the system until the whole operation is completed.

(b) Single Withdrawal - Only a fixed number of tubes is used in this procedure. When the fundamental procedure has been completed, the last tube is withdrawn and replaced by an empty one. After proceeding one more step, the last tube is again withdrawn and replaced. This process may be continued indefinitely, or until a standard separation is achieved.

(c) Double Withdrawal - The fundamental procedure is again completed, followed by withdrawal and replacement of both the first and last tubes. This process gives three series of tubes. The ones withdrawn from the first contain only the lower layer, the ones remaining in the system contain both layers, and the ones withdrawn from the last contain only the upper layer.

(d) Alternate Withdrawal - This procedure is a modification of the last. After the fundamental procedure with, say 10 transfers, has been completed, tube 0 is completely withdrawn and a new tube added to the end. Following the next equilibration and transfer, tube 11 is withdrawn and replaced by another new tube. On the next transfer, tube 1 is withdrawn and a new tube introduced at position 12. Tube 12 is set aside on the next step and replaced by a new one. This process can be continued indefinitely and can be represented by three curves. This method is most advantageous for separating complicated mixtures and substances having closely related partition coefficients.

Counter-current distribution of Lycopodium alkaloids has been used extensively by Anet and co-workers (22,25) and by Burnell and co-workers (26-28). Both groups distributed the alkaloids between chloroform and phosphate buffers.

6. Paper Chromatography (36,39) - The resolution of mixtures of solutes on filter paper may depend on surface adsorption, on ion exchange or on partition between two immiscible solvents. The last factor is the predominant one. Fundamentally, the only difference between partition chromatography and paper chromatography is that in the latter case, a strip of filter paper acts as the inert support for

the stationary aqueous phase, and the solution is drawn into the paper by capillary action. In principle, there is no difference between the separations achieved by the two methods.

Paper chromatography is more of a qualitative technique because generally only small amounts of material (about 10-50 μg) are used. From the number of spots found on a developed chromatogram, one can determine the complexity of a mixture, or the purity of a supposedly pure alkaloid.

This technique has been used to only a limited extent in the investigation of Lycopodium alkaloids.

7. Other Techniques with Limited Application - Paper electrophoresis (39,40,41) is essentially the same as paper chromatography, except that an electric potential is used to induce migration of the solute.

Partition chromatography (36) is a fractional liquid-liquid extraction technique, where a solution of a mixture is placed on a column packed with some solid capable of holding water, e.g., silica gel, and eluted with water-immiscible solvents.

Ion exchange (42) is based on an exchange of ions in solution with those attached to, or incorporated within, the molecular structure of insoluble substances called ion exchangers. Since alkaloids form strongly basic cations, their isolation from large volumes of highly dilute aqueous extracts may be accomplished by ion exchange techniques.

None of these techniques have been applied in the study of Lycopodium alkaloids.

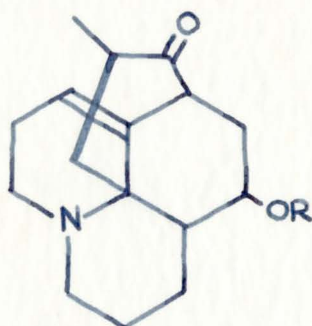
Pharmacological Action

The Lycopodium alkaloids have been found to induce a variety of pharmacological actions, none of which have any medical applications. Lethal doses for small animals have been determined by Oficjalski (45), Lee and Chen (46) and Achmatowicz and Rodewald (47). Other effects have been studied by Achmatowicz and Uzieblo (6), Lee and Chen (46), Nikonorow (48), DeEspanes (49), Marier and Bernard (50) and Muszynski (51).

Structure and Stereochemistry of Lycopodium Alkaloids

Acrifoline (Ia, Ib)

Bertho and Stoll (17) were the first to report degradative work on this alkaloid. Intensive study by French and MacLean (52,53) resulted in the elucidation of skeletal structure Ia for acrifoline. The stereochemistry was later established by Anet (54) and Burnell and Taylor (55).



Ia	R=H	Ib
IIa	R=$COCH_3$	IIb

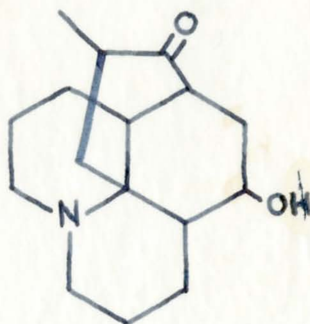
O-acetylacrifoline (L.12)(IIa, IIb)

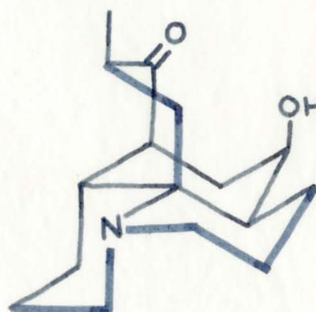
This alkaloid was first shown to be a derivative of acrifoline by Perry and MacLean (20). The stereochemistry was established by

Burnell and Taylor (55)

Annofoline (IIIa, IIIb)

The structure of a dihydroacrifoline was proposed for this alkaloid by Anet and Khan (56). Anet (54) also determined the stereochemistry (IIIb) of annofoline, later confirmed by Burnell and Taylor (57).

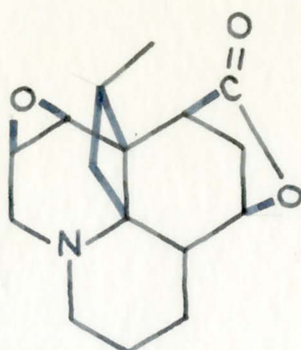




IIIa IIIb

Annotinine (IV)

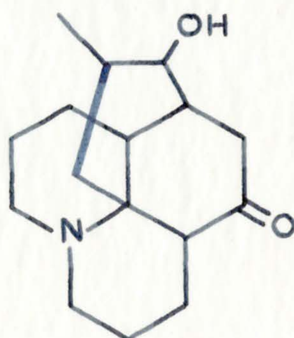
Of all the *Lycopodium* alkaloids investigated, none have been examined as extensively as annotinine. The preliminary investigation of the structure of annotinine was begun by Manske and Marion (14). The degradative studies have been carried out by three Canadian groups led by Marion (58-60) at the National Research Council in Ottawa, by MacLean (61-67) in these laboratories, and by Wiesner (68-77) at the University of New Brunswick. The correct structure (IV) for annotinine was first proposed by Wiesner *et al.* (74) in 1956 and confirmed the following year by the same group (76). About the same time, Marion *et al.* (78) proposed another, but incorrect structure. The structure and stereochemistry were later confirmed by X-ray (79,80) and chemical (77) means.



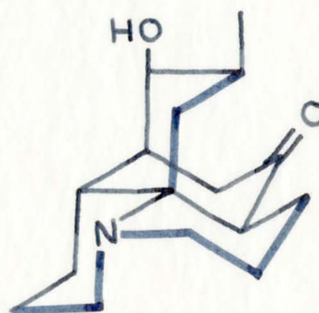
IV

<u>Clavolonine</u> (Va, Vb) <u>and Fawcettiine</u> (VIa, VIb)

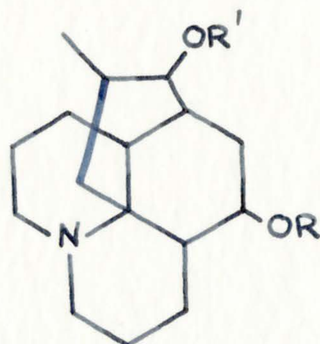
By correlation with annofoline, Burnell and Taylor were able to elucidate the structures (81) and stereochemistry (57) of clavolonine (Va and Vb) and fawcettiine (VIa and VIb).



Va



Vb



VIa	R=$COCH_3$, R'=H	VIb
VIIa	R=R'=$COCH_3$	VIIb
VIIIa	R=R'=H	VIIIb

Acetylfawcettiine (Base K) (VIIa, VIIb)

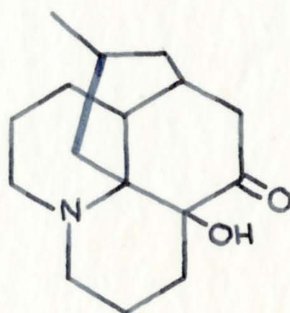
Burnell *et al.* showed this alkaloid to be an acetyl derivative of fawcettiine (27) with structure VIIa (81) and stereochemistry VIIb (57).

Deacetylfawcettiine (Base D) (VIIIa, VIIIb)

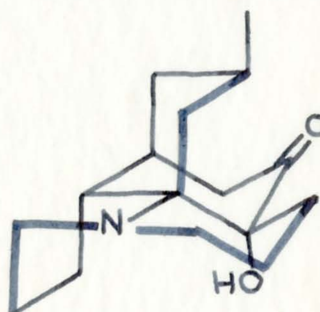
Burnell and Taylor (57,81) were also able to relate this alkaloid to fawcettiine.

Flabelliformine (IXa, IXb)

Structure IXa and stereochemistry IXb have been proposed just recently for flabelliformine by Curcumelli-Rodostamo and MacLean (32).



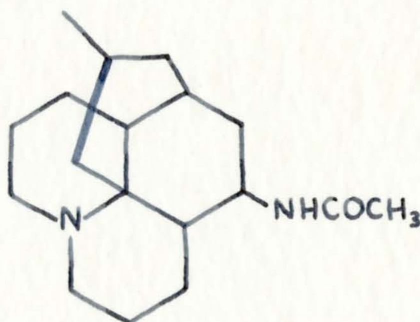
IXa



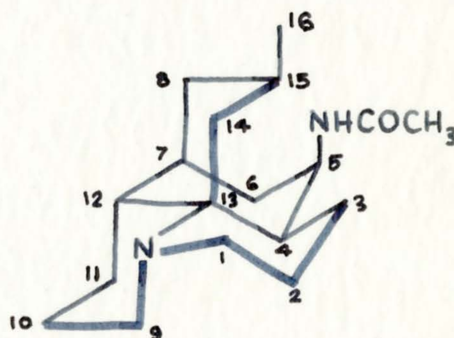
IXb

Flabelline (Xa, Xb)

The elucidation of the structure of flabelline is described in this thesis.



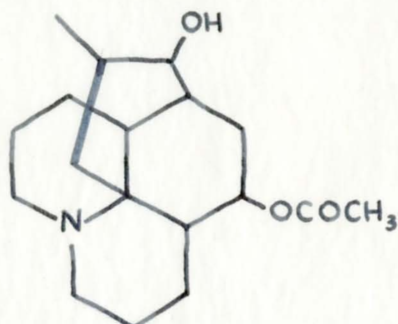
Xa

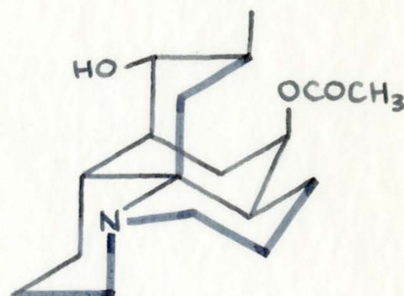


Xb

Lofoline (XIa, XIb)

Lofoline, an epimer of fawcettiine, has been assigned structure XIa and stereochemistry XIb by Burnell and Taylor (57).

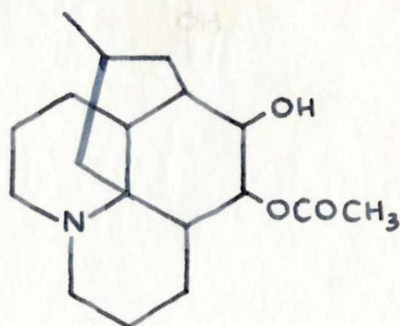


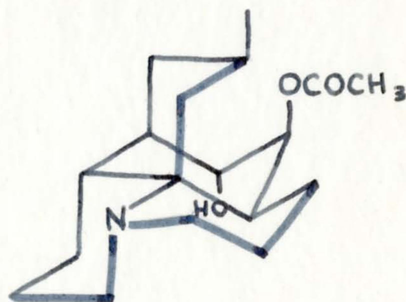


XIa XIb

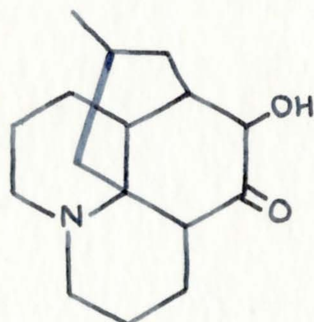
Lycoclavine (XIIa, XIIb) and L.20 (XIIIa, XIIIb)

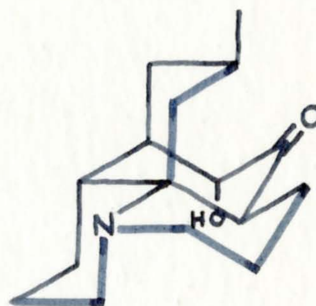
The structures and stereochemistry of these two alkaloids were recently determined by Anet and Law (31) by transformation from lycopodine.





XIIa XIIb

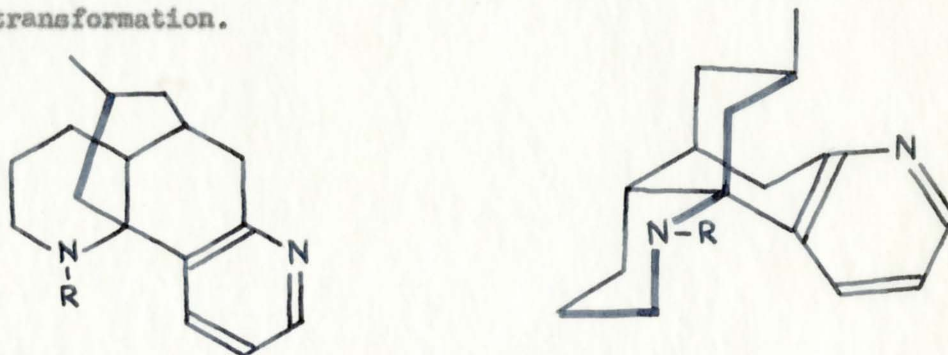




XIIIa XIIIb

Lycodine (XIVa, XIVb)

The nature of the 2,3-substituted pyridine portion of the structure was established by Anet and Eves (22) while Ayer and Iverach (23) later proposed total structure XIVa for lycodine. About the same time, Anet and Rao (24) converted lycopodine to lycodine, thus confirming structure XIVa. Stereochemistry XIVb is deduced from this transformation.



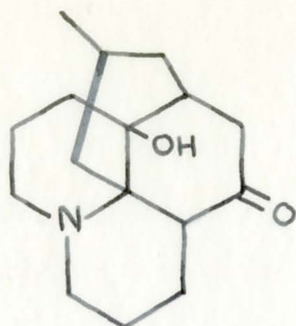
XIVa	R=H	XIVa
XVa	R=CH_3	XVa

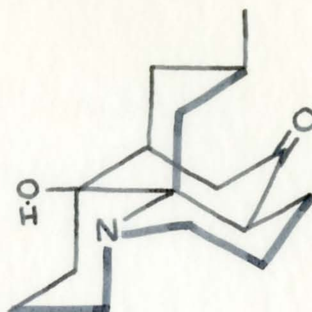
N-Methyllycodine (XVa, XVb)

Structure XVa for N-methyllycodine necessarily follows from structure XIa for lycodine.

Lycodoline (L.8) (XVIa, XVIb)

This alkaloid was shown by Marion et al. (82) to be identical with alkaloid L.30, and preliminary investigation by Perry and MacLean (20) revealed the nature of the functional groups. Recent investigations by Ayer and Iverach (83) have led these workers to propose structure XVIa and stereochemistry XVIb for lycodoline.

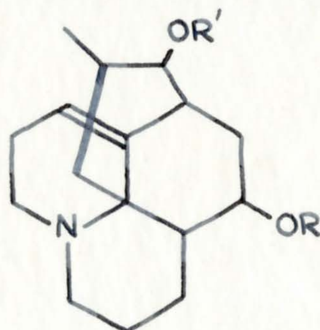


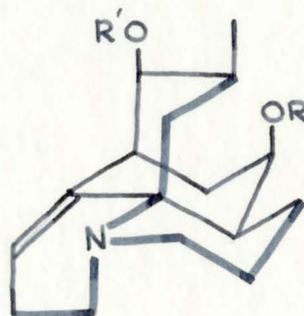


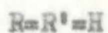
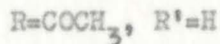
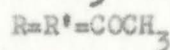
XVIa XVIb

Lycofoline (XVIIa, XVIIb)

The nature of the functional groups was first investigated by Anet and Khan (25). Within the past year, Anet *et al.* (84) and Burnell and Taylor (55) have independently deduced the structure (XVIIa) and stereochemistry (XVIIb) of lycofoline.





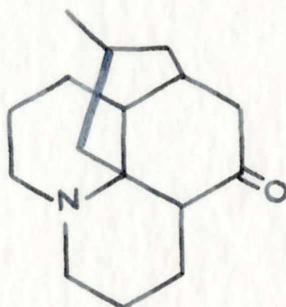
XVIIa	 R=R'=H	XVIIb
XVIIIa	 R=$COCH_3$, R'=H	XVIIIb
XIXa	 R=R'=$COCH_3$	XIXb

Monoacetyllycofoline (Base M) (XVIIIa, XVIIIb) and Diacetyllycofoline (Base N) (XIXa, XIXb)

Burnell and Taylor (55) related these two bases with lycofoline, assigning structures XVIIIa and XIXa to the monoacetate and diacetate derivatives, respectively.

Lycopodine (XXa, XXb)

This major Lycopodium alkaloid was extensively investigated before structure XXa was proposed by Harrison and MacLean (85,86) in 1960. The initial degradative work was done by Manske, Marion and co-workers (82,87,88) at the National Research Council, while the remaining structural investigations were carried out by MacLean and co-workers (61,85,86,89-92) in these laboratories. The stereochemistry (XXb) was later deduced by Anet (54).

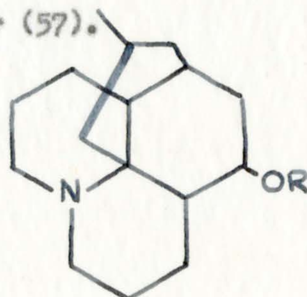


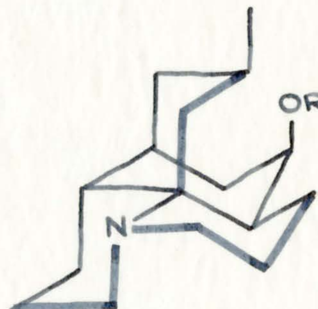
XXa

XXb

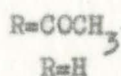
Acetyldihydrolycopodine (L.2) (XXIa, XXIb)

This alkaloid was first shown to be an acetyl derivative of dihydrolycopodine by Douglas, Lewis, and Marion (82). The structure (XXIa) became apparent with the elucidation of the structure of lycopodine, while the stereochemistry (XXIb) was deduced by Burnell and Taylor (57).





XXIa
XXIIIa

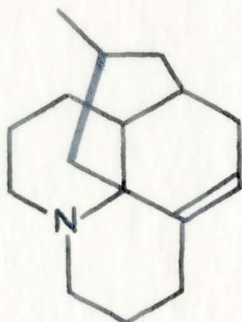


R=$COCH_3$
R=H

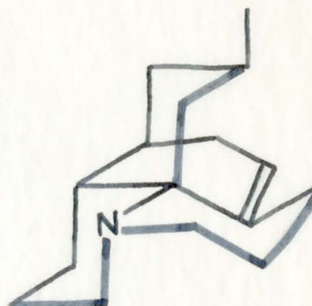
XXIb
XXIIIb

Anhydrodihydrolycopodine (L.14) (XXIIa, XXIIb)

Douglas, Lewis and Marion (82) also were able to relate this base to lycopodine. The stereochemistry XXIIb has been assigned by Anet (54).



XXIIa



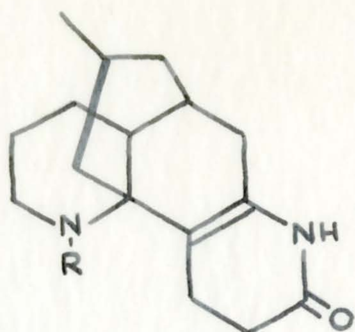
XXIIb

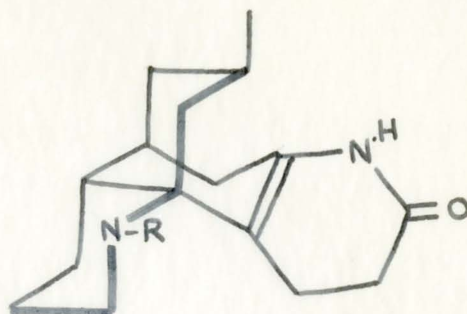
Dihydrolycopodine (L.1) (XXIIIa, XXIIIb)

The relationship between lycopodine and this derivative was first established by Marion et al. (82,88). Structure XXIIIa for dihydrolycopodine was automatically furnished with the establishment of structure XXa for lycopodine. The stereochemistry (XXIIIb) was determined by Burnell and Taylor (57).

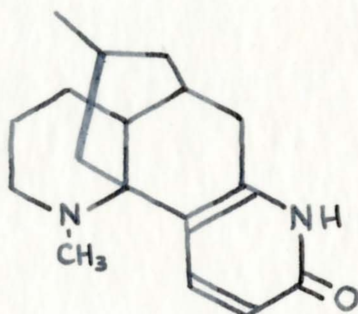
α-Obscurine (XXIVa, XXIVb) and β-Obscurine (XXVa, XXVb)

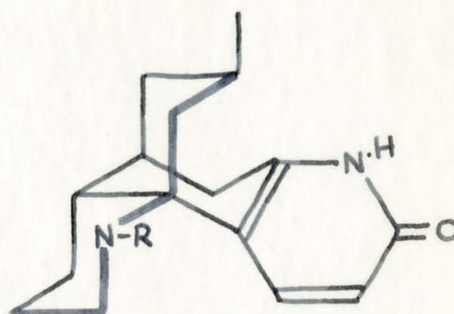
Moore and Marion (43) showed obscurine to be a mixture of two bases, α-obscurine and β-obscurine. In this initial study, they were able to deduce the nature of the α-pyridone rings. Total structures XXIVa and XXVa were later assigned to α- and β-obscurine, respectively, by Ayer and Iverach (93). In a recent paper, Ayer et al. (30) assigned the stereochemistry of the obscurines. The configuration of the N-methyl group is still uncertain and has been tentatively assigned the more favourable equatorial conformation.





XXIVa	R=CH_3	XXIVb
XXVIa	R=H	XXVIb





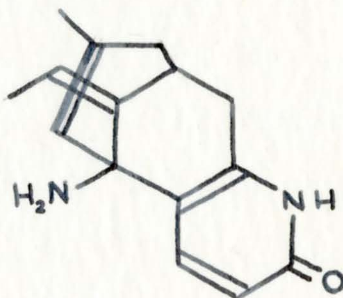
XXVa XXVb

Des-N-methyl-α-obscurine (XXVIa, XXVIb)

This derivative of α-obscurine has structure XXVIa.

Selagine (XXVII)

The structure of selagine was shown by Wiesner et al. (29,94) to be as represented by XXVII.



XXVII

Absolute Stereochemistry of the *Lycopodium* Alkaloids

The relative stereochemistry of the lycopodine-type alkaloids was first elucidated by Anet (54).

The absolute stereochemistry of the *Lycopodium* alkaloids has been determined recently by Wiesner *et al.* (77). From optical rotatory dispersion measurements on lycopodine, application of Djerassi's Octant Rule (95) suggests the absolute stereochemistry to be as shown in formula XXb. This is the mirror-image of structures postulated in previous publications. The absolute stereochemistry of annotinine was confirmed by two additional unrelated methods. From optical rotatory dispersion curves of lycopodine and clavolonine, Burnell and Taylor (57) have independently deduced the same absolute stereochemistry for the *Lycopodium* alkaloids.

Figure 3 shows the inter-relationship of the *Lycopodium* alkaloids. The direction of the arrows indicates conversions which have been achieved.

Biogenesis of *Lycopodium* Alkaloids

Shortly after the structure of annotinine was established, Leete (96) proposed a biogenetic scheme for this alkaloid. This scheme involves initial condensation between β-δ-diketocaproic acid and mevalonic lactone followed by condensation with $HN(CH_2CH_2CHO)_2$ at a later step. However, this pathway cannot be applied to the other *Lycopodium* alkaloids.

A more promising scheme has been proposed by Conroy (97) involving condensation of two unbranched eight-carbon polyacetate

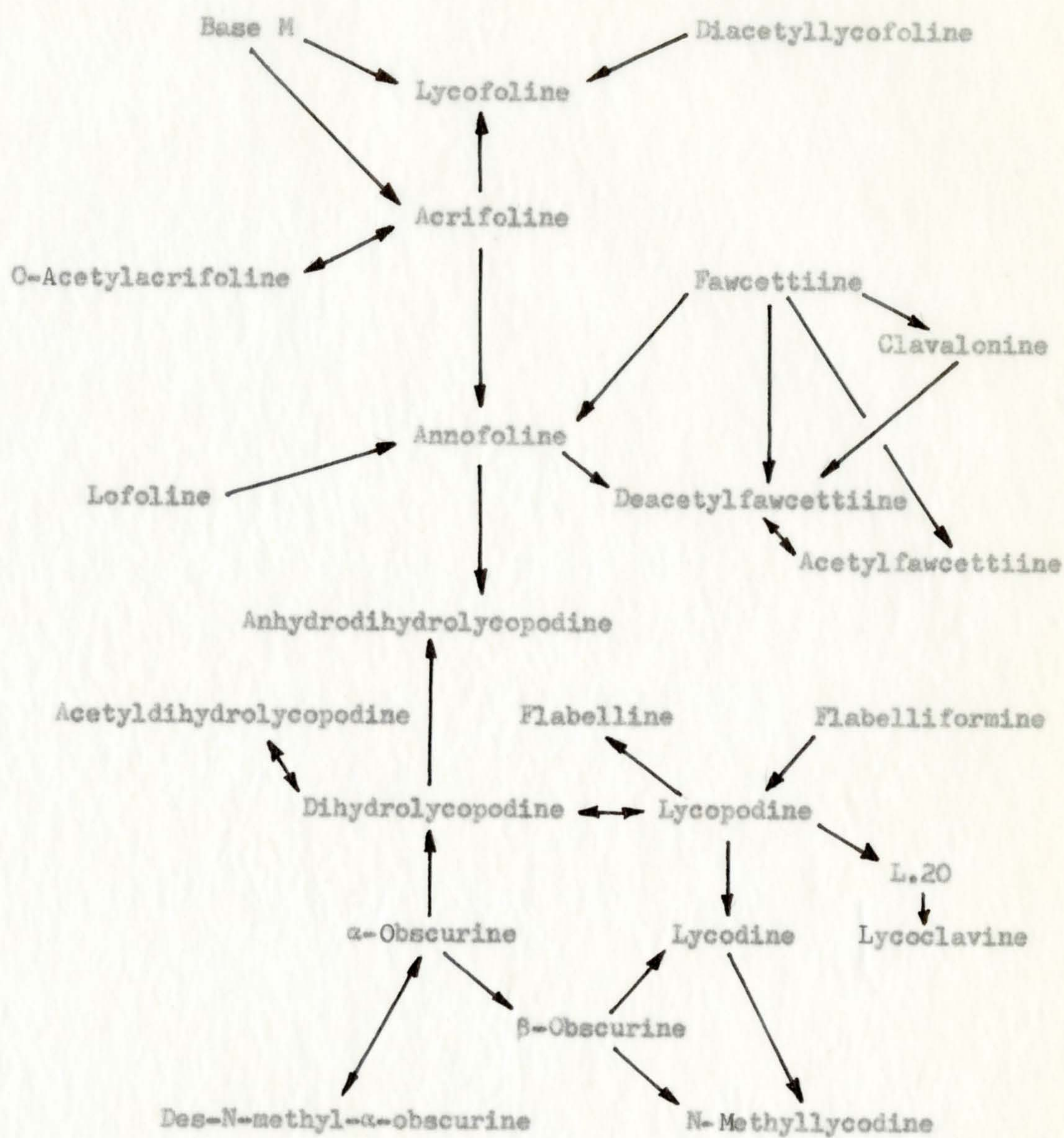
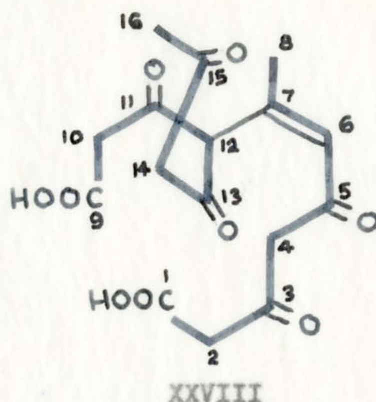


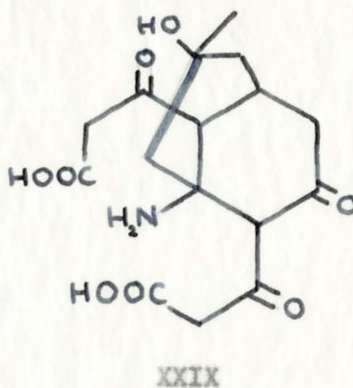
Fig. 3. Inter-relationship of Lycopodium Alkaloids

chains. The first step involves condensation of two molecules of 3,5,7-triketo-octanoic acid, or biological equivalent, to give the common intermediate XXVIII.



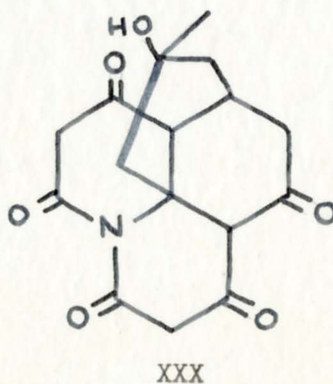
XXVIII

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, gives intermediate XXIX



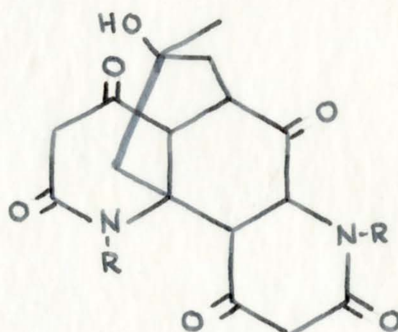
XXIX

For the lycopodine-type alkaloids, two lactamizations give XXX, which can proceed via various steps to the desired base.



XXX

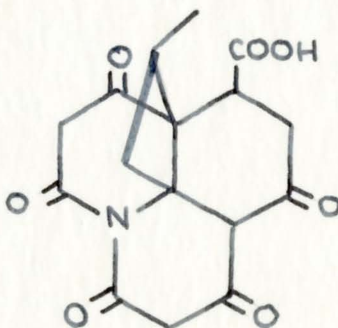
Addition of ammonia to XXIX at C-5, followed by lactamizations at the two amino sites gives XXXI, which can undergo further transformations to yield the obscurines or lycodine.



XXXI

Decarboxylation of C-9 prior to lactamization of the second amine affords a precursor of selagine.

The pathway to annotinine involves oxidation of C-8 followed by condensation of C-12 and C-15 to give the cyclobutane ring. Mannich reaction with ammonia and lactamization gives XXXII



XXXII

Appropriate reduction, epoxide formation with the oxygen at C-11 and lactonization results in annotinine.

The Lycopodium alkaloids have been reviewed by Henry (98), Manske (99), Saxton (100) and Wiesner (101).

DISCUSSION OF RESULTS

Isolation of Alkaloids

Manske and Marion (7) were the first to report an investigation of L. flabelliforme Fern. for its alkaloidal content. They isolated nine alkaloids: lycopodine, acetyldihydrolycopodine, anhydrodihydrolycopodine (L.4, L.14), dihydrolycopodine, nicotine, α-obscurine, β-obscurine, L.3, and L.5; of which only lycopodine and nicotine were known at that time. Since then, the structures of all but L.3 and L.5 have been elucidated.

L. flabelliforme var. ambiguum has been used as a source of lycopodine by other workers in these laboratories. In this study, the mother liquors from several of their extractions were combined and investigated for their contained alkaloids.

The crude alkaloid mixture was first subjected to an acid extraction and filtered through celite to remove resinous material. Non-basic material was then removed by ether extraction. The first chromatography of crude alkaloids was carried out on about 75 g. of material. The column was first eluted with chloroform, effecting removal of the bulk of the crude bases. The more strongly adsorbed bases were removed by successively eluting with 2% methanol in chloroform and 5% methanol in chloroform.

The mixture of crude bases obtained by elution with chloroform partially crystallized. Separation of these crystals yielded material melting at 192.5-196.5°. The infrared spectrum in nujol showed hydroxyl absorption at 3125 $cm.^{-1}$ and carbonyl absorption at 1705 $cm.^{-1}$ Purification of this crystalline material by formation and recrystallization of the perchlorate salt, followed by regeneration of the free base yielded crystals melting sharply at 210°. A change in crystalline form was noted at 150°. The analysis of these crystals fits the formula $C_{16}H_{25}O_2N$. This minor alkaloid of L. flabelliforme had not been reported, and was given the name flabelliformine. It was thought that flabelliformine might be identical with clavatine isolated from L. clavatum by Achmatowicz and Uzieblo (6). However, a comparison of the specific rotation of flabelliformine ($[\alpha]_D^{24}$ = -25.1° (c. 1.106 g./100 ml. acetone) and $[\alpha]_D^{24}$ = -38.4° (c. 3.827 g./100 ml. chloroform)) with that of clavatine ($[\alpha]_D^{20}$ = -365.7° (acetone)) clearly showed that these are two distinct alkaloids. Curcumelli-Rodostamo and MacLean (32) have recently elucidated the structure of flabelliformine, and have shown it to be represented by structure IXa. It was found difficult to separate flabelliformine from traces of dihydrolycopodine by this method. Treatment of the flabelliformine-dihydrolycopodine mixture with hydrobromic acid readily afforded pure flabelliformine hydrobromide, dihydrolycopodine being recovered from the mother liquors.

The mother liquor obtained from the separation of crude flabelliformine was distributed between pH 6.0 citrate-phosphate buffer solution and chloroform in a twelve separatory funnel distribution. The bulk of the more strongly basic alkaloids was partitioned among

the first five funnels, whereas the bulk of the more weakly basic alkaloids was distributed among the last four funnels.

In working up the various fractions, most of the crystalline or semi-crystalline materials obtained were characterized by means of their infrared spectra.

Dihydrolycopodine was isolated from the first four fractions of the first distribution. An alkaloid melting at 186-187.5° was isolated from buffer fractions 3-5. Analysis of this base agrees with the formula $C_{18}H_{30}ON_2$. This new alkaloid was given the name flabelline. The elucidation of the structure of flabelline is described below. The fifth buffer fraction also yielded α-obscurine.

The last three chloroform fractions have been investigated by Alam (33) and found to contain lycodine, annotinine, and α-obscurine. Although lycodine and annotinine are known, this is the first recorded isolation of these alkaloids from L. flabelliforme. Furthermore, this is the first recorded isolation of annotinine from a Lycopodium species other than L. annotinum.

Alam has also investigated the mixture of crude bases obtained by elution with 2% methanol in chloroform. By means of column chromatography and separatory funnel distribution, he has isolated des-N-methyl-α-obscurine, clavolonine, α-obscurine, and a base, the analysis of which points to a hydroxy-α-obscurine ($C_{17}H_{26}O_2N_2$). Of these alkaloids, α-obscurine was the only one known to occur in L. flabelliforme.

A second batch of the crude alkaloid mixture was chromatographed and worked up as described above. Flabelliformine could not be isolated

from the chloroform eluate as after the first chromatography.

The chloroform eluate was distributed between pH 6.0 buffer solution and chloroform as in the first distribution. Dihydrolycopodine was obtained from the first two buffer fractions and flabelline, from the first three. Flabelliformine was found in the first five buffer fractions. Fractions 3-9 yielded α-obscurine, and lycopodine was obtained from the last three buffer fractions.

Distribution of the chloroform eluate between pH 4.9 buffer solution and chloroform yielded the same alkaloids as did SD2, however, dihydrolycopodine, flabelline, and flabelliformine were found only in the first three buffer fractions. This made their separation more laborious than in SD2.

The fundamental procedure was followed for all distribution performed in this study. To conserve time, the separatory funnel distributions were carried out with a large amount of material, the concentration of the crude bases in the initial chloroform solution being in the order of 0.35 M (based on an average molecular weight of 275). It is not surprising then that the pH of the buffer solutions increased by as much as 0.6 pH units while being shaken with the basic chloroform solutions. Since the mixtures distributed in this study were very complex and the number of transfers small, one cannot expect to achieve much more than a separation of the weak from the strong bases. The observed change in pH will not detract seriously from the usefulness of this preliminary separation, provided all distributions of crude bases are performed under the same conditions. Distribution between chloroform and pH 6.0 buffer solution was found to give a

fairly clear-cut separation.

If a more complete separation of individual alkaloids is desired on the first distribution, the use of high crude base concentrations must be avoided. This can be achieved to a certain degree, by dividing and placing the mixture into the first two separatory funnels. A dozen or sc transfers would not be enough to effect the desired separation. However, by completing the fundamental procedure with the number of tubes available and then performing one of the withdrawal procedures, many more transfers can be achieved. Thus a more complete separation with a minimum of equipment can be effected.

The tendancy of the alkaloids to decompoase while in solution was a constant annoyance throughout the course of this work. Flabelline was difficult to purify because of this. The decomposition products could be removed by chromatography, but the eluted bases tended to decompose if left in solution. The salts of the alkaloids were not so prone to decompose as were the free bases.

It was found that at best, only 75% of the original total mass of each buffer fraction could be isolated as crystalline or semi-crystalline material. The remaining mass that apparently was lost during the isolation of the alkaloids much have been comprised of solvent incompletely removed and decomposition products.

Many of the buffer fractions yielded dark resinous residues after the bulk of the alkaloids had been removed by fractional crystallization. The residues which could not be induced to yield additional crystalline material were not worked up further.

Base I, isolated by Burnell _et al_. (27) from _L_. _fawcettii_, and

hydroxy-α-obscurine isolated from L. flabelliforme appear to be identical. The infrared spectrum of hydroxy-α-obscurine in nujol shows absorption at 3500 $cm.^{-1}$ in the hydroxyl region, a band at 3220 $cm.^{-1}$ in the NH region, and absorption at 1660 $cm.^{-1}$ attributed to a lactam carbonyl. Base I shows infrared absorption bands arising from the same functional features at 3480 $cm.^{-1}$, 3200 $cm.^{-1}$ and 1662 $cm.^{-1}$, respectively. Furthermore, hydroxy-α-obscurine shows ultraviolet absorption at λ_{max} 254 mμ (log ε = 3.78) and base I shows λ_{max} 252 mμ (log ε = 3.77). Finally, both alkaloids melt at 300°.

Alkaloid L.5 was isolated from L. flabelliforme by Manske and Marion (7) as a perchlorate salt, and from its analysis was incorrectly assigned formula $C_{18}H_{28}O_2N_2 \cdot HClO_4$*. The analysis reported by Manske and Marion can, however, be accommodated by $C_{18}H_{30}ON_2 \cdot HClO_4$, the formula for flabelline perchlorate. Furthermore, the melting point reported for L.5 perchlorate (282°) coincides with that found for flabelline perchlorate (280-282°). It is therefore quite likely that L.5 and flabelline are identical.

The Structure of Flabelline

The presence of a secondary amide group is suggested from the infrared spectra of Flabelline in nujol and chloroform.

The infrared spectrum of flabelline in nujol (see figure 4) shows a split band at 3250 $cm.^{-1}$ and 3180 $cm.^{-1}$ in addition to a weak

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* The calculated elemental percentages given by Manske and Marion do not correspond with either $C_{18}H_{28}O_2N_2 \cdot HClO_4$ or $C_{18}H_{28}ON_2 \cdot HClO_4$.

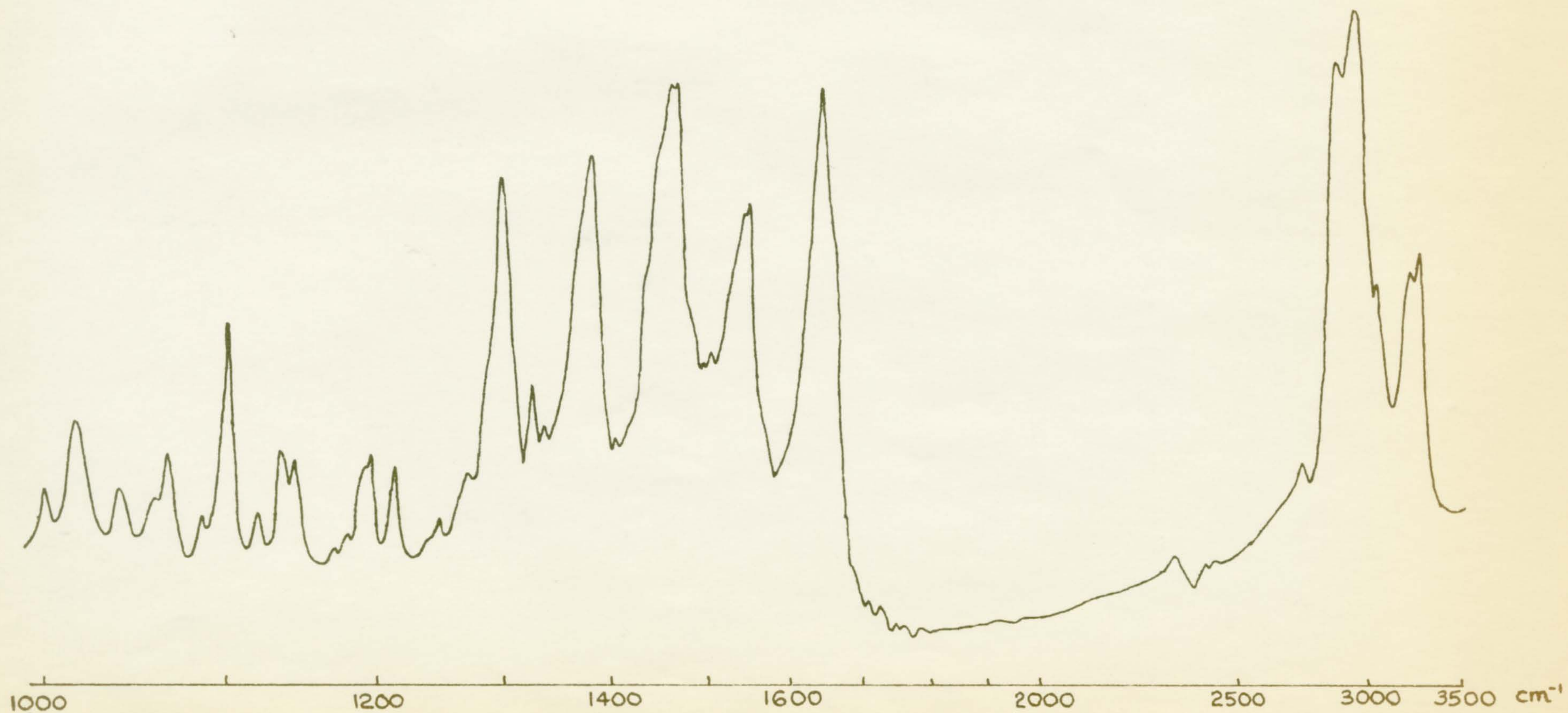
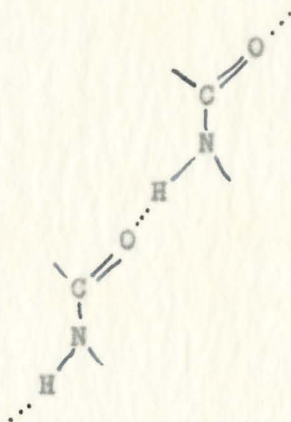
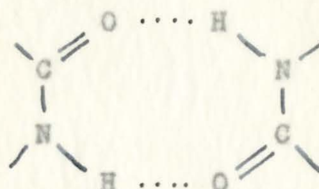


Figure 4. Infra-red Spectrum of Flabelline in Nujol

band at 3050 cm.$^{-1}$, all attributed to amide NH stretching. The absorption at 3250 cm.$^{-1}$ has been assigned by Darmon and Sutherland (102) to intermolecular hydrogen bonding in the trans-form XXXIII whereas the 3180 cm.$^{-1}$ absorption arises from the cis-configuration XXXIV.





XXXIII

XXXIV

The intensity of the 3250 cm.$^{-1}$ band is greater than that of the 3180 cm.$^{-1}$ band, indicating that in the solid state, flabelline is predominantly trans hydrogen bonded. The absorption bands at 1645 cm.$^{-1}$, 1550 cm.$^{-1}$, and 1300 cm.$^{-1}$ are designated as the amide I, II and III bands, respectively. The amide I band has been assigned to carbonyl absorption (103a) whereas the origins of the amide II and III bands have not been definitely established (103b).

In dilute chloroform solution (see figure 5) there is a sharp, concentration-independent, absorption band of medium intensity at 3415 cm.$^{-1}$. This band is attributed to non-hydrogen bonded amide NH stretching (103c). As the concentration of the solution is increased, a band shifting continuously from 3315 cm.$^{-1}$ to 3270 cm.$^{-1}$ appears. This

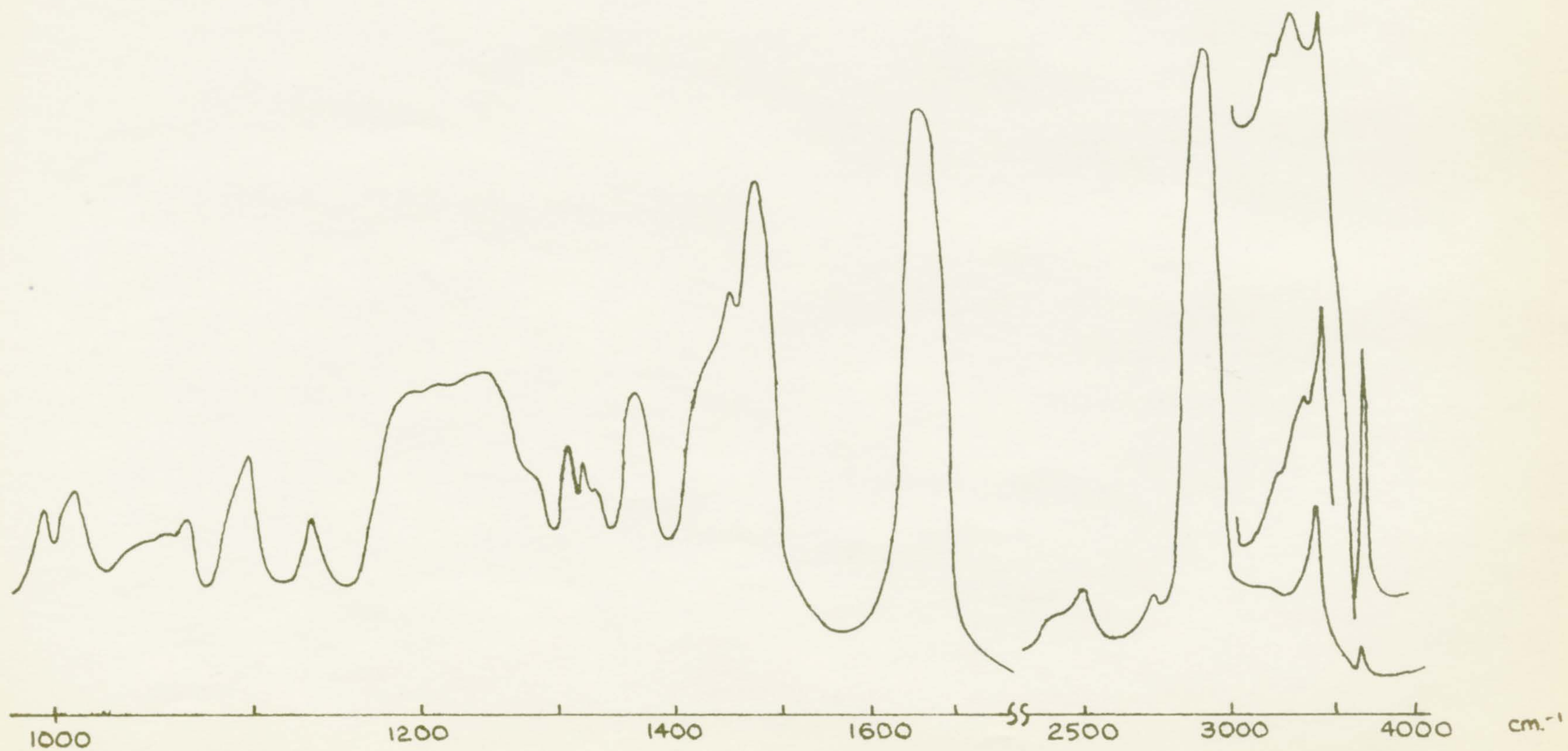


Figure 5. Infra-red Spectrum of Flabelline in Chloroform

band has been assigned by Mizushima et al. (104) to trans intermolecular hydrogen bonding and is taken as evidence that variations in the length of the polymer chain are taking place with change in concentration. The intensity of the hydrogen bonded band increases greatly, relative to the non-bonded band, with increase in concentration. A shoulder at 3170 $cm.^{-1}$ also appears at higher concentration. This concentration-independent absorption has been assigned to a hydrogen bonded cis-configuration. The amide I, II and III absorption bands are found at 1656 $cm.^{-1}$, 1475 $cm.^{-1}$, and 1308 $cm.^{-1}$, respectively. There is also a very sharp, concentration-independent, absorption band of weak intensity at 3670 $cm.^{-1}$ The origin of this band is unknown. It is unlikely that this band arises from an enolic -OH (i.e., from $CH_3C(OH)=N-$), for results of X-ray studies on secondary amides support the keto form.

The nuclear magnetic resonance spectrum (60 Mc/sec) of flabelline in deuterochloroform (see figure 6) shows a single peak of intensity corresponding to one proton shifting continuously from $\tau = 2.72$ at higher concentration to $\tau = 3.39$ at lower concentration. This peak is indicative of an amide NH. The chemical shift of 0.67 τ is undoubtedly associated with the change in degree of intermolecular hydrogen bonding with change in concentration.

A single peak at $\tau = 7.98$ with intensity corresponding to three protons suggests the presence of a methyl group attached to a carbonyl.

The NMR spectrum of flabelline also shows a doublet (splitting 6 c/s) at $\tau = 9.11$ of intensity corresponding to four protons. This band indicates the presence of $>CH-CH_3$ grouping, where the methyl proton peak is split into a doublet by spin-spin coupling with the

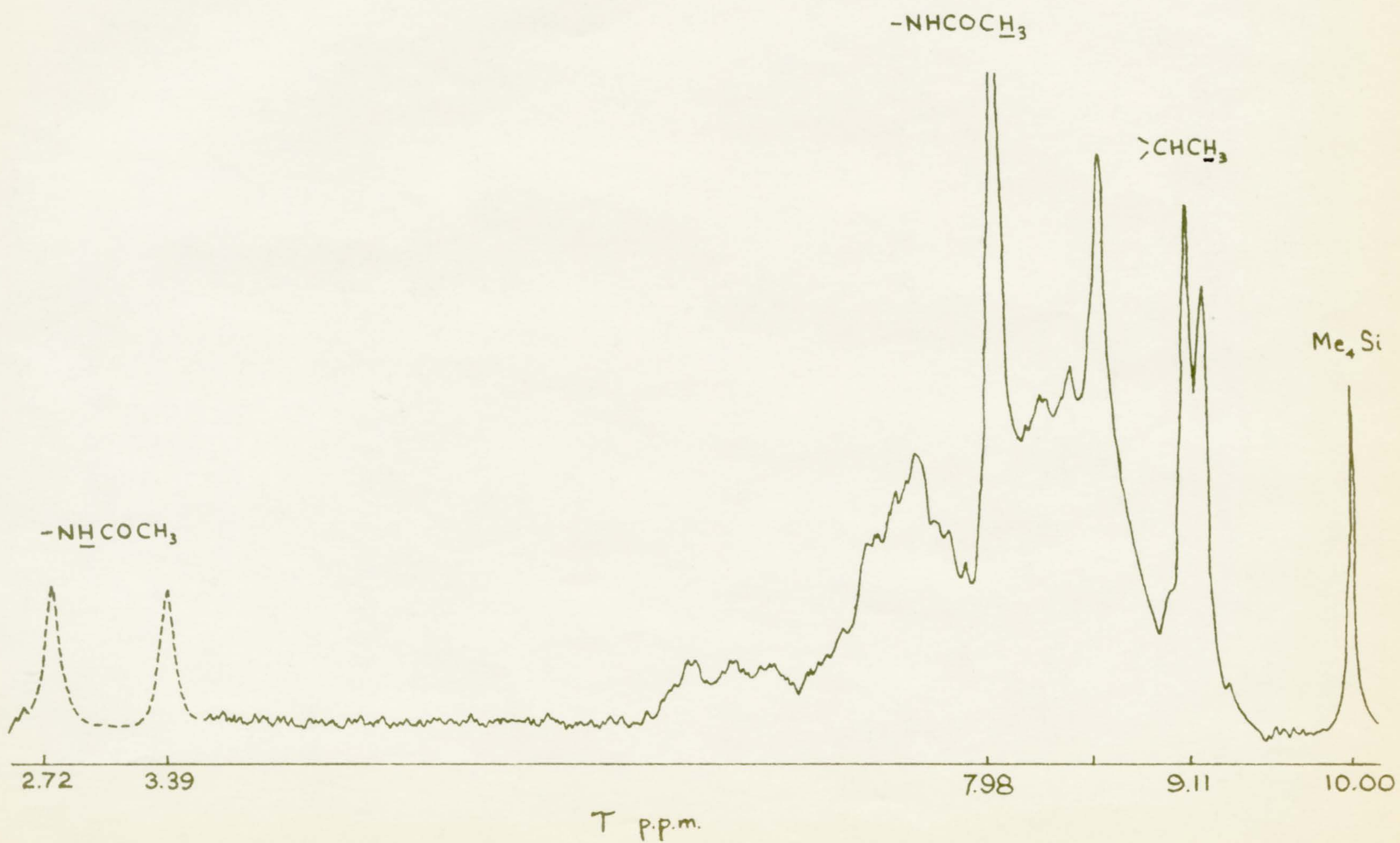


Figure 6. NMR Spectrum of Flabelline

adjacent proton. One would expect a doublet with intensity corresponding to only three protons for this grouping. Itis possible that there is underlying absorption, as indicated by shoulders on this peak.

From the infrared and NMR spectra and the elemental analysis, it was reasoned that flabelline might conceivably be accommodated by structure Xa. Structure Xa was then shown to be correct by synthesis of flabelline from lycopodine.

There are several methods for converting a carbonyl to an amine. Since lycopodine oxime was readily obtainable from lycopodine, it was decided to carry out the synthesis from the oxime. Lycopodine oxime did not reduce on treatment with hydrogen over platinum oxide in acetic anhydride. Treatment with lithium aluminum hydride in dry tetrahydrofuran yielded an oil, which resisted all attempts at crystallization. The infrared spectrum of the oil showed a strong broad absorption band at 3280 $cm.^{-1}$ in the amine NH stretching region. and another broad band at 1635 $cm.^{-1}$ in the amine NH deformation region.

The lycopodine oxime reduction product was treated with acetyl-chloride-pyridine complex to give a dark oil which resisted all attempts to obtain a crystalline product. The oil showed an infrared spectrum bearing some similarity to that of flabelline. This was the first indication that structure Xa was correct for flabelline.

Since the attempted reduction of lycopodine oxime with platinum oxide and hydrogen at low pressure (48 p.s.i.g.) was unsuccessful, it was hoped that reduction under more forcing conditions would yield the desired product. Treatment of lycopodine oxime in acetic anhydride with Raney nickel and hydrogen at high pressure

(1160 p.s.i.g.) and temperature (100°) yielded a crystalline product showing infrared and NMR spectra identical with those of naturally occurring flabelline. There was no depression of the melting point on admixture of the reaction product with flabelline isolated from *L. flabelliforme*. This simple conversion confirmed structure Xa for flabelline.

Flabelline is the first *Lycopodium* alkaloid shown to contain an acetamide group and also the first alkaloid containing two nitrogen atoms shown to possess the hexahydrojulolidine ring system.

Stereochemistry of Flabelline

The conversion of lycopodine to flabelline involved reaction only at C-5, and therefore both alkaloids must have the same configuration at all other sites. Since all of the other alkaloids with a functional group (other than a carbonyl) at C-5 have the axial configuration at this centre, it would be reasonable to expect that the amide group in flabelline has the same configuration. However, this evidence is only circumstantial.

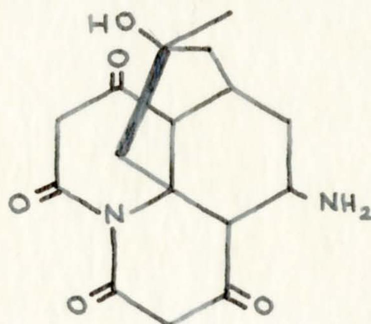
The stereochemistry of lycopodine oxime is such that when this compound is adsorbed on Raney nickel, a hydrogen atom would saturate C-5 in the equatorial position, pushing the NH_2 group into the axial position. Acetylation of the amine would not affect the configuration at C-5.

Acid hydrolysis of flabelline proceeded slowly, further indicating that the amide group is in the more hindered axial position.

On the basis of these three arguments, the amide group at C-5 has been assigned the axial conformation as shown in structure Xb.

Biogenesis of Flabelline

The structure of flabelline is compatible with the biogenetic scheme proposed by Conroy (97). Once intermediate XXIX has formed (*vide supra*), there are several pathways leading up to the total biogenesis of flabelline. One may involve formation of intermediate XXX which undergoes addition of ammonia at C-5 to give XXXV.



XXXV

Another possible pathway involves addition of ammonia to XXIX at C-5, followed by two lactamizations with the amine on C-13 to give XXXV. Intermediate XXXV might then be reduced and acetylated to yield flabelline.

EXPERIMENTAL

Apparatus, Methods, and Materials

All melting points were taken on a Kofler Micro Hot Stage unless otherwise specified. Infrared spectra were measured in nujol mull unless otherwise specified and were determined on a Beckman Model IR-5 recording spectrophotometer with a sodium chloride prism. Ultraviolet spectra were measured in methanol on a Perkin-Elmer Model 4000 Spectracord. The nuclear magnetic resonance spectra were measured on a Varian V-4300 B high-resolution spectrometer at a frequency of 60 Mc/sec. The NMR spectra were measured in concentrated deuterochloroform solutions with tetramethylsilane as an internal standard. Chemical shifts are given on the τ scale of Tiers (105).

The analyses were done by Schwarzkopf Microanalytical Laboratory, Woodside, N.Y.; E. Thommen, Basel, Switzerland; and Galbraith Laboratories, Inc., Knoxville, Tennessee.

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* var. *ambiguum* collected in the Wentworth Valley of Nova Scotia. The dry, ground plant material was extracted by the procedure of Manske and Marion (7). Lycopodine was isolated by column

chromatography as described by Barclay and MacLean (89). The other more strongly adsorbed alkaloids were eluted and combined to give the mixture of crude alkaloids used in these experiments.

Preliminary Purification of Crude Alkaloids

To a collection of the crude alkaloids obtained after removal of lycopodine was added several liters of water and several hundred grams of tartaric acid. The resultant solution was steam distilled to remove organic solvents used in the original extractions. Several more liters of water were added to the distillation residue, and the aqueous solution was shaken with about 60 grams of powdered celite. Suction filtration through a pad of celite yielded a dark red filtrate which was then extracted twice with ether to remove 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, yielding about 200 grams of a black, tar-like residue.

First Chromatography of Crude Alkaloids

About 75 grams of the crude alkaloid extract was taken up in a minimum of warm chloroform and adsorbed on a 5 x 45 cm. column of alumina. The column was 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, yielding dark resinous fractions weighing 14 grams and 3 grams respectively. These last two fractions have been examined by Alam (33).

Isolation of Flabelliformine

The mixture of crude bases obtained by elution with chloroform partially crystallized and the semi-solid mass was shaken with cold ether. The insoluble crystalline material (13.0 g.) was separated by filtration and melted at 192.5-196.5°. The infrared spectrum showed a broad band at 3125 $cm.^{-1}$ in the hydroxyl region, a sharp band at 1705 $cm.^{-1}$ with shoulders at 1720 $cm.^{-1}$ and 1665 $cm.^{-1}$ in the carbonyl region, and a band at 1410 $cm.^{-1}$ attributed to a methylene adjacent to a carbonyl.

To 12.6 g. of the impure bases isolated above dissolved in a minimum of warm 10% aqueous acetic acid was added 5.5 ml. of perchloric acid. A perchlorate salt precipitated immediately and was filtered on cooling. The salt was recrystallized from 10% aqueous acetic acid yielding 17.7 g. of long straw coloured needles which melted at 261-264°, after drying in vacuo at 110°.

Calc. for $C_{16}H_{25}O_2N \cdot HClO_4 \cdot H_2O$: C, 50.33; H, 7.39; N, 3.67%.

Found: C, 50.38; H, 7.53; N, 3.22%.

The infrared spectrum showed a split band at 3600 $cm.^{-1}$ and 3470 $cm.^{-1}$ in the hydroxyl region, a sharp band at 1715 $cm.^{-1}$ in the carbonyl region, a band at 1418 $cm.^{-1}$ attributed to a methylene adjacent to a carbonyl, and a band at 1595 $cm.^{-1}$

Concentration of the flabelliformine perchlorate filtrates yielded brown plates showing an infrared spectrum similar to dihydrolycopodine perchlorate. The free base was regenerated from the perchlorate salt and recrystallized several times from acetone to yield elongated crystals melting sharply at 210°. A change in crystalline

form occurred at 150°.

Calc. for $C_{16}H_{25}O_2N$: C, 72.96; H, 9.57; N, 5.32%.

Found: C, 73.04; H, 9.68; N, 5.33%.

The infrared spectrum in nujol showed a sharp band in the hydroxyl region at 3435 $cm.^{-1}$, carbonyl absorption at 1700 $cm.^{-1}$, and a band at 1414 $cm.^{-1}$ assigned to a methylene group adjacent to the carbonyl. In chloroform solution, the above bands were at 3560 $cm.^{-1}$, 1705 $cm.^{-1}$ and 1415 $cm.^{-1}$ respectively.

$[\alpha]_D^{24}$ = -25.1° (c. 1.106 g./100 ml. acetone)

$[\alpha]_D^{24}$ = -38.4° (c. 3.827 g./100 ml. chloroform)

Preparation of Flabelliformine Methiodide

When treated with methyl iodide in acetone solution, flabelliformine gave a crystalline methiodide which was recrystallized from methanol. The salt did not melt under 335°.

Calc. for $C_{16}H_{25}O_2N \cdot CH_3I$: C, 50.37; H, 6.96; N, 3.45%.

Found: C, 50.75; H, 7.08; N, 3.35%.

First Separatory Funnel Distribution of Crude Bases (SD1)

The mother liquour obtained from the separation of crude flabelliformine was evaporated to dryness and the resultant residue was dissolved in 350 ml. of chloroform. The chloroform solution was then shaken with 350 ml. of a pH 6.00 citrate-phosphate buffer solution (106) for 15 minutes in a one liter separatory funnel. The chloroform phase, after separation was complete, was transferred to a second separatory funnel containing 350 ml. of fresh buffer solution, and 350 ml. of fresh chloroform was added to the first separatory funnel. Both funnels were then shaken for 15 minutes each. This procedure was

continued until 12 transfers had been completed.

The resulting extracts were worked up as outlined below. The chloroform fractions were separated from their respective buffer fractions and each washed with ammonia to basify any salts which may have formed. The chloroform extracts were then dried over anhydrous sodium sulfate and evaporated to dryness in tared flasks. The buffer fractions were basified with ammonia and extracted with chloroform. The chloroform extracts were then dried over anhydrous sodium sulfate and evaporated to dryness in tared flasks. Each flask was weighed, the weights of the bases in each flask being recorded (see Table XV) and plotted against flask number (see Figure 7).

TABLE XV

Distribution of Alkaloids in SD1

Flask No.	Buffer Fraction (Wt. in grams)	Chloroform Fraction (Wt. in grams)
1	3.631	0.155
2	4.426	0.365
3	4.021	0.365
4	2.622	0.347
5	1.56	0.186
6	0.972	0.235
7	0.531	0.386
8	0.529	0.831
9	0.778	2.361
10	1.273	3.704
12	0.670	6.867

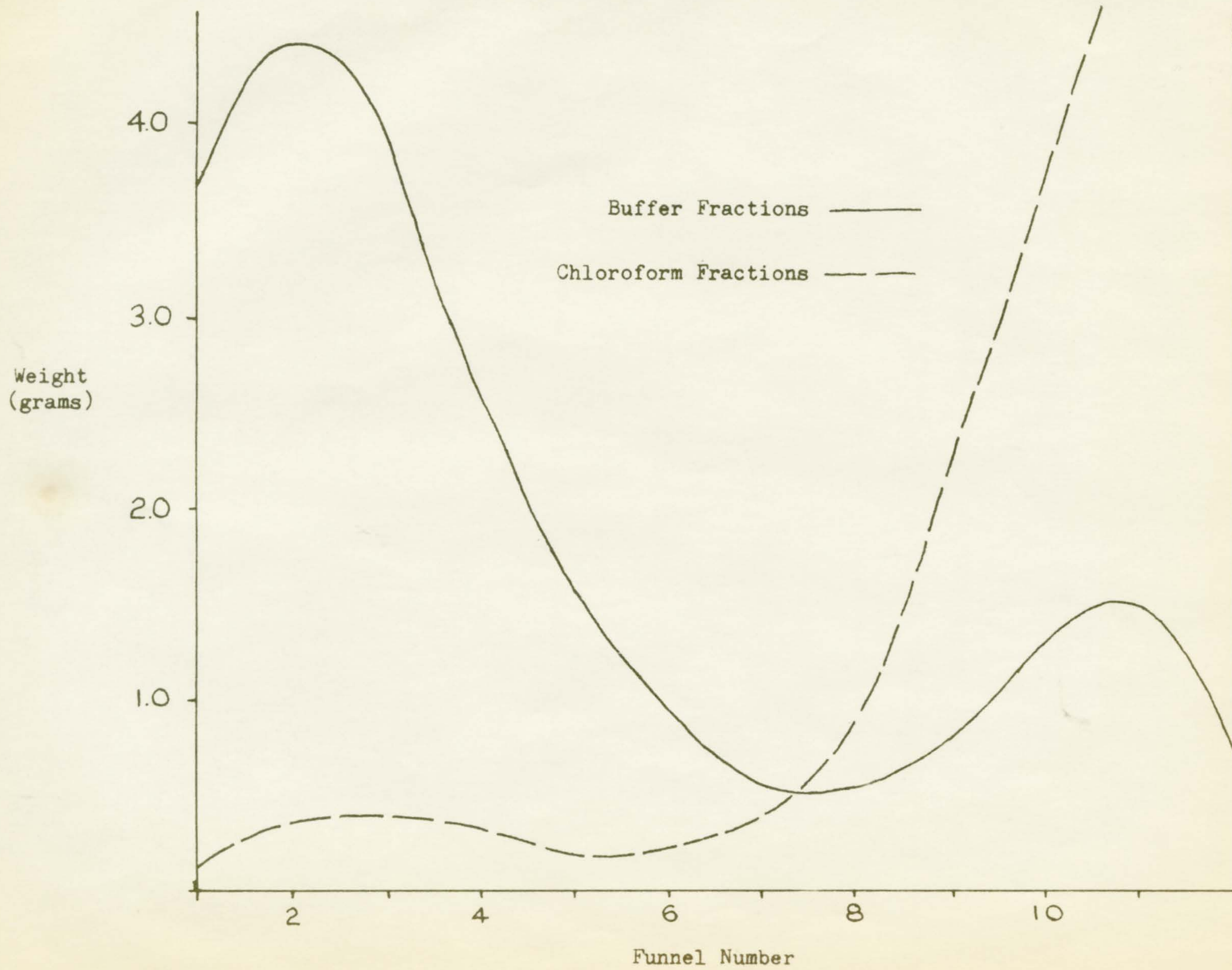


Figure 7. Separatory Funnel Distribution SD1

Resolution of SD1.1B*

Crystalline material formed on treatment with acetone. Recrystallization from acetone yielded 3.0 g. of dihydrolycopodine.

Resolution of SD1.2B

This fraction was worked up in the same manner as SD1.1B, yielding 4.0 g. of dihydrolycopodine.

Resolution of SD1.3B

Crystalline material formed on treatment with acetone. Recrystallization from acetone yielded two crops of dihydrolycopodine weighing 2.0 and 0.5 g. The filtrate was evaporated to dryness, dissolved in a minimum of chloroform, and adsorbed on a column of alumina. Elution with 0.5% methanol in chloroform effected removal of a yellow band giving 1.26 g. of a yellow oil which resisted all attempts at crystallization. The oil was taken up in acetone, made slightly acid with perchloric acid and treated with water until the solution was turbid. Cooling in a refrigerator yielded crystals which were recrystallized from acetone-methanol to give small white crystals melting above 258° (with decomposition and effervesence). The infrared spectrum showed bands at 3480 $cm.^{-1}$, 3300 $cm.^{-1}$, a shoulder at 3150 $cm.^{-1}$ and bands at 1650 $cm.^{-1}$ and 1515 $cm.^{-1}$ The mixture of salts was taken up in water, basified with ammonia, and extracted with chloroform. The combined extract was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was combined with fraction SD1.4B and

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* SD refers to a separatory funnel distribution and its number while B refers to the buffer layer and its tube number.

distributed between chloroform and buffer solution (*vide infra*).

Resolution of SD1.4B

This fraction was taken up in a minimum of chloroform, adsorbed on a column of alumina, and eluted with 0.5% methanol in chloroform. A yellow band passed through the column from which a yellow oil was obtained. Treatment with acetone-methanol gave yellow crystals which melted at 150-163°. The infrared spectrum showed bands in the NH region at 3250 $cm.^{-1}$, and the amide carbonyl region at 1650 $cm.^{-1}$ and 1515 $cm.^{-1}$ The crystalline material and filtrates were combined with the residue from SD1.3B and distributed as described in the next section.

Counter-current Distribution of SD1.3B Residues and SD1.4B

The residue remaining after removal of dihydrolycopodine from fraction SD1.3B and fraction SD1.4B were combined and dissolved in 50 ml. of chloroform. The chloroform solution was divided and placed in the first two tubes of a 49 tube Craig counter-current extractor. The alkaloid mixture was then distributed between chloroform and pH 6.0 citrate-phosphate buffer solution. The machine was agitated for 15 minutes for each transfer, the buffer layer being the moving phase. When the 49 transfers had been completed, the contents of each tube were made alkaline with ammonia, shaken, and the chloroform layer removed. The remaining aqueous layers were then extracted with fresh chloroform. The chloroform extract for each tube was dried over anhydrous sodium sulfate and evaporated to dryness in tared flasks. The weight of material in each flask was recorded (see Table XVI) and plotted against tube number (see Figure 8).

TABLE XVI

Distribution of Alkaloids in CD1

Tube No.	Weight (mg.)	Tube No.	Weight (mg.)
1	34	33	63
2	60	34	67
3	21	35	110
5	8	36	160
7	7	37	158
9	4	38	66
11	7	39	139
13	6	40	159
15	2	41	182
17	1	42	134
19	1	43	234
21	3	44	102
23	5	45	101
25	4	46	150
27	6	47	92
29	8	48	139
31	22	49	76
32	44		

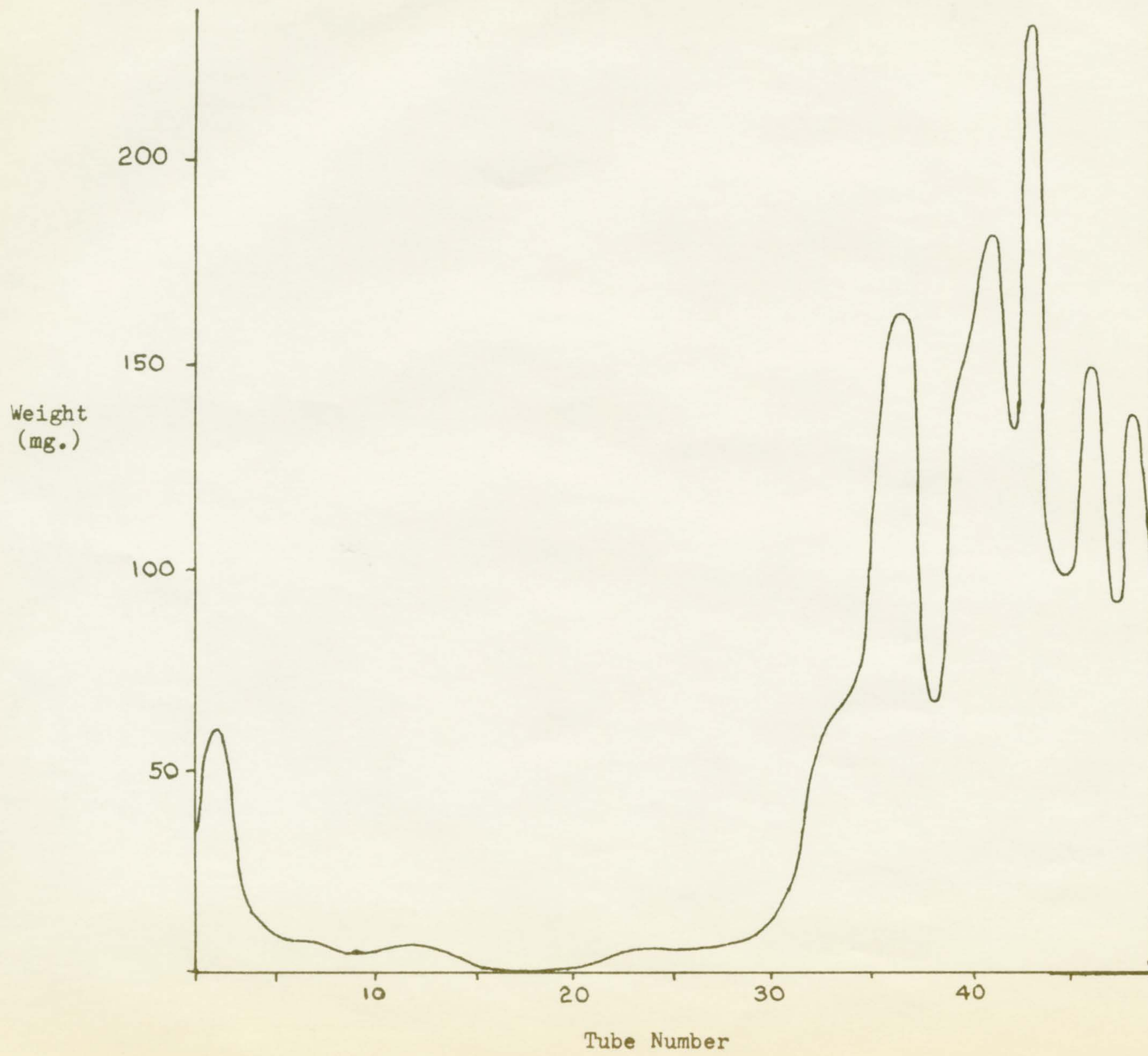

Figure 8. Counter-current Distribution CD1

Fractions 35-38 were taken up in acetone and combined. After initial concentration of the solution, ether was added and the solution further concentrated. Yellow rhombic crystals formed on cooling. These crystals (250 mg.), after filtering and washing with 2:1 ether-acetone, melted at 185-187.5°.

Calc. for $C_{18}H_{30}ON_2$: C, 74.48; H, 10.34; N, 9.66%.

Found: C, 74.39; H, 9.94; N, 9.97%.

The infrared spectrum of flabelline showed a split band at 3250 $cm.^{-1}$ and 3180 $cm.^{-1}$ in the NH region, and absorption at 1645 $cm.^{-1}$, 1550 $cm.^{-1}$, and 1300 $cm.^{-1}$ attributed to the amide I, II and III bands.

Fractions 39-43 were combined, adsorbed on a column of alumina and eluted with 1:1 benzene-chloroform. A wide yellow band passed through the column from which was obtained a yellow oil which crystallized on treatment with 2:1 ether-acetone. Recrystallization from 2:1 ether-acetone yielded about 400 mg. of flabelline.

Fractions 44-47 were combined, adsorbed on a column of alumina and eluted with 1.5% methanol in benzene. A fluorescent band quickly passed through the column from which light yellow crystals were obtained. Recrystallization from ether-petroleum ether gave about 250 mg. of dihydrolycopodine.

Fraction 48 was adsorbed on a column of alumina and eluted with 1% methanol in benzene. A colourless band rapidly passed through the column from which material showing an infrared spectrum identical with that of dihydrolycopodine was obtained.

Resolution of SD1.5B

This fraction was taken up in benzene, adsorbed on a column of

alumina and successively eluted with 1:1, 1:2 and 1:3 benzene-chloroform. The resultant 15 fractions were collected into 3 groups on the basis of their infrared spectra. Treatment of the first group with ether gave an amorphous precipitate which was then crystallized from 2:1 ether-acetone, yielding flabelline. The filtrate was taken up in 10% aqueous acetic acid and treated with excess perchloric acid. A dark solid and a crystalline material came down on standing in a refrigerator. Filtration yielded 125 mg. of dark solid and 108 mg. of crystals, both showing infrared spectra similar to that of flabelline perchlorate. After the filtrate was concentrated and allowed to stand, a second crop (59 mg.) of flabelliformine perchlorate separated. The second group resisted all attempts to obtain a crystalline compound. The third group was taken up in a 2:1 ether-acetone and yielded long needles on standing in a refrigerator. Several recrystallizations from 2:1 ether-acetone gave needle-like crystals of α-obscurine.

Second Chromatography of Crude Alkaloid Extracts

About 120 grams of the remaining crude alkaloid extract was taken up in a minimum of warm chloroform, divided into four equal portions and adsorbed on four chromatographic columns, each packed with two pounds of alumina. The columns were eluted with chloroform until the eluates were almost colourless. The eluates were combined and evaporated to dryness, yielding 90 g. of dark resinous material. The columns were then eluted with 2% methanol in chloroform and 5% methanol in chloroform, yielding dark resinous fractions weighing 13 and 3.5 g. respectively.

Second Separatory Funnel Distribution of Crude Bases (SD2)

About 45 g. of the mixture of crude bases obtained by elution with chloroform was taken up in 400 ml. of chloroform. The chloroform solution was then shaken with 400 ml. of a pH 6.00 citrate-phosphate buffer solution for 10 minutes in a one liter separatory funnel. The fundamental counter-current distribution procedure was carried out for 12 transfers. The resulting extracts were worked up as outlined for SD1. The weight of material in each flask was recorded (see Table XVII) and plotted against flask number (see Figure 9).

Resolution of SD2.1B

Crystalline material formed on treatment with acetone. Recrystallization from acetone yielded 2.377 g. of dihydrolycopodine. Concentration of the filtrate gave a second crop (541 mg.) of dihydrolycopodine. An amorphous salt formed when perchloric acid was added to the filtrate, and on filtration, 110 mg. of flabelline perchlorate was obtained. The acidic filtrate was then basified, and the regenerated bases chromatographed on a column of alumina. The eluates were combined and evaporated to dryness. Treatment with 4:1 acetone-methanol gave a white, finely divided suspension of flabelliformine hydrobromide (345 mg.). Concentration of the filtrate yielded 40 mg. of crystalline flabelliformine hydrobromide.

Resolution of SD2.2B

Treatment with acetone gave crystalline material which, when recrystallized from acetone, yielded 1.263 g. of dihydrolycopodine. Crystallization of the filtrate gave a binary mixture of crystals which was separated manually, after filtration, to yield 118 mg. of impure

TABLE XVII

Distribution of Alkaloids in SD2

Flask No.	Buffer Fraction (Wt. in grams)	Chloroform Fraction (Wt. in grams)
1	6.468	0.174
2	4.393	0.257
3	3.773	0.359
4	2.000	0.394
5	1.217	0.316
6	0.623	0.343
7	0.477	0.635
8	0.634	1.012
9	0.705	1.493
10	0.856	2.432
11	0.687	2.996
12	0.329	8.550

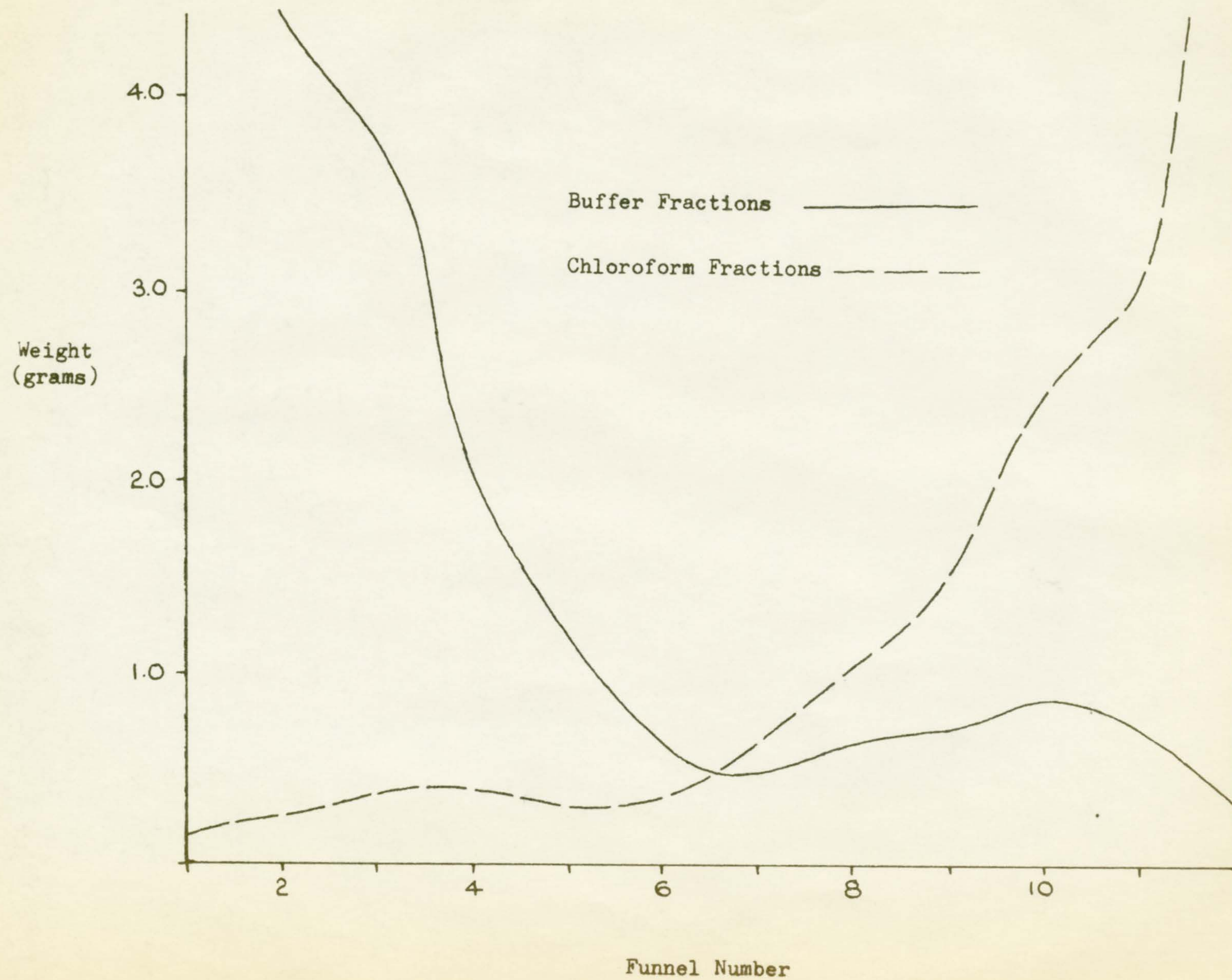

Figure 9. Separatory Funnel Distribution SD2

dihydrolycopodine and 15 mg. of impure flabelliformine. The filtrate was treated with perchloric acid until slightly acid. The resultant amorphous salt was crystallized from acetone to give three crops of flabelline perchlorate weighing 326, 128 and 20 mg. The free bases were regenerated from the filtrate from which the amorphous salt was separated and chromatographed on a column of alumina. The eluates were combined, evaporated to dryness and taken up in acetone. The solution was made slightly acid with hydrobromic acid giving finely divided flabelliformine hydrobromide (267 mg.).

Resolution of SD2.3B

Treatment with acetone gave an amorphous precipitate which was recrystallized from the same solvent. The resulting binary mixture of crystals was filtered and separated manually to give 112 mg. of α-obscurine and 370 mg. of impure flabelliformine. The filtrate was made slightly acid with perchloric acid, giving an amorphous salt which, when crystallized from acetone, yielded 305 mg. of flabelline perchlorate. The filtrate gave a second crop of crystals (52 mg.) showing an infrared spectrum similar to that of flabelline perchlorate. The free bases, regenerated from the acidic filtrate, were adsorbed on a column of alumina and eluted with chloroform. The eluates were combined, concentrated, and taken up in acetone. Addition of hydrobromic acid resulted in the precipitation of a finely divided salt (114 mg.) showing an infrared spectrum similar to that of flabelliformine hydrobromide.

Resolution of SD2.4B

Treatment with acetone yielded both crystalline and amorphous

material, which were manually separated after filtration. The crystalline material (96 mg.) showed an infrared spectrum similar to that of α-obscurine, while the amorphous material (239 mg.) showed an infrared spectrum similar to that of flabelliformine. Concentration of the filtrate again yielded a mixture of amorphous and crystalline material which was separated as above. This gave 40 mg. of crystalline flabelliformine and 187 mg. of amorphous material showing an infrared spectrum similar to that of flabelliformine. Concentration of the filtrate yielded a third crop of amorphous material (63 mg.) showing an infrared spectrum similar to that of flabelliformine.

Resolution of SD2.5B

Treatment with acetone gave an amorphous precipitate (266 mg.) which was filtered and crystallized from acetone to yield two different kinds of crystals. Filtration and manual separation gave 82 mg. of flabelliformine and 10 mg. of material which could not be characterized. Concentration of the filtrate from the recrystallization gave two crops of amorphous material (51 and 25 mg.) showing infrared spectra very similar to that of flabelliformine. The first filtrate was concentrated, giving two crops of finely divided material (75 and 66 mg.) showing infrared spectra very similar to that of flabelliformine. The filtrate was concentrated and acidified with perchloric acid, giving two crops (58 and 164 mg.) of flabelliformine perchlorate.

Resolution of Fractions SD2.6B to SD2.9B

Fractions SD2.6B to SD2.9B, inclusive, were combined and treated with acetone. The resultant amorphous precipitate was crystallized from methanol to give 146 mg. of α-obscurine. The filtrate could not

be induced to crystallize.

Resolution of SD2.10B

This fraction did not precipitate or crystallize on treatment with acetone, and did not form a crystalline perchlorate salt. It was then adsorbed on a column of alumina and eluted with chloroform. The eluates were combined, concentrated, and taken up in acetone. Addition of hydrobromic acid afforded an amorphous salt (141 mg.) which showed an infrared spectrum identical with that of lycopodine hydrobromide. The filtrate was saved and worked up as described in section SD2.12B.

Resolution of SD2.11B

An amorphous salt (154 mg.) was obtained by acidifying with hydrobromic acid an acetone solution of this fraction. The salt had an infrared spectrum almost identical with that of lycopodine hydrobromide. The filtrate was saved and worked up as described in the following section.

Resolution of SD2.12B

This fraction was dissolved in acetone and acidified with hydrobromic acid, giving a dark oil. Crystalline material, (57 mg.) showing an infrared spectrum almost identical with that of lycopodine hydrobromide, was obtained by adding methanol, concentrating and allowing the acidic solution to stand in a refrigerator. The filtrate was combined with the filtrates from SD2.10B and SD2.11B and basified, the regenerated bases being chromatographed on a column of alumina. The eluates resisted all attempts to obtain crystalline material.

Third Separatory Funnel Distribution of Crude Bases (SD3)

The remaining 45 g. of crude bases obtained by chromatography

of the crude plant extracts (eluted with 100% chloroform) was distributed between chloroform and pH 4.9 buffer solution and worked up as in SD2. As before, dihydrolycopodine, flabelline, flabelliformine, α-obscurine and lycopodine were the only alkaloids that could be isolated from the buffer fractions. In this distribution, the alkaloids were isolated from buffer fractions numbered lower than those from the pH 6.0 buffer distribution. The mixture of alkaloids in each of the first three buffer fractions was more complex, and the resolution of these mixtures was more laborious than in SD2.

Preparation of Flabelline Methiodide

A solution of flabelline (79 mg.) in 4 ml. of acetone and an excess of methyliodide was heated under reflux for 3 hours. Crystallization was induced by concentration and scratching. After filtration and recrystallization from acetone-methanol, flabelline methiodide melted sharply at 281-282°.

Calc. for $C_{18}H_{30}ON_2 \cdot CH_3I$: C, 52.78; H, 7.66; N, 6.48%.

Found: C, 53.15; H, 7.34; N, 6.14%.

The infrared spectrum had N-H absorption at 3200 $cm.^{-1}$ and carbonyl absorption at 1653 $cm.^{-1}$

Preparation of Flabelline Perchlorate

Several drops of perchloric acid were added to a solution of flabelline (40 mg.) in a minimum of warm 10% acetic acid. The resultant 37 mg. of short white needles were removed by filtration and melted at 280-282° (with decomposition).

Calc. for $C_{18}H_{30}ON_2 \cdot HClO_4$: C, 55.31; H, 7.94; N, 7.17%.

Found: C, 54.76, 55.44; 7.32, 7.55; 7.00, 7.50%.

The infrared spectrum showed amide NH absorption at 3300 $cm.^{-1}$ and bands at 1658 $cm.^{-1}$ and 1524 $cm.^{-1}$ in the amide I and II regions, respectively.

Attempted Hydrogenation of Flabelline

Flabelline (25 mg.) was dissolved in 50 ml. of methanol and treated with hydrogen (47 psig.) and platinum (20 mg. PtO_2) for 3½ hours at room temperature. The catalyst was removed by filtration and the solvent evaporated. The residue showed an infrared spectrum identical with that of the starting material.

Attempted Hydrolysis of Flabelline

A solution of flabelline (20.55 mg.) in 5.00 ml. of 4 N H_2SO_4 was heated under reflux for 5 hours. The solution was then distilled, 2.55 ml. of distillate being collected. The distillate (2.00 ml.) required 0.98 ml. (after blank correction) of 0.0253 N sodium hydroxide solution to give a potentiometrically determined equivalence point. This corresponds with a recovery of 43.7% of the theoretical amount of acetic acid resulting from complete hydrolysis. Distillation and titration of a known amount of acetic acid under similar conditions gave an 82% recovery of acetic acid in the distillate.

The distillation residue was basified with sodium hydroxide solution and extracted with chloroform. The purple coloured extract was dried over anhydrous sodium sulfate and evaporated to dryness, leaving 15.9 mg. of a dark oil which could not be induced to crystallize.

Preparation of Lycopodine Oxime

To lycopodine (600 mg.) dissolved in 10 ml. of 95% aqueous ethanol was added a solution of hydroxylamine hydrochloride (600 mg.)

in 10 ml. of 95% aqueous ethanol. Addition of potassium hydroxide pellets (3.0 g.) and more ethanol (20 ml.) resulted in the formation of a white fluffy precipitate. The reaction mixture was then heated under reflux for 3 hours and cooled. After the ethanol had been removed by evaporation, the residue was taken up in water and slowly treated with hydrochloric acid until the solution was only slightly basic. The resultant precipitate was filtered to give 440 mg. of lycopodine oxime. Recrystallization from ethanol yielded crystals melting at 275-277°.

Attempted Platinum Oxide Reduction of Lycopodine Oxime

A solution of lycopodine oxime (25 mg.) in 30 ml. of redistilled acetic anhydride containing about 50 mg. of platinum oxide was shaken with hydrogen (49 psig.) for 13 hours. The acetic anhydride was decomposed with ice and the catalyst removed by filtration. The filtrate was basified with ammonia and extracted with chloroform. After the extract was dried over anhydrous sodium sulfate, it was evaporated to dryness under reduced pressure to yield crystalline material. Recrystallization from acetone gave crystals which showed no depression of the melting point on admixture with the starting material.

Lithium Aluminum Hydride Reduction of Lycopodine Oxime

Evolution of hydrogen accompanied addition of lithium aluminum hydride (27 mg.) to a solution of lycopodine oxime (26.8 mg.) in 5 ml. of dry, purified tetrahydrofuran. The mixture was heated under reflux for an hour, and an additional 27 mg. of lithium aluminum hydride was added to the reaction mixture. The mixture was heated under reflux for 4½ hours longer, cooled, and allowed to stand at room temperature

overnight. Excess hydride was destroyed by addition of wet tetrahydrofuran, and the resultant gelatinous hydroxides were filtered and washed with chloroform. The filtrate was dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure leaving 29 mg. of a yellow oil. The infrared spectrum showed a strong broad band at 3280 $cm.^{-1}$ in the amine region. All attempts to obtain a crystalline product were unsuccessful. The residue was adsorbed on a column of alumina and eluted with chloroform. Most of the material rapidly passed through the column from which a brown oil, showing an infrared spectrum similar to that of the residue before chromatography, was obtained. The residue also resisted all attempts to obtain crystalline material.

Acetylation of Lycopodine Oxime Reduction Product with Acetyl Chloride-Pyridine Complex

A solution of the lycopodine oxime reduction product and acetyl chloride-pyridine complex (prepared by mixing together 2 ml. of pyridine and 0.5 ml. of acetyl chloride) in about 10 ml. of pyridine was heated under reflux for 9½ hours. The deep reddish-brown solution was treated with water to destroy excess acetyl chloride, and then basified with ammonia. The basic solution was extracted several times with chloroform. The chloroform solution was extracted with dilute aqueous acid and the acid extract made alkaline with ammonia and extracted with chloroform. The latter chloroform extract was dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure, leaving a dark oil which showed an infrared spectrum vaguely similar to that of flabelline. The residue was adsorbed on a column

of alumina and successively eluted with 4:1, 1:1, and 1:2 benzene-chloroform. The 1:1 and 1:2 benzene-eluates were combined and evaporated to dryness, leaving a brown oil which showed an infrared spectrum similar to that of flabelline. All attempts to obtain a crystalline product were unsuccessful.

High Pressure Reduction and Acetylation of Lycopodine Oxime with Raney Nickel and Acetic Anhydride

Lycopodine oxime (248 mg.), dissolved in 40 ml. of redistilled acetic anhydride was treated with hydrogen (1160 psig.) and Raney Nickel (several hundred milligrams) at 100° for 24 hours. Excess acetic anhydride was destroyed with ice, and the catalyst removed by filtration. The filtrate was made alkaline with ammonia and extracted with chloroform. The extract was dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure, leaving a yellow oil which showed an infrared spectrum almost identical with that of flabelline. The residue was adsorbed on a column of alumina and eluted with 1:1 benzene-chloroform. A small amount of lycopodine quickly passed through the column, whereas the major product passed very slowly through the column. The eluates containing the bulk of the product were evaporated to dryness under reduced pressure to give about 100 mg. of crystalline material showing an infrared spectrum identical with that of flabelline. Recrystallization from acetone-ether gave rectangular plates which showed no depression of the melting point on admixture with naturally occurring flabelline.

The infrared spectrum showed amide NH absorption at 3250 $cm.^{-1}$ and 3180 $cm.^{-1}$ and absorption at 1645 $cm.^{-1}$, 1550 $cm.^{-1}$ and 1300 $cm.^{-1}$

attributed to the amide I, II and III bands respectively.

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