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A STUDY OF

THE ALKALOIDS OF LYCOPODIUM ANNOTINUM L.

By

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2. PREFACE

This research problem was suggested by Professor F.A.L. Anet, the candidate's supervisor, who recognized the need for a more efficient, reliable and reproducible method of separating and isolating the alkaloids of Lycopodium annotinum L. from the "crude" alkaloid. Former methods of separation were dependent upon the tendency of the component alkaloids and their salts to crystallize in various solvents. Aside from the problem of isolation, a study of any new alkaloids of Lycopodium annotinum L. isolated was to be undertaken by the candidate.

First and foremost the candidate is greatly indebted to Professor F. A. L. Anet, whose intelligent direction, high standards, endless patience and personal friendship left nothing to be desired. Special thanks are also due to Professor R.U.Lemieux, Chairman, Dept. of Chemistry, who supervised the writer's initial research during the summer of 1955, in the absence of Professor Anet. Very tangible assistance during the early part of my research was also received from Professor Ludovic Ouellet and Dr. P.M.G.Bavin, then a N.R.C. Post-doctorate Fellow, and is gratefully acknowledged.

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6. ABSTRACT

A more efficient, reliable and reproducible scheme for the quantitative concentration and separation of the alkaloids of Lycopodium annotinum L. has been developed. This scheme involves successive countercurrent distributions using the system chloroform-buffer. The pH values of the buffers are selected, utilizing determined or calculated partition data of the alkaloids. The use of chromatography on buffered paper, as well as by ion-exchange and buffered Celite partition columns, for the separation of the alkaloids of Lycopodium annotinum L. has also been studied.

A new alkaloid, lycodine, $C_{17}H_{24}N_2$, m.p. 118°, has been isolated from Lycopodium annotinum L.. Lycodine is a tetracyclic diacidic base containing a secondary nitrogen, a 2,3-disubstituted pyridine ring and a C-methyl group. The 2,3-disubstitution is in the form of a saturated six-membered ring. It is believed that the carbon in the C8 position, adjacent to the pyridine ring, has two hydrogen atoms attached.

7. INTRODUCTION

(a) Description of the problem.

The term "alkaloid" was originally applied to all organic nitrogenous bases which were alkali-like. Now, however, the use of the name has narrowed to the class of relatively complex, physiologically active bases occurring naturally in plants, but excluding the amino acids and the purines. Apart from their intrinsic value, such as their use in medicine, alkaloids are of great importance because so much of their chemical history is closely associated with the history and study of organic chemistry as a whole.

In the introduction to "The Plant Alkaloids" by Henry (1), a suggested division of the literature of alkaloids is provided as follows:

(i) the occurrence and distribution of these substances in plants;

(ii) biogenesis, or, the methods by which alkaloids are produced in the course of plant metabolism;

(iii) analysis, ranging from the commercial and industrial estimation of particular alkaloids to the separation, purification and description of the individual components of the natural mixture of alkaloids which normally occurs in plants;

(iv) determination of structure; and,

(v) pharmacological action.

The problem with which this thesis deals falls within divisions (iii) and (iv), above. More particularly, the problem was to investigate systematic methods of separation and of isolation and the characterization of the minor alkaloids of the club-moss, Lycopodium annotinum L.. In addition, structural studies of some of these alkaloids were to be made.

(b) Consideration of the pertinent literature.

1) General botanical background. Lycopodium annotinum L. is one species of about one hundred species of the genus Lycopodium (2). Well represented in North America, these plants are small and live mainly in shady woods. They are variously known as "Club Mosses", "Ground Pines", or, "Christmas Greens". Most of the plants have creeping underground stems (rhizomes) from which emerge the aboveground portions of the plants. The largest species, Lycopodium cernuum L., a tropical form, is only two or three feet high.

The Lycopodium species are interesting from the standpoint of the evolutionary history of plants. The genus Lycopodium is included in the group Pteridophyta, comprising the ferns and their allies. The Pteridophyta, together with the Bryophyta (mosses and liverworts), constitute the second great division of plants (3). They share, therefore, a middle position between the essentially aquatic Thallophytes (algae and fungi) and the essentially terrestrial Spermato-

phytes, to which all the higher plants belong (4). Their geological history, as shown through their appearance as fossils, also supports the conclusion that they occupy a middle position in the evolutionary progression of plant-life from life in water to life on land (4).

As pointed out by Marion (5) it is of interest that nicotine is the only alkaloid which has bridged the gap between the flowering plants (Spermatophytes) and the vascular cryptogams (Pteridophytes). D'Alcontus (6), on the other hand, reported the extraction of a white substance from the spores of Lycopodium clavatum L. which bore many of the properties of fish-sperm protamines.

2) Non-alkaloidal constituents. Compared with the study of the alkaloids of the Lycopodium species, the study of the non-alkaloidal constituents has received little attention. Manske and Marion (7) reported a preliminary investigation of the water-insoluble polysaccharides of Lycopodium complanatum L. An appreciable amount of galactose was obtained from the hydrolysis with boiling dilute sulphuric acid. Fucose and mannose, sugars present in the polysaccharides of marine algae, were not found, however. Marked accumulation of aluminum, usually a rare constituent of living matter (8), has been noted in Lycopodium species (9) (10). Towers and Gibbs (11) have made reference to Lycopodium complanatum L. in their study of

lignin chemistry and the taxonomy of higher plants.

The "lycopodium powder" of commerce (12) (13), consisting of spores of Lycopodium clavatum L. has yielded, on small scale extractions by Riebsomer and Johnson (14), 46-47% of oil. They found that the fatty acids of lycopodium oil consisted mainly of oleic acid (ca 55-60%) and 9,10-palmitoleic acid (ca 30-35%). Palmitic, linoleic and 9,10-dihydroxystearic acids were also identified.

3) Alkaloidal constituents.

(i) Methods of isolation. The amounts of dried plant material used by the various workers varied between 1.6 kg. and 108 kg. In most cases, after air-drying, the plant material was ground to suitable size and extracted with methanol in a Soxhlet apparatus. After removing the methanol, the "crude alkaloid" was separated from fats and other materials by a series of acid-base extractions procedures.

The separation of the "crude alkaloid" into the pure component alkaloids was effected by fractional distillations followed by procedures based on the different solubilities of the bases and their salts in various solvents. This method of separation was dependent upon the tendency of various alkaloids to crystallize. Aside from the length of time sometimes required for crystallization, the conditions for crystallization were often difficult to reproduce. It

was also suspected that the method used was not highly efficient in separating minor alkaloids from the "crude alkaloid".

The purpose of the present investigation was, therefore, to find a faster, more efficient, reliable and reproducible method of separating and isolating the alkaloids of Lycopodium annotinum L.

(ii) Alkaloids in Lycopodium species. The alkaloidal contents of some twelve Lycopodium species, which have been determined by the various workers shown, are listed hereunder. To avoid cumbersome and perhaps inappropriate names, Marion and Manske (7) adopted the expedient of designating the individual alkaloids by a number, following the letter "L". In those cases where the purity and homogeneity of the Lycopodium alkaloids did not seem to be in doubt, a trivial name was sometimes also given. The relative concentrations of the alkaloids (as the free base), where known, are expressed hereunder as percentages, or, parts per million (p.p.m.) of the dried plant material.

Alkaloids of Lycopodium annotinum L.

"Stiff Club-moss". This lycopod is dominant in the cold parts of Quebec, Canada (15). This species is regarded as the most primitive of the genus Lycopodium (9).

Manske and Marion - 1943 (16) extracted 103 kg. of dried plant material.

Annotinine (L-7)		$C_{16}H_{21}O_3N$	0.084%
Lycopodine		$C_{16}H_{25}ON$	0.007%
Obscurine (L-6)	α	$C_{17}H_{26}ON_2$ (17)	0.009%
	β	$C_{17}H_{24}ON_2$ (17)	
Alkaloid L-8		$C_{16}H_{25}O_2N$	
Alkaloid L-9	Mixture of alkaloids:		
		$C_{20}H_{31}O_4N$ and	
		$C_{16}H_{23}ON$	0.010%
Alkaloid L-10		$C_{16}H_{27}O_2N$	
Alkaloid L-11		$C_{16}H_{21}O_3N$	
Alkaloid L-12		$C_{18}H_{25}O_3N$	

Manske and Marion - 1947 (18) extracted 67 kg. of dried plant material.

Lycopodium annotinum var. acrifolium Fern.

(Lycopodium acrifolium (Fern) N. Comb.)

Annotinine		0.089%
Lycopodine		0.004%
Acrifoline (L-27)	$C_{16}H_{23}O_2N$	0.015%
Alkaloid L-28	$C_{17}H_{27}O_2N$	0.001%
Alkaloid L-29	$C_{16}H_{23}O_2N$	
Alkaloid L-30	(Alkaloid L-8)(17)	0.003%
Alkaloid L-31	$C_{20}H_{29}O_4N$	

Bertho and Stoll - 1952 (19) extracted 52 kg. of dried plant material.

Annotinine

Lycopodine

Acrifoline

Annotine - found later to be alkaloid L-11 (20).

Annotoxine - found later to be $C_{32}H_{44}O_5N_2$ (20).

Alkaloid $C_{16}H_{25}ON$ (as methiodide and picrate).

Alkaloid $C_{10}H_{19(20)}ON$

Achmatowicz and Rodewald - 1955 (20) extracted 108 kg. of dried plant material.

Annotinine	0.084%
α-Obscurine	0.002%
β-Obscurine	0.001%
Lycopodine	0.001%
Alkaloid L-8	0.006%
Annotine (Alkaloid L-11)	0.031%
Acrifoline	0.038%
Annotoxine (annotine-acrifoline complex)	
Isolycopodine (possibly L-13) $C_{16}H_{25}ON$	0.001%

Anet and Eves - 1958 (21)

Lycodine $C_{17}H_{24}N_2$

Achmatowicz and Rodewald - 1958 (22)reported the continuation of crystallizations from the final mother-liquors remaining from the previous crystallizations (see above (20)).

In addition to the amounts reported previously (20), the following additional yields were obtained:

Obscurine		0.5 g.
Isolycopodine	(as methiodide)	1.5 g.
Acrifoline	(as methiodide)	2 g.

The additional alkaloids, isolated as their methiodides, were made up of the following:

Nicotine	$C_{10}H_{14}N_2$	4 p.p.m.
Alkaloid	$C_{16}H_{23}ON$	5.3 p.p.m.
-believed to be the same as $C_{16}H_{25}ON$ of Bertho and Stoll (19).		
Alkaloid L-28		6.1 p.p.m.
Alkaloid L-29		14.4 p.p.m.
Alkaloid L-31		9.9 p.p.m.
Alkaloid	$C_{16}H_{21}O_3N$	7.3 p.p.m.
Alkaloid	$C_{17}H_{25}O_2N$	4.9 p.p.m.
Alkaloid	$C_{17}H_{25}O_3N$	5.6 p.p.m.
Alkaloid	$C_{18}H_{25}O_3N$	7.6 p.p.m.
Alkaloid	$C_{18}H_{25}O_4N$	5.8 p.p.m.

These authors indicated that the alkaloids reported in their papers (20)(22) account for 93.3% of the total alkaloids in Lycopodium annotinum L.

Alkaloids of Lycopodium cernuum L.

This species is native to the West Indian archipelago (23).

Marion and Manske - 1948 (23) extracted 9.1 kg. of dried plant material.

Cernuine (L-32)	$C_{16}H_{26}ON_2$	0.002% (chief constituent)
Nicotine	$C_{10}H_{14}N_2$	ca 0.1 p.p.m.
Alkaloid L-33	uncharacterized.	

Note: Lycopodine, if present, probably amounts to less than 0.1 p.p.m. Total "crude alkaloid" was only 0.01% of dried plant material.

Alkaloids of Lycopodium clavatum L.

"Common Club-moss". Of all the Canadian Lycopodium species, this is the largest and best known. It furnishes the "lycopodium powder" of pharmacy (15).

Achmatowicz and Uzieblo - 1938 (24).

Lycopodine	$C_{16}H_{25}ON$
Clavatine	$C_{16}H_{25}O_2N$
Clavatoxine	$C_{17}H_{27}O_2N$

Marion and Manske - 1944 (25) extracted 1.515 kg. of dried plant material.

Lycopodine		0.087%
Nicotine	$C_{10}H_{14}N_2$	

Alkaloid L-13	$C_{16}H_{25}ON$
Alkaloid L-18	$C_{11}H_{19}ON$ (as picrate only).
Alkaloid L-19	-insufficient for complete analysis.

Note: Total content of alkaloids comprised 0.145% of dried plant material. About 60% of the alkaloid content was lycopodine; the other bases being present in only very small quantities.

Alkaloids of Lycopodium complanatum L.

"Flattened Club-moss". This is a species found in Canada (15).

Bodeker - 1881 (26)

Lycopodine	$C_{16}H_{25}ON$ (24) - Original formula by Bodeker was $C_{32}H_{52}O_3N_2$.

Alkaloids of Lycopodium densum Labill.

The plants of this species which were studied were of New Zealand origin (27).

Manske - 1953 (27) extracted 2.365 kg. of dried plant material.

Lycopodine	$C_{16}H_{25}ON$
Alkaloid L-34	$C_{16}H_{25}O_2N$
Alkaloid L-35	$C_{14}H_{21}ON$

Note: Total alkaloid content was only 0.07% of dried plant material.

Alkaloids of Lycopodium flabelliforme Fern

"Fan-shaped Club-moss". It is a purely American species (15).

Marion and Manske - 1942 (7) extracted 65 kg. of dried plant material. (Submitted originally as Lycopodium complanatum L.) (28).

Lycopodine		$C_{16}H_{25}ON$
Complanatine (L-1)		$C_{16}H_{27}ON$ - identical with dihydrolycopodine (29).
Alkaloid L-2		$C_{18}H_{29}O_2N$ - shown to be O-acetyldihydrolycopodine (30).
Alkaloid L-3		$C_{18}H_{31}O_2N$ - as perchlorate only.
Alkaloid L-4		$C_{16}H_{27}N$ - as perchlorate only.
Alkaloid L-5		$C_{18}H_{28}O_2N_2$ - as perchlorate only.
Obscurine (L-6)	α-	$C_{17}H_{26}ON_2$ (17)
	β-	$C_{17}H_{24}ON_2$ (17)
Nicotine		$C_{10}H_{14}N_2$

Alkaloids of Lycopodium lucidulum Michx (Urostachys lucidulus Heter)

"Shining Club-moss".(15). With the exception of Lycopodium annotinum L. this species is regarded as a more primitive plant than the other species of this genus investigated by Manske and Marion (9).

Manske and Marion - 1946 (9) extracted 91.6 kg. of dried plant material.

Lycopodine	$C_{16}H_{25}ON$	0.032%
Nicotine	$C_{10}H_{14}N_2$	trace.
Alkaloid L-13	$C_{16}H_{25}ON$	0.015%
Alkaloid L-20	$C_{17}H_{27}O_2N$	0.001%
Alkaloid L-21	$C_{13}H_{21}ON$	- as perchlorate and picrate only.
Alkaloid L-22	$C_{16}H_{27}ON$	0.003%
Alkaloid L-23	$C_{16}H_{25}O_2N$	
Alkaloid L-24	$C_{16}H_{25}ON$	
Alkaloid L-25	$C_{16}H_{25}O_2N$	

Note: While the total quantity of ether-soluble bases amounted to 0.23% of the dried plant material, the crystalline alkaloids isolated represent only about 0.05%.

Alkaloids of Lycopodium obscurum L.

"Ground Pine". This species is remarkable for its geographic distribution which embraces Eastern America and Eastern Asia (15). The common variety of this species, viz. Lycopodium obscurum var. dendroideum (Michx) D.C.Eaton, is found in the region from North Carolina to Newfoundland and northward (31).

Manske and Marion - 1944 (31) extracted 38.2 kg. of dried plant material.

Lycopodine		$C_{16}H_{25}ON$	0.058%
Obscurine	α-	$C_{17}H_{26}ON_2$ (13)	-second to lyco-
	β-	$C_{17}H_{24}ON_2$ (13)	podine in amount.
Alkaloid L-13		$C_{16}H_{25}ON$	- as perchlorate only.
Alkaloid L-16		$C_{16}H_{25}ON$	- as perchlorate only.
Alkaloid L-17		$C_{18}H_{27}O_3N$	- as perchlorate only.

Alkaloids of Lycopodium sabinaefolium Wild

"Cedar-like Club-moss". This is a species found in Canada (15)(32).

Marion and Manske - 1946 (32) extracted 1.6 kg. of dried plant material.

Lycopodine	$C_{16}H_{25}ON$	0.108%
Nicotine	$C_{10}H_{14}N_2$	-trace only as picrate.
Alkaloid L-13	$C_{16}H_{25}ON$	0.022%
Alkaloid L-26	$C_{15}H_{25}ON$	0.012%

Alkaloids of Lycopodium saururus

This species is native to Argentina and other South American countries as well as Africa (33)(34).

Arata and Canzoneri - 1892 (33)(34).

Pillijanine	$C_{15}H_{20}ON_2$

Deulofeu and De Langhe - 1942 (34) extracted 7.4 kg. of dried plant material.

Saururine	$C_{10}H_{19}N$	0.018%
Sauroxine	$C_{17}H_{26}ON_2$	0.007%

(Marion and Manske have suggested (31)(23) the formula $C_{16}H_{24}ON_2$).

Alkaloids of Lycopodium selago L.

The plants of this species which were studied were of Polish origin (35).

Achmatowicz and Rodewald - 1956 (35).

Lycopodine	$C_{16}H_{25}ON$	0.053%
Acrifoline	$C_{16}H_{23}O_2N$	0.002%
Alkaloid L-8	$C_{16}H_{25}O_2N$	0.001%
Pseudoselagine (possibly L-23)	$C_{16}H_{25}O_2N$	0.015%

Alkaloids of Lycopodium tristachyum Pursh

"Three-spiked Club-moss". This is a species found in Canada in sunny, sandy locations (15).

Marion and Manske - 1944 (28) extracted 15.7 kg. of dried plant material.

Lycopodine	$C_{16}H_{25}ON$	0.174%
Nicotine	$C_{10}H_{14}N_2$	

Alkaloid L-13	$C_{16}H_{25}ON$ - as perchlorate	less than 0.01%
Alkaloid L-14	$C_{16}H_{25}N$ - as perchlorate	
-shown to be anhydrodihydrolycopodine (30)		
Alkaloid L-15	$C_{20}H_{31}O_4N$ - as perchlorate	

(iii) Physical properties of Lycopodium alkaloids. The physical properties of the Lycopodium alkaloids have been listed in Table No. 1 in the order in which they were reported in the literature pertaining to the Lycopodium species. Tables 1(a) to 1(o), inclusive, describe those alkaloids of Table No. 1 which have been more fully characterized. For the sake of completeness and possible future reference, physical properties determined during the present work have been included in these tables. Solvents of crystallization and other miscellaneous data, as shown in the literature, have been included in the "Remarks" column of Table No. 1.

(iv) Structures and Reactions of Lycopodium alkaloids. With the exception of nicotine, which has been found in only trace amounts, annotinine is the only alkaloid of the Lycopodium species whose structure has been established (42). In addition to annotinine, the structural studies and reactions of lycopodine, acrifoline, α-obscurine, β-obscurine and annotine are reviewed hereunder.

1 Annotinine ($C_{16}H_{21}O_3N$). As indicated above, annotinine is the principal alkaloid of Lycopodium annotinum L. It is also the only alkaloid of the Lycopodium alkaloids (with the exception of Nicotine) whose structure has been established. Wiesner and co-workers (42) have now published a detailed account of the experiments that permit the derivation of the complete structure for annotinine and the assignment of configuration to most of the asymmetric centers.

In this thesis, the contributions of the many workers in the field of annotinine chemistry have been reviewed from the time of the isolation of this new alkaloid reported by Marion and Manske in 1943. Such a review, it was felt, might contribute to an understanding of the chemistry of other Lycopodium alkaloids.

This review is historical in its approach -- outlining the step-by-step elucidation of the structure of annotinine in terms of the determination of the functional groups present, of the position of the functional groups and of the integration of the functional groups and ring structure. To permit, however, a simultaneous comparison of the reactions which formed the basis of this historical development and of these same reactions in the light of present knowledge of the alkaloid's structure, a flow chart (Fig.1) has been prepared. This chart correlates the work of the various workers and shows the probable structures of

some of the key reaction products. Physical data of the various products and their probable structures are contained in Table No. 2.

In 1943, Manske and Marion (16) reported the isolation of a new alkaloid, which they called annotinine (I) from Lycopodium annotinum L. This alkaloid (I), best represented by the formula $C_{16}H_{21}O_3N$, was found to be a lactonic base since it yielded a water-soluble salt when heated with ethanolic potassium hydroxide. Regeneration of the base by heating the acidified solution of this salt followed by the addition of ammonia yielded not annotinine (I) but annotinine hydrate (II), $C_{16}H_{23}O_4N$, containing an added molecule of water.

The first detailed work on the structure of annotinine was reported by Manske and Marion (18) in 1947. It was found that annotinine hydrate (II) could not be reconverted to the parent base, annotinine (I). Oxidation of (II) with permanganate gave a small amount of (III), $C_{16}H_{21}O_4N$. It was not possible to oxidize (II) with periodic acid.

Oxidation of annotinine (I) by the slow addition of potassium permanganate to a suspension of this alkaloid in water produced a new compound (IV), $C_{16}H_{19}O_4N$, with the lactone ring intact and believed at that time to be a base. As the Clemmensen reduction of (IV) yielded a saturated base (V), $C_{16}H_{23}O_2N$, it was believed that compound (IV) probably

contained two carbonyl groups.

Clemmensen reduction was applied to annotinine (I) to reduce a possible carbonyl group but instead the strong hydrochloric acid present under the reaction conditions added to (I) giving a compound (VI), $C_{16}H_{22}O_3NCl$, which was not reduced by amalgamated zinc in acid solution. A similar addition compound was obtained by boiling (I) with hydrobromic acid. The addition compound (VI) was considered to be closely related to annotinine hydrate (II) as the former was converted to the latter by treatment with alcoholic alkali.

The chlorohydrin (VI) was dechlorinated on reduction with chromous chloride to an unsaturated base (VII), $C_{16}H_{21}O_2N$, which was easily reduced catalytically to the saturated base (V), above. The chromous chloride reduction was considered anomalous since, in addition to replacement of the chlorine with hydrogen, a molecule of water was lost. As will be seen, this reaction was studied later in greater detail (43)(44)(45). Oxidation of (VI) with permanganate gave the compound (VIII), $C_{16}H_{20}O_4NCl$.

With the evidence then available, Manske and Marion made the following interpretations:

(a) annotinine (I) contained an ether bridge;

(b) the hydrate (II) was a dihydroxy compound but not a 1,2-diol because of its inertness to periodate oxidation;

(c) the hydrogen chloride addition product (VI) was a chlorohydrin which on reduction with chromous chloride yielded an alcohol which dehydrated easily to the unsaturated compound (VII);

(d) the oxidation product (IV) was a diketone corresponding to the dihydroxy compound (II) which would therefore be disecondary and as this diketone was colourless and quite stable, it was probably not a 1,2- nor a 1,3-diketone; and,

(e) the ether bridge was therefore thought to be part of a 5- or 6-membered ring; however, it was not clear why such a large ring should be so easily opened with alkali.

Further support for the presence of a lactone ring in annotinine (I) was reported by Marion, Ramsay and Jones (37) in 1951 in the occurrence of a strong absorption peak at 1776 $cm.^{-1}$ in the infrared spectrum; the position of this peak being characteristic of a 5-membered lactone.

Bertho and Stoll (19) reported that treatment of annotinine with selenium dioxide, Grignard reagent and 2,4-dinitrophenylhydrazine left the alkaloid unchanged as did a von Braun degradation. Likewise, annotinine (I) did not react with benzaldehyde in the presence of sodium ethoxide. While the base was presumed to be tertiary a methiodide could not be obtained from it. From the foregoing and other reactions it was concluded that the alkaloid did not contain any unsaturation, N-methyl, O-methyl or hydroxyl groups.

In 1953 MacLean and Prime (46) reported a re-examination of the oxidation product (IV) of annotinine (I) and found (IV) to be a neutral amide rather than a base as had been previously reported. They reasoned that the formation of the amide could be achieved: (a) by the oxidation of the 5- or 6-membered ether ring to a 1,4- or 1,5-diketone, thereby fixing one end of the ether ring adjacent to the nitrogen, or, (b) by the oxidation of a methylene group adjacent to the nitrogen, leaving the original ether ring intact. The results of their work, as reported hereunder, indicated to them that the latter alternative applied.

A chlorohydrin (IX), $C_{16}H_{20}O_4NCl$, was readily obtained by the oxidation of the amide (IV). Treatment of (IX) with phosphorus oxychloride, or, prolonged treatment with hydrochloric acid yielded the anhydrochloro-compound (X), $C_{16}H_{18}O_3NCl$. Treatment of (IX) and (X) with dilute permanganate disclosed that (IX) was unreactive whereas (X) was reactive. This confirmed the unsaturation in (X). The unreactivity of (IX) was interpreted at that time as suggesting that the hydroxyl group was tertiary. Refluxing (IX) with sodium bicarbonate in acetone effected ring closure of the ether ring and reformation of (IV). Catalytic reduction of (IX) using hydrogen with Adam's catalyst resulted in the formation of two products (XI), $C_{16}H_{21}O_4N$, and (XII), $C_{16}H_{21}O_3N$. The chloro-group was replaced with hydrogen in

the formation of (XI) whereas both the chloro and hydroxyl groups were eliminated in the formation of (XII). The compound (XII) alone was formed by a similar catalytic reduction of (X) apparently by the saturation of the double bond and hydrogenolysis of the chloro group. Resistance of (XI) and (XII) to dilute permanganate was interpreted as indicating saturation in both these compounds and a tertiary hydroxyl in (XI). Compound (XI) underwent dehydration on refluxing with phosphorus oxychloride to yield (XIII), $C_{16}H_{19}O_3N$; however, (XI) was virtually unaffected after prolonged boiling with concentrated hydrochloric acid. The presence of a double bond in (XIII) was indicated with the reaction with permanganate. Treatment of (XI) with hydrogen over Adam's catalyst left (XI) unchanged which indicated that the removal of the elements of water in the similar treatment of (IX) was probably contingent upon the presence of the chloro group.

MacLean and Prime compared the reactions of (IX), the amide chlorohydrin, with those of (VI), the annotinine chlorohydrin. While the former reacted readily on treatment with sodium bicarbonate in acetone, concentrated hydrochloric acid, phosphorus oxychloride and hydrogen over Adam's catalyst, as outlined above, in the case of the latter (VI), the starting material was recovered quantitatively in all four cases except with phosphorus oxychloride. In this

latter reaction presumably some loss of starting material was caused through side-reactions of the lactone ring.

These workers reasoned that as the only structural differences between the two chlorohydrins (VI) and (IX) was the replacement of a $-CH_2-N\lessgtr$ by a $-CO-N\lessgtr$ group, the increased lability of the chloro and hydroxyl groups in (IX) must be dependent upon this factor.

This suggested that the ether ring was attached adjacent to the carbonyl of the amide group. Furthermore, the effect that the chloro and hydroxyl groups appeared to exert on one another in the reactions described, suggested that the chloro and hydroxyl groups were adjacent. The ether ring would be, therefore, an epoxide ring. The relationship of the nitrogen and ether ring was considered, therefore, to be $\gtrless N-CH_2-\underset{\diagdown O \diagup}{CH}-C-$ in the case of annotinine (I) and $\gtrless N-CO-\underset{\diagdown O \diagup}{CH}-C-$ in the case of the amide (IV).

The formation of the chlorohydrins (VI) and (IX), respectively, from each of these compounds (I) and (IV) would be expected to proceed by "trans" addition with inversion at the carbon carrying the chlorine atom. The hydrogen on the carbon bearing the chloro group and the hydroxyl on the adjacent carbon would not therefore be in the proper orientation for elimination and both compounds would dehydrate with difficulty. In the case of the amide chlorohydrin (IX), however, the dehydration was accounted for by isomerization

at the carbon carrying the chloro group (see a suggested mechanism, Fig. 2). In the case of the chlorohydrin (VI), however, such isomerization was not possible and elimination did not occur on similar treatment. When hydrogen replaced the chloro group easy dehydration occurred, as would be expected. The conversion of the chlorohydrin (VI) to the unsaturated base (VII) upon treatment with chromous chloride and hydrochloric acid, as previously reported by Manske and Marion (18) was interpreted as proceeding through replacement of the chloro group by hydrogen followed by dehydration in the acid medium; the hydrogen being in the proper steric orientation to permit easy elimination.

In those reactions where dehydration did not occur -- as in the treatment of (VI) with hydrochloric acid and phosphorus oxychloride -- due to the lack of reactivity of the hydroxyl group to displacement reactions, it was suggested that it might be of the type where the carbon atom was attached to a rigid ring structure with the hydroxyl fixed in space. Compounds of this type were known not to participate in displacement reactions which proceed through inversion. Reactions of this type (i.e. with concentrated hydrochloric acid and phosphorus oxychloride) would be expected to proceed through a nucleophilic displacement mechanism with inversion. The unreactivity of the hydroxyl group

would thus be accounted for and these workers pointed out that it was not unlikely that such a configuration (e.g. a bridge-head attached to the carbon bearing the hydroxyl) could exist in the multimembered ring system of the alkaloid. This explanation appeared probable at that stage as it was then believed that the hydroxyl was tertiary, which hydroxyl, as will be seen, was later shown to be secondary.

The postulation of an epoxide ring in annotinine lead to the conclusion that the hydrate (II) would be a 1,2-diol and hence should have been susceptible to periodate oxidation which, as previously reported, it was not. These workers offered as possible explanations, the non-reactivity of a cyclic "trans"-diol and possible steric impediments. As it is now known that annotinine hydrate (II) is not a 1,2-diol, despite the existence of an epoxide ring in annotinine (I), this lack of reactivity towards periodate is understandable. Another possible objection raised against this arrangement by these workers was "the assumption that dehydration must proceed in the direction of the nitrogen atom".

The Clemmensen reduction of (IV) previously reported by Manske and Marion (16) was re-examined by MacLean and Prime (46) and it was found that besides the saturated base (V), the non-basic compounds (XI) and (XII) were also formed and in much higher yield than (V). As attempts to isolate

compound (IX) were unsuccessful even after a short reaction time, it was suggested that the oxide fission possibly proceeded by hydrogenolysis as well as by the action of hydrochloric acid. Separate examinations of the Clemmensen reduction of (XI) and (XII) revealed the formation of only small amounts of the saturated base (V). Similar treatment of the amide chlorohydrin (IX) yielded a mixture of (XI) and (XII) whereas the anhydrochloro compound (X) was rapidly converted to (XII).

The results of these reactions indicated that the Clemmensen reduction of those compounds involved a direct reduction of an amide to an amine, which circumstance was not usually associated with this type of reaction. Analogies were also referred to with regard to the above reactions involving the chloro and hydroxyl groups and reductions of double bonds by the Clemmensen reduction. These reactions tended to confirm the supposition of an epoxide situated adjacent to the carbonyl of the amide group.

In 1954, Meier, Meister and Marion (43) reported further studies which they had undertaken to gain insight into the nature of the functional groups of annotinine. In the course of this work most of the degradation reactions reported up to that time were reinvestigated.

The neutral amide (IV) from the oxidation of annotinine

(I) was confirmed as such by its infrared absorption spectrum which contained in addition to the usual lactone band (1768 cm.$^{-1}$) a new strong peak at 1640 cm.$^{-1}$ characteristic of a -CO-N< group present in a 6-membered ring. As compound (IV) was a lactam, it followed that annotinine must contain a methylene group next to the nitrogen.

The compound (IX), identical with (VIII), was also established as a lactam chlorohydrin from its infrared spectrum. This compound was also obtained, although in small yield, by the oxidation of annotinine chlorohydrin (VI).

The infrared absorption spectrum of compound (V) obtained previously via two routes (viz. from (I) to (IV) to (V) and from (I) to (VI) to (VII) to (V)) disclosed the lactone ring intact. This compound (V) had no epoxide or lactam carbonyl remaining and was called "dihydrolactone A". The reduction of the amide group was understandable only if strong activation of the lactam carbonyl by the close proximity of one of the other functional groups were assumed. By contrast, annotinine subjected to the same conditions was only affected by the hydrochloric acid and converted to the chlorohydrin (VI). It was found, however, that if the Clemmensen reduction were continued for ten hours it did go further and gave rise to the unsaturated lactone-A (VII) and a hydroxylactone (XIV), $C_{16}H_{23}O_3N$. This comparison of the

reactions of (IV) and (I) illustrated that as well as the lactam carbonyl having increased reactivity, as shown by the results of the Clemmensen reduction, this carbonyl also enhanced the reactivity of the cyclic ether.

It had been previously reported (18) that annotinine chlorohydrin (VI) reacted with chromous chloride to produce the unsaturated lactone-A (VII). Reinvestigation proved that the reaction was more complex, producing additional compounds depending particularly upon the solvent used and the concentration of hydrochloric acid. Treatment of (VI) with an alcoholic solution of chromous chloride and hydrochloric acid yielded unsaturated lactone-A (VII) and the hydroxylactone (XIV) in the ratio of 2:1. When (VI) was treated with an aqueous solution of chromous chloride and hydrochloric acid the product included, in addition to (VII) and (XIV), an unsaturated lactone-B (XV), with formula later established (44) to be $C_{16}H_{23}O_2N$. It was considered that the formation of the unsaturated lactone-B was a secondary reaction as treatment of (XIV) with a dilute aqueous acid solution of chromous chloride remained unchanged, whereas treatment with a more concentrated solution of the reagent transformed (XIV) into (XV) in good yield. No unsaturated lactone-A (VII) was obtained from (XIV) and it was concluded that (VII) must have arisen from the chlorohydrin (VI)

directly by a different mechanism of dehalogenation and dehydration.

The hydroxyl group in annotinine chlorohydrin (VI) and in the hydroxylactone (XIV) were found resistant to oxidation with chromic acid and to O-acetyl formation. Thionyl chloride, even under drastic conditions, did not react with (VI), nor did phosphorus halides -- although treatment with phosphorus pentachloride resinified some of the starting material. Treatment of (XIV) with thionyl chloride under mild conditions yielded, after chromatography, an unstable, colourless oil containing chlorine. This oil on treatment with a dilute solution of chromous chloride (which dilute solution would not have affected (XIV)) gave rise to unsaturated lactone-B (XVI). Catalytic hydrogenation of the latter yielded dihydrolactone-B (XVI), $C_{16}H_{25}O_2N$, with a sharp band at 3325 $cm.^{-1}$ in the infrared absorption spectrum, indicating the presence of an imino group which was later (47)(44) confirmed chemically.

On surveying the infrared spectra of annotinine and its derivatives, Meier, Meister and Marion noted that in some of them the absorption band of the lactone carbonyl was slightly shifted towards lower frequencies such as in compounds (XV) and (XVI). They felt that such shifts might be due to either structural changes near the lactone carbonyl,

or, relactonization with a hydroxyl other than the original. As most of these compounds were prepared under acidic conditions, however, it was felt that such shifts were probably not due to hydrolysis of the lactone with subsequent relactonization. In this connection annotinine hydrate (II) was again prepared and reinvestigated. As this compound was prepared under alkaline conditions it was felt that the chances of hydrolysis of the lactone ring and relactonization with another hydroxyl resulting from the cleavage of the cyclic ether were considerable. Annotinine hydrate (II) did, however, contain the lactone carbonyl band in the same position as in annotinine. If relactonization with another hydroxyl had occurred with the formation of another 5-membered lactone, the carbonyl absorption band would probably be at the same frequency. It was not possible, therefore, to draw any conclusions from the infrared data.

Chromic acid oxidation of annotinine hydrate (II) produced a new compound (XVII), $C_{16}H_{21}O_4N$, which from its infrared spectrum was found to be a hydroxy ketone. It was concluded, therefore, that of the two hydroxyls of annotinine hydrate (II) one was certainly a secondary and the other probably a tertiary. The preparation of an oxime (XVIII), $C_{16}H_{22}O_4N_2$, from (XVII) confirmed the presence of a keto group. Attempts to reconvert the carbonyl of (XVII) to a secondary hydroxyl by the use of hydrogen over a platinum

catalyst failed.

Treatment of annotinine hydrate (II) with thionyl chloride at room temperature converted it into an unsaturated chlorolactone (XIX), $C_{16}H_{20}O_2NCl$, which contained no hydroxyl absorption in the infrared spectrum, indicating replacement of one hydroxyl with a chloro group and the elimination of the other with the formation of a double bond. Catalytic reduction of (XIX) produced the dihydrochlorolactone (XX), $C_{16}H_{22}O_2NCl$. This compound was in turn treated with chromous chloride to form a dihydrolactone-C (XXI), $C_{16}H_{23}O_2N$, which contained an absorption band at 3355 $cm.^{-1}$ in the infrared spectrum which, like the dihydrolactone-B (XVI), was interpreted as a possible imino group.

It was pointed out by Meier, Meister and Marion that the presence of an epoxide ring next to a $\rangle N\text{-}CH_2\text{-}$ group in annotinine, as proposed by MacLean and Prime, still had not been definitely established.

Again in 1954, Meier and Marion (48) reported work which was designed to provide some information concerning the way in which the 5-membered lactone ring was attached to the rest of the molecule and its position relative to the nitrogen or the cyclic ether.

A limited quantity of lithium aluminum hydride in dioxane solution reduced the lactone function of annotinine (I) to give a dihydroxy ether (XXII), $C_{16}H_{25}O_3N$. The infra-

red spectrum of this compound showed the absence of carbonyl absorption but the presence of two peaks at 3455 cm.$^{-1}$ and at 3375 cm.$^{-1}$ in the hydroxyl region. The same reagent in excess in tetrahydrofuran produced a trihydroxy compound (XXIII), $C_{16}H_{27}O_3N$, in which not only had the lactone been reduced but the ether ring had been opened. This same compound (XXIII) was obtained from the reaction of the chlorohydrin (VI) with lithium aluminum hydride in ether solution. Hence it was apparent that the hydroxyl group in the trihydroxy compound (XXIII) arising from the ether function must be in the same position as the hydroxyl group of the chlorohydrin (VI) arising from the same function. Lithium aluminum hydride and hydrochloric acid, therefore, must have opened the ether ring in the same way.

Bertho and Stoll (19) had previously reported that annotinine was inert to lithium aluminum hydride in ether solution. Since the lactone ring in the chlorohydrin (VI) was reduced in ether whereas annotinine was not affected under those conditions it appeared that the lactone was more stable when the cyclic ether was intact. These authors surmised from this observation that the two functions might well be near to one another.

Conversion of the dihydroxy ether (XXII) to the corresponding dichloro ether (XXIV), $C_{16}H_{23}ONCl_2$, was accomplished, but in small yield, by phosphorus pentachloride.

On the other hand, thionyl chloride did not effect chlorination but gave rise to a cyclic sulfite ester (XXV), $C_{16}H_{23}O_4NS$; the infrared spectrum showing a strong absorption band at 1206 cm.$^{-1}$ characteristic of the S-O frequency. In the case of the trihydroxy compound (XXIII) with thionyl chloride, a similar reaction occurred except for the hydroxyl group from the ether function which was presumably replaced by a chloro group. This uncrystallizable product (XXVI), when refluxed with chromous chloride and hydrochloric acid, gave rise to an unsaturated dihydroxy compound (XXVII), $C_{16}H_{25}O_2N$, with two absorption bands in the hydroxyl region. Catalytic reduction of (XXVII) with the absorption of one mole of hydrogen gave a dihydro-dihydroxy compound (XXVIII) which did not crystallize but was converted to an unstable crystalline sulfite ester by thionyl chloride. On the other hand, when (XXVI) was refluxed with amalgamated zinc and hydrochloric acid instead of chromous chloride, an unsaturated dihydroxy compound (XXIX) was formed which was isomeric with (XXVII).

It was thought unlikely that more than two hydroxyls would have the required configuration for the easy formation of a sulfite ester. By analogy with the formation of the unsaturated lactones (VII) and (XV) described previously (43) it was assumed that it was the hydroxyl arising from the cyclic ether that was chlorinated and eventually eliminated

with the formation of a double bond. Attempts were made to remove the elements of water from (XXVII) without success and treatment with thionyl chloride produced a sulfite ester. Opening the lactone ring of annotinine was also effected by treatment with phenyl lithium producing the diphenyl derivative (XXX), $C_{28}H_{35}O_4N$, rather than the expected $C_{28}H_{33}O_3N$. The addition of the elements of water was accounted for by the fact that the reaction mixture had been poured onto ice and it was probable that the lithium hydroxide had cleaved the ether resulting in the formation of a glycol. Compound (XXX) contained no absorption in the carbonyl region. Oxidation of (XXX) with chromic acid in acetic acid caused the loss of two hydrogens with no increase in the oxygen present. The oxidation product (XXXI), $C_{28}H_{33}O_4N$, was still basic and contained several absorption bands in the hydroxyl region and a strong peak at 1720 $cm.^{-1}$ indicative of a keto carbonyl in the infrared spectrum. The above reaction indicated the oxidation of a secondary hydroxyl to a ketone group. It was assumed that this hydroxyl occupied the same position as the one in annotinine hydrate (II) which had been oxidized to a ketone in compound (XVII). While this latter assumption was in fact correct enough, the authors assumed at the same time that the hydroxyl in question originated from the ether function rather than from the hydrolysis of the original lactone function, as was later shown to be the

case when the structure of annotinine hydrate (II) was established. Consequently, as it was thought that the hydroxyl in (XXXI) (from the breaking of the lactone ring) had not been oxidized, it was believed to be tertiary.

Henderson, Stonner, Valenta and Wiesner (49) also in 1954 oxidized the anhydrochloro compound (X) with potassium permanganate in acetone at room temperature giving a good yield of the amino acid (XXXII), $C_{14}H_{19}O_4N$. Acetylation of (XXXII) gave a quantitative yield of an acid which was esterified with diazomethane to give an oily N-acetyl methyl ester (XXXIII). This amino acid was expected to provide an avenue for further deep-seated degradation of annotinine (I).

In agreement with a vinyl chloride structure adjacent to the lactam carbonyl within a 6-membered ring, the anhydrochloro compound (X), as proposed by these authors, they found that mild reflux with alcoholic alkali did not liberate chlorine but gave the corresponding hydroxy acid (XXXIV), $C_{16}H_{20}O_4NCl$. The two absorption bands at 1645 and 1617 $cm.^{-1}$ in the carbonyl region of the infrared spectrum was ascribed to the conjugated amide group in (XXXIV); a similar pair (1657 and 1616 $cm.^{-1}$) having been present in the spectrum of the anhydrochloro compound (X). The ultraviolet absorption spectrum of (XXXIV) also showed a maximum at 230mμ (log ε, 4.02) in agreement with the above. Treatment of the acid

(XXXIV) with a catalytic amount of p-toluenesulphonic acid gave a neutral lactone (XXXV), $C_{16}H_{20}O_4NCl$, isomeric with (XXXIV). The infrared spectrum of this compound showed a a prominent hydroxy band, a 5-membered lactone carbonyl at 1760 cm.$^{-1}$ and a single amide carbonyl band at 1660 cm.$^{-1}$; the ultraviolet spectrum containing only end absorption. The lactone ring of the relactonized product (XXXV) was again opened by the action of alkali with the reintroduction of the double bond giving a second isomeric acid (XXXVI), $C_{16}H_{20}O_4NCl$, whose infrared and ultraviolet spectra were very similar in principle to those of the acid (XXXV) although the infrared spectrum shows distinct non-identity.

Because of the foregoing reactions these authors, as had the previous authors, suspected that annotinine hydrate (II) had a lactone group which was not the same as the original one in annotinine (I) but rather a new one resulting from the interaction of a hydroxy group from the oxide ring with the carboxy group from the lactone. This hypothesis was tested by hydrolysis of annotinine (I) with methanolic potassium hydroxide, neutralization with methanolic hydrochloric acid followed by methylation with diazomethane. In addition to the production of annotinine hydrate (II), a methyl ester (XXXVII), $C_{17}H_{25}O_4N$, was formed. This result was interpreted as indicating that on mild treatment with alkali, the lactone ring was opened in all molecules, where-

as opening of the oxide ring occurred in only some of the molecules. On subsequent treatment with diazomethane (known to converty γ-hydroxy acids to γ-lactones) it was interpreted that relactonization occurred only in those molecules where the oxide ring had been opened; whereas, those molecules still containing the oxide ring intact, formed the methyl ester (XXXVII) instead of reforming annotinine. Hence this was interpreted as evidence that the original lactone ring of annotinine was difficult to reform and that annotinine hydrate (II) contained a new lactone. Prolonged refluxing of annotinine with alkali produced a quantitative yield of annotinine hydrate (II) which was consistent with the above interpretation.

Henderson, Stonner, Valenta and Wiesner (50) supplemented their earlier communication with tentative partial structures showing the positions of the lactone functions and functions from the hydrolysis of the lactone relative to the oxide and lactone functions of compounds (X), (XXXIV) and (XXXV). These structures, consistent with the now established structure of annotinine (I) were reported by these authors as being the only plausible way to account for the base-catalyzed opening of the lactone group in (XXXV) with the reformation of the double bond. A tentative partial structure for annotinine hydrate (II) was proposed also

showing the carboxyl group lactonized with one of the hydroxyls originating from the oxide ring.

An isomer of annotinine hydrate (II) was obtained on refluxing annotinine with 28% sulphuric acid for 26 hours. This compound (XXXVIII), $C_{16}H_{23}O_4N$, was thought to contain the original lactone group of annotinine. Acetylation gave a diacetyl product (XXXIX), $C_{20}H_{27}O_6N$, m.p. 168°. A re-examination of these reactions was made later (see Martin-Smith, Greenhalgh and Marion (51)). Acetylation of annotinine hydrate (II) gave a diacetyl product (XL), $C_{20}H_{27}O_6N$, m.p. 187°.

Bankiewicz, Henderson, Stonner, Valenta and Wiesner (52) reported the results of selenium dehydrogenation of annotinine. After chromatography on alumina, three main products were obtained. The basic compound (XLI), $C_{12}H_{13}N$, which was formed was established as 8-n-propylquinoline. The second basic compound (XLII), $C_{15}H_{13}N$, appeared from its ultraviolet spectrum to be a substituted α- or β-naphthoquinoline. A third product (XLIII), $C_{15}H_{11-15}N$, was neutral. These authors were the first to publish very tentative structures for annotinine as a working hypothesis providing for the existence of a 5-membered ring in the annotinine molecule.

Anet and Marion (44)(47) reported in 1954 and 1955 that they had obtained, via a different route, the same

amino acid (XXXII) as Wiesner et al had obtained via the anhydrochloro lactam (X). Anet and Marion clarified the relationship between annotinine chlorohydrin (VI) and the two unsaturated lactones (VII) and (XV) which provided support for MacLean and Prime's suggestion (46) of the existence of the system $\rangle N\text{-}CH_2\text{-}CH(O)\text{-}C\text{-}$ and, also, the modification by Wiesner et al to $\rangle N\text{-}CH_2\text{-}CH(O)\text{-}CH\text{-}$ in annotinine.

In the case of annotinine chlorohydrin (VI), the group-$\rangle N\text{-}CH_2\text{-}CHCl\text{-}CHOH\text{-}$, as originally suggested by MacLean and Prime (46) was found to permit a simple interpretation for the chromous chloride reduction products (VII) and (XV) not possible with the alternate opening of the oxide ring which would have given the product $\rangle N\text{-}CH_2\text{-}CHOH\text{-}CHCl\text{-}$.

Anet and Marion (44)(47) found that the hydroxylactone (XIV)which, on more vigorous treatment with chromous chloride in strongly acidic solution gave the unsaturated lactone-B (XV), was unsaturated and yielded on hydrogenation a dihydroxylactone (XLIV), $C_{16}H_{25}O_3N$. Unlike (XIV), the compound (XLIV) was unaffected by more vigorous treatment with chromous chloride in strongly acidic solution. Ozonolysis of the hydroxylactone (XIV) yielded formaldehyde. Treatment of (XIV) with with acetic anhydride produced a neutral O,N-diacetyl derivative (XLV), $C_{20}H_{27}O_5N$.

The unsaturated lactone-A (VII) behaved as a cyclic allylamine. The pKa of (VII) was 7.06, whereas its dihydro

derivative (V) was 8.48. The methosulphate (XLVI), $C_{16}H_{21}O_2N \cdot (CH_3)_2SO_4$, of (VII) reacted smoothly with two moles of hydrogen in the presence of Adam's catalyst to form a tertiary base (XLVII), $C_{17}H_{25}O_2N$. Permanganate oxidation of (VII) yielded the amino acid (XXXII) previously obtained by Wiesner et al.

The unsaturated lactone-B (XV) showed no >NH band in its infrared spectrum although its dihydro derivative (XVI) did; however, (XV) formed a neutral N-acetyl derivative (XLVIII), $C_{18}H_{25}O_3N$, which produced formaldehyde on ozonolysis. The presence of a terminal methylene group was indicated by the easy reduction of (XV) to (XVI) and the low intensity of its ultraviolet absorption at short wavelengths. An N-acetyl derivative (XLIX), $C_{18}H_{27}O_3N$, was obtained from the acetylation of (XVI). The infrared spectra of (XV) and (XVI) were very similar except for an indication of a vinyl group in (XV). Partial structures of (XV) and (XVI) were presented consistent with the above evidence and with the confirmation of the empirical formula of (XV) as $C_{16}H_{23}O_2N$.

The "normal" reduction product with chromous chloride would be simply the replacement of the halogen by hydrogen. Anet and Marion suggested that (VII) and (XIV) were formed by 1,2-elimination analogous to the reduction of vic-dibromides to alkenes. In the hydroxylactone (XIV) a chlorine and a β-nitrogen, whereas in the unsaturated lactone-A (VII)

a chlorine and a β-hydroxyl group were involved. Recently Anet and Isabelle (45) reported the study of some simple β-halogenated alcohols and amines to obtain analogies for the reduction of (VI). While "normal" reduction was observed as well, nevertheless the same type of reduction was found in the simple model compounds as occurred in annotinine chlorohydrin (VI).

Chromous chloride reduction of the lactam chlorohydrin (IX) gave as the main two products (XI) and (XIII) previously prepared by MacLean and Prime (46). These authors had dehydrated (XI) with phosphorus oxychloride to give (XIII). On this basis and consistent with the lactam chlorohydrin structure, partial structures were proposed by Anet and Marion for (XI) and (XIII). It was noted that (XI) had not been dehydrated to (XIII) under the chromous chloride conditions but nevertheless (XIII) had been formed directly from (IX) under these conditions in agreement with the mechanisms which they had proposed. As it had been found (46) that catalytic or Clemmensen reduction of annotinine lactam chlorohydrin (IX) gave (XI) and (XII), it was thought probable that (XI) and (XIII) were the primary reduction products and that (XIII) was further reduced to (XII). Ultraviolet spectra of (X) and (XIII) confirmed the presence of an α,β-unsaturated lactam system. This system was also indicated in the infrared spectra with two bands in the carbonyl

region whereas saturated lactams contain only one carbonyl band.

Dehydrogenation of the amino acid (XXXII) with palladium on barium sulphate at 200-250° yielded an acid (L), $C_{14}H_{15}O_3N$. This new acid show no γ-lactone band in the infrared spectrum (Nujol mull) but did contain bands at 1715, 1620 and 1575 $cm.^{-1}$ consistent with the presence of a carboxyl, an amide group and a benzene ring. The acid (L) was decarboxylated to a neutral compound (LI), $C_{13}H_{15}ON$, by heating with copper powder. The ultraviolet spectrum of (LI), compared with that of (L), indicated that the carboxyl group was directly attached to the chromophoric system in (L). Although there was no indication of an $\geq$N-H band in the infrared spectrum of (LI), it was thought probable that an $\geq$N-H was present, as the presence of a benzene ring in (L) would require one less ring than the acid (XXXII). Hence, it was reasoned that, a carbon-nitrogen fission being more probable than a carbon-carbon fission, an N-H group would be present. The ultraviolet spectrum of (LI) was very similar to that of strychnine and, like that alkaloid, (LI) gave an intense violet colour with 80% sulphuric acid and a trace of chromic acid (Otto reaction). Reduction of (LI) with lithium aluminum hydride in ether solution gave a weak base (LII) which formed a crystalline picrate, m.p. 172°.

Anet and Marion (44)(47) dehydrogenated annotinine

itself at 300° over palladium yielding a small amount of 7-methylquinoline, isolated as the picrate. The fact that 8-n-propylquinoline had been isolated by Wiesner et al (52) the formation of 7-methylquinoline under different conditions at this stage in the development was puzzling. A tentative structure for annotinine (I) suggested by these authors did not, as they pointed out, explain the formation of 8-methylquinoline but did permit the representation of the dehydrogenated acid (L) by a structure consistent with its ultraviolet spectrum and colour reactions.

Some doubt was raised as to the theory of Wiesner et al (50) whereby certain annotinine derivatives were changed into compounds containing a new γ-lactone. According to this theory the acid (XXXII) should contain the grouping -O-CO-C=C-COOH. It was felt, therefore, that dehydrogenation of (XXXII) would have given an o-phthalic acid derivative as one of the carboxyls had been shown to be attached to the benzene ring in acid (L). This, however, was not the case as an amide was formed involving the other carboxyl and the nitrogen attached to the benzene ring. It was mentioned, however, that these difficulties concerning the acceptance of the relactonization theory could only be overcome by assuming either a change in the carbon skeleton in the formation of (L), or, that the lactone ring in some of the derivatives of annotinine is larger than a 5-membered

ring despite the infrared evidence.

Valenta, Wiesner, Bankiewicz, Henderson and Little (53) in 1956 repeated the dehydrogenation reaction of the amino acid (XXXII) as carried out by Anet and Marion (44)(47) obtaining the lactam carboxylic acid (L). The neutral material of the dehydrogenated product was carefully chromatographed and the compound (LI), which had been obtained by Anet and Marion by the decarboxylation of (L), was separated. In addition, a compound (LIV), $C_{13}H_{13}ON$, was obtained which proved to be identical with a previously known compound obtained by the condensation of ethyl acetoacetate and the tetrahydroquinoline (LVIII) according to the method of Reissert (54). Treatment of the lactam (LI) with palladium charcoal at 270° gave an almost quantitative yield of (LIV). When the synthetic compound (LIV) was reduced with sodium amalgam, the compound (LV), $C_{13}H_{15}ON$, was formed which was a synthetic racemate, of which (LI) was one of the optical antipodes.

Wiesner et al found that the lactam carboxylic acid (L) had an ultraviolet absorption spectrum very similar to that of m-acetylaminobenzoic acid and not that of the p-isomer. With this information, (L) had to be represented by one of two structures (LVI) or (LVII), representing the two meta positions of the carboxyl groups with reference to the position of the nitrogen group attached to the benzene ring.

Synthesis of (LVII) commenced with 5-carboxytetrahydroquinoline (LIX) which was converted into the oily methyl ester (LX). This was reduced with lithium aluminum hydride to the corresponding alcohol (LXI). The alcohol was condensed with ethyl acetoacetate to give (LXII) in low yield. A two-stage oxidation with chromic acid in pyridine followed by permanganate in acetone yielded the crude acid (LXIII). This was reduced with sodium amalgam to give (LVII), $C_{14}H_{15}O_3N$. The infrared spectrum of the methyl ester of (LVII) in chloroform was similar but definitely non-identical with the spectrum of the "natural" ester (i.e. ester of (L)).

The synthesis of (LVI) was analogous to that of (LVII) commencing with 7-carboxytetrahydroquinoline (LXIV) through the steps to (LXVIII) and thence to (LVI). The ultraviolet and infrared spectra of the methyl ester of (LVI) were found to be identical in all respects with the optically active "natural" ester (i.e. ester of (L)). As a point of interest it was noted by the authors that the ultraviolet spectra of (LVI) and (LVII) were slightly but distinctly different and this was considered probably due to the steric interaction of the carboxyl and methyl in (LVI). Hence (L) was shown to be an optical antipode of the racemate (LVI) and the structure of (L) was therefore established as that shown in Fig. 1, and not the tentative structure suggested by the previous authors.

Valenta, Stonner, Bankiewicz and Wiesner (55) reported that the treatment of the amino acid (XXXII) with diazomethane produced the crystalline methyl ester (LXIX), $C_{15}H_{21}O_4N$. The amino acid itself had pKa values of 4.03 and 9.3 whereas the methyl ester (LXIX) had the pKa value 6.8. These values were, therefore, in agreement with the expected values for a β-amino acid derivative. The resistance of the ester (LXIX) to saponification indicated that the carbomethoxy group was tertiary.

The investigation concerning the existence of two different lactone rings, as in compounds (IV) and (XXXV) was continued by these workers. Treatment of the hydroxy acid (XXXVI) with acetic acid in pyridine for 48 hours gave the acetoxy γ-lactone (LXX), $C_{18}H_{22}O_5NCl$. This was considered to be the O-acetyl derivative of the hydroxy lactone (XXXV). When (XXXVI) was acetylated for three hours at 100°, the corresponding acetoxy acid (LXXI), $C_{18}H_{22}O_5NCl$ was formed; thereby blocking the hydroxy group in the hydroxy acid. Relactonization to the compound (LXX) was then effected quantitatively by heating (LXXI) with a trace of p-toluenesulphonic acid. Due to the remote possibility of acetyl migration it was acknowledged that the above did not constitute rigorous evidence of the relactonization theory but it did give strong support for same.

Compound (XII) previously reported by MacLean and

Prime (46) also helped to elucidate some of the properties of the lactone ring. When refluxed under mild conditions with potassium hydroxide in methanol, (XII) was changed to a hydroxymethyl ester (LXXII), $C_{17}H_{25}O_4N$. Saponification of this ester with an excess of ethanolic potassium hydroxide produced the corresponding acid (LXXIII), $C_{16}H_{23}O_4N$, which could be converted back to (LXXII) by treatment with ethereal diazomethane.

When the lactone (XII) was treated with ethanolic potassium hydroxide direct saponification took place with the formation of the hydroxy acid (LXXIV) characterized through its methyl ester (LXXV), $C_{17}H_{25}O_4N$. When (LXXV) was treated with methanolic potassium hydroxide, the ester (LXXII), above, was formed quantitatively.

Wiesner et al presented a rationalization of the above transformations. It was pointed out that methanolic potassium hydroxide contained a considerably higher concentration of methoxide than hydroxide ion. The lactone ring of (XII) was opened by the methoxide ions giving the ester (LXXV) which was immediately isomerized under the conditions used to (LXXII). In the case of ethanolic potassium hydroxide, the lactone of (XII) was opened by a hydroxyl ion rather than an ethoxide ion. The corresponding hydroxy acid (LXXIV) so produced was present as a carboxylate ion which was stable to epimerization. Treatment with diazomethane gave the

unstable ester (LXXV). This sequence established that the lactone carboxyl was secondary and, further, that the original configuration of the carboxyl group in annotinine is less stable than the epimeric one.

Acetylation of the two hydroxy esters (LXXII) and (LXXV) produced the corresponding acetoxy derivatives (LXXVI) $C_{19}H_{27}O_5N$, and its isomer (LXXVII), respectively. As the difference in molecular rotations in (LXXII) and (LXXVI) was identical in sign and magnitude with that of (LXXV) and (LXXVII), it followed that the only difference between the two esters (LXXII) and (LXXV) was the configuration of the ester groups.

Oxidation of these latter two esters with chromium trioxide in pyridine gave the corresponding keto esters (LXXVIII), $C_{17}H_{23}O_4N$, and the isomeric (LXXIX), respectively. This, therefore, corroborated the secondary nature of the hydroxyl group contained in the original lactone of annotinine (I).

A direct correlation between the unsaturated hydroxy acid-B (XXXVI) and the two saturated hydroxy acids (LXXIII) and (LXXIV) was obtained by the conversion of (XXXVI) to (LXXIII) by hydrogenation; further substantiation of the identification being the conversion of (LXXIII) into the methyl ester (LXXII).

The relationship of the carboxyl to the conjugated

amide group was further clarified by the following transformations commencing with the conversion of the acid (XXXVI) by oxidation with chromium trioxide in pyridine to an oily product from which the ketolactone (LXXX), $C_{16}H_{18}O_4NCl$, was obtained. This compound showed only end absorption in the ultraviolet and a single amide peak at 1665 cm.$^{-1}$ in the infrared as well as a ketonic carbonyl band at 1725 cm.$^{-1}$, indicative of a keto group in a 6-membered ring and a γ-lactone band at 1790 cm.$^{-1}$. In alkaline solution the ketolactone (LXXX) had an ultraviolet spectrum almost identical with the acid (XXXVI) which indicated that the α,β-unsaturated amide group had been regenerated. On boiling several hours in alkali the spectrum remained unchanged and the corresponding keto acid (LXXXI) could be reconverted to the ketolactone (LXXX) by p-toluenesulfonic acid in benzene. This was evidence, therefore, that the keto group was not in the α- or β- position with respect to the lactam carboxyl and its logical position was at the site of the original lactone hydroxyl. Rigorous evidence supporting this conclusion was obtained by the direct conversion of (LXXX) into the keto ester (LXXVIII). Considering the alternate route of formation of (LXXVIII) there remained no doubt that the keto group in (LXXX) occupied the site of the original lactone hydroxyl.

With regard to the formation of the lactam carboxylic

acid (L) from the amino acid (XXXII) these authors remarked on the difficulty in rationalizing this conversion in a simple unexceptional manner bearing in mind all the limitations imposed by their particular work.

The partial structure of annotinine hydrate (II) by a consideration of the various alternatives was determined with the γ-lactone and hydroxyl groups occupying the relative positions indicated in the flow chart, Fig. 1. This partial structure was adduced mainly from the absence of α-ketol properties in the oxidation product (XVII) of annotinine hydrate (II) described previously by Meier, Meister and Marion (43).

Wiesner, Valenta, Ayer and Bankiewicz (56)(57) reported again in 1956 the dehydration of the hydroxy methyl ester (LXXXII), $C_{17}H_{22}NO_4Cl$, obtained from the hydroxy acid (XXXVI). Treatment of (LXXXII) with phosphoric acid in boiling xylene produced an anhydroester (LXXXIII), $C_{17}H_{20}O_3NCl$, and an isomeric α-pyridone ester (LXXXIV). The anhydro ester (LXXXIII) gave the corresponding acid (LXXXV), $C_{16}H_{18}O_3NCl$ on saponification. On treatment of this acid with p-toluenesulphonic acid in benzene a γ-lactone (LXXXVI), $C_{16}H_{18}O_3NCl$, was formed --reportedly by the addition of the carboxyl across the double-bond conjugated with the amide group.

Hydrolysis of the pyridine ester (LXXXIV) gave the

pyridine acid (LXXXVII), $C_{16}H_{18}O_3NCl$, which in turn took up two moles of hydrogen in alcohol with platinum oxide to give a pyridone acid (LXXXVIII), $C_{16}H_{21}O_3N$, a tricyclic compound formed by the opening of a ring and formation of a double-bond.

The anhydro ester (LXXXIII) was dehydrogenated with palladium charcoal at 290° for two hours giving a good yield of the quinolone acid (LXXXIX), $C_{13}H_{11}O_3N$. The structure of this compound was determined from its decarboxylation to the quinolone (XC), $C_{12}H_{11}ON$, identified by comparison with an authentic sample and also from the reduction of (LXXXIX) to the dihydroquinolone acid (XCI), $C_{13}H_{13}O_3N$, by sodium amalgam. The ultraviolet spectrum of (XCI) was identical and the infrared spectrum very similar to the spectra of (LVI) previously synthesized by the authors (53).

A tentative structure for annotinine (I) was proposed by these authors on the basis of the foregoing and assuming that the same hexahydrojulolidene skeleton was present in annotinine as appeared in the dehydrogenation products. This structure lacked only the specific detail concerning the ring size and attachment of one of the rings when compared with the now established structure of annotinine.

In 1957, Betts and MacLean (58) drew attention to the

fact that insofar as certain structural features of annotinine were concerned, such as the size of the oxide ring and the lactone ring, unequivocal evidence had not as yet been obtained. The matter of rearrangements in the dehydrogenation studies was also considered. Evidence was adduced for the presence of the epoxide function and the size of the nitrogen ring carrying the epoxide. Other reactions and conclusions were reported as reviewed hereunder.

Annotinine lactam (IV) when treated with boiling aqueous sulphuric acid was converted to the annotinine lactam diol (XCII), $C_{16}H_{21}O_5N$, which had two hydroxyl bands at 3530 cm.$^{-1}$(sharp) and 3300 cm.$^{-1}$(broad) in the infrared spectrum. It was expected that (XCII) contained the arrangement $\rangle$N-CO-CHOH-CHOH- and would be susceptible to glycol cleavage reagents; however, periodate did not react. Compound (XCII) was, however, slowly oxidized with the consumption of 3 moles of lead tetraacetate. Permanganate or chromic acid oxidation in acetic acid gave two products in low yield, one neutral (XCIII), $C_{15}H_{17}O_4N$, and the other acidic (XCV), $C_{15}H_{19}O_5N$.

The neutral product (XCIII) had three strong carbonyl absorption bands in the infrared spectrum at 1708 cm.$^{-1}$ (γ-lactam), 1760 cm.$^{-1}$ (5-membered cyclic ketone) and 1780 cm.$^{-1}$ (lactone). Treatment of (XCIII) with sodium borohydride in ethanol gave compound (XCIV), $C_{15}H_{19}O_4N$. This compound had

two carbonyl bands and lacked the band at 1760 $cm.^{-1}$ which was replaced by a new band at 3335 $cm.^{-1}$ in the hydroxyl region. The presence of a 6-membered nitrogen ring was therefore supported by the formation of a γ-lactam with the loss of a single carbon atom.

The acidic product (XCV) had three carbonyl absorption peaks in its infrared absorption spectrum at 1610 $cm.^{-1}$ (amide), 1705 $cm.^{-1}$ (carboxylic acid) and 1775 $cm.^{-1}$ (γ-lactone). Treatment of a methanolic solution of the acid (XCV) with diazomethane gave the methyl ester (XCVI), $C_{16}H_{21}O_5N$. Treatment of (XCV) with dilute sulphuric acid produced formic acid in equimolar amount and the amino acid (XXXII). It was concluded, therefore, that (XCV) was the N-formyl derivative of (XXXII).

Betts and MacLean explained the formation of the products (XCIII) and (XCV) (see Fig. 3). In the case of (XCIII) initial fission was believed to occur between the lactam carbonyl and the adjacent hydroxyl of the diol. This would result in the formation of a carbamic acid which would lose carbon dioxide to yield the secondary amine. Oxidation of the other hydroxyl of the diol would form an α-keto acid which cyclized with the secondary amine to yield (XCIII). Initial fission between the two hydroxyls of the diol producing an N-oxalyl carboxylic acid which under the reaction conditions would decarboxylate to form (XCV). The amino acid

(XXXII) could be formed from either (XCIII) or (XCV) by hydrolysis or oxidation or a combination of the two.

An allylamine structure had been assigned by Anet and Marion (44)(47) to the unsaturated lactone-A (VII). Due, however, to its lack of reactivity with cyanogen bromide, Betts and MacLean (58) reinvestigated the barium permanganate oxidation of (VII). The amino acid (XXXII) was the major oxidation product as had been established by Anet and Marion. Another product proved to be identical with the N-formyl amino acid (XCV). The third product (XCVII) was isolated as its methyl ester (XCVIII), $C_{17}H_{21}O_6N$. This ester had four bands in the carbonyl region; a double peak at 1633 and 1645 $cm.^{-1}$ (amide), bands at 1710 $cm.^{-1}$ (keto), 1743 $cm.^{-1}$ (carbomethoxy) and 1780 $cm.^{-1}$ (γ-lactone). On hydrolysis of (XCVIII) a small amount of (XCIII) was recovered together with formic acid, thereby indicating the structure of (XCVIII).

Betts and MacLean pointed out that the inertness of (VII) towards cyanogen bromide, together with the formation of the N-formyl derivatives on oxidation of (VII), suggested that (VII) might contain a vinylamine rather than an allylamine structure. Ozonolysis of (VII) was therefore carried out to clarify this point. If a vinylamine structure were present, it would be expected that an N-formyl derivative

would be formed and, if an allylamine structure, an amino dicarboxylic acid. Instead, the conjugated lactam (XIII) was formed, the formation of which was more readily rationalized from an allylamine structure than from the alternate vinylamine structure.

The formation of (XIII) was considered to proceed via the formation of a hydroperoxide at the methylene carbon and its subsequent decomposition. On the assumption that (XIII) was an intermediate in the permanganate oxidation, then (XCV) could be formed by hydroxylation to the lactam diol followed by cleavage, as outlined above with (XCII). The amino acid (XXXII) was the product of complete oxidation. A mechanism for the oxidation of (VII) to (XCVII) was proposed.

Reduction of annotinine (I) with hydrogen over Raney nickel yielded two products, the dihydrolactone-A (V) and a hydroxylactone (XCIX), $C_{16}H_{23}O_3N$, formed by the opening of the oxide ring. The two derivatives of annotinine which had been used in dehydrogenation studies were (a) the amino acid (XXXII), obtained by the oxidation of (VII) and of (X), and, (b) the methyl ester (LXXXIII) derived also from (X). Both (VII) and (X) had been obtained by dehydration reactions in acid media where rearrangement of the carbon skeleton of annotinine was possible, if not probable. As (V) was the dihydro derivative of (VII), the three compounds (V), (VII)

and (X) were therefore related as to skeletal structure. Compound (V), however, from the reaction described at the beginning of this paragraph, was shown to be of the same skeletal arrangement as annotinine itself. Hence, it was established that the original skeletal arrangement was preserved in (VII) and (X).

Betts and MacLean also found that reduction of the lactam (IV) over platinum catalyst in neutral or basic media yielded the hydroxy lactam (XI). In the presence of a small amount of acid, however, annotinine (I) and the lactam chlorohydrin (IX) were formed. While the formation of (IX) was normal, the unusual reduction of (IV) back to annotinine (I) was believed to be a peculiarity of the α,β-epoxy lactam system in (IV).

In 1957, Martin-Smith, Greenhalgh and Marion (51) subjected annotinine (I) to prolonged treatment with boiling dilute sulphuric acid. These workers failed to obtain the compound (XXXVIII), m.p. 75°, obtained by Wiesner et al (49) (50) under similar treatment but instead obtained three products. One was present only in traces while the other two were annotinine hydrate (II)(8%) and the isomeric annotinine diol, (C), $C_{16}H_{23}O_4N$, (67%). Acetylation of (C) gave the diacetyl derivative (XXXIX) which was the same as the diacetyl derivative of (XXXVIII) which Wiesner et al (49)

(50) had obtained. It was thought probable that the annotinine diol (C) contained the same γ-lactone as annotinine (I) itself.

Boiling annotinine (I) with aqueous barium hydroxide for several hours gave rise to a trihydroxy acid (CI), $C_{16}H_{25}O_5N$, isolated as the sulphate. On treatment with concentrated hydrochloric acid, (CI) gave rise to the chlorohydrin (VI) and, on treatment with 25% sulphuric acid, it was converted to annotinine diol (C). Oxidation of (C) with barium permanganate gave rise to the amino acid (XXXII). On the other hand, it had been shown (43) that the oxidation of annotinine hydrate (II) gave rise to a hydroxyketo lactone (XVII).

Boiling annotinine (I) with aqueous barium hydroxide for 40 minutes produced a good yield of a hydroxy acid (CII), $C_{16}H_{23}O_4N$, containing in the infrared spectrum a diffuse peak at 1575 -1568 $cm.^{-1}$, typical of an ionic carboxyl group, and a strong hydroxyl peak at 3330 $cm.^{-1}$. This acid, (CII), with the epoxy ring intact was designated as annotininic acid. Treatment of (CII) with concentrated hydrochloric acid yielded annotinine chlorohydrin (VI).

Annotininic acid (CII) was smoothly converted to its methyl ester (CIII), $C_{17}H_{25}O_4N$, on treatment with diazomethane. Boiling the ester (CIII) with barium hydroxide

regenerated annotininic acid (CII). When methyl annotinate (CIII) was treated with potassium methoxide in absolute methanol, it was converted into the isomeric ester, methyl epiannotininate (XXXVII). This latter ester was also formed by the treatment of annotinine (I) with an equivalent of potassium methoxide in methanol at room temperature. The action of barium hydroxide on the epiester (XXXVII), however, converted it to annotinine hydrate (II) rather than regenerating the acid, as in the case of (CIII). It was pointed out by Marion et al (51) that when methyl epiannotininate (XXXVII) had been prepared previously (49)(50) by the action of methanolic potassium hydroxide on annotinine (I), followed by methylation with diazomethane, the essential nature of the reaction, which was a methanolysis of the lactone followed by an epimerization, had been masked. It also appeared unlikely that epiannotininic acid could be isolated as such as it changed rapidly into the hydrate (II).

The above transesterification with epimerization was similar to that observed by Wiesner et al (55) with unsaturated lactamic derivatives of annotinine, as dealt with previously in this review.

The same transesterification took place when annotinine lactam (IV) was converted to the methyl epiannotininate lactam (CIV), $C_{17}H_{23}O_5N$, which compound was also obtained by the

oxidation of methyl epiannotininate (XXXVII). Similarly, the methyl ester (LXIX) of the amino acid (XXXII) was found to undergo transesterification under the influence of potassium methoxide and gave rise to a diester (CV), $C_{16}H_{25}O_5N$.

Martin-Smith, Greenhalgh and Marion (51) proposed a tentative structure for annotinine, incorporating a 4-membered ring and a 7-membered ring containing the nitrogen and bearing the epoxide ring. On the basis of this structure for annotinine it was felt that the derivation of such compounds as the lactam carboxylic acid (L) from the amino acid (XXXII) and the derivation of the quinolone acid (LXXXIX) from the hydroxy epiester (LXXXIII) could be more easily explained than on the basis of the structure proposed by Wiesner et al (56). For example, the formation of (LXXIX) was rationalized by a contraction of a 7-membered ring in (LXXXII), a wandering of a carboxyl group and aromatization followed by elimination of the side chain.

Also in 1957, Wiesner, Ayer, Fowler and Valenta (59) published a short account of their work on the definition of the periphery of annotinine (I). This work was centered mainly on reactions of the lactam keto ester (LXXVIII), the partial formula of which had been previously formulated (55) and the periphery of which was now elucidated (59).

This compound (LXXVIII) was refluxed with selenium

dioxide--dioxane resulting in the oxidation to four products, compound (CVI), $C_{17}H_{21}O_4N$; compound (CVII), $C_{17}H_{21}O_5N$; compound (CVIII), $C_{17}H_{21}O_5N$, epimeric with (CVII), and, compound (CIX), $C_{17}H_{19}O_4N$.

Treatment of compound (LXXVIII) with bromine in acetic acid produced the dibromide (CX), $C_{17}H_{21}O_4NBr_2$. Hydrolysis of (CX) with sodium hydroxide in dioxane, followed by re-esterification of the acid, produced (CVIII) in 60% yield.

Vigorous hydrogenation of (CIX) over platinum oxide in acetic acid gave the hydroxy ester (LXXV) previously described (55). Reduction of the keto group of (CVI)with sodium borohydride gave the corresponding alcohol (CXI), $C_{17}H_{23}O_4N$. Isomerization of the alcohol (CXI) back to the lactam keto ester (LXXVIII) was effected by the use of sodium methoxide in boiling methanol (see Fig. 4 for probable mechanism). Oxidation of (CVIII) by periodate-permanganate method (60) gave formic and succinic acids, isolated by partition chromatography on silica gel.

Compound (LXXVIII) also gave a quantitative yield of the benzylidene derivative (CXII), $C_{24}H_{27}O_4N$. Similarly, an amorphous furfurilidene derivative (CXIII) was prepared and ozonized. On decomposition in acetic acid the ozonide was changed into an amorphous acid (CXIV) which, by heating to 110-160°, lost one mole of carbon dioxide to give the

dicarboxylic acid (CXV), $C_{15}H_{21}O_5N$.

It was at this stage that Wiesner et al (59) proposed the now established structure for annotinine. Confirmation of this molecular structure followed shortly thereafter in a publication by Przybylska and Marion (61) through an X-ray diffraction study of annotinine bromohydrin, $C_{16}H_{22}O_3NBr$. This study disclosed that in this molecule the carbon atom of the methyl group was directed towards the carbonyl oxygen of the lactone. To keep these atoms at a distance equal to the sum of their van der Waals' radii, some of the bond angles varied considerably from the value of 109.8°, thus decreasing the stability of the molecule. In agreement with chemical evidence, the bromine atom was found to be "trans" with respect to the hydroxyl group.

From the photograph of the molecular model of annotinine bromohydrin published by these authors (61), it appeared that the conformation was such that the bromine and hydroxyl groups were in the "trans-diequatorial" positions (see Fig.5) as would be anticipated. Initial opening of the epoxide ring of annotinine (I), however, would have placed these two groups in the "trans-diaxial" conformation, followed by a change to the more stable "trans-diequatorial" conformation. From this knowledge the relative configuration of annotinine, as indicated in the above figure, can be derived.

More recently, in 1958, Betts and MacLean (62) published the results of their studies on reactions centering about the dihydrolactone-A (V) which these authors called desoxodihydroannotinine. Compound (V) reacted readily with phenyl lithium to yield diphenyldesoxodihydroannotinine (CXVI), $C_{28}H_{35}O_2N$. On treatment with boiling aqueous hydrochloric acid, (CXVI) dehydrated readily giving the anhydro derivative (CXVII), $C_{28}H_{33}ON$. This latter compound had an ultraviolet spectrum characteristic of a 1,1-diphenylethylene although (CXVII) would not absorb hydrogen at low pressures nor oxidize with ease.

When oxidized with aluminum isopropoxide in the presence of cyclohexanone, (CXVI) was converted to the hydroxyketone (CXVIII), $C_{28}H_{33}O_2N$; however, when oxidized with chromic acid in acetic acid, (CXVI) was converted to the dihydroxyketone (CXIX), $C_{28}H_{33}O_3N$. As the new hydroxyl function in (CXIX) was not oxidized under the conditions used, it is probably tertiary in character. Introduction of this hydroxyl group could be effected at one of the two activated positions, either adjacent to the carbonyl to form an α-hydroxy ketone, or, adjacent to the nitrogen to form a carbinolamine. Tests for the carbinolamine were negative. Anhydronium salts of (CXIX) were not formed, the methiodide of (XCIX) was normal in character and hydride reduction of

(CXIX) or its hydrochloride produced a trihydroxy compound (CXX), $C_{28}H_{35}O_3N$. While (CXIX) or (CXX) did not react with periodate, (CXIX) did react with three moles of lead tetra-acetate. In the infrared spectra of (CXVIII) and (CXIX), the carbonyl absorption frequencies shifted from 1700 $cm.^{-1}$ to 1720 $cm.^{-1}$, respectively, implying a close structural relationship between the hydroxyl and carbonyl groups. Similarly in the case of diphenylannotinine (XXX), it was found that the introduction of the new hydroxyl function was dependent upon the presence of the carbonyl group. It was indicated, therefore, that compound (CXIX) contained an α-hydroxyketone structure.

The products of the oxidation of (CXVI) with potassium tertiary butoxide were dependent upon the refluxing solvent used. When carried out in benzene, a ketone (CXXI), $C_{28}H_{33}O_2N$, was formed, whereas in toluene, a conjugated ketone (CXXII), $C_{15}H_{21}ON$, was isolated. This conjugated ketone was also obtained from both (CXXI) and (CXVIII) by treatment with potassium tertiary butoxide in refluxing toluene. Elimination of the diphenyl carbinol group from (CXVI), (CXVIII) or (CXXI) to form (CXXII) was found to occur as the elimination of benzhydrol which in the presence of air was converted, to some extent, to benzophenone. The mechanism of the elimination was considered to be a "reverse-Michael"

reaction (see Fig. 6).

Partial conversion of (CXVIII) to (CXXI) by prolonged refluxing with sodium ethoxide was consistent with these two forms being epimers. These two ketones behave differently towards chromic acid in glacial acetic acid; the former being converted to (CXIX), as previously noted, and (CXXIX) remaining unchanged. The difference in this behaviour was considered as probably dependent upon the conformation of the tertiary hydrogen in these two compounds.

The presence of a methylene group at the C6 position (ref. Fig. 1) in annotinine (I) was supported by the ultraviolet spectrum of (CXXII), characteristic of an α,β-unsaturated ketone. The isomeric ketones (CXVIII) and (CXXI) could be epimeric only at position C8, while (CXIX) carried its hydroxyl function at the same position. These findings were in accord, therefore, with the established structure of annotinine.

Wiesner et al (42) have given one "from several plausible" mechanisms for the conversion of the amino acid (XXXII) to the lactam carboxylic acid (L). Another plausible mechanism, involving an acid anhydride intermediate, is proposed, as shown in Fig. 7.

2 Lycopodine ($C_{16}H_{25}ON$). Lycopodine (I) is the major alkaloid of a number of Lycopodium species. The above formula was assigned in 1938 by Achmatowicz and Uzieblo (24) and confirmed by Marion and Manske (63) in 1942.

This alkaloid, like all other alkaloids of the Lycopodium species, with the exception of annotinine and nicotine, is still of undetermined structure. A chronological review of the structural investigations and reactions carried out to date on lycopodine (I) is presented. To supplement this review, a flow chart, Fig. 8, has been prepared embodying the development of the partial structure, insofar as the functional groups and partial ring structure are concerned.

The absence of methoxyl and N-methyl groups, as well as active hydrogen (Zerewetinoff), as determined by Achmatowicz and Uzieblo (24), indicated that the nitrogen was probably common to two rings and that the oxygen was present either in a cyclic ether, or, in a ketone group. Marion and Manske (63), in 1942, found lycopodine (I) inert to phenylmagnesium bromide at 100° and to hydrogenation over Raney nickel at 200° and 2000 p.s.i. It appeared, therefore, that the oxygen was probably present in a cyclic ether group.

Selenium dehydrogenation of lycopodine (I) yielded a complex mixture from which five bases were separated as their picrates. These bases included 7-methylquinoline (II),

$C_{10}H_9N$, (picrate, m.p. 242°; styphnate, m.p. 242°); 5,7-dimethylquinoline (III), $C_{11}H_{11}N$, (picrate, m.p. 246°); (IV), $C_{11}H_{15}N$, (picrate, m.p. 167°); (V), $C_{16}H_{19}ON$, (picrate, m.p. 169°) and (VI), $C_{16}H_{21}N$, (picrate, m.p. 151°). The 7-methylquinoline (II) was obtained, also, by heating (I) with palladium - barium sulphate in an atmosphere of nitrogen and by heating (I) with phthalic anhydride in an evacuated tube. These reactions indicated the probability that lycopodine (I) contained a quinoline nucleus completely hydrogenated.

In 1950, MacLean, Manske and Marion (36) published evidence showing that the oxygen of lycopodine (I) was present in a carbonyl group rather than in an ether linkage. An absorption band at 1693 $cm.^{-1}$ in the infrared absorption spectrum indicated the presence of a carbonyl which was confirmed by the formation of a hydrazone (VII), $C_{16}H_{27}N_3$, (m.p. 208-210°; perchlorate, m.p. 257°(dec.)). Additionally, the keto group was reduced by lithium aluminum hydride to the alcohol dihydrolycopodine (VIII), $C_{16}H_{27}ON$, (m.p. 168°; I.R. absorption band at 3625 $cm.^{-1}$ (-OH); perchlorate, m.p. 223-224°). Conversion of lycopodine (I) with phenyl lithium to the tertiary carbinol (IX), $C_{22}H_{31}ON$, (m.p. 154-155°) constituted additional confirmation of the presence of a ketone group.

Lycopodine (I) was inert to a number of degradation reactions, such as the Emde and Hofmann degradations. Treatment with cyanogen bromide (von Braun degradation), however, yielded two isomeric, non-basic compounds: α-cyanobromolycopodine (X), $C_{17}H_{25}ON_2Br$, (m.p. 140°; yield 50-60%) and β-cyanobromolycopodine (XI), $C_{17}H_{25}ON_2Br$, (m.p. 108-109°). Treatment of (X) with silver acetate in benzene and by silver oxide in acetone failed to remove the bromine; however, complete removal was effected by the action of potassium acetate in ethanol. The resulting products were α-cyanoacetoxylycopodine (XII), $C_{19}H_{28}O_3N_2$, (m.p. 112-113°) and the corresponding α-cyanohydroxylycopodine (XIII), $C_{17}H_{26}O_2N_2$, (m.p. 97°). Acetylation of (XIII) with boiling acetyl chloride containing a trace of pyridine, produced (XII). Deacetylation of (XII) was effected by refluxing with potassium hydroxide in methanol. The amorphous acid (XIV), produced by the oxidation of (XIII) with chromic acid, yielded a crystalline methyl ester (XV), $C_{18}H_{26}O_3N_2$, (m.p. 82-83°). Oxidation of (XIII) to the acid (XIV) apparently involved the conversion of the alcoholic group, which must have been primary, into a carboxylic acid group.

When the same reagent, potassium acetate, was used in glacial acetic acid, α-cyanobromolycopodine (X) was reconverted to lycopodine (I) with small quantities of two isomeric

bases being formed in side-reactions. The α-cyanobromolycopodine (X) was then boiled with methanolic potassium hydroxide removing the elements of hydrogen bromide and yielding the non-basic compound (XVI), $C_{17}H_{24}ON_2$ (m.p. 143-144°). An isomeric, non-basic compound (XVII), (m.p. 102°) was formed by treatment of β-cyanobromolycopodine with potassium acetate in ethanol. Resistance of (XVI) and (XVII) to oxidation with potassium permanganate, chromic acid or ozone and to catalytic hydrogenation, indicated an absence of unsaturation. It was suggested that removal of the elements of hydrogen bromide may have been accompanied by cyclization; however, cyclization did not occur when (X) and (XI) were hydrogenated catalytically. Hydrogenolysis occurred with the formation of α-cyanolycopodine (XVIII), $C_{17}H_{26}ON_2$, (m.p. 130-131°) and β-cyanolycopodine (XIX), $C_{17}H_{26}ON_2$, (m.p. 73-93°), respectively.

In order to attempt another type of degradation, the α-cyanobromolycopodine (X) was treated with trimethylamine resulting in the quaternary salt, cyanotrimethylaminolycopodine bromide (XX), $C_{20}H_{34}ON_3Br$, (m.p. 259-260°). This salt was converted to the corresponding quaternary base by treatment with silver oxide and this base pyrolyzed in vacuo. Two products were obtained, the previously obtained neutral com-

pound (XVI) and a base (XXI), $C_{19}H_{31}ON_3$, (perchlorate, m.p. 230-231°(dec.)) formed from the quaternary base by the loss of methanol.

In 1953, Douglas, Lewis and Marion (30) reported the preparation of the oxime (XXII), $C_{16}H_{26}ON_2$, (m.p. 262-264°) from lycopodine (I). Beckmann rearrangement of the oxime (XXII) under the usual conditions did not occur.

These workers reported that lycopodine had a pKa 7.44, obtained by titration of the perchlorate in 50% methanol. Later, however, Barclay and MacLean (48) determined the pKa of lycopodine by titration of the perchlorate and the hydrobromide in 50% aqueous methanol. Values obtained were 9.06 and 9.07, respectively. Lycopodine (I) failed to condense with benzaldehyde to form a benzylidene derivative. Oxidation of dihydrolycopodine (VIII) with chromic acid back to lycopodine (I) was reported by Douglas, Lewis and Marion.

These same authors determined the relationship which existed between the two minor alkaloids L-14 and L-2 of the Lycopodium species and lycopodine (I). Treatment of dihydrolycopodine (VIII) with phosphorus pentachloride removed the elements of water yielding anhydrodihydrolycopodine (XXIII), $C_{16}H_{25}N$, (perchlorate, m.p. 238-239°; $[\alpha]_D^{26}$ -107° (c, 1.10 in methanol)). The hydroxyl absorption band in the infrared

spectrum of (VIII) was not present in (XXIII). It was then definitely established that alkaloid L-14 was identical with the anhydrodihydrolycopodine (XXIII).

Acetylation of dihydrolycopodine (VIII) (pKa 9.2 in 50% aqueous methanol) took place readily with trifluoracetic anhydride and acetic acid producing O-acetyldihydrolycopodine (XXIV), $C_{18}H_{29}O_2N$, (m.p. 90-95°; pKa, 8.4 in 50% aqueous methanol; I.R. absorption band at 1730 $cm.^{-1}$ (carbonyl); perchlorate, m.p. 246-247° - when immersed at 205°). Alkaloid L-2 was shown to be identical with O-acetyldihydrolycopodine (XXIV). Distillation of (XXIV) at atmospheric pressure resulted in partial decomposition to an oily base which proved to be 7-methylquinoline (II).

In an effort to obtain more information concerning the ring structure of the alkaloid, Barclay and MacLean (39), in 1956, extended the study of the reactions of α-cyanobromolycopodine (X). Treatment of α-cyanolycopodine (XVIII) with 2M hydrochloric acid hydrolyzed the cyanamide to the secondary base α-des-dihydrolycopodine (XXV), $C_{16}H_{27}ON$, (m.p. 49°; pKa 9.05 (50% aqueous methanol); I.R. spectrum, no nitrile bands, 1694 $cm.^{-1}$ (carbonyl) and 3330 $cm.^{-1}$ (>NH); hydrochloride, m.p. above 300°). This secondary base (XXV) was acetylated with acetic anhydride to a neutral amorphous

compound (XXVI) (I.R. spectrum - no absorpiton in >NH region, 1640 cm.$^{-1}$ (carbonyl of N-acetyl), 1700 cm.$^{-1}$ (keto carbonyl)). Methylation of (XXV) was effected by treatment with formaldehyde and formic acid to a tertiary base (XXVII) (I.R. spectrum - no bands in the 3300-3400 cm.$^{-1}$ region, band at 1694 cm.$^{-1}$ (carbonyl)). Treatment of (XXVII) with concentrated hydrochloric acid converted it to the salt (XXVIII), $C_{17}H_{29}ON$, $HCl \cdot H_2O$ (m.p. 256°); while treatment with methyl iodide converted (XXVII) to the quaternary salt (XXIX), $C_{18}H_{32}ONI$, (m.p. 192.5°) but in low yield. This quaternary salt (XXIX) was inert to reductive fission. Compared with the inability of (XXVII) to form a methiodide readily, lycopodine (I) formed a methiodide rapidly and quantitatively under similar conditions.

The secondary base, α-dcs-dihydrolycopodine (XXV) was reduced by lithium aluminum hydride to the corresponding carbinol (XXX), $C_{16}H_{29}ON$, (m.p. 168-169°; pKa 9.80 (50% aqueous methanol); I.R. spectrum - 3150 cm.$^{-1}$ and no carbonyl absorption). Direct reduction of α-cyanobromolycopodine (X) with lithium aluminum hydride also produced the carbinol (XXX), involving reduction of the carbonyl, hydrogenolysis of the bromine and conversion of >N-CN to >N-H. In addition to the carbinol, in this latter reaction, the corresponding neutral

cyanamide (XXXI), $C_{17}H_{28}ON_2$, (m.p. 191-192°) was produced. Compound (XXI) was also produced slowly and inefficiently by the Meerwein-Ponndorf reduction (aluminum isopropoxide) of α-cyanolycopodine (XVIII) and readily and in good yield by the reduction of α-cyanobromolycopodine (X) with sodium borohydride.

These authors studied the bromination of α-cyanolycopodine (XVIII) involving the ready consumption of bromine with the deposition of a solid amorphous precipitate. From this reaction product, a monobromo derivative (XXXII), $C_{17}H_{25}ON_2Br$, (m.p. 129°; I.R. spectrum - 2210 cm.$^{-1}$ (-C≡N), 1710 cm.$^{-1}$ (carbonyl)) was separated. The displacement of the carbonyl absorption in the infrared spectrum was 10 cm.$^{-1}$ in comparison with its position of 1700 cm.$^{-1}$ in the starting material (XVIII). This shift was considered to be consistent with an α-bromoketone. Compound (XXXII) was very unstable when in contact with water or aqueous alkali, losing its bromine and forming a mixture of compounds probably alcoholic or olefenic in nature. The main amorphous bromination product was likewise unstable in aqueous alkali. Treatment of this amorphous residue with alkaline aqueous dioxane produced a product from which a crystalline derivative was obtained. This compound (XXXIII), $C_{17}H_{24}O_2N_2$, (m.p. 157-158°; I. R.

spectrum bands at 1640 cm.$^{-1}$, 1660 cm.$^{-1}$, 2210 cm.$^{-1}$ and 3340 cm.$^{-1}$; U.V. spectrum - 280 mμ (log ε, 4); deep blue colouration with ferric chloride in aqueous ethanol) had the properties of an enol. The infrared spectrum indicated the presence of a conjugated carbonyl group, an olefinic double-bond and a hydroxyl group. The ultraviolet spectrum was consistent with a hydroxylated α,β-unsaturated carbonyl chromophore, as indicated in the flow chart, Fig. 8. Such a structure would have arisen from an α,α-dibrominated ketone, $\geq CH-CBr_2-CO-$. It appeared, therefore, that bromination of (XVIII) proceeded to give a mixture of mono- and di-brominated ketones. While this was at variance with the failure of lycopodine (I) to form a benzylidene derivative, it was found that α-cyanolycopodine (XVIII) formed a monobenzal derivative (XXXIV), $C_{24}H_{30}ON_2$, (m.p. 179.5 - 180.5°; I.R. spectrum in CS_2 solution - 3050 cm.$^{-1}$, 3017 cm.$^{-1}$ and 1617 cm.$^{-1}$(aromatic); 1690 cm.$^{-1}$ (carbonyl); 2205 cm.$^{-1}$ (nitrile); in Nujol - 1607 cm.$^{-1}$ (aromatic); 1681 cm.$^{-1}$ (carbonyl); 2205 cm.$^{-1}$ (nitrile)) and the presence of a methylene group adjacent to the carbonyl was confirmed. This indicated that this methylene group was in a hindered position in lycopodine and the hindrance was relieved on ring fission in the formation of α-cyanolycopodine (XVIII).

Bromination of lycopodine (I) produced monobromo-

lycopodine (XXXV), $C_{16}H_{24}ONBr$, (hydrobromide, m.p. 290-295° (dec.); pKa 7.30 (50% aqueous methanol); I.R. spectrum - 1710 cm.$^{-1}$ (carbonyl), 2500-2700 cm.$^{-1}$(amine salt)). Bromination of the α-des-dihydrolycopodine (XXV) produced the monobromo derivative (XXXVI), $C_{16}H_{26}ONBr$, (hydrobromide, m.p. 275-280° (dec.); pKa 7.20 (50% aqueous methanol); I.R. spectrum - 1713 cm.$^{-1}$ (carbonyl), 1585 cm.$^{-1}$ (ionized salt of secondary amine)). Attempted conversion of (XXXV) and (XXXVI) resulted in decomposition. As each gave a Fehling's test, it was concluded that they were α-bromoketones.

From the pKa's of the bromo compounds (XXXV) and (XXXVI), compared with those of the parent bases and dihydrobases (I), (VIII), (XXV) and (XXX) (see above and flow chart, Fig. 8) the following inferences were drawn: (a) the similarity of the pKa's of lycopodine (I) and of α-des-dihydrolycopodine (XXV) suggested that in the ring fission reaction the relationship of the nitrogen and carbonyl groups had not been altered; and, (b) the greatly decreased basicity of the brominated bases (XXXV) and (XXXVI) implied a rather close proximity of the nitrogen and carbonyl functions in the molecule.

On the basis of the above review a tentative partial structure for lycopodine (I) has been incorporated in the flow chart, Fig. 8.

3 Acrifoline (L-27) ($C_{16}H_{23}O_2N$) A flow chart, Fig. 9, has been prepared showing some of the reactions of acrifoline and the probable functional groups of this base and its derivatives. In 1952, Bertho and Stoll (19) reported the absence of N-methyl and -OH groups in acrifoline. The lack of reaction with selenium dioxide and the absence of epoxide formation with peracetic acid indicated to them that the base was saturated. While acrifoline (I) failed to react with Grignard reagents and 2,4-dinitrophenylhydrazine, it did react with phenyl lithium to form (II), $C_{22}H_{29}O_2N$, (m.p. 230-231°; methiodide, 282°). While acrifoline (I) did not undergo Hofmann degradation, the methiodide of compound (II) did with the formation of (III), $C_{23}H_{31}O_2N$, (m.p. 192-193°). Acrifoline (I) failed to react with benzaldehyde in the presence of sodium ethylate.

In 1956, Perry and MacLean (41) made a further study of the nature of the functional groups in acrifoline (I), $C_{16}H_{23}O_2N$, (m.p. 99-104°; $[\alpha]_D^{19}$ = -266.2° (c, 1% chloroform); pKa 8.34 (50% aqueous methanol)). The infrared spectrum of acrifoline in Nujol showed very strong absorption in the hydroxyl region but very weak absorption in the carbonyl region. Another band at 1675 $cm.^{-1}$ was assigned by these workers as indicative of an unsaturated linkage.

Catalytic reduction of acrifoline (I) over Adams' catalyst at 50 p.s.i. yielded dihydroacrifoline (IV), $C_{16}H_{25}O_2N$, (m.p. 162.5 - 169.5°; I.R. absorption spectrum - 3500 cm.$^{-1}$ (-OH) and 1679 cm.$^{-1}$ (carbonyl); pKa 9.13 (50% aqueous methanol)) analyzed as its hydrochloride and hydrobromide salts. Reduction of dihydroacrifoline (IV) with lithium aluminum hydride yielded α-dihydroacrifolinol (V), $C_{16}H_{27}O_2N$, (m.p. 195.2°; I.R. spectrum - strong band at 3300 cm.$^{-1}$ (-OH), no bands attributable to carbonyl or an unsaturated linkage).

Treatment of acrifoline (I) with lithium aluminum hydride in boiling tetrahydrofuran converted (I) to acrifolinol (VI), $C_{16}H_{25}O_2N$, (m.p. 192°; I.R. spectrum - strong band at 3266 cm.$^{-1}$ (-OH) and weak absorption at 1425 cm.$^{-1}$ attributable to unsaturation). When (VI) was treated with hydrogen over Adams' catalyst it yielded an isomeric β-dihydroacrifolinol (VII), $C_{16}H_{27}O_2N$, (m.p. 165.5 - 167.5°; mixed m.p. with (V) depressed 40°; I.R. spectrum - strong band at 3290 cm.$^{-1}$ (-OH), spectrum almost identical with (V)). Perry and MacLean concluded that (V) and (VII) were stereoisomers with the isomerism being either about the newly-formed hydroxyl group, or, about the centers of the original double-bond in acrifoline (I).

Acrifoline was inert to attempted dehydration with boiling hydrochloric acid or phosphorus oxychloride. Treat-

ment of (I) with acetic anhydride yielded a monoacetate (VIII) $C_{18}H_{25}O_3N$, (m.p. 119-120°; I.R. spectrum - no hydroxyl but two new bands at 1740 cm.$^{-1}$ and 1230 cm.$^{-1}$, attributable to acetoxy group, and a band at 1715 cm.$^{-1}$(carbonyl group)). in admixture with a sample of alkaloid L-12, there was no depression in melting point. Also, the infrared spectra of compound (VIII) and L-12 were superimposable, Therefore, O-acetylacrifoline and alkaloid L-12 were the same alkaloid. Alkaline hydrolysis of (VIII) gave back the original free base (I).

These authors pointed out that the increase in basic strength on reduction of acrifoline (I), with pKa 8.34, to dihydroacrifoline (IV), with pKa 9.13, was consistent with an allylamine structure for acrifoline.

4 Obscurines

α-Obscurine ($C_{17}H_{26}ON_2$) Moore and Marion (17), in 1953, reported the separation of α- and β- obscurines from "obscurine" by chromatography on alumina. From a number of analyses of the bases and their picrates the following formulae were proposed as being the most probable:

α-obscurine - $C_{17}H_{26}ON_2$ (m.p. 283-284° (dec.))

β-obscurine - $C_{17}H_{24}ON_2$ (m.p. 322-323° (dec.))

The presence of a secondary amino group in α-obscurine was indicated by an absorption peak at 3411 $cm.^{-1}$ and the presence of a carbonyl group by a broad band at 1667 $cm.^{-1}$ in the infrared spectrum. The ultraviolet absorption spectrum of α-obscurine (I) (λ_{max} 255mμ, log ε, 3.75) seemed to indicate the presence of a cyclic lactam containing unsaturation.

Dehydrogenation of α-obscurine (I) with palladium charcoal yielded 7-methylquinoline (II) and 6-methyl-α-pyridone (III), C_6H_7ON, (m.p. 158.5°; I.R. spectrum - 3387 $cm.^{-1}$(>NH), double carbonyl bands at 1662-1651 $cm.^{-1}$ and smaller bands at 1628 and 1613 $cm.^{-1}$ usually attributable to a pyridone structure; U.V. spectrum - $\lambda_{max.}$ 229mμ, log ε, 3.87 and $\lambda_{max.}$ 304 mμ, log ε, 3.83).

It was found that α- and β-obscurines were very similar not only in their empirical formulae but in their properties.

Compound (III) and both α-obscurine (I) and β-obscurine produced an orange-red colour with aqueous ferric chloride and coupled with diazotized sulphanilic acid to produce a deep orange product. An attempt to convert α-obscurine (I) to β-obscurine by bromination of (I) followed by dehydrobromination altered (I) but did not form β-obscurine.

<u>β-Obscurine</u> ($C_{17}H_{24}ON_2$) Moore and Marion (17) reported that the infrared absorption spectrum of β-obscurine contained an absorption peak at 3385 $cm.^{-1}$, indicative of a secondary amino group and a strong double peak at 1659-1651 $cm.^{-1}$ with smaller peaks at 1620 $cm.^{-1}$ and 1613 $cm.^{-1}$, suggestive of an α-pyridone. The similarity between the absorption peaks of β-obscurine and those of 6-methyl-α-pyridone (see α-obscurine, derivative (III), above) indicated that an α-pyridone ring must be present in β-obscurine. This was substantiated by a comparison of the ultraviolet spectrum of β-obscurine ($\lambda_{max.}$ 232 mμ, log ε, 3.78 and $\lambda_{max.}$ 315mμ, log ε, 3.89) and that of 6-methyl-α-pyridone.

The partial structures of the obscurines are illustrated in Fig. 10.

5 Annotine (L-11) ($C_{16}H_{21}O_3N$) A flow chart, Fig. 11, has been prepared showing some of the reactions of annotine and the possible functional groups of this base and its derivatives.

Bertho and Stoll (19), in 1952, reported the absence of N-methyl, O-methyl and -OH groups in annotine. These authors assigned the formula, $C_{16}H_{23}O_4N$, to annotine which was later shown by Achmatowicz and Rodewald (20) to be identical with alkaloid L-11. Annotine (I) was found by Bertho and Stoll (19) to react with phenyl lithium to form the phenyl lithium derivative (II) (m.p. 215-216°), the formula for which was complicated by the lack of knowledge of the correct formula for annotine. Treatment of annotine (I) with lithium aluminum hydride in an ether solution yielded the compound (III), $C_{16}H_{23}O_3N$, (m.p. 162-163°). Here again the interpretation of this reaction was complicated by the lack of knowledge of the correct formula. It was reported that annotine (I) failed to react with benzaldehyde in the presence of sodium ethylate, with hydrogen, with selenium dioxide or with hydrazine. Attempted Hofmann degradation of the methiodide of annotine produced no satisfactory results as did the attempted von Braun bromocyanogen reaction.

In 1956, Perry and MacLean (41) made a further study

of the nature of the functional groups of annotine (I), $C_{16}H_{21}O_3N$, (m.p. 174-176°; pKa 7.50 (50% aqueous methanol); $[\alpha]_D^{19} = -114.5°$ (c, 1% chloroform); I.R. spectrum - in Nujol shows weak absorption at 3300 cm.$^{-1}$ (-OH) and strong band at 1712 cm.$^{-1}$ in the carbonyl region. - in CS_2 solution shows strong absorption at 3550 cm.$^{-1}$. - in chloroform solution shows strong absorption at 1720-1730 cm.$^{-1}$ (carbonyl) and at 1644 cm.$^{-1}$ (unsaturation)).

Attempts by Perry and MacLean to reduce annotine (I) with lithium aluminum hydride did not result in the formation of (III) reported by Bertho and Stoll (19). However, treatment of (I) with sodium borohydride in alcohol yielded annotinol (IV), $C_{16}H_{23}O_3N$. This base (IV) was crystallized as $C_{16}H_{23}O_3N \cdot 2C_2H_5OH$, (m.p. 131.5 - 132.5°; I.R. spectrum - broad band at 3550 cm.$^{-1}$ (-OH) and weak bands at 1650 cm.$^{-1}$ and 1403 cm.$^{-1}$ (unsaturation); in carbon disulphide solution, two bands in the hydroxyl region at 3510 cm.$^{-1}$ (str.) and 3380 cm.$^{-1}$ (broad) and a band at 3022 cm.$^{-1}$ attributed to unsaturation. (No carbonyl absorption was present.)). Catalytic reduction of annotinol (IV) yielded dihydroannotinol (V), $C_{16}H_{25}O_3N$, (m.p. 185.8°; I.R. spectrum in CS_2 solution - strong band at 3500 cm.$^{-1}$ (-OH) and a broad band at 3290 cm.$^{-1}$).

Annotine (I) absorbed one mole of hydrogen at 50 p.s.i.

over Adams' catalyst producing dihydroannotine (VI), $C_{16}H_{23}O_3N$, (m.p. 201.5 - 202.5°; pKa 8.61 (methanol); I.R. spectrum - strong band at 3200 cm.$^{-1}$ (-OH) and at 1728 cm.$^{-1}$ (carbonyl) but no bands attributable to unsaturation).

Perry and MacLean confirmed the presence of a single carbonyl group in annotine (I) by its reaction with a single mole of phenyl lithium, producing phenylannotine (II), $C_{22}H_{27}O_3N$, (m.p. 214°; I.R. spectrum - no bands in the carbonyl region, strong band at 3470 cm.$^{-1}$ (-OH), weak band at 1640 cm.$^{-1}$ (non-benzenoid unsaturation) and strong bands in the 600-1200 cm.$^{-1}$ region (benzene ring)). These workers also demonstrated the presence of a single hydroxyl function in annotine (I) by its conversion with acetic anhydride to the monoacetate, O-acetylannotine (VII), $C_{18}H_{23}O_4N$, (m.p. 180.7 - 182.2°; I.R. spectrum - strong bands at 1732 cm.$^{-1}$ and at 1250 cm.$^{-1}$ (acetoxy group), a band at 1715 cm.$^{-1}$ (carbonyl group) but no bands attributable to a hydroxyl). Alkaline hydrolysis of (VII) yielded annotine (I).

As two of the oxygens present in annotine (I) were accounted for as being present in a single carbonyl and a single hydroxyl group, the third oxygen was apparently present as an ether. This ether, unlike the ether in annotinine, was found to be inert. Treatment of annotine (I) with boiling hydrochloric acid did not form a chlorohydrin but instead

formed an isomeric compound (VIII), $C_{16}H_{21}O_3N$, isolated as its hydrochloride (m.p. greater than 300°; I.R. spectrum - strong band at 3220 cm.$^{-1}$ (-OH), a strong band at 1740 cm.$^{-1}$ with a shoulder at 1695 cm.$^{-1}$ (carbonyl) and a weak absorption at 1420 cm.$^{-1}$ (probably unsaturation)). While the nature of this isomerization reaction could not be determined, these workers tentatively assumed that the ether ring present in annotine was larger than 3-membered.

Additionally, the increase in basic strength on the reduction of annotine (I), with pKa 7.5, to dihydroannotine (VI), with pKa 8.61, was, as in the case of acrifoline, consistent with an allylamine structure.

8. EXPERIMENTAL

The alkaloid mixture used in the experiments reported hereunder was the very viscous, reddish-brown mother-liquors remaining from the crystallization of annotinine from the "crude alkaloid" of Lycopodium annotinum L.

(a) Paper Chromatography

Paper chromatography was first examined as a means of analyzing mixtures of alkaloids in order to establish a method for following the extent of separation of alkaloids effected by other means of separation.

1) Preliminary tests

(i) Buffering the filter paper- Preliminary tests were run using Whatman No. 1 and No. 3 chromatographic paper (18 1/4 in. x 22 1/2 in.) which had been buffered at seven different pH values, ranging from pH2 to pH8. Papers buffered at various pH values were used because of the influence of pH on the Rf values of alkaloids, as reported by Brindle, Carless and Woodhead (64) in the case of Solanaceous alkaloids.

Buffers at the various pH's were prepared from 0.2 M Na_2HPO_4 and 0.1 M citric acid using a Beckman Model H2 pH meter. Initially, the papers were dipped in the buffer, blotted to remove the excess buffer and air-dried. Later,

it was found more convenient to spray the paper uniformly with buffer and air-dry.

(ii) Application of alkaloids to buffered paper - After preliminary tests, a uniform arrangement for placement of the alkaloids on the paper was adopted. Descending chromatograms were run along the lengthwise direction of the paper with the alkaloid spots (eleven per sheet) being located three inches from that end of the paper which was to be dipped in the solvent.

Tests to determine the amount of alkaloid to be applied were made by applying various concentrations of individual alkaloids and mixtures of alkaloids, running the chromatograms and, after air-drying, "developing" the spots to a red or rose-red colour with Dragendorff-Munier reagent (65). These tests indicated that approximately 0.020 mg. per individual alkaloid and 0.500 mg. per mixture of alkaloids gave satisfactory results. These spots were applied with a 0.005 ml. pipette.

(iii) Solvent system and descending development - The developing solvent system used in the descending chromatograms was n-butanol saturated with water. Initially, the suspended papers in the "Chromatocab" were equilibrated in an atmosphere of water saturated with butanol for periods

ranging from one to six hours prior to the addition of the developing organic phase. As the duration of the "equilibration" period was found to have little or no effect, these periods were discontinued. Chromatograms which were run on No.1 Whatman paper for 17 1/2 hours had a solvent front located approximately 42 cm. from the starting point of the alkaloids. After air-drying, the chromatograms were sprayed with Dragendorff-Munier reagent, as indicated above. The general effect of pH on the Rf value of individual alkaloids is illustrated in Fig. 12A.

(iv) Two-dimensional paper chromatograms - Two-dimensional paper chromatograms of the alkaloid mixture were run on both No.1 and No.3 Whatman papers (18 1/4" x 22 1/2"), buffered at pH 5.85 using both the ascending and descending techniques. The development was carried out in one direction with n-butanol saturated with water. After air-drying, development with methyl ethyl ketone, saturated with water, was carried out at right angles to the initial direction of development.

As illustrated in Fig. 12B, two-dimensional development was successful in this instance in resolving the alkaloid mixture into fourteen or fifteen areas. The chromatogram illustrated was run using the descending technique on No.3

Whatman paper, buffered at pH 5.85. Development in one direction with n-butanol was continued for seven hours, the solvent front reaching a distance of 36 cm. from the starting point. Development in the direction at right-angles to the first with methyl ethyl ketone was continued for a period of two hours, the solvent front reaching a distance of 47 cm. from the starting point.

(v) Tests for reproducibility of Rf values - After running approximately eighty descending paper chromatograms, it was found that the Rf values of the individual alkaloids were subject to considerable variations. This sensitivity of Rf values to many extraneous variables was well reported in the literature (66)(67)(68). Variations were noted even when individual alkaloids were run concurrently on different sheets in the same "Chromatocab" under similar conditions.

Thinking that perhaps variation in the paper itself and in the treatment of same might be the cause of the variability of Rf values, a rather detailed study was made of factors such as the effect of the:

1 direction of fibres in the paper;

2 smooth and rough sides of the paper;

3 variations in thickness of the paper within the one grade;

4 methods of buffering (i.e. dipping and rolling

as opposed to spraying);

5 distance of the solvent from the starting point;

6 time for equilibration before running; and,

7 placement of sheets in the "Chromatocab".

While no direct correlation was observed between any of the above factors and the variation in Rf values, the preparation and running of subsequent chromatograms were standardized as much as possible bearing the above factors in mind. As the time for "equilibration" had no obvious effect, the subsequent chromatograms were not "equilibrated" before running. Where possible, reference standards were run on the same sheet.

2) Known Lycopodium alkaloids - Rf values - After the above preliminary tests, descending paper chromatograms were run using a n-butanol/water solvent system on Whatman No.1 chromatographic paper (18 1/4" x 22 1/2") buffered at pH3.0, 4.0, 5.0, 6.0 and 7.0. The alkaloids used were annotinine, lycopodine, β-obscurine, α-obscurine (pH3 and pH6, only), annotine (alkaloid L-11), O-acetylacrifoline (alkaloid L-12) and acrifoline. These chromatograms were run for 17 1/2 hours and, after air-drying, the alkaloids were made visible by spraying with the Dragendorff-Munier reagent. The Rf values obtained were recorded in the appropriate sections of

Table No.1, above. The Rf values of these alkaloids were plotted against the pH values of the buffered papers (see Fig.13) (Note: Rf values for lycodine, determined later, were also plotted in Fig.13). It was observed from this plot that the optimum pH for the separation of a mixture of these alkaloids was approximately pH6. As might be anticipated, annotoxine, the molecular complex of annotine and acrifoline, gave two spots with Rf values corresponding to those of annotine and acrifoline.

(b) Ion-exchange Column Chromatography

1) Large scale run using Dowex 50-X12 resin - A complete run was made with Dowex 50-X12 (200-400 mesh), a sulfonic acid cation exchange resin of low porosity. A column of one-inch diameter, containing 153 g. of resin, was followed in series by a smaller column (50 ml. burette) containing 33 g. of resin, as recommended by Brimley and Barrett (69).

The columns were regenerated by elution with 1.625 l. of 10% HCl followed by washing with 1.445 l. of distilled water until the eluant was neutral to litmus. The water was then replaced with 50% aqueous methanol. Six grams of "crude alkaloid", dissolved in 60 ml. of 50% aqueous methanol were

added to the column. Elution was effected with a 0.2 N solution of sodium hydroxide. The flow through the column was very slow (ca 13 ml. per hour, under pressure). The entire run occupied a period of more than two weeks, running almost continuously. As the alkaloid material commenced to appear in the eluant, 168 fractions were collected using an interval fraction collector (Misco Interval Timer No.651 and Fraction Collector No.650).

Descending paper chromatograms were run on a representative number of these fractions, using No.1 Whatman chromatographic paper buffered at pH 5.85. The chromatograms indicated that no separation of the alkaloids had been effected by the ion-exchange columns.

2) Small scale runs using Dowex 50-X12 resin - Prior to packing the columns, the Dowex 50-X12 resin was cycled with 2 N sodium hydroxide and 2 N hydrochloric acid. Distilled water used in washing and other operations was degassed under vacuum to avoid the evolution of gas during the change from water to 50% aqueous methanol in the packed column.

The cycled resin (approx. 4 g.) was packed into a five ml. graduated pipette and regenerated with 35 ml. of 10% HCl. The columns were washed with distilled water until the eluant

was neutral to litmus. A volume (25 ml.) of 50% aqueous methanol was then passed through the column.

Two columns were prepared as above and, after the addition of the "crude alkaloid", elution was effected with 0.04 N sodium hydroxide in one of the columns and 0.2 N NaOH in the other. Paper chromatography of the eluted fractions indicated that the alkaloid mixture had not been separated.

3) Qualitative comparison of Dowex 50-X12 and Dowes 50-X4 resins - The Dowex 50-X12, a sulfonic acid type resin with 12% cross-linkage which had been used in the previous runs was compared with the Dowex 50-X4 resin, the same type of resin but with 4% cross-linkage. Both resins were rated with a capacity of 5±0.3 meq. per dry gram. This simple qualitative experiment consisted of dividing equally between two 400-ml. beakers a solution of the "crude alkaloid" mixture in 50% aqueous methanol. Both cross-linkage types of the ion-exchange resins had been cycled previously with 2 N NaOH and 2 N HCl and washed thoroughly with distilled water while in the hydrogen form. Approximately equivalent amounts of -X4 and -X12 resins were added to the respective beakers containing the solution of alkaloids. Prior to the addition of the resin and after each equivalent addition of resins to the solutions, the pH values of the solution of alkaloids was read, using a Beckman pH meter.

At the start, prior to the addition of the resins, both solutions were recorded at pH9.8. After five additions of the -X4 resin, the pH value of the solution was pH5.2; whereas, after five similar additions of the -X12 resin, the pH value of the solution was pH9.2. Even after a final large addition of -X12 resin, the pH value dropped to only pH9.1; however, after the addition of a small amount of -X4 resin to this latter solution, the pH value was lowered significantly.

4) Small and moderate scale runs with Dowex 50-X4 resin - A series of runs using 5 ml. graduated pipettes and 50 ml. burettes as columns packed with -X4 resin were carried out. A variety of conditions were used -- varying the solvent composition from 50% aqueous methanol to 95% aqueous methanol and the eluting solutions from 0.3 N to 0.037 N NaOH. In all cases marked contraction of the resin occurred during the elution with NaOH resulting in serious channeling. Paper chromatography of the fractions showed in some runs a slight tendency to separate the alkaloids, In general, however, results were unsatisfactory.

5) "Rubber-tubing" column with Dowex 50-X4 resin - In an effort to counteract the effect of marked contraction during the elution process, a column was prepared from a

length of firm but elastic rubber tubing with a 1 cm. internal diameter. A tube with a sintered glass base was placed in one end of the rubber tubing and wired into position. The column was then packed with Dowex 50-X4 resin in the "contracted" (i.e. sodium) phase. Another sintered glass exit tube was placed in the other end of the rubber tubing with the sintered glass resting against the top of the resin column. The sintered glass tubes at either end were then fixed in position with the packed 9 cm. rubber column stretched between. The resin column was then regenerated with 2N HCl. During the regeneration to the hydrogen phase, the rubber tubing expanded radially as the HCl passed down the column. After regeneration, the column was washed with 80% aqueous methanol, all solutions used in the column having been de-gassed under vacuum prior to use.

The "crude alkaloid" mixture (0.55 g.) was dissolved in 80% aqueous methanol. Elution was effected with 0.075 N NaOH. The rate of flow was approximately 38 ml. per hour. After 283 ml. of eluant had passed through the column, the alkaloid material commenced coming from the column. Paper chromatography of the fractions collected indicated that no separation had taken place.

(c) Partition Column Chromatography

1) Preliminary runs with buffered Celite column - A partition chromatographic technique, as reported by Neish (70) and by Lemieux and Charanduk (71), was applied using acid-washed Celite No.535, dried at 500°. The technique was modified to meet the requirements of the present problem by buffering the Celite column.

A phosphate-citrate buffer of pH5.65 was prepared, as for the paper chromatography, above. This buffer (500 ml.) was equilibrated with n-butanol (1500 ml.). A glass column, 55 cm. in length and with a 26 mm. internal diameter, was used with a cotton plug at the bottom of the column. A 3 g. portion of dry Celite was first placed in the column, occupying 16 cm. in column height after it was packed down with a cork plunger. This was followed by a filter-paper disc slightly larger than the inner diameter of the tube. The buffered Celite portion was prepared by thoroughly mixing in a beaker 25 g. of dry Celite with 25 ml. of the aqueous buffer phase which had been equilibrated with n-butanol. The buffered Celite was then made into a slurry by the addition of the n-butanol phase and added to the column. Using a mercury-pressure flask, n-butanol was forced through the column, packing the buffered Celite column. Final uniform packing was accomplished by the use of the cork plunger

providing 17.1 cm. of column height for the buffered Celite portion. Another paper disc was added, followed by the addition of 1 g. of dry Celite with which the crude alkaloid mixture (155 mg.), dissolved in 1 cc. of n-butanol, had been uniformly mixed. After packing, this alkaloid-impregnated portion occupied 6 mm. of column height. A final filter-paper disc was added to the top of the column. Care was taken throughout the packing that no portion of the column ran dry. The moving n-butanol phase was added to the top of the column, as required.

After allowing 80 ml. of liquid to pass through the column, sixty-six 10-ml. fractions were collected. Paper chromatography of these fractions, together with those from a second preliminary run indicated that some separation of the alkaloid mixture had taken place.

2) Series of runs with Celite columns buffered at pH5.65.- The technique used in the preliminary Celite columns was repeated in a run using 177 mg. of crude alkaloid mixture. The same type of column was used as previously described; however, a more firmly packed column was used with the 3 g. dry Celite occupying 15 mm. of column height, the 25 g. of buffered (pH5.65) Celite occupying 13.5 cm. and the 1 g. alkaloid-bearing Celite occupying 2mm. in height.

Thirty 10ml. fractions were collected. Fraction 31 consisted of 64 ml. of n-butanol phase, followed by fraction 32, consisting of 64 ml. of aqueous phase passed through the column.

Paper chromatograms buffered at pH5.65 and using n-butanol, as in the column, were run on alternate fractions. The extent of the separation is shown by plotting the Rf values, as determined from the paper chromatograms, against the volume of effluent which had passed through the column (see Fig. 14). The fractions were divided into four groups, A, B, C and D, as indicated on Fig. 14.

To obtain a more quantitative separation, nine runs, duplicating the above conditions and using 150-200 mg. of crude alkaloid mixture for each run, were carried out. In each case, the first 60 ml. of effluent, containing no alkaloid, was discarded. Group A fraction consisted of the next 50 ml., group B, the next 80 ml., group C following with 110 ml. and group D the final 150 ml. of both the n-butanol and aqueous phases passed through the column.

Paper chromatography of the four groups using buffers of various pH values indicated that the alkaloid components of group A were separated more completely on paper buffered at pH3.3.

3) Partition chromatography of group A fraction using a Celite column buffered at pH3.28 - A Celite column prepared in the manner described above was prepared using a glass column of 4 cm. internal diameter. The bottom portion consisted of 9 g. of dry Celite occupying 2 cm. of column height. The buffered portion consisted of 75 g. of Celite thoroughly mixed with 75 ml. of buffer of pH3.28, previously equilibrated with n-butanol, and occupied 22 cm. in column height. The top portion consisted of 3 g. of Celite uniformly impregnated with the group A fraction from the previous series of runs at pH5.65.

N-butanol (600 ml.) which had been previously equilibrated with buffer of pH3.28 (200 ml.) was passed through the column. Forty 10 ml. fractions were collected initially and were numbered A-1 to A-40, inclusive. Fraction A-41 consisted of the next 200 ml. of n-butanol combined with 200 ml. of water passed through the column. The water phase of fraction A-41 was extracted with chloroform. When the chloroform extractions were taken to dryness under vacuum at low temperature, the residue crystallized readily. This material was identified as annotinine by melting point and paper chromatography.

The fractions A-19 to A-42, inclusive, were taken down to dryness and then dissolved in a small amount of methanol.

Paper chromatograms, with paper buffered at pH3.3, were run on these fractions. The chromatograms disclosed that, within the fractions A-19 to A-38, each fraction consisted of two or three alkaloid components.

4) Ultraviolet absorption spectra of fractions from Celite columns - Using a Beckman DK-2 spectrophotometer, the ultraviolet absorption spectra of the fractions of group A were recorded with those of the gross fractions, groups B, C and D. The spectrum of fraction A-36, from the previous Celite run, disclosed a very marked absorbance in the region 263 to 275 mμ, characteristic of pyridine-like structures and unlike any spectra of Lycopodium alkaloids previously isolated.

A paper chromatogram, buffered at pH2.28, containing two separate applications of fraction A-36, was run using n-butanol/water development. After running and air-drying, the two "runs" were separated and the one portion was made visible by spraying with Dragendorff-Munier reagent. Three well separated areas appeared. Visual examination of the unsprayed portion of the chromatogram with a portable ultraviolet (Mineralight) source in a darkened room, disclosed that the spot with the lowest Rf value absorbed ultraviolet light and appeared as a dark area. This unsprayed spot was cut out of the paper and eluted with water. The ultraviolet

absorption spectrum of the eluted solution disclosed a marked absorbance at 268.2 mμ with a shoulder at 275.7mμ. On addition of acid, the absorbance shifted to 272 mμ without the appearance of the shoulder.

Steam-distillation of a small portion of the original crude alkaloid mixture, followed by ultraviolet absorption spectroscopy of the distillate disclosed that the pyridine-like alkaloid was steam-distillable. Many of the other alkaloids of the crude mixture, however, were also found to be steam-distillable -- as was shown by paper chromatography of the distillates.

(d) Countercurrent Distribution Method

1) Preliminary tests -

(i) Determination of apparent partition coefficients

1 Lycopodine - between chloroform/buffer pH6 - Lycopodine (12.8 mg.) was dissolved in chloroform (10 ml.) and mixed in a separatory funnel with buffer pH6 (10 ml.). The chloroform and buffer pH6 had been previously equilibrated. After mixing and equilibrating, the chloroform phase was evaporated to dryness, taken up in methanol and diluted to make 50% aqueous methanol. Microtitration of the lycopodine in the 50% aqueous methanol was carried out using 0.5018 N HCl. The apparatus used in the titration was a

small, three-necked, pear-shaped flask, a Beckman pH meter using a calomel--glass electrode arrangement, a microburette and a stream of nitrogen bubbling through the solution for uniform stirring. The lycopodine in the chloroform phase required 0.066 ml. of 0.5018 N HCl. The amount in the buffer phase, determined by difference, would be 0.049 ml. of the HCl. The apparent distribution coefficient:

$$k' = \frac{\text{Lycopodine in chloroform phase}}{\text{Lycopodine in buffer (pH6) phase}} = \frac{0.066}{0.049} = 1.3.$$

2 Annotinine - between chloroform/buffer pH5 - Using 11.2 mg. of annotinine and following the same procedure as above, the apparent distribution coefficient (k') of annotinine was found to be 3.8 at pH5.

2) Countercurrent distribution and partition chromatography sequence

(i) Countercurrent distribution between chloroform and buffer pH5.2 - The annotinine mother-liquors received from N.R.C. were treated with 1 N HCl and shaken in an agitator to put into solution. The solutions were filtered through a Buchner funnel containing some Celite, made alkaline to pH10 with ammonium hydroxide and extracted with ether (4 l. - total extract). On taking this ether extract down to approximately one liter some annotinine crystallized out. The alkaline aqueous solution from the ether extractions were

then extracted with chloroform. The combined ether and chloroform extracts were evaporated to 68 g. of heavy viscous alkaloid mixture.

The countercurrent scheme of Bush and Densen (72), as diagrammed in Fig. 15, was carried out using 2-liter separatory funnels partitioning the 68 g. of alkaloid mixture between chloroform (500 ml.) and concentrated citrate-phosphate buffer pH5.2 (500 ml.). At each transfer, the pH of the buffer was checked and corrected to pH5.2, where necessary.

1 Chloroform fractions - The chloroform fractions C1, C2 and C3 (see Fig. 15) were combined and extracted with four 500 ml. portions of 0.1 N citric acid. The citric acid extracts were then made alkaline with ammonium hydroxide, extracted with chloroform and the chloroform extracts taken to dryness. The remaining residue was dissolved in methanol from which approximately 4 g. of annotinine was crystallized.

2 Buffer fractions - The buffer fractions, B1, B2, and B3 (see Fig. 15) were examined only in a cursory fashion. These aqueous solutions were made alkaline with ammonium hydroxide and extracted with three 500 ml. portions of chloroform. The extracts were evaporated under vacuum to a heavy viscous material, the apparent distribution coefficient (k') of which was 1.1 between benzene and buffer pH 6.2. This material was dissolved in acetone and set aside for some time in the cold to crystallize out annotoxine,

however, results were negative in this regard. Hydrobromic acid was added to the acetone solution. On standing, white acrifoline hydrobromide crystallized out. Approximately 2.6 g. of the hydrobromide was obtained from six crystallizations.

The mother-liquors were made alkaline with ammonium hydroxide and extracted six times with benzene. Examination of the extracts by ultraviolet spectroscopy did not disclose the presence of the obscurines which were being sought. Tannins, which, it had been hoped, would be excluded from the benzene extracts, were present in the extracts, as indicated by their characteristic ultraviolet absorption around 325 mμ to 330 mμ.

3 Central fraction - The aqueous phase of the central fraction of the countercurrent scheme was made alkaline with ammonium hydroxide and equilibrated with the chloroform phase. This latter phase was separated and taken down under vacuum to give the concentrated chloroform solution, L-38, (see Fig.15). A few cc.'s of L-38 were taken to dryness and dissolved in methanol for ultraviolet absorption spectroscopy which disclosed the presence of the new pyridine-like alkaloid referred to previously. Two-dimensional ascending paper chromatography of this fraction was carried out using n-butanol/water as the solvent system in one direction and methyl ethyl ketone/water in the other. This chromatogram disclosed that, in addition to the main com-

ponent, presumably the pyridine-like alkaloid, there were at least five other minor components in the solution, L-38.

(ii) Partition columns of Celite, buffered at pH3.3 with n-butanol (large scale). - Small scale attempts were made to separate out the pyridine-like alkaloid from L-38 using a Celite column buffered at pH 5.2 and chloroform as the developing phase. This was followed by chromatography of the fraction, rich in the pyridine-like alkaloid, from the preceding run, through a Celite column buffered at pH 3.3, with n-butanol as the developing phase.

As a result of the foregoing small-scale tests, a large scale run was carried out using two columns, in series. The first column, 108 cm. in length and with a 26 cm. internal diameter was prepared, as previously described in detail for Celite columns. This Celite column consisted of 50 g. of dry Celite at the bottom of the column (4 1/2 cm. in column height), 825 g. of Celite buffered at pH 3.3 (85 cm. in column height) and 8 g. of Celite at the top of the column intimately mixed with 1.96 g. of the evaporated L-38 solution. The second Celite column, placed below the larger column was 96 cm. in length with a 6.4 cm. internal diameter. The dry Celite at the bottom of the smaller column weighed 10 g. (5 1/2 cm. in column height) and the Celite portion buffered at pH 3.3 contained 125 g. of Celite (76 1/2 cm. in column height). The n-butanol and buffer solutions were equili-

brated before use in the ratio of n-butanol to buffer 3:1.

Some 6.175 liters of n-butanol were passed through the column at the rate of about 1.7 ml. per hour under a pressure of approximately 13 cm. of mercury. Examination of the fractions by ultraviolet spectroscopy and by paper chromatography indicated that the pyridine-like alkaloid was concentrated with one or two other alkaloids in about 1 liter of effluent. This fraction commenced coming off the column after approximately 3.5 liters of n-butanol had passed through the column. After extraction of the fraction with 0.1 N HCl, the aqueous extracts were made alkaline to pH 10 with ammonium hydroxide and extracted with ether. After washing with water and drying over anhydrous sodium sulfate, the ether extracts were taken to dryness and the residue dissolved in a small amount of methanol.

This methanol solution was treated with an excess of picric acid in methanol. The mixture was then concentrated to some extent by evaporation under vacuum and allowed to stand. The first two crystallizations yielded ca 300 mg. of picrate, S-17, m.p. 230-235°(dec.) which was identified by ultraviolet spectroscopy as that of the pyridine-like alkaloid. By successive concentrations of the picrate mother-liquors, a fourth crystallization yielded shiny yellow platelets of a picrate, S-20, m.p. 240-246°, quite unlike

S-17 in appearance.

(iii) Electrometric titration of pyridine-like alkaloid picrate (S-17) - Titration of 11.1 mg. of the picrate (S-17) with 0.1184 N NaOH in 50% aqueous methanol disclosed the probable presence of two slopes (see Fig. 16) which suggested that the picrate was probably a dipicrate. The second end-point was not sharply defined, however, probably due to dilution during titration. Assuming a dipicrate, this titration indicated the equivalent weight of the free base (based on the strongly basic nitrogen) to be near 263 g.

(iv) Attempted formation of other crystalline derivatives of pyridine-like alkaloid - Attempts were made at this stage to crystallize the pyridine-like alkaloid as the free base, without result. Similarly attempts to form the monopicrate, diperchlorate, dihydrochloride, dihydroiodide, di-p-toluene sulfonate, or, the distyphnate in a crystalline state were unsuccessful.

(v) Characterization of the pyridine-like alkaloid dipicrate - The dipicrate, S-17, was recrystallized from hot methanol into characteristic radiating clusters of prisms, m.p. 229-233° (dec.). Heating the recrystallized dipicrate in a drying pistol at 100° (water) under a vacuum of 0.3 to 0.4 mm. Hg and again at 118° (acetic

acid) for 2 1/4 hours at the same vacuum, did not alter its weight. There did not appear, therefore, to be any methanol-of-crystallization present in the dipicrate. Analysis indicated the probable formula to be $C_{17}H_{24}N_2 \cdot 2C_6H_3O_7N_3$, as shown hereunder: Calculated - C,48.74; H,4.23; N,15.68%

Found - C,48.20; H,4.02; N,15.91%

- C,48.47; H,4.03; N,15.64%

The spectrophotometric method for the micro-determination of the molecular weights of picrates, as reported by Cunningham, Dawson and Spring (73), was applied using 1.648 mg. of piperidine picrate (molecular weight 314 g.) and a value of 310 g. was obtained, representing an error of -1.3%. However, using this technique for the pyridine-like alkaloid dipicrate (1.273 mg.), a value of 757 g. was obtained, as compared with 714.6 g. calculated for $C_{17}H_{24}N_2 \cdot 2C_6H_3O_7N_3$. Electrometric titration of the dipicrate, referred to in a previous section, had given a value of 721.6 g. for the equivalent weight (based on the strongly basic nitrogen).

3) Countercurrent distribution (Craig machine)
Chloroform - Buffer pH6

The distribution of the alkaloid mixture (anno-tinine mother-liquors from the N.R.C.) between chloroform and buffer pH6, using a 60-tube Craig countercurrent machine

(74) was studied. The original alkaloid mixture was dissolved in 1 N HCl. After making the solution alkaline to pH 10 with ammonium hydroxide, it was extracted three times with chloroform. In an attempt to remove tannins from the mixture, these chloroform solutions were extracted extensively with 1 N NaOH, back-washing the latter with chloroform in each instance. The chloroform solutions were dried over anhydrous sodium sulphate and then taken to dryness under vacuum at 27° providing 2.6 g. of alkaloid mixture for the countercurrent run.

The buffer of pH6 was prepared using 1 M K_2HPO_4 and 0.5 M citric acid. The alkaloid mixture, dissolved in 120 ml. of chloroform, was placed in the initial three tubes (40 ml. each) of the 60-tube countercurrent machine and partitioned through 60 transfers between chloroform and buffer pH6 (40 ml. in each phase).

After the transfers had been completed, 20 ml. of 2.1 N NaOH was added to each of the 60 tubes of the Craig machine and the solutions were mixed. The chloroform solution (40 ml.) was removed from each tube and placed in a separate Erlenmeyer flask containing 2 g. of anhydrous sodium sulphate. After drying, each chloroform solution was evaporated to dryness under reduced pressure and then dissolved in 5 ml. of methanol.

Paper chromatograms of the methanol solutions of alkaloids from alternate tubes of the Craig machine were run using n-butanol and No.1 Whatman paper buffered at pH6. Known alkaloids were run also on these chromatograms for reference purposes. A chart, included in Fig. 17, indicating the Rf values of the alkaloid components of the Craig countercurrent tubes examined was prepared. This chart indicated the extent of the distribution achieved by the Craig machine. Prior to spraying with Dragendorff-Munier reagent, the chromatograms, after development with n-butanol and air-drying, were examined in a darkened room with ultraviolet radiation. Each series of spots which were visible under these conditions were indicated with the notation "UV" on the chart (see Fig. 17).

Again using alternate tubes, one drop of methanol solution of the alkaloid mixture was diluted to 3 ml. with methanol. The ultraviolet spectra of these solutions disclosed an absorption at 229 mμ in tubes 1 to 7, at 268 mμ in tubes 8 to 15 and at 234 mμ in tubes 37 to 51. The absorption at 268 mμ was that of the pyridine-like alkaloid; however, the absorptions in the region of 234 mμ was not related to any known alkaloid and is the region associated with α,β-unsaturated ketones. Figure 17, referred to above, also included a chart showing the distribution of these absorptions rel-

ative to the tube numbers of the Craig countercurrent machine.

Infrared spectra of tubes numbered 17, 23, 34, 37, 40, 44, 46, 52, 57 and 58 were measured on a Perkin-Elmer single-beam double-pass instrument using a 1 mm. micro-cell. Carbon tetrachloride solutions were prepared by evaporating 2 ml. of the methanol solutions obtained from the above tubes to dryness and dissolving the residue in 0.1 ml. of CCl_4. The concentration in the case of tube 48, used as a preliminary run, was 10.6 mg. per 0.1 ml., or, ca 10% weight/volume. The spectra were measured within the region 3600-1640 $cm.^{-1}$ and the relative intensities (S - strong; M - medium; W - weak) of the absorption peaks and their wave-numbers were plotted against the tube numbers of the Craig machine and included in Fig. 17. Placing the charts of the infrared, paper chromatography and ultraviolet data in juxtapostion, as per Fig. 17, permitted a study of the distribution of the crude alkaloid mixture effected by this method.

(e) Isolation of Lycodine

From the countercurrent distribution experiments with buffer of pH6 and chloroform, it was observed that most of the alkaloids have a high partition coefficient (concentration in buffer / concentration in chloroform) with this system. However, annotinine, lycopodine, O-acetylacrifoline

(alkaloid L-12) and the new pyridine-like alkaloid, lycodine, have low partition coefficients. Alkaloids with a partition coefficient close to unity were present to only a minor extent in the batches of alkaloids used. Annotine (alkaloid L-11), which has such a partition coefficient (75)(see Table No. 3) is not present to a significant extent (75) in the crude mixture which was available to us. On the basis of the above results, the lycodine was concentrated in several stages (see Fig. 18).

1) Countercurrent distribution (large scale) - The mother-liquors from the crystallization of annotinine (ca 40 g. of crude alkaloid) were first partitioned by countercurrent distribution between chloroform and concentrated citrate-phosphate buffer pH6 (600 ml. in each phase), using six 2 l. separatory funnels. The extraction scheme (see Fig. 18) used was that of Bush and Densen (72). At each transfer the pH of the buffer was checked and corrected to pH6 where necessary. Of the six chloroform phases (C-1 and C-6) and the six buffer phases (B-1 to B-6) obtained, the four containing lycodine (C-1 to C-4) (i.e. 94% of lycodine) were mixed and concentrated for the next step. The remaining fractions were retained for the separation of other alkaloids.

2) Countercurrent distribution (Craig machine) - The chloroform fractions, C-1 to C-4, (see Fig. 18) concentrated to 120 ml., were placed in the initial three tubes (40 ml. each) of a 60-tube Craig countercurrent machine (74) and partitioned through 60 transfers between chloroform and citrate-phosphate buffer pH5.2 (40 ml. in each phase). Examination of the fractions by their ultraviolet absorption spectra disclosed the presence of lycodine in tubes 30 to 40 with the maximum around tubes 35 and 36. The alkaloids contained in both phases of these 11 tubes were then isolated in the usual manner. Paper chromatography of the material by the descending technique with Whatman No.1 paper buffered to pH 2.5, with n-butanol saturated with water as solvent, showed the presence of alkaloids in addition to lycodine, after spraying with the Dragendorff-Munier reagent.

3) Adsorption chromatography on activated alumina - Using a column of 25 mm. inner diameter, the alkaloid mixture (2.6 g.) from tubes 30 to 40 of the Craig machine was then chromatographed on activated alumina (37 cm. in column height) using 6.7 l. of n-pentane/benzene (1:1), followed by 7.3 l. of benzene. After passing 450 ml. of n-pentane/benzene through the column, the first alkaloid eluted was the known alkaloid, lycopodine, The second alkaloid was

the new alkaloid, lycodine, which commenced being eluted after approximately 1.3 l. of *n*-pentane/benzene (1:1) had passed through the column. A third alkaloid component, as yet uncharacterized, followed lycodine from the column upon elution with benzene. The fractions containing lycodine were again detected by their ultraviolet absorption spectra and paper chromatography.

(f) Characterization of Lycodine

As indicated in a previous section, lycodine was isolated initially as the dipicrate, m.p. 229-233°(dec.) with a possible formula, $C_{17}H_{24}N_2 \cdot 2C_6H_3O_7N_3$. Lycodine dipicrate was converted to the base which was vacuum distilled at 100-110° (air-bath)(0.18 mm. Hg) to give a colourless distillate which crystallized spontaneously. The lycodine so obtained had a m.p. 118°, $[\alpha]_D = -10 \pm 2°$ (c, 1.01 in ethanol). Calculated for $C_{17}H_{24}N_2$: C, 79.64; H, 9.43; N, 10.93; C-Me, 5.85%; M.W. 256.4. Found: C, 79.50, 79.46; H, 9.44, 9.38; N, 11.16; C-Me, 3.56%; M.W. (Rast), 246. Titration of lycodine (0.01059 g.) in 50% aqueous methanol gave an equivalent weight of 255.8 (based on the strongly basic nitrogen) and pKa's 3.97 and 8.08 (see Fig. 16). Titration of freshly distilled nicotine under the same conditions disclosed pKa's of 3.35 and 7.70 (see Fig. 16).

Later, it was found that lycodine could be crystallized from n-pentane, when seeded. This method was used to crystallize the base from the alumina fractions, above, purification of the alkaloid in a number of instances being effected by means of recrystallizations of lycodine dipicrate. In all, about 400 mg. of lycodine was obtained. From the position of lycodine in the countercurrent distribution mentioned above, the distribution coefficient of the alkaloid in the system chloroform-buffer pH5.2 was calculated to be 1.4 (concentration in buffer / concentration in chloroform).

The ultraviolet absorption spectra of lycodine (see Fig. 19) was measured on a Beckman DK-2 spectrophotometer using a solution of 3.603 mg. of lycodine in 90 ml. of methanol: free base - λ_{max} 268.2 mμ, log ε, 3.728 and λ_{max} 275.7 mμ, log ε, 3.615; salt - λ_{max} 272 mμ, log ε, 3.926. The infrared spectra of lycodine (see Fig. 20) were measured on a Perkin-Elmer double-beam spectrometer, model 21B, with sodium chloride optics. The nuclear magnetic resonance spectrum of lycodine (see Fig. 21), as concentrated carbon tetrachloride solutions, was recorded (76) with a Varian V-4300 NMR spectrometer, equipped with field stabilizer, at a fixed frequency of 40 Mc/sec., with spinning sample tubes. Signal separations were measured by the side-band technique in the usual manner.

(g) Reactions of Lycodine and Related Compounds

1) Acetylation of lycodine - Lycodine (39.5 mg.) was refluxed in an excess of acetic anhydride under nitrogen for two hours. The solution was cooled and methanol added to react with the remaining acetic anhydride. After removal of the methyl acetate by distillation, the residue was acidified with 5% HCl and washed with ether. The aqueous solution was then made basic with sodium hydroxide solution and extracted with ether to obtain the base. N-acetyl lycodine was isolated as the monopicrate, which crystallized from water as yellow needles, m.p. 180-182.5° (dec.). Calculated for $C_{19}H_{26}ON_2 \cdot C_6H_3O_7N_3$: C, 56.92; H, 5.54; N, 13.28%. Found: C, 56.58; H, 5.39; N, 13.69%. The free base was not obtained crystalline. The infrared absorption spectrum (in Nujol) of N-acetyl lycodine contained a band at 1661 $cm.^{-1}$, characteristic of an amide carbonyl, which band was not present in the spectrum of lycodine. By means of descending paper chromatography, using Whatman No.1 paper buffered at pH3, and n-butanol saturated with water as the moving phase, lycodine (Rf 0.35) was easily distinguished from N-acetyl lycodine (Rf 0.65). Titration of N-acetyl lycodine monopicrate (12 mg.) with 0.1184 N NaOH (see Fig. 16) displayed the presence of only one basic center (pKa 4.95) as would be expected after acetylation of the secondary nitrogen.

2) Hydrolysis of N-acetyl lycodine - N-acetyl lycodine obtained from the monopicrate was refluxed in conc. HCl for 6 hours. After cooling, the solution was rendered basic with NaOH solution and extracted with ether. The ether extractions were each washed with water and dried over anhydrous sodium sulphate before being evaporated to dryness. The residue was distilled around 125° (air bath)(0.8 mm. Hg) and the clear distillate crystallized on standing. These crystals had a m.p. 118° either alone or mixed with lycodine.

3) Attempted acetylation of β-obscurine - β-obscurine (ca 1 mg.) was subjected to the same reaction conditions as used for the acetylation of lycodine. After removal of the remaining acetic anhydride, the product gave only one spot which corresponded to that of β-obscurine when it was paper-chromatographed. The same result was obtained when β-obscurine was treated with acetic anhydride, using anhydrous sodium acetate as catalyst, for double the length of time. The neutral fraction of the product did not contain any substance having an α-pyridone chromophore.

4) Preparation of α-stilbazole from α-picoline (77) - A mixture of ca 0.1 mole quantities of α-picoline, benzaldehyde and acetic anhydride was refluxed in a 100 ml. round-bottom flask in an atmosphere of nitrogen for 28 hours. The

excess α-picoline and acetic anhydride were removed by distillation. Steam-distillation of the mixture after the addition of water was carried out to remove much of the benzaldehyde. When poured into cold water, a heavy white precipitate formed. The precipitate was dissolved in 5% HCl and extracted with ether. As α-stilbazole is a weak base, a modified countercurrent procedure was carried out on each ether extraction, using 5% HCl. The acid extracts were made alkaline with conc. NaOH and again extracted with ether. After drying over anhydrous sodium sulphate, the ether solutions were taken to dryness. The product was twice crystallized from ethanol. The ultraviolet absorption spectrum was measured of the product.

5) Picrate of benzylidene derivative of 5,6,7,8-tetrahydroquinoline - After refluxing 5,6,7,8-tetrahydroquinoline (0.1078 g.) with excess acetic anhydride and benzaldehyde for 32 hours, the benzylidene derivative was recovered using the same procedure as for α-stilbazole, above. The solution of the free base was converted to 0.058 g. of crystalline picrate, m.p. 148-150°, after recrystallization from ethanol. Calculated for $C_{16}H_{15}N.C_6H_3O_7N_3$: N, 12.44. Found: N, 12.60. The ultraviolet absorption spectrum of the free base displayed maxima at 269.2 mμ and 314.2 mμ, while

in the salt form, the maxima appeared at 269.2 mμ and 345 mμ (see Fig. 22, UV-76).

6) Attempted formation of a benzylidene derivative of lycodine using acetic anhydride as catalyst - Lycodine (0.089 g.) was refluxed for 30 hours in an atmosphere of nitrogen with an excess of acetic anhydride and freshly distilled benzaldehyde. Steam-distillation of the product removed a portion of the remaining benzaldehyde. Methanol was then added and the mixture distilled to remove the excess acetic anhydride as low-boiling methyl acetate. As it was expected that the lycodine would be in the weakly basic N-acetyl form, modified countercurrent acid-base extractions with ether were carried out, as for α-stilbazole, to isolate the product.

As previous attempts to crystallize the product as the free base or picrate had failed, the product mixture was passed through a column of activated alumina (21 cm. in length, 2" internal diameter) using a mixture of petroleum ether (30-65°): benzene in the ratio 1:2. After passing through 5 l. of the mixture, sixty-six 50 ml. fractions were collected. These fractions were taken to dryness and dissolved in a methanol-pentane mixture to permit the measurement of their ultraviolet absorption spectra. After a detailed ex-

amination of the spectra and paper chromatograms of these fractions, it was determined that a non-volatile impurity (0.0009%) in the reagent-grade anhydrous ether, used in the extractions, had a characteristic absorption in the ultraviolet which interfered with the measurement of the other spectra. Only re-distilled anhydrous ether was used for later extractions.

The spectra of the products were also compared with the spectrum of the amide of cinnamic acid, m.p. 147°. This amide was prepared by refluxing 1 g. of cinnamic acid with thionyl chloride for one half hour and pouring the mixture over cold concentrated NH_4OH. After separation of the neutral cinnamamide from the excess acid, the amide was recrystallized from hot water.

7) Attempted formation of a benzylidene derivative of lycodine using conc. HCl as a catalyst - Lycodine (0.0013 g.) was refluxed with 5 ml. conc. HCl and 0.05 ml. of freshly distilled benzaldehyde in an atmosphere of nitrogen for 41 hours. After the usual extraction procedures, examination of the ultraviolet absorption spectra of the product indicated that no benzylidene derivative had been formed.

8) Attempted formation of a benzylidene derivative of lycodine using zinc chloride as catalyst -

(i) Preliminary run - Lycodine (0.0028 g.) was refluxed with 8 ml. of freshly distilled benzaldehyde and a small amount of zinc chloride in an atmosphere of nitrogen for 19 hours, the flask being heated by an oil bath (145°). The product was recovered by acid-base extractions as outlined above. The ultraviolet absorption spectrum (see Fig. 22, UV-111) of the crude product displayed maxima which corresponded roughly with those of the benzylidene derivative of 5,6,7,8-tetrahydroquinoline (see Fig. 22, UV-76) including the corresponding shift to a longer wavelength maximum in acid media. Descending paper chromatograms, using n-butanol and Whatman No.1 paper buffered at pH3, were run on the product. Several applications of the same product were run concurrently on the same chromatogram (D-134), those specimens on the outside edges of the paper being sprayed with Dragendorff-Munier reagent to locate the areas having the alkaloid. Each of the two developed marginal specimens displayed three alkaloidal spots in addition to the spot corresponding to the unreacted lycodine. The unsprayed central part of the chromatogram was then cut into areas corresponding to the above four areas (including lycodine). The location of the areas was also determined by exposing the

chromatograms to ultraviolet light in a darkened room. These separated areas were individually extracted with 5% HCl, filtered through Celite and converted to the free base. The ether extractions of the free bases were evaporated to dryness and dissolved in methanol. Ultraviolet absorption spectra identified the one spot (lowest Rf value) as lycodine. The most intense spot, however, displayed maxima (see Fig. 22, UV-112) corresponding to those of the crude product, above, and those of the benzylidene derivative of 5,6,7,8-tetrahydroquinoline (see Fig. 22, UV-76), but the longer wavelength maximum was less sharply defined.

(ii) 1st Run - Lycodine dipicrate (0.057 g.) was converted to the free base. Using an 8 ml. flask immersed in an oil-bath (130°) and fitted with a reflux condenser, the lycodine (ca 0.020 g.) was refluxed for 30 hours with 8 ml. of freshly distilled benzaldehyde and a small amount of zinc chloride as catalyst. The product was recovered by the usual acid-base extractions as the free base.

1 Celite column buffered at pH3 - 1st pass - A partition column (24 mm. inside diameter) was prepared using 3 g. of dry Celite, at the bottom (2.3 cm. in column height) followed by 25 g. of Celite thoroughly mixed with 25 ml. of buffer pH3 (equilibrated with n-butanol). The buffered portion occupied 19.2 cm. in column height. The product (free

bases) from the reaction was dissolved in 1 ml. of n-butanol, mixed well with 1 g. Celite and added to the top of the column. Using n-butanol as the moving phase, forty 10 ml. fractions were collected from the column which was eluted at the rate of 1 ml. per 3.5 minutes. The ultraviolet absorption spectrum of fraction 5 (see Fig. 22, UV-114(5)) resembled the benzylidene-type spectrum described above (see Fig. 22, UV-76 and UV-111). The spectrum of fraction 7 (see Fig. 22, UV-114(7)) was quite different to that of fraction 5. Fractions 9 to 12, inclusive, displayed the characteristic absorption spectrum of lycodine.

2 Celite column buffered at pH3 - 2nd pass - In order to collect smaller fractions, the fractions 4 to 8, inclusive, were combined and, after concentration, were passed a second time through the above Celite column. On this run, after collecting 30 ml. of effluent, some sixty-nine 1 ml. fractions were collected. The benzylidene-type ultraviolet absorption spectrum appeared in fractions 17 to 24, inclusive. The spectrum of fraction 7 of the 1st Celite pass (ref. Fig. 22, UV-114(7)) did not appear in any of the of the fractions of this 2nd Celite pass. In its place, fractions 32 to 35 of the 2nd run (i.e. after 62 ml. had passed through the column) had ultraviolet absorption spectra as shown in Fig. 22, UV-122, when run as the free base and in acid media.

3 Ultraviolet irradiation of Celite fraction 19 - Fraction 19 of the Celite column (2nd pass), whose ultraviolet spectrum as the free base and salt resembled the benzylidene-type spectrum (see Fig. 22, UV-128), was diluted to approximately 3 1/2 times its original volume and placed as a shallow layer in a petri dish. Using a portable ultraviolet source, the solution was irradiated for three hours. The ultraviolet absorption spectrum of the irradiated solution (see Fig. 22, UV-128) was comparable to that shown in Fig. 22, UV-112, of the solution extracted from one area of the paper chromatogram (D-134).

(iii) 2nd Run - Lycodine dipicrate (0.059 g.) was converted to the free base. Using an 8 ml. flask, immersed in an oil-bath (145°), the lycodine (ca 0.020 g.) was refluxed for 40 hours with 5 ml. of freshly distilled benzaldehyde and a small amount of zinc chloride as catalyst. The product was recovered by the usual acid-base extractions as the free base.

1 Celite column buffered at pH3 - A partition column was prepared as in the first run. Using n-butanol as the moving phase, thirty-seven ml. was passed through the column prior to the collection of fifty-nine 2 ml. fractions. Elution was at the rate of 1 ml. per 3.5 minutes. Ultraviolet absorption spectra of the fractions disclosed two main

types of spectra in addition to the characteristic spectrum of the starting material (lycodine). Fractions 13 to 16, inclusive, (i.e. 41 ml. to 49 ml. from the start of elution) displayed the characteristic benzylidene-type spectrum (see Fig. 22, UV-140). Fractions 22 to 26, inclusive, (i.e. 59 ml. to 69 ml. from the start of elution) displayed the ultra-violet absorption spectrum as shown in Fig. 22, (UV-142). Fractions 32 to 50 displayed the spectrum of lycodine (see Fig. 22, UV-143). As the "holdup" of the Celite column was determined to be ca 40 ml., the benzylidene-type material apparently travelled along with the solvent front.

2 Attempted isolation of benzylidene derivative - Fractions 13 to 16 were combined and extracted several times with 5% HCl solutions each of which were in turn washed with ether. The acid extractions were then made alkaline with 50% NaOH solution and extracted a number of times with ether. Each ether extraction, after it was washed with water, was dried over anhydrous sodium sulphate and then taken to dryness.

The free base was taken up in a small amount of ether and transferred to a small distilling bulb prepared from 5 mm. glass tubing. After evaporating the ether, the residue in the bulb was vacuum distilled at 140-150° (air-bath)(0.5 mm. Hg). The distillate was fractionated, in a rough way, by recovering the distillate in different areas of the

condensing portion of the tubing. The ultraviolet absorption spectra indicated the distillate to be relatively homogeneous. The spectrum itself was that of a benzylidene-type spectrum.

As the distillate did not crystallize spontaneously, attempts were made to crystallize it from various solvents and solvent combinations, such as n-pentane, pentane-ether, pentane-benzene, hexane-benzene, benzene, toluene and toluene -pentane, without success.

(iv) 3rd Run (sealed tube) - Lycodine (0.019 g.) was placed in a length of glass tubing (5 mm. bore) sealed at one end. After the addition of 0.1 ml. of freshly distilled benzaldehyde and a small amount of zinc chloride, the mixture was frozen in the closed end of the tubing by immersing it in liquid nitrogen. While frozen, the tube was evacuated to 0.6 mm. Hg and sealed off while under vacuum. The tube was placed in an oven maintained at 140° for four hours and at 162° for forty-four hours.

The product, which was dark and tar-like in appearance, was removed from the tube using hot 5% HCl and ether, in which solvents the material was only sparingly soluble. The remaining residue in the tube was readily removed with chloroform. After removal of the chloroform, the HCl solutions were washed several times with ether, each ether extraction being washed in turn with 5% HCl. The material was converted

to the free base and the ether extractions of the free base were taken to dryness.

The free base was passed through a Celite column, prepared as in the previous runs and buffered at pH3. Ultraviolet absorption spectra of the fractions indicated the presence of mainly one product (see Fig. 22, UV-147). This product commenced being eluted after 42 ml. of n-butanol had passed through the column.

(h) Nuclear Magnetic Resonance Spectrum of 5,6,7,8-tetrahydroquinoline (free base)

The 5,6,7,8-tetrahydroquinoline hydrochloride (0.2567 g.) was dissolved in water, made alkaline with 5% NaOH and extracted five times with ether. The ether extractions, after washing with water and drying over anhydrous sodium sulphate, were taken to dryness. The free base (0.1785 g.) was dissolved in ether and placed in a small distilling bulb. After evaporation of the ether, the free base was vacuum distilled at 100° (air-bath)(2-3 mm. Hg) as a very clear liquid. The NMR spectrum of the free base was recorded with a Varian V-4300 NMR spectrometer, equipped with field stabilizer, at a fixed frequency of 40 Mc/sec., with spinning sample tubes. Signal separations were measured by the side-band technique in the usual manner.

9. DISCUSSION OF RESULTS

(a) Paper Chromatography

The paper chromatography of mixtures of strongly basic Solonaceous alkaloids and Ergot alkaloids using papers impregnated with buffers of various pH values has been studied, from an analytical viewpoint, by Brindle, Carless and Woodhead (64). Of the solvents used, these authors found that only ether and butanol gave reasonable spots (i.e. with little or no "tailing"). Schmall, Wollish and Shafer (78) have also reported the chromatography of organic bases on multi-buffered paper. These workers utilized a filter paper strip impregnated in individually marked zones with various buffers in sequence of decreasing pH. The mixture of organic bases was chromatographed on such a strip using a single solvent in the presence of water vapour, the bases tending to form salts at zones, depending upon their pK values.

Descending paper chromatograms, using papers buffered at various pH values in the manner of Brindle, et al (64) and the solvent system, n-butanol/water, were run of known alkaloids of Lycopodium annotinum L. The results of these runs (see Fig. 13) indicated that satisfactory separations of most of the known alkaloids of this plant could be effected by the use of paper buffered at pH6. This technique was adopted to follow the extent of separation accomplished by

more quantitative means of separation. Identifications of known alkaloids separated were made by running reference standards of these alkaloids on the same chromatograms, or by using instrumental methods in conjunction with paper chromatography in the manner of Pozdera and co-workers (79).

It will be noted from Fig. 13 that the alkaloids β-obscurine and O-acetylacrifoline (alkaloid L-12) were not separated at any of the pH values used. It was of interest that the alkaloid annotoxine, the molecular complex of annotine (alkaloid L-11) and acrifoline, gave, as would be expected, two spots corresponding to these latter two alkaloids.

Two-dimensional paper chromatography of the alkaloid mixture (see Fig. 12) did effect a greater separation than the one-dimensional. The second solvent system (methyl ethyl ketone / water), used in the two-dimensional run, distributed the alkaloids in a sequence similar to the first solvent system (n-butanol / water). It would have been preferable if a second solvent system could have been found which distributed the alkaloids in a different sequence to the first solvent system.

(b) Ion-exchange Column Chromatography

Appleweig (80), Appleweig and Ronzone (81) and Sussmann, Mindler and Wood (82) have described methods of isolating alkaloids from plant material utilizing suitable cation-exchange material. These methods, however, were used to isolate alkaloids from plant material rather than separating individual alkaloids of a mixture one from another. Van Etten, Earle, McGuire and Senti (83) and Jindra and Böswart (84) have described methods of separating morphine from non-phenolic opium alkaloids by means of suitable anion-exchange resins.

Their work on the separation of adjacent rare-earths on ion-exchange columns lead Spedding and co-workers (85) to the separation of nitrogen isotopes by ion-exchange. The method used by these workers involved the elution of an adsorbed band down a column in an "equilibrium-type" band. Such a displacement technique appeared to be one which might be applied to the separation of alkaloid mixtures.

It was felt that the following type of separation might be carried out using a cation-exchange resin, such as Dowex-50 (a sulfonic acid resin). Representing the alkaloids as B and B', the reaction inside the resin particle would be expected to be:

$$R\text{-}SO_3^- H^+ + B \longrightarrow R\text{-}SO_3^- BH^+ \qquad (I)$$

As the bases would not diffuse down the column until all the

acid groups above it were neutralized, a sharp lower boundary would be formed. If now a 0.2 N solution of NaOH in 50% aqueous methanol were slowly passed through the column, the following reaction would be expected to take place inside the resin particle:

$$R\text{-}SO_3^- BH^+ + OH^- + Na^+ \longrightarrow R\text{-}SO_3^- Na^+ + H_2O + B \qquad (II)$$

Since B is unprotonated and soluble in aqueous methanol, it will diffuse out of the resin and down the column where the following equilibrium would take place:

$$R\text{-}SO_3^- BH^+ + B' \rightleftharpoons R\text{-}SO_3^- B'H^+ + B \qquad (III)$$

Since OH^- is a much stronger base than any of the alkaloids, reaction (II) goes to completion and should give a sharp rear boundary to the alkaloid band. As a result of (III), the alkaloids should be separated as they proceed head to tail down the column.

It should perhaps be emphasized that although a simple or gradient elution technique might separate the alkaloids on an ion-exchange resin, the amount of substance which can be separated on a given weight of resin is quite small (e.g. the work of Moore and Stein (86) with amino acids). This is in contrast to the displacement technique, as described above, where (if separation occurs) the full exchange capacity of the resin is used.

Large and small scale runs of the alkaloid mixture with Dowex 50-X12 effected no separation of the individual alka-

loids. A qualitative comparison was made of the Dowex 50-X12 and the Dowex 50-X4 resins with regard to their abilities to adsorb the alkaloid mixture. It was apparent from this experiment that while the -X4 resin, of lower cross-linkage and hence of greater porosity, readily adsorbed the alkaloid mixture, the -X12 resin did not.

Small and moderate scale runs were then carried out using the Dowex 50-X4 resins. On conversion of the -X4 resin from the hydrogen phase to the sodium phase, marked contraction of the resin bed occurred which resulted in serious channeling. While there was a slight indication of separation in this run, the results were unsatisfactory.

While this work was being done, Byrne and Lapidus published a note (87) concerning concentration profiles in packed-bed ion-exchange systems. Their results indicated two distinct types of concentration profiles. A flat profile was intimately connected with the swelling of the resin and an inverted paraboloid profile connected with the shrinking of the resin as the exchange processes take place. In the latter type of profile, these workers found that it was possible for basic solutions to pass through the entire column almost unreacted because of the accelerated flow near the walls of the column. The authors concluded, therefore, that the assumption of a flat concentration profile in an ion-exchange column would seem to be valid only if the exchange

reaction is such that swelling of the resin takes place.

In an attempt to overcome the effect of shrinkage of the resin bed, an expansible column of firm but elastic rubber tubing was tried. The column was packed in the sodium phase (i.e. the contracted phase) and then regenerated with hydrochloric acid. The progressive expansion of the column during regeneration and contraction during elution was clearly evident. The elution lasted overnight and the first twenty fractions collected did not contain sodium (from the eluting agent) suggesting possible absence of channeling. However, paper chromatograms of the fractions containing the alkaloids disclosed that no separation of the individual alkaloids had occurred.

Theoretically, it should be possible to separate the individual alkaloids of a mixture in accordance with the principles outlined earlier in this section. However, in practice, it is apparent that the pore-size of the ion-exchange resin must be sufficiently large to permit the entry of the relatively large alkaloid molecule and, at the same time, be of such a character that it does not contract on elution. (See also (88)). These requirements cannot be met by available ion-exchange resins.

(c) Partition Column Chromatography

The discovery of the technique of partition column chromatography has been credited by subsequent authors (89) (90) to Martin and Synge (91). These latter workers conceived the idea of absorbing water in silica gel and then using the water-saturated solid as one phase of a chromatogram, the other being some fluid immiscible with water. The silica acted merely as a mechanical support. Separations in a chromatogram of this type thus depended upon differences in the partition between two liquid phases of the substances to be separated, and not on differences in adsorption between liquid and solid phases as in previous chromatograms.

Celite, a brand of diatomaceous earth manufactured by Johns-Manville Company, New York, has since been used widely as the mechanical support for partition columns. Neish (70), employing a slurry technique to pack his columns, used this material in the resolution of glycerol and related compounds present in fermentation solutions. Lemieux and Charanduk (71), who modified Neish's procedure for preparing the columns, utilized the Celite technique for separating the hydrolysis products of ustilagic acid.

The use of partition columns, containing aqueous buffer as the stationary phase, for the separation of alkaloids has been reported by various authors (92)(93)(94). Small amounts

of alkaloid starting material (e.g. 40 mg. in (93)) were used in these studies.

The inter-relationships which exist between the apparent distribution coefficient (k') (i.e. the coefficient of the fully or partially protonated base), the true distribution coefficient (k) (i.e. the coefficient of the unprotonated base) of an alkaloid in a particular solvent system, the pKa of the alkaloid and the pH of the buffer phase can be derived readily from first principles (see Appendix "A"). The relationship, as reported by Goulombic and Orchin (95), is as follows: $\log k' = \log k - pKa + pH$. This relationship is useful, of course, not only in partition column chromatography but also in simple partition procedures and countercurrent distribution procedures (see below).

Bottomley and Mortimer (92) characterized the partition behaviour of Tropane alkaloids by the pH at which $\log k' = 0$ (i.e. where the alkaloid was equally distributed between the two phases which were of equal volume). The symbol "$pH_{\frac{1}{2}}$" was used for this value. Bottomley and Mortimer had hoped that chromatography of the Tropane alkaloids on buffered paper would provide information on the relative $pH_{\frac{1}{2}}$ values of the alkaloids. It was apparent from their results, however, that the order of movement of these bases on buffered paper differed from the order of their $pH_{\frac{1}{2}}$ values.

As the most satisfactory separations of the mixture of

alkaloids from Lycopodium annotinum L. had been obtained by paper chromatography on paper buffered in the range pH 5.65 to pH 6, the Celite columns used in the initial runs were buffered at pH 5.65. Using small amounts of crude alkaloid, ranging from 150-200 mg. for each run, these buffered Celite columns were developed with n-butanol, saturated with water, as the moving phase. Paper chromatography of the fractions collected from the partition columns disclosed that some separation of the alkaloid mixture had taken place but that the fractions obtained were still rather complex mixtures of alkaloids.

It was apparent, also, as in the case of Bottomley and Mortimer (92), that the sequence of elution of alkaloids from a buffered Celite partition column could not be accurately predicted by running a "pilot" run with micro amounts of alkaloids using the same solvent system. This was well illustrated, in a qualitative way, in Fig. 14. In their work, Bottomley and Mortimer (92) suggested that this might be evidence that paper chromatography is not true partition chromatography. On the other hand, Lemieux, Bishop and Pelletier (89), in a paper describing preparative partition chromatography of carbohydrates on Celite columns, reported that their "Rv" value, a measure of the relative mobilities of materials on Celite No.535 columns, bore "a rough but useful relationship to the Rf term of paper chromatography".

A series of ten duplicate runs on Celite columns buffered at pH 5.65 yielded four major fractions. The component alkaloids of the first of these four fractions were more completely separated by chromatography on paper buffered at pH 3.3. This first major fraction was therefore passed through a Celite column buffered at pH 3.28. The early fractions from this column, when taken to dryness, yielded annotinine. Later fractions, however, when examined by ultraviolet absorption spectroscopy possessed ultraviolet absorption of a pyridine chromophore more or less submerged behind general absorption. Much of the subsequent work was directed towards the isolation, characterization and partial determination of structure of a new alkaloid (lycodine) which was responsible for this characteristic absorption.

(d) Countercurrent Distribution Method

Craig, Hogeboom, Carpenter and du Vigneaud (97) reported the separation and characterization of some penicillins by the method of countercurrent distribution. As these authors pointed out, the procedure of countercurrent distribution represented an attempt to make the partition coefficient a more useful physical constant in organic chemistry. It is based on a certain systematization of multiple extractions or transfers so that the mathematics of the binomial expansion

may be applied directly in the interpretation of the results.

Bush and Densen (72) applied systematic multiple fractional extraction procedures to the separation of organic mixtures. These workers demonstrated that for a mixture of two substances having partition constants K_a and K_b, the greatest fractional separation for a given number of extractions using their type of procedure was attained if the ratio of the volumes of the two solvents, X and Y, was maintained at:

$$\frac{V_X}{V_Y} = \sqrt{\frac{1}{K_a K_b}}$$

From this relationship it is apparent that where the phase volumes are equal, the most efficient separation of any two bases is obtained where the geometric mean of the apparent partition coefficients is unity. This latter condition occurs where the buffer pH is the average of the $pH_{\frac{1}{2}}$ values (92) of the two bases (see Appendix "B"), provided that the concentration of alkaloid is low enough not to raise the pH of the buffer appreciably.

The equation relating the partition coefficient and ionization constant of an organic base involving the distribution of an organic base between an organic solvent and an immiscible buffer phase was stated by Goulombic and Orchin (95), as referred to previously, to be:

$\log k' = \log k - pKa + pH$ (see Appendix "A")

where k' is the apparent partition coefficient, k is the true

partition coefficient and pKa is the acidic ionization constant. This equation is limited to the circumstances where pH << pKa, where no association occurs in the organic phase, and where the protonated base is insoluble in the organic phase. Bottomley and Mortimer (92) applied the foregoing equations to the partition separation of Tropane alkaloids. In this present work these equations were used in the countercurrent distribution studies of the alkaloids of Lycopodium annotinum L.

Partition data for the alkaloids, lycopodine, annotinine, acrifoline, annotine (alkaloid L-11), and lycodine have been calculated and recorded in Table No.3. These data were calculated utilizing constants obtained both from the present work and other sources, as shown in Appendices "C" and "D".

The average of the $pH_{\frac{1}{2}}$ values of lycopodine (5.9) and annotinine (4.4) is pH 5.2. Efficient separation of the two alkaloids should be effected by a countercurrent scheme using chloroform and buffer of pH5.2. Using the scheme shown in Fig. 15 and the system chloroform-buffer pH5.2, the theoretical percentage distributions of the alkaloids annotinine, lycopodine, lycodine and acrifoline were calculated. These calculations (ref. Fig. 15), made some time after the actual runs were completed, indicated that annotinine would be concentrated (96%) in the chloroform fractions (C-1, C-2,

and C-3), whereas lycopodine would be concentrated (93%) in the buffer fractions (B-1, B-2 and B-3), as would acrifoline (100%). The central fraction would contain 26% of the original lycodine (see below) present.

When the crude alkaloid mixture (68 g.) from the annotinine mother-liquors was subjected to the countercurrent scheme illustrated in Fig. 15, annotinine (4 g.) was recovered from the chloroform fractions (C-1, C-2 and C-3) and acrifoline hydrobromide (2.6 g.) was obtained from the buffer fractions (B-1, B-2 and B-3). This was consistent, therefore, with the theoretical calculations referred to above.

Examination of the ultraviolet absorption spectrum of the free base recovered from the central fraction disclosed the characteristic pyridine-like absorption referred to previously. It was later calculated that this central fraction (L-38) would contain, theoretically, 4% of the annotinine, 5% of the lycopodine, 0% of the acrifoline and 26% of the lycodine (see below) present in the initial crude alkaloid mixture (see Fig. 15) -- as well as unknown amounts of other minor alkaloids initially present.

The fraction L-38 was then chromatographed through two large Celite columns buffered at pH 3.3, using n-butanol as the moving phase. The resulting fractions were examined by ultraviolet absorption spectroscopy and paper chromatography and those fractions containing lycodine were isolated.

Fractional crystallization from methanol of the picrates from these latter fractions yielded a picrate (prisms), m.p. 230-235° (dec.) (300 mg.) and a small amount of another picrate, S-20, m.p. 240-246°, as yet unidentified and uncharacterized. Electrometric titration (see Fig. 16) of the first base picrate (11.1 mg.) indicated that the base was diacidic. This picrate was identical with that isolated later by a different method and which was shown to be the picrate of a new alkaloid. This new alkaloid was named "lycodine". Attempts to form other crystalline derivatives of lycodine, at this stage, were unsuccessful. Lycodine dipicrate, m.p. 229-233° (dec.), as recrystallized from hot methanol, analyzed as $C_{17}H_{24}N_2 \cdot 2C_6H_3O_7N_3$.

From preliminary countercurrent distribution experiments (Craig machine) with buffer pH6 and chloroform, it was observed that most of the alkaloids have a high partition coefficient (concentration in buffer / concentration in chloroform) but that annotinine, lycopodine, O-acetylacrifoline (alkaloid L-12) and lycodine have low partition coefficients. Alkaloids with a partition coefficient close to unity are present to only a minor extent in the batches of alkaloids used. Annotine (alkaloid L-11) which has such a partition coefficient (see Table No. 3) is not present to a significant extent (75) in the crude alkaloid mixture which was available to us. This distribution of the alkaloid mixture under the

conditions described, as detected by paper chromatography as well as by ultraviolet and infrared absorption spectroscopy, is illustrated in the composite chart, Fig. 17. The ultraviolet absorption at 268 mμ, shown in Fig. 17, is that of lycodine; that at 234 mμ, which is the region associated with α,β-unsaturated ketones, was not related to any known Lycopodium alkaloids.

(e) Isolation of Lycodine

On the basis of the above results, lycodine was concentrated by countercurrent distribution of the alkaloid mother-liquors from the crystallization of annotinine. Two successive countercurrent distributions were carried out, first, in six large separatory funnels with the system chloroform-buffer pH6 and then, in a 60-tube Craig machine with the system chloroform-buffer pH5.2. From these countercurrent distributions an alkaloid fraction was obtained which had the correct ultraviolet absorption for lycodine. By chromatography of this fraction on activated alumina, pure crystalline lycodine was obtained.

A schematic chart showing the three-step procedure outlined above, is shown in Fig. 18. The theoretical distribution of some of the alkaloids in the first step, calculated either by the method illustrated in Fig. 15, or, by the

method of Bush and Densen(72), is shown in the inset table in Fig. 18. This first step theoretically eliminated the acrifoline and retained 41% lycopodine, 100% annotinine, 36% annotine (alkaloid L-11) and 94% lycodine originally present in the crude mixture. The theoretical distributions of the alkaloids in the second countercurrent step, using the 60-tube Craig machine, has been calculated, as illustrated in Appendix "E" and plotted in Fig. 18. This plot illustrates the extent of theoretical separation of lycodine from annotinine, lycopodine and annotine (alkaloid L-11) remaining from the first step. When this scheme was carried out, some lycopodine still remained with the lycodine after the second step and was separated from lycodine by the third step, absorption chromatography on activated alumina. A trace of another alkaloid which was not characterized was indicated in the final fractions from the alumina column by an infrared absorption in the carbonyl region associated with ketones. In all, about 400 mg. of lycodine was obtained by this isolation procedure and the preliminary isolation procedures.

(f) Lycodine - Characterization and Structural Studies

Lycodine, m.p. 118°, $[\alpha]_D$ = -10°, has the formula, $C_{17}H_{24}N_2$, and gave a characteristic dipicrate, m.p. 229-233° (dec.), which was sparingly soluble in methanol. Titration of the base in 50% aqueous methanol showed that the pKa's of the base were 3.97 and 8.08. One C-methyl group was found to be present by the Kuhn-Roth method. The ultraviolet spectra of the base in neutral and acid solutions are shown in Fig. 19 and correspond to an unconjugated pyridine chromophore in the base and salt forms, respectively. A fuller discussion of these spectra will be left till after consideration of other results (see below).

The infrared spectrum of the base (in Nujol) is shown in Fig. 20; the sharp band at 3270 $cm.^{-1}$ must be due to a N-H group (37). As often happens (37) this band is of very low intensity in the spectrum (Fig. 20) of the carbon tetrachloride solution of the compound. The presence of a pyridine ring is confirmed by the band (99) at 1580 $cm.^{-1}$ (in Nujol) and that of a C-methyl group by a band (100) at 1382 $cm.^{-1}$ (in CCl_4). Acetylation of lycodine with acetic anhydride gave a N-acetyl derivative, isolated as the monopicrate. Titration of the picrate showed that the acetylated base had only one basic center (pKa 4.95). Hydrolysis of N-acetyl lycodine gave back lycodine, thus confirming the

presence of a secondary nitrogen atom in the alkaloid.

In order to determine the substitution on the pyridine ring, the nuclear magnetic resonance (NMR) spectrum (Fig. 21) of lycodine was examined. Under moderately high resolution there are three peaks of equal area at low field. These are probably due to protons on the pyridine ring (76). The sharp peak at highest field has three times the area of any of the peaks at lowest field and must correspond to the C-methyl group already found. It may be observed that the low value (61% of theoretical) found in the Kuhn-Roth estimation and the fact that the C-methyl peak in the NMR spectrum is unsplit both suggest that the methyl group is attached to a quaternary carbon atom. The other peaks in the spectrum correspond to the remaining protons in the molecule.

The spectrum of the low field part is shown at higher resolution above the main spectrum, in Fig. 21. Each peak is split into four peaks, not always very well resolved. Bernstein, Pople and Schneider (76)(101)(102) have examined the spectra of pyridine and various substituted pyridines and discussed the effects of chemical shifts and coupling constants on the spectra. From their analysis it can be seen that lycodine must be a 2,3-disubstituted pyridine, and in fact the spectrum of lycodine is very similar to that of 2,3-lutidine except for a difference in the relative chemical shift of the γ-proton. The three peaks at low field

(Fig. 21) may be assigned, from left to right, to the α-, γ-, and β-protons of the pyridine ring.

The relative chemical shifts and coupling constants of the pyridine protons of lycodine have been calculated (see Appendix "F") and are compared with those of 2,3-lutidine (76) and pyridine (102) in Table No. 4. The observed intensities and energies of the signals from the pyridine protons of lycodine are in satisfactory agreement (see Table No. 5) with those calculated from the above parameters (see Appendix "F") by the method of Bernstein, Schneider and Pople (76). This confirms that the three peaks at low field indeed originate from the protons on the pyridine ring, and not from any other protons. The reason for the abnormal shift of the γ-proton of lycodine may be due to the nearness of the secondary nitrogen atom. Although, as will be shown later (see below), the secondary nitrogen atom is most probably separated from the pyridine ring by more than one carbon atom, it may still be fairly close in space to the γ-proton. The abnormal shift is not due to the fact that in lycodine, as will be shown below, the 2,3-substitution is in the form of a saturated six-membered ring, since 5,6,7,8-tetrahydroquinoline gave a NMR spectrum closely similar to that of 2,3-lutidine.

We may be certain, therefore, of the presence of a 2,3-disubstituted pyridine ring in lycodine. Godar and

Mariella (103) have recorded the ultraviolet absorption spectra of 2,3-lutidine, 2,3-trimethylene-, 2,3-tetramethylene-, and 2,3-pentamethylene-pyridine both as free bases and as salts. Their data have been replotted in Fig. 19 using a linear ϵ scale, instead of the original log scale, which facilitates the comparison of these curves with those of lycodine. It is clear that lycodine shows the greatest similarity to 2,3-tetramethylenepyridine (5,6,7,8-tetrahydroquinoline) and therefore that lycodine has in fact a saturated six-membered ring fused 2,3- to the pyridine ring.

The pKa's (3.97 and 8.08) of lycodine are appreciably different from those of nicotine (3.35 and 7.70) determined under identical conditions. The difference in pKa's of the non-pyridine nitrogen atoms may be due to the secondary nature of the nitrogen atom in one case and the tertiary nature in the other. The difference in the pKa's of the pyridine nitrogen atoms, however, cannot be due to such an effect, but is readily understood if the saturated nitrogen atom is further (i.e. at least β-) from the pyridine ring in the case of lycodine than in the case of nicotine. Wepster (104) in his study of phenylalkylamines of the general type, $C_6H_5(CH_2)_nNH_2$, noted experimental values of pKa 9.34, 9.83 and 10.20 where n was 1, 2 and 3, respectively. The increment in pKa value is therefore 0.49 where there were two, rather than one, carbon atoms between the nitrogen atom and

the aromatic ring. Although, admittedly, it is very approximate, this increment is consistent with the increments in pKa values for lycodine and nicotine.

Lycodine does not seem to contain unsaturation apart from the pyridine ring, as far as is shown by UV, IR and NMR spectra. Therefore lycodine contains four rings and the facts known about its constitution may be summarized by the partial structure shown as an inset with the ultraviolet absorption spectrum of lycodine in Fig. 22.

In order to determine whether the carbon in the "8" position, adjacent to the pyridine ring of lycodine, had two hydrogen atoms attached, attempts were made to prepare and isolate the benzylidene derivative of lycodine. The ultraviolet absorption spectrum of the benzylidene derivative of 5,6,7,8-tetrahydroquinoline (see Fig. 22, UV-76) was used to assess whether or not a benzylidene derivative of lycodine may have been formed in the various reactions which were carried out.

Acetic anhydride was used as the catalyst in the preparation of the benzylidene derivative of 5,6,7,8-tetrahydroquinoline. This catalyst, after trial, was considered unsuitable for use with lycodine due to the formation of the N-acetyl derivative and the expected reaction of the N-acetyl portion with benzaldehyde to form a cinnamamide derivative.

The ultraviolet absorption spectrum of the cinnamamide portion of the lycodine derivative would be expected to interfere with the benzylidene portion, if formed. The use of concentrated hydrochloric acid as a catalyst was also found to be unsatisfactory for the preparation of a benzylidene derivative of lycodine.

Due to the small quantity of lycodine available, the reactions of lycodine with benzaldehyde and zinc chloride (as catalyst) were confined to ca 20-30 mg. quantities of lycodine as starting material. Assessment of the reaction products was therefore made by examination of the ultraviolet absorption spectra of the products separated either by paper chromatography on buffered paper, or, by partition chromatography using buffered Celite. Of the runs made, the second run wherein lycodine (from 0.059 g. of lycodine dipicrate) was refluxed with freshly distilled benzaldehyde (5 ml.) and a small amount of zinc chloride catalyst for forty hours at 145° appeared to be most successful from the standpoint of production of a benzylidene-like derivative (see Fig. 22, UV-140).

From the variety of products (see Fig. 22) separated by both paper chromatography and partition chromatography of the reaction products it would appear that these reactions are quite complex, no doubt involving rearrangements as have

been encountered in the chemistry of annotinine (42). Condensations between the pyridine ring and the benzene ring of a benzylidene derivative of lycodine are conceivable from a theoretical standpoint to form highly conjugated systems. The spectra of some reaction products (e.g. Fig. 22, UV-142, and UV-114(7)) have been compared with those of quinoline (Fig. 22, UV-117) and other related materials with only general similarities being noted.

The possibility of cis-trans isomerism in a benzylidene derivative of lycodine could also contribute to the formation of a number of reaction products. In the case of the benzylidene derivative of 5,6,7,8-tetrahydroquinoline, it would be expected that one isomer (e.g. trans isomer) would predominate. Under varying reaction conditions and due to the more complex nature of the lycodine molecule, either isomer, or, a mixture of both isomers might result. A very brief study of the change which occurred in the benzylidene-like absorption spectrum of one of the reaction products after irradiation was made (ref. Fig. 22, UV-128). The partial elimination of the maximum at the higher wavelength after irradiation made the spectrum somewhat comparable to that of the product whose ultraviolet spectrum appeared in Fig. 22, UV-112. This latter product had been extracted from an area on a paper chromatogram which in turn had been subjected to

ultraviolet radiation in order to locate the positions of the products on the chromatogram without the use of a "colour" developer.

While a benzylidene derivative of lycodine has not been isolated and characterized, the similarity between spectra, particularly Fig. 22, UV-140, and the benzylidene derivative of 5,6,7,8-tetrahydroquinoline (Fig. 22, UV-76), indicates a strong probability that a benzylidene derivative of lycodine can be formed. It is probable, therefore, that the carbon in the "8" position, adjacent to the pyridine ring of lycodine (ref. Fig. 22) has two hydrogen atoms attached.

Finally, it may be mentioned that there appears to be a close similarity between lycodine and the Lycopodium alkaloid, β-obscurine, $C_{17}H_{24}ON_2$, which has been shown by Marion and Moore (17) to have an α-pyridone chromophore. Although the empirical formula of β-obscurine is correct for it to be the α-pyridone analogue of lycodine, the relationship between the two alkaloids cannot be so simple, for the basic nitrogen atom of β-obscurine appears to be tertiary, as the alkaloid could not be acetylated.

(g) Appendices

1) Appendix "A" - The derivation of the relationship in the partition of a monoacidic alkaloid between the organic and buffer phases:

$$\log k' = \log k - pKa + pH$$

where, k' is the apparent partition coefficient,

k is the true partition coefficient,

pKa is that of the alkaloid, and,

pH is that of the buffer.

Note: This relationship is given by Goulombic and Orchin (95). These authors indicated that the derivation was analogous to that for a previously derived equation relating the partition coefficient and ionization constant of an organic acid (96).

Derivation:

In the buffer, the equilibrium, $BH^+ \rightleftharpoons B + H^+$, exists where B represents the free, non-protonated base or alkaloid.

Hence, $$K_a = \frac{[B]_b\,[H^+]}{[BH^+]}$$ where $[B]_b$ is the concentration of the non-protonated alkaloid in the buffer phase.

or, $$[B]_b = \frac{K_a\,[BH^+]}{[H^+]}$$

The true distribution coefficient, $k = \frac{[B]_o}{[B]_b}$

where $[B]_o$ is the concentration of the non-protonated alkaloid in the organic phase.

Hence, $k = \frac{[B]_o\ [H^+]}{K_a\ [BH^+]}$ or, $[B]_o = \frac{k \times K_a \times [BH^+]}{[H^+]}$

But the apparent partition coefficient, $k' = \frac{[B]_o}{[BH^+]+[B]_b}$

Hence, $k' = \frac{k \times K_a \times [BH^+]}{[H^+]} \times \frac{1}{[BH^+] + \frac{K_a\ [BH^+]}{[H^+]}}$

$$k' = \frac{k}{1 + \frac{[H^+]}{K_a}}$$

Approximation:

Where $\frac{[H^+]}{K_a} >> 1$ (i.e. where $[H^+] >> K_a$, or, pH is much lower than the pKa of the alkaloid.)

then $k' = \frac{k \times Ka}{[H^+]}$

Hence, $\log k' = \log k + \log Ka - \log [H^+]$

or, $\underline{\log k' = \log k - pKa + pH}$

This latter relationship exists where the base occurs in the organic phase as the non-protonated and unassociated base

only and the pH values of the buffers are much lower than the pKa of the base.

It is also apparent that when the pH value of the buffer is the same as the pKa of the base that $k' = \frac{1}{2}k$. Also, when the pH value of the buffer is much greater than the pKa, then $k' = k$.

Another useful relationship may be derived from $k' = \dfrac{k}{1 + \dfrac{[H^+]}{Ka}}$ as follows:

Since k and Ka are constants of the alkaloid,

then k' varies as $\dfrac{1}{[H^+]}$ OR log k' varies as the pH.

At a particular pH value, say pH_a, the apparent partition coefficient will be k'_a.

Doubling the apparent partition coefficient (k'_a),

$k'_b = 2k'_a$. Hence, $\log k'_b = \log k'_a + \log 2.$

$= \log k'_a + 0.3010$

But $\log k'_a$ varies as pH_a; hence, $\log k'_b$ varies as $pH_a + 0.3010$

and $pH_b = pH_a + 0.3010$.

Therefore, for <u>each increase of 0.3</u> (approx.) in pH value, the apparent partition coefficient (k') is <u>doubled</u>.

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2) Appendix "B" - When the pH of the buffer is the average of the $pH_{\frac{1}{2}}$ values of the two bases, the geometric mean of the apparent partition coefficients (k' values) of the bases is unity.

Note: This relationship was stated by Bottomley and Mortimer (92); however, these authors did not include the proof in their paper.

Proof:

$\log k' = \log k - pKa + pH$ (see Appendix "A")

Considering alkaloids "a" and "b":

$pH_a = \log k'_a + \text{constant}$ and $pH_b = \log k'_b + \text{constant}$.

$$\frac{pH_a + pH_b}{2} = \frac{\log k'_a + \log k'_b}{2} + \text{constant}.$$

When $pH_a = (pH_{\frac{1}{2}})_a$ then $k'_a = 1$ (by definition)

When $pH_b = (pH_{\frac{1}{2}})_b$ then $k'_b = 1$ (by definition)

But the term, $\frac{\log k'_a + \log k'_b}{2} = \log (k'_a \times k'_b)^{\frac{1}{2}}$ (i.e. log of G.M. of k'_a and k'_b)

Hence, when the pH of the buffer is $\frac{(pH_{\frac{1}{2}})_a + (pH_{\frac{1}{2}})_b}{2}$, then the geometric mean of k'_a and k'_b is unity.

Example: (see over).

Example:

Lycopodine - $pH_{\frac{1}{2}}$ = 5.87 (see Table No.3)

Annotinine - $pH_{\frac{1}{2}}$ = 4.43 (see Table No.3)

Average of $pH_{\frac{1}{2}}$ values is 5.15

Lycopodine - $\log k' = \log k - pKa + pH$ (see Appen. "A")

$= 1.57 - 7.44 + 5.15$ (see Tables No.

$k' = 0.19$ 1(a) and No.3)

Annotinine - $\log k' = 2.074 - 6.5 + 5.15$ (see Tables No.

$k' = 5.30$ 1(f) and No. 3)

Geometric Mean of k'(lycopodine) and k'(annotinine) at pH 5.15 is:

$$(5.30 \times 0.19)^{\frac{1}{2}} = 1.$$

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3). Appendix "C" - Calculation of partition data (chloroform - buffer) from the relationship

$\log k' = \log k - pKa + pH$ (see Appendix "A")

Example : Annotinine.

Calculation of the true partition coefficient (k):

Apparent partition coefficient $(k') \frac{(\text{chloroform})}{(\text{buffer pH5})} = 3.8$ (see Exptl)

pKa = 6.5 (see Table No. 1(f)).

Hence, $\log k = \log 3.8 + 6.5 - 5$

or, $k = 120$

Hence, true partition coefficient (k) $\frac{(\text{chloroform})}{(\text{buffer})} = 120.$

Calculation of apparent distribution coefficient (k') at pH 5.2:

$\log k' = \log 120 - 6.5 + 5.2$

$k' = 6.0$ (i.e. 86% in chloroform phase)

at pH 6.0:

$\log k' = \log 120 - 6.5 + 6.0$

$k' = 38$ (i.e. 97% in chloroform phase)

Calculation of $pH_{\frac{1}{2}}$ value:

$pH_{\frac{1}{2}}$ value is where $\log k' = 0$ (by definition)

Hence, $pH_{\frac{1}{2}} = pKa - \log k = 6.50 - \log 120 = 4.4.$

Similarly, the partition data (Table No. 3) of the following alkaloids were calculated using the following data:

Lycopodine:

$k' \frac{(\text{chloroform})}{(\text{buffer pH6})} = 1.3$ (see Experimental)

pKa = 7.44 (see Table No. 1(a))

Acrifoline:

$k' \frac{(\text{chloroform})}{(\text{buffer pH7})} = 0.86$ (75)

pKa = 8.34 (see Table No. 1(k))

Annotine (alkaloid L-11):

$k' \frac{(\text{chloroform})}{(\text{buffer pH6})} = 1.2$ (75)

pKa = 7.50 (see Table No. 1(h))

Lycodine:

$k' \frac{(\text{chloroform})}{(\text{buffer pH5.2})} = 0.71$ (see Appendix "D")

pKa = 8.08 (see Table No. 1(o))

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4) Appendix "D" - Calculation of apparent partition coefficient (k') of lycodine from the countercurrent distribution (Craig machine) in the system chloroform - buffer pH5.2.

From the examination of the ultraviolet curves, the maximum of the absorption associated with lycodine occurred around tubes 35 and 36 (see Experimental). As the starting material was placed in tubes 0, 1 and 2 of the Craig machine, the maximum was taken, for purposes of calculation, at tube 35.

From Craig and Craig (98) it is noted that: $N = \frac{n\ K\ r}{Kr + 1}$

where: n - total number of transfers.

N - position of maximum.

r - ratio of volumes, upper and lower.

K - partition coefficient $\frac{\text{(upper phase)}}{\text{(lower phase)}}$

Hence, in the case of lycodine:

$$35 = \frac{60\ K}{K + 1} \quad \text{or,} \quad K = \frac{35}{25} \quad \frac{\text{(buffer pH5.2)}}{\text{(chloroform)}}$$

Hence, the apparent distribution coefficient (k'):

$$k' \ \frac{\text{(chloroform)}}{\text{(buffer pH5.2)}} = \frac{25}{35} = 0.71.$$

5) Appendix "E" - Calculations to determine the theoretical distributions of alkaloids in the Craig countercurrent machine (chloroform - buffer pH 5.2) as plotted in Fig. 18.

Example : Annotinine.

At pH5.2, apparent partition coefficient (k') = 6.0 (see Table No.3).

or, $K = \frac{1}{6.0} \quad \frac{(\text{buffer pH5.2})}{(\text{chloroform})}$

From Craig and Craig (98) it is noted that $N = \frac{n\,K\,r}{Kr + 1}$

where: n - total number of transfers.

N - postion of maximum.

r - ratio of volumes, upper and lower.

K - partition coefficient $\frac{(\text{upper phase})}{(\text{lower phase})}$

Substituting $K = \frac{1}{6.0}$, n = 60 and r = 1,

then, N = 8.6.

Hence, the maximum would occur around tube 9.

From Craig and Craig (98) it is also noted that:

$$y = \frac{1}{(2\pi rK/(K+1)^2)^{\frac{1}{2}}}\; e^{-X^2 \Big/ \frac{2nK}{(K+1)^2}}$$

where: K and n - as described above.

y - the fraction of substance in a given tube.

X - the distance from the maximum of the tube in question.

The value of the term $\frac{2nK}{(K+1)^2}$ is calculated to be 14.75.

Hence, $y_0 = \frac{1}{(14.75\pi)^{\frac{1}{2}}} = 0.146$ (or, 14.6% in tube 9).

$y_1 = 0.146 \; e^{-(1)^2/14.75}$

$\log y_1 = \log 0.146 - \frac{1}{14.75} \log 2.718$

$= \log 0.146 - 0.0294$

Hence, $y_1 = \frac{0.146}{\text{antilog } 0.0294} = 0.137$ (or, 13.7% in tubes 8 and 10).

$y_2 = \frac{0.146}{\text{antilog } (2^2 \times 0.0294)} = 0.111$ (or, 11.1% in tubes 7 and 11).

$y_3 = \frac{0.146}{\text{antilog } (3^2 \times 0.0294)} = 0.080$ (or, 8.0% in tubes 6 and 12).

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y_4 to y_7, inclusive, were calculated in a similar fashion. The y values (as percentages) were plotted as the ordinates against the tube numbers to provide the theoretical distri-

bution curve for annotinine (see Fig. 18).

Similar theoretical values were determined and plotted for lycopodine, annotine (alkaloid L-11) and lycodine (see Fig. 18).

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6) Appendix "F" - An analysis of the nuclear magnetic resonance (NMR) spectrum of lycodine. (see Tables No. 4 and No. 5).

The NMR spectrum of lycodine (see Fig. 21) appears to be of the ABX type as described by Bernstein, Pople and Schneider (76). Assuming a 2,3-substitution of the pyridine ring in lycodine, the signals from the α-proton (i.e. the X-position of Bernstein et al) would be well separated from those of the other two, the β- and γ-protons (i.e. the B and A positions, respectively, of Bernstein et al). There is, however, a greater chemical shift between the β- and γ-protons of lycodine than of 2,3-lutidine.

All twelve non-combination lines (ref. Table VII (76)) are clearly visible and the values in cycles per second (c/sec.) are indicated in Fig. 21. Since it is to be expected that the coupling between protons attached to neighbouring carbon atoms would be dominant, the β-proton is assigned to the position in the spectrum, as indicated in Fig. 21, with

the γ-proton occupying the central position. The splittings in the βγ-region are 5.1, 5.2, 1.6 and 1.9 c/sec. The corresponding separations in the α-region are 5.0, 5.0, 1.7 and 1.7 c/sec. in good agreement with theory (76).

Bernstein and co-workers pointed out that there are two possible assignments which will be considered in turn.

Assignment 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12. -

Note: The numbers in this first assignment correspond to the non-combination lines of Table VII of (76).

From the average values of the splitting we have, from the β-pairs (i.e. 2 - 1, and, 4 - 3):

$$\tfrac{1}{2}(J_{\gamma\alpha} + J_{\beta\alpha}) + (D_- - D_+) = 5.2 \qquad \text{(i)}$$

and from the γ-pairs (i.e. 6 - 5, and, 8 - 7):

$$\tfrac{1}{2}(J_{\gamma\alpha} + J_{\beta\alpha}) - (D_- - D_+) = 1.8 \qquad \text{(ii)}$$

Adding (i) and (ii),

$$J_{\gamma\alpha} + J_{\beta\alpha} = 7.0 \qquad \text{(iii)}$$

From Table VII (76) it is apparent that the coupling constant, $J_{\beta\gamma}$, is equal to the separation between the lines 1 and 3 (8.8 c/sec.), or, between lines 5 and 7 (8.4 c/sec.); the mean value of $J_{\beta\gamma}$ being 8.6 c/sec. The coupling constant ($H_\beta \cdot H_\gamma$) is 8.6 c/sec. (see Table No.4).

The value of D_+ is equal to half the separation between lines 2 and 6 (16.85 c/sec.), or, between lines 4 and 8 (16.75 c/sec.); the mean value of D_+ being 16.8 c/sec.

The value of D_- is equal to half the separation between lines 1 and 5 (18.6 c/sec.), or, between lines 4 and 8 (18.4 c/sec.); the mean value of D_- being 18.5 c/sec.

By definition (76):

$$D_+\cos 2\phi_+ = \tfrac{1}{2}\eta H_o(\sigma_\beta - \sigma_\gamma) + \tfrac{1}{4}(J_{\gamma\alpha} - J_{\beta\alpha}) \qquad \text{(iv)}$$

$$D_+\sin 2\phi_+ = \tfrac{1}{2}J_{\gamma\beta} \qquad \text{(v)}$$

$$D_-\cos 2\phi_- = \tfrac{1}{2}\eta H_o(\sigma_\beta - \sigma_\gamma) - \tfrac{1}{4}(J_{\gamma\alpha} - J_{\beta\alpha}) \qquad \text{(vi)}$$

$$D_-\sin 2\phi_- = \tfrac{1}{2}J_{\gamma\beta} \qquad \text{(vii)}$$

Adding (i) and (ii):

$$\eta H_o(\sigma_\beta - \sigma_\gamma) = (D_+\cos 2\phi_+) + (D_-\cos 2\phi_-) \qquad \text{(viii)}$$

Taking (v): $D_+\sin 2\phi_+ = \tfrac{1}{2}J_{\gamma\beta}$

$$\sin 2\phi_+ = \frac{8.6}{2 \times 16.8} = 0.25595$$

Hence, $2\phi_+ = 14^\circ 50'$ and $\phi_+ = 7^\circ 25'$

and $\cos 2\phi_+ = 0.96667$.

Similarly, taking (vii):

$$\sin 2\phi_- = \frac{J_{\gamma\beta}}{2D_-} = \frac{8.6}{2 \times 18.5} = 0.23243$$

Hence $2\phi_- = 13^\circ 26'$ and $\phi_- = 6^\circ 43'$

and $\cos 2\phi_- = 0.97264$.

Taking (viii):

$$\eta H_o(\sigma_\beta - \sigma_\gamma) = (16.8 \times 0.96667 + 18.5 \times 0.97264)$$

$$= 34.2 \text{ c/sec.} \qquad \text{(ix)}$$

Therefore, chemical shift $(H_\gamma - H_\beta)$ = 34.2 c/sec. (see Table 4)

Subtracting (vi) from (iv):

$$J_{\gamma\alpha} - J_{\beta\alpha} = 2(D_+ \cos 2\phi_+ - D_- \cos 2\phi_-)$$
$$= 2(16.8 \times 0.96667 - 18.5 \times 0.97264)$$
$$= -4.6 \text{ c/sec.} \qquad \text{(x)}$$

The addition of lines 2, 3, 6, and 7 (Table VII (76)) gives the term:

$$2\eta H_o(2 - \sigma_\gamma - \sigma_\beta) = 98.3$$
$$\eta H_o(2 - \sigma_\gamma - \sigma_\beta) = 49.2 \qquad \text{(xi)}$$

Adding (ix) and (xi):

$$\eta H_o(2 - 2\sigma_\gamma) = 83.4$$
$$\eta H_o(1 - \sigma_\gamma) = 41.7 \qquad \text{(xii)}$$

Subtracting (ix) from (xi):

$$\eta H_o(2 - 2\sigma_\beta) = 15.0$$
$$\eta H_o(1 - \sigma_\beta) = 7.5 \qquad \text{(xiii)}$$

The value of $\eta H_o(1 - \sigma_\alpha)$ is equal to half the sum of lines 9 and 12 (61.9 c/sec.), or, of lines 10 and 11 (61.9 c/sec.); the value being 61.9 c/sec.

$$\text{i.e. } \eta H_o(1 - \sigma_\alpha) = 61.9 \qquad \text{(xiv)}$$

Subtracting (xii) from (xiv):

$$\eta H_o(\sigma_\gamma - \sigma_\alpha) = 20.2 \text{ c/sec.}$$

i.e. Chemical shift $(H_\gamma - H_\alpha)$ = 20.2 c/sec. (see Table No.4)

Subtracting (xiii) from (xiv):

$$\eta H_o(\sigma_\beta - \sigma_\alpha) = 54.4 \text{ c/sec.}$$

i.e. Chemical shift $(H_\beta - H_\alpha) = 54.4$ c/sec. (see Table No. 4)

Since $J_{\gamma\alpha} + J_{\beta\alpha} = 7.0$ (see (iii))

and $J_{\gamma\alpha} - J_{\beta\alpha} = -4.6$ (see (x))

Adding (iii) and (x):

$$J_{\gamma\alpha} = 1.2 \text{ c/sec.}$$

i.e. Coupling constant $(H_\alpha \cdot H_\gamma) = 1.2$ c/sec. (see Table 4)

Subtracting (x) from (iii):

$$J_{\beta\alpha} = 5.8 \text{ c/sec.}$$

i.e. Coupling constant $(H_\beta \cdot H_\alpha) = 5.8$ c/sec. (see Table 4)

The chemical shifts and coupling constants for lycodine, as calculated above, are included in Table No. 4.

<u>Calculation of Energy and Relative Intensity Values for NMR spectrum of lycodine.</u>

1. Calculation of terms common to various lines (ref. Table VII (76)):

(a) From (xi) $\frac{1}{2}\eta H_o(2 - \sigma_\gamma - \sigma_\beta) = 24.6$ c/sec. (xv)

(b) $\frac{1}{4}(2J_{\gamma\beta} + J_{\gamma\alpha} + J_{\beta\alpha}) = \frac{1}{4}(2 \times 8.6 + 1.2 + 5.8)$

$= 6.1$ c/sec. (xvi)

(c) $\frac{1}{4}(2J_{\gamma\beta} - J_{\gamma\alpha} - J_{\beta\alpha}) = \frac{1}{4}(2 \times 8.6 - 1.2 - 5.8)$

$= 2.6$ c/sec. (xvii)

(d) $\frac{1}{2}(J_{\gamma\alpha} + J_{\beta\alpha}) = \frac{1}{2}(1.2 + 5.8)$

$= 3.5$ c/sec. (xviii)

2. Calculation of energy values of various lines (ref Table VII (76):

Line 1 -

$\frac{1}{2}\eta H_o(2 - \sigma_\gamma - \sigma_\beta) - \frac{1}{4}(2J_{\gamma\beta} + J_{\gamma\alpha} + J_{\beta\alpha}) - D_-$

i.e. 24.6 - 6.1 - 18.5 = <u>0 c/sec.</u>

Line 2 -

$\frac{1}{2}\eta H_o(2 - \sigma_\gamma - \sigma_\beta) - \frac{1}{4}(2J_{\gamma\beta} - J_{\gamma\alpha} - J_{\beta\alpha}) - D_+$

i.e. 24.6 - 2.6 - 16.8 = <u>5.2 c/sec.</u>

Line 3 -

$\frac{1}{2}\eta H_o(2 - \sigma_\gamma - \sigma_\beta) + \frac{1}{4}(2J_{\gamma\beta} - J_{\gamma\alpha} - J_{\beta\alpha}) - D_-$

i.e. 24.6 + 2.6 - 18.5 = <u>8.7 c/sec.</u>

Line 4 -

$\frac{1}{2}\eta H_o(2 - \sigma_\gamma - \sigma_\beta) + \frac{1}{4}(2J_{\gamma\beta} + J_{\gamma\alpha} + J_{\beta\alpha}) - D_+$

i.e. 24.6 + 6.1 - 16.8 = <u>13.9 c/sec.</u>

Line 5 -

$\frac{1}{2}\eta H_o(2 - \sigma_\gamma - \sigma_\beta) - \frac{1}{4}(2J_{\gamma\beta} + J_{\gamma\alpha} + J_{\beta\alpha}) + D_-$

i.e. 24.6 - 6.1 + 18.5 = <u>37.0 c/sec.</u>

Line 6 -

$\frac{1}{2}\eta H_o(2 - \sigma_\gamma - \sigma_\beta) - \frac{1}{4}(2J_{\gamma\beta} - J_{\gamma\alpha} - J_{\beta\alpha}) + D_+$

i.e. 24.6 - 2.6 + 16.8 = <u>38.8 c/sec.</u>

Line 7 -

$\frac{1}{2}\eta H_o(2 - \sigma_\gamma - \sigma_\beta) + \frac{1}{4}(2J_{\gamma\beta} - J_{\gamma\alpha} - J_{\beta\alpha}) + D_-$

i.e. 24.6 + 2.6 + 18.5 = 45.7 c/sec.

Line 8 -

$\frac{1}{2}\eta H_o(2 - \sigma_\gamma - \sigma_\beta) + \frac{1}{2}(2J_{\gamma\beta} + J_{\gamma\alpha} + J_{\beta\alpha}) + D_+$

i.e. 24.6 + 6.1 + 16.8 = 47.5 c/sec.

Line 9 -

$\eta H_o(1 - \sigma_\alpha) - \frac{1}{2}(J_{\gamma\alpha} + J_{\beta\alpha})$

Substituting (xiv):

i.e. 61.9 - 3.5 = 58.4 c/sec.

Line 10 -

$\eta H_o(1 - \sigma_\alpha) + D_+ - D_-$

i.e. 61.9 + 16.8 - 18.5 = 60.2 c/sec.

Line 11 -

$\eta H_o(1 - \sigma_\alpha) - D_+ + D_-$

i.e. 61.9 - 16.8 + 18.5 = 63.6 c/sec.

Line 12 -

$\eta H_o(1 - \sigma_\alpha) + \frac{1}{2}(J_{\gamma\alpha} + J_{\beta\alpha})$

i.e. 61.9 + 3.5 = 65.4 c/sec.

3. Calculation of relative intensity values (ref. Table VII (76):

Line 1 -

$(\cos \phi_- - \sin \phi_-)^2$

i.e. $(\cos 6^\circ 43' - \sin 6^\circ 43')^2$ = 0.77

Line 2 -

$(\cos \phi_+ - \sin \phi_+)^2$

i.e. $(\cos 7^{\circ}25' - \sin 7^{\circ}25')^2 = \underline{0.74}$

Line 3 -

$(\cos \phi_- + \sin \phi_-)^2$

i.e. $(\cos 6^{\circ}43' + \sin 6^{\circ}43')^2 = \underline{1.23}$

Line 4 -

$(\cos \phi_+ + \sin \phi_+)^2$

i.e. $(\cos 7^{\circ}21' + \sin 7^{\circ}21')^2 = \underline{1.26}$

Line 5 -

(Same as line 3) 1.23

Line 6 -

(Same as line 4) 1.26

Line 7 -

(Same as line 1) 0.77

Line 8 -

(Same as line 2) 0.74

Line 9 -

Relative intensity = 1

Line 10 -

$\cos^2(\phi_+ - \phi_-)$

i.e. $\cos^2(7^{\circ}25' - 6^{\circ}43') = \underline{1.0}$

Line 11 -

(Same as line 10) 1.0

Line 12 -

Relative intensity = 1

Assignment 1, 2, 3, 4, 6, 5, 8, 7, 10, 9, 12, 11.

Note: The numbers in this second assignment correspond to the non-combination lines of Table VII (76).

From the average values of the splittings we have, from the β-pairs (i.e. 2 - 1, and, 4 - 3):

$$\tfrac{1}{2}(J_{\gamma\alpha} + J_{\beta\alpha}) + (D_- - D_+) = 5.2 \qquad 2(i)$$

and from the γ-pairs (i.e. 6 - 5, and, 8 - 7):

$$\tfrac{1}{2}(J_{\gamma\alpha} + J_{\beta\alpha}) - (D_- - D_+) = -1.8 \qquad 2(ii)$$

Adding 2(i) and 2(ii):

$$J_{\gamma\alpha} + J_{\beta\alpha} = 3.4 \text{ c/sec.} \qquad 2(iii)$$

From Table VII (76) it is apparent that the coupling constant, $J_{\beta\gamma}$, is equal to the separation between lines 1 and 3 (8.8 c/sec.), or, between lines 5 and 7 (8.7 c/sec.); the mean value of $J_{\beta\gamma}$ being 8.75 c/sec.
The coupling constant $(H_\beta \cdot H_\gamma)$ is 8.7 c/sec. (see Table No. 4)

The value of D_+ is equal to half the separation between lines 2 and 6 (16.05 c/sec.), or, between lines 4 and 8 (15.8 c/sec.); the mean value of D_+ being 15.9 c/sec.

The value of D_- is equal to half the separation between lines 1 and 5 (19.4 c/sec.), or, between lines 3 and 7 (19.4 c/sec.); the mean value of D_- being 19.4 c/sec.

As in the calculation of the first assignment:

$$\eta H_o(\sigma_\beta - \sigma_\gamma) = (D_+ \cos 2\phi_+) + (D_- \cos 2\phi_-) \qquad 2(viii)$$

also, $D_+ \sin 2\phi_+ = \frac{1}{2} J_{\gamma\beta}$

$$\sin 2\phi_+ = \frac{8.7}{2 \times 15.9} = 0.27358$$

Hence, $2\phi_+ = 15^\circ 53'$ and $\phi_+ = 7^\circ 56'$

also, $\cos 2\phi_+ = 0.96182$.

Similarly, $\sin 2\phi_- = \frac{J_{\gamma\beta}}{2D_-} = \frac{8.7}{2 \times 19.4} = 0.22423$

Hence, $2\phi_- = 12^\circ 57'$ and $\phi_- = 6^\circ 29'$

also, $\cos 2\phi_- = 0.97457$.

Taking 2(viii):

$$\eta H_o(\sigma_\beta - \sigma_\gamma) \doteq (15.9 \times 0.96182 + 19.4 \times 0.97457)$$
$$= 34.2 \text{ c/sec.}$$

Therefore, chemical shift $(H_\gamma - H_\beta) = 34.2$ c/sec. 2(ix)

As in the calculation of the first assignment:

$$J_{\gamma\alpha} - J_{\beta\alpha} = 2(D_+ \cos 2\phi_+ - D_- \cos 2\phi_-)$$
$$= 2(15.3 - 18.9)$$
$$= -7.2 \text{ c/sec.} \qquad 2(x)$$

The addition of lines 2, 3, 6, and 7 (Table VII (76)) gives the term:

$$2\eta H_o(2 - \sigma_\gamma - \sigma_\beta) = 98.6$$
$$\eta H_o(2 - \sigma_\gamma - \sigma_\beta) = 49.3 \text{ c/sec.} \qquad 2(xi)$$

Adding 2(ix) and 2(xi):

$$\eta H_o(2 - 2\sigma_\gamma) = 83.5$$

$$\eta H_o(1 - \sigma_\gamma) = 41.7 \qquad 2(xii)$$

Subtracting 2(ix) from 2(xi):

$$\eta H_o(2 - 2\sigma_\beta) = 15.1$$

$$\eta H_o(1 - \sigma_\beta) = 7.5 \qquad 2(xiii)$$

The value of $\eta H_o(1 - \sigma_\alpha)$ is equal to half the sum of lines 9 and 12 (61.85 c/sec.), or, of lines 10 and 11 (61.85 c/sec.); the value taken as 61.9 c/sec.

i.e. $\eta H_o(1 - \sigma_\alpha) = 61.9$ c/sec $\qquad 2(xiv)$

Subtracting 2(xii) from 2(xiv):

$$\eta H_o(\sigma_\gamma - \sigma_\alpha) = 20.2 \text{ c/sec.}$$

Therefore, chemical shift $(H_\gamma - H_\alpha) = 20.2$ c/sec.

Subtracting 2(xiii) from 2(xiv):

$$\eta H_o(\sigma_\beta - \sigma_\alpha) = 54.4 \text{ c/sec.}$$

i.e. Chemical shift $(H_\beta - H_\alpha) = 54.4$ c/sec.

Adding 2(iii) and 2(x):

$$2J_{\gamma\alpha} = -3.8$$

$$J_{\gamma\alpha} = -1.9$$

i.e. Coupling constant $(H_\alpha \cdot H_\gamma) = -1.9$ c/sec.

Subtracting 2(x) from 2(iii):

$$2J_{\beta\alpha} = 10.6 \quad \text{and,} \quad J_{\beta\alpha} = 5.3$$

i.e. Coupling constant $(H_\alpha \cdot H_\beta) = 5.3$ c/sec.

Calculation of Energy and Relative Intensity Values for NMR spectrum of lycodine

1. Calculation of terms common to various lines (ref. Table VII (76)):

(a) From 2(xi) $\frac{1}{2}\eta H_o(2 - \sigma_\gamma - \sigma_\beta) = 24.6$ c/sec. 2(xv)

(b) $\frac{1}{4}(2J_{\gamma\beta} + J_{\gamma\alpha} + J_{\beta\alpha}) = \frac{1}{4}(2 \times 8.7 - 1.9 + 5.3)$

$= 5.2$ c/sec 2(xvi)

(c) $\frac{1}{4}(2J_{\gamma\beta} - J_{\gamma\alpha} - J_{\beta\alpha}) = \frac{1}{4}(2 \times 8.7 + 1.9 - 5.3)$

$= 3.5$ c/sec. 2(xvii)

(d) $\frac{1}{2}(J_{\gamma\alpha} + J_{\beta\alpha}) = \frac{1}{2}(-1.9 + 5.3)$

$= 1.7$ c/sec. 2(xviii)

2. Calculation of energy values of various lines (ref. Table VII (76)):

Line 1, $24.6 - 5.2 - 19.4 =$ 0 c/sec.

Line 2, $24.6 - 3.5 - 15.9 =$ 5.2 c/sec.

Line 3, $24.6 + 3.5 - 19.4 =$ 8.7 c/sec.

Line 4, $24.6 + 5.2 - 15.9 =$ 11.9 c/sec.

Line 5, $24.6 - 5.2 + 19.4 =$ 38.8 c/sec.

Line 6, $24.6 - 3.5 + 15.9 =$ 37.0 c/sec.

Line 7, $24.6 + 3.5 + 19.4 =$ 47.5 c/sec.

Line 8, $24.6 + 5.2 + 15.9 =$ 45.7 c/sec.

Line 9, $61.9 - 1.7 =$ 60.2 c/sec.

Line 10, $61.9 + 15.9 - 19.4 = \underline{58.4 \text{ c/sec.}}$

Line 11, $61.9 - 15.9 + 19.4 = \underline{65.4 \text{ c/sec.}}$

Line 12, $61.9 + 1.7 = \underline{63.6 \text{ c/sec.}}$

3. Calculation of relative intensity values (ref. Table VII (76)):

Line 1, $(\cos 6^{\circ}29' - \sin 6^{\circ}29')^2 = \underline{0.78}$

Line 2, $(\cos 7^{\circ}56' - \sin 7^{\circ}56')^2 = \underline{0.73}$

Line 3, $(\cos 6^{\circ}29' + \sin 6^{\circ}29')^2 = \underline{1.22}$

Line 4, $(\cos 7^{\circ}56' + \sin 7^{\circ}56')^2 = \underline{1.27}$

Line 5, (same as Line 3) = 1.22

Line 6, (same as Line 4) = 1.27

Line 7, (same as Line 1) = 0.78

Line 8, (same as Line 2) = 0.73

Line 9, Relative intensity = 1

Line 10, $\cos^2(7^{\circ}56' - 6^{\circ}29') = \underline{1.0}$

Line 11, (same as Line 10) = 1.0

Line 12, Relative intensity = 1

10. CLAIMS TO ORIGINAL RESEARCH

(a) The application of paper chromatography of the alkaloids of Lycopodium annotinum L. on buffered papers to effect the separation of and identification of the alkaloidal components.

(b) The determination and recording of Rf values of some of the known alkaloids of Lycopodium annotinum L. by chromatography of these individual alkaloids on papers buffered at various pH values.

(c) Research was conducted into the possible application of ion-exchange column chromatography, utilizing an equilibrium-type band, for the quantitative separation of the alkaloids of Lycopodium annotinum L.

(d) The extent of separation of the alkaloids of Lycopodium annotinum L. which was accomplished by means of partition chromatography using buffered Celite was studied. This technique was later utilized in the separation of products obtained in reactions with lycodine.

(e) A more efficient, reliable and reproducible scheme was developed for concentrating, quantitatively, selected alkaloids of Lycopodium annotinum L. in countercurrent distribution steps utilizing partition data of the alkaloids in the system chloroform-buffer (as illustrated in the isolation

of the new alkaloid, lycodine).

(f) The isolation, characterization and partial structure determination of a new alkaloid "lycodine", $C_{17}H_{24}N_2$, m.p. 118° from Lycopodium annotinum L.

(g) The utilization of the nuclear magnetic resonance spectrum of lycodine in the determination of the 2,3-disubstitution of the pyridine ring contained in the lycodine molecule.

(h) A thorough review of the alkaloids of the Lycopodium species has been carried out.

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TABLE NO. 1.

Physical Properties Of

Alkaloids Contained in Lycopodium Species

Date	Alkaloid	Formula	m.p.	Remarks	References
1881	Lycopodine	$C_{16}H_{25}ON$	116°	✱ Table 1(a)	
1892	Pillijanine	$C_{15}H_{20}ON_2$	65°		(33)(34)
1938	Clavatine	$C_{16}H_{25}O_2N$	213°	$[\alpha]_D^{20}$ -365.7° (acetone)	(24)
	-methiodide		318°		
	Clavatoxine	$C_{17}H_{27}O_2N$	186°		(24)
1942	Saururine	$C_{10}H_{19}N$		viscous syrup	(34)
	-picrate		202°	from acetone	
	-methiodide		244°	from water	

Sauroxine	$C_{17}H_{26}ON_2$	198°	$[\alpha]_D^{20}$ -71.8°	(31)(34)
or,	$C_{16}H_{24}ON_2$		(alcohol)	
-methiodide		258°	from aq.acetone	
Complanatine (L-1)	$C_{16}H_{27}ON$	169°	from aq.acetone	(7)(29)
(dihydrolycopodine)				
-perchlorate		194°	from EtOAc	
O-acetyldihydro-lycopodine (L-2)	$C_{18}H_{29}O_2N$	95°	※ Table 1(b)	
Alkaloid L-3	$C_{18}H_{31}O_2N$			(7)
-perchlorate		246°	from hot water	
Alkaloid L-4	$C_{16}H_{27}N$			(7)
-perchlorate		225°	from hot water	
Alkaloid L-5	$C_{18}H_{28}O_2N_2$			(7)
-perchlorate		282°	from hot water	
α-Obscurine (L-6)	$C_{17}H_{26}ON_2$	284°	※ Table 1(c)	

	β-Obscurine (L-6)	$C_{17}H_{24}ON_2$	323°	✻ Table 1(d)	
	Nicotine	$C_{10}H_{14}N_2$		✻ Table 1(e)	
1943	Annotinine (L-7)	$C_{16}H_{21}O_3N$	232°	✻ Table 1(f)	
	Alkaloid L-8 (L-30)	$C_{16}H_{25}O_2N$	180°	✻ Table 1(g)	
	Alkaloid L-9			prob. a mixture (see paper)	(16)
	Alkaloid L-10	$C_{16}H_{27}ON$			(16)
	-perchlorate		223°	from acetone - EtOAc.	
	Annotine (L-11)	$C_{16}H_{21}O_3N$	174°	✻ Table 1(h)	
	O-acetylacrifoline (L-12)	$C_{18}H_{25}O_3N$	244°	✻ Table 1(i)	
1944	Alkaloid L-13	$C_{16}H_{25}ON$	130°	may be isolycopodine.	(25)(28) (28)
	- perchlorate		274°	from hot acetone	

	Anhydrodihydro-lycopodine (L-14)	$C_{16}H_{25}N$		* Table 1(j)	
	Alkaloid L-15 -perchlorate	$C_{20}H_{31}O_4N$	231°	from MeOH-EtOAc	(28)
	Alkaloid L-16 -perchlorate	$C_{16}H_{25}ON$	221°	from acetone-EtOAc	(31)
	Alkaloid L-17 -perchlorate	$C_{18}H_{27}O_3N$	285°	from MeOH-EtOAc	(31)
	Alkaloid L-18 -picrate	$C_{11}H_{19}ON$	195°	from MeOH	(25)
	Alkaloid L-19	uncharac-terized.	231°	from hot MeOH Analysis: Found C,60.22; H,4.25%	(25)
1946	Alkaloid L-20 -perchlorate	$C_{17}H_{27}O_2N$	259°	from hot MeOH	(9)
			271°	from MeOH	

Alkaloid L-21	$C_{13}H_{21}ON$			(9)
-perchlorate		201°	from MeOH-ether	
-picrate		107°	from MeOH	
Alkaloid L-22	$C_{16}H_{27}ON$	108°	from ether (cold)	(9)
-perchlorate		254°	from hot MeOH	
Alkaloid L-23	$C_{16}H_{25}O_2N$	162°	from pet. ether	(9)
-perchlorate		300°	from MeOH-EtOAc. Could be pseudo-selagine.	
Alkaloid L-24	$C_{16}H_{25}ON$		(Not lycopodine)	(9)
-perchlorate		278°	from MeOH	
Alkaloid I-25	$C_{16}H_{25}O_2N$		(Not L-23)	(9)
perchlorate		297°	from hot MeOH	
Alkaloid L-26	$C_{15}H_{25}ON$	171°	from dry ether	(32)
Acrifoline (L-27)	$C_{16}H_{23}O_2N$	97°	✱ Table 1(k)	

Alkaloid L-28	$C_{17}H_{27}O_2N$			(18)(22)
-perchlorate		211°	from acetone-EtOAc	
-methiodide		304°	$[\alpha]_D^{20}$ -66.0°	
-methochloride		258°		
-methoperchlorate		321°		
-methopicrate		oily		
Alkaloid L-29	$C_{16}H_{23}O_2N$			(18)(22)
-perchlorate		274°	from acetone-EtOAc.	
-methiodide		294°	$[\alpha]_D^{20}$ -13.9°	
-methochloride		263°		
-methoperchlorate		287.5°		
-methopicrate		163°		
Alkaloid L-30			(See L-8)	(30)
Alkaloid L-31	$C_{20}H_{29}O_4N$			(18)(22)
-perchlorate(hydrated)		132°	from hot water	

	Alkaloid L-31 (cont'd.)				
	-perchlorate			after drying	
	(anhydrous)		217°	hydrated.	
	-methiodide		292°	$[\alpha]_D^{20}$ -11.1°	
	-methochloride		261°		
	-methoperchlorate		295°		
	-methopicrate		oily		
1948	Cernuine (L-32)	$C_{16}H_{26}ON_2$	106°	from hot hexane	(23)
	-perchlorate				
	$\cdot 1.5\ H_2O$		110°	from warm water	
	Alkaloid L-33	uncharac-			
		terized	218°	from hot ether	(23)
1952					
	Annotoxine	$C_{32}H_{44}O_5N_2$	197°	✱ Table 1(1)	
	Base	$C_{16}H_{25(23)}ON$			(19)(22)
	-picrate		241°(d)	from alcohol	

	-methiodide		261°(d)	from alcohol	(19)
			265°	$[\alpha]_D^{20}$ -150.1°	(22)
	-perchlorate		234°(d)	from water	
	-methochloride		210°		
	-methoperchlorate		270°		
	-methopicrate		230-232°		
	Base	$C_{10}H_{19(21)}ON$			(19)
	-picrate		121°	from small volume of alcohol	
	-methiodide		290°		
1953	Alkaloid L-34	$C_{16}H_{25}O_2N$	236°	from acetone	(27)
	Alkaloid L-35	$C_{14}H_{21}ON$	133°	from ether	(27)
1955	Isolycopodine	$C_{16}H_{25}ON$	136°	✱ Table 1(m)	
1956	Pseudoselagine	$C_{16}H_{25}O_2N$	163°	✱ Table 1(n)	

1958	Lycodine	$C_{17}H_{24}N_2$	118°	* Table 1(o)	
	Base	$C_{16}H_{21}O_3N$			(22)
	-methiodide		216-217°		
	-methochloride		255°		
	-methoperchlorate		234-236°		
	-methopicrate		134-136°		
	Base	$C_{17}H_{25}O_2N$			(22)
	-methiodide		324°	$[\alpha]_D^{20}$ -49.8°	
	-methochloride		274°		
	-methoperchlorate		316°		
	-methopicrate		78-80°		
	Base	$C_{17}H_{25}O_3N$			(22)
	-methiodide		315°	$[\alpha]_D^{20}$ -30.9°	
	-methochloride		269°		
	-methoperchlorate		335°		
	-methopicrate		151°		

Base	$C_{18}H_{25}O_3N$			(22)
-methiodide		272°	$[\alpha]_D^{20}$	-95.8°
-methochloride		250°		
-methoperchlorate		267-268°		
-methopicrate		oily		

Base	$C_{18}H_{25}O_4N$			(22)
-methiodide		283°	$[\alpha]_D^{20}$	-168.8°
-methochloride		270°		
-methoperchlorate		259-260°		
-methopicrate		219°		

Note: ✱ - indicates that reference should be made to table shown for more complete data.

Table No. 1(a)

NAME	FORMULA
LYCOPODINE	$C_{16}H_{25}ON$

CRYSTALS

m.p. 116° (1. dry ether 2. hexane)

DERIVATIVES

perchlorate	prisms	283°(d)	from hot water
methiodide	plates	296°(d)	from EtOH
nitrate	pyramids	270°(d)	
picrate	rosettes	207°	from little EtOH
hydrobromide		360°	

pKa

7.44 (50% aqueous MeOH)

STRUCTURE

See Fig. 8.

ROTATION

$[\alpha]_D^{26} = -24.5°$ (c, 1.1 EtOH)

PAPER CHROMATOGRAPHY n-butanol — water

Buffer pH	3	4	5	6	7
Rf	0.44	0.45	0.46	0.50	0.71

INFRARED (cm.$^{-1}$)

1693	carbonyl

SOLUBILITIES

ULTRAVIOLET

280mμ log ε, 1.8

REFERENCES

(7) (16) (19) (20) (24) (26) (30) (36) (37) (38) (39)

Table No. 1(b)

NAME	FORMULA
O-ACETYLDIHYDROLYCOPODINE (L-2)	$C_{18}H_{29}O_2N$

CRYSTALS flat prisms m.p. 95° from pentane

DERIVATIVES

perchlorate 247° (when immersed at 205°)

pK_a	STRUCTURE
8.4 (50% aqueous MeOH)	See lycopodine Fig. 8.
ROTATION	

PAPER CHROMATOGRAPHY n-butanol — water

Buffer pH	3	4	5	6	7
Rf					

INFRARED ($cm.^{-1}$)

1730	carbonyl
1241 1228	probably acetoxy

SOLUBILITIES

ULTRAVIOLET

REFERENCES

(7) (30)

Table No. 1(c)

NAME
α-OBSCURINE

FORMULA
$C_{17}H_{26}ON_2$

CRYSTALS
colourless prisms m.p. 284° from MeOH

DERIVATIVES

monopicrate	needles	135°	from MeOH-ether
perchlorate	prisms	255°(d)	
hydriodide		307°	

pKa

ROTATION

STRUCTURE
See Fig. 10.

PAPER CHROMATOGRAPHY n-butanol — water

Buffer pH	3	4	5	6	7
Rf	0.23			0.44	

INFRARED cm.$^{-1}$

3411	secondary amino
1667	carbonyl

ULTRAVIOLET
255mμ, log ε, 3.75 (abs. EtOH)

SOLUBILITIES at 17°

$CHCl_3$	1:22
MeOH	1:148
Benzene	1:263
Acetone	1:1000
Ligroin	1:2500

REFERENCES
(7) (17) (20) (38)

Table No. 1(d)

NAME	FORMULA
β-OBSCURINE	$C_{17}H_{24}ON_2$

CRYSTALS

colourless prismatic rods m.p. 323° from MeOH

DERIVATIVES

monopicrate	plates	254°(d)	from MeOH
perchlorate	needles	322°(d)	

pKa

ROTATION

STRUCTURE

See Fig. 10

PAPER CHROMATOGRAPHY n-butanol — water

Buffer pH	3	4	5	6	7
Rf	0.29	0.33	0.39	0.61	0.86

INFRARED ($cm.^{-1}$)

3385	secondary amino
1659), 1651), 1620), 1613)	α-pyridone

ULTRAVIOLET

232mμ, log ε, 3.98 abs. EtOH

315mμ, log ε, 3.89 abs. EtOH

SOLUBILITIES

REFERENCES

(7) (17) (38)

Table No. 1(e)

NAME	FORMULA
NICOTINE	$C_{10}H_{14}N_2$

CRYSTALS

colourless oily liquid b.p. 247°(d)

DERIVATIVES

dipicrate	yellow prisms	266°	from hot MeOH
dipicrolonate	needles	228°	from aq. acetone
rac. dimethiodide		220-222°	

pK_a 1) 7.7
2) 3.35 (50% aq. MeOH)

STRUCTURE

See Fig. 16.

ROTATION

$[\alpha]_D^{20} = -169°$

PAPER CHROMATOGRAPHY *n-butanol — water*

Buffer pH	3	4	5	6	7
Rf					

INFRARED

ULTRAVIOLET

SOLUBILITIES

Very soluble in EtOH, $CHCl_3$, ether and pet. ether.

REFERENCES

(7) (22) (40)

Table No. 1(f)

NAME	FORMULA
ANNOTININE (L-7)	$C_{16}H_{21}O_3N$

CRYSTALS

brilliant colourless prisms m.p. 232° (MeOH-$CHCl_3$)

DERIVATIVES

perchlorate		267°	from hot water
nitrate	needles	223 (d)	from water
methiodide		185	
chloroplatinate		238	
hydriodide		242	

pKa

6.5 (50% aqueous MeOH)

ROTATION

$[\alpha]_D^{21} = -27.5°$ (c, 1% $CHCl_3$)

STRUCTURE

See Fig. 1.

PAPER CHROMATOGRAPHY n-butanol — water

Buffer pH	3	4	5	6	7
Rf	0.20	0.24	0.40	0.68	0.87

INFRARED (cm.$^{-1}$)

1776	γ-lactone

ULTRAVIOLET

325mμ, log ε, 1.25

SOLUBILITIES at 19°

$CHCl_3$	1:2
Benzene	1:6
Acetone	1:17
MeOH	1:34
Ligroin	1:1867

REFERENCES

(16) (19) (20) (37) (38)

Table No. 1(g)

NAME	FORMULA
Alkaloid L-8 (same as L-30)	$C_{16}H_{25}O_2N$

CRYSTALS

brilliant pearly plates m.p. 180° from MeOH-ether

DERIVATIVES

perchlorate	318°(d)	from MeOH-EtOAc
hydriodide	305	
methiodide	317	
methochloride	313	

pKa	STRUCTURE
	from IR only,
ROTATION $[\alpha]_D^{16}$ = -121.3° (c, 1% acetone)	-OH and C=O

PAPER CHROMATOGRAPHY *n-butanol — water*

Buffer pH	3	4	5	6	7
Rf					

INFRARED ($cm.^{-1}$)

3220	(strong)	-OH
1710	(strong)	C=O

SOLUBILITIES at 17°

acetone	1:51
ether	1:143
ligroin (60-80°)	1:250

ULTRAVIOLET

REFERENCES (16) (18) (20) (30)

Table No. 1(h)

NAME
ANNOTINE (Alkaloid L-11)

FORMULA
$C_{16}H_{21}O_3N$

CRYSTALS
brilliant colourless rhoms. m.p. 174° from methanol.

DERIVATIVES

perchlorate		239°	from hot water
hydriodide		297	
methiodide	prisms	317	from water
methochloride		245	
nitrate	rod or needle	173	

pKa
7.50 (50% aqueous MeOH)

ROTATION
$[\alpha]_D^{19} = -114.5°$ (c, 1% $CHCl_3$)

STRUCTURE
See Fig. 11

PAPER CHROMATOGRAPHY *n-butanol — water*

Buffer pH	3	4	5	6	7
Rf	0.13	0.13	0.13	0.20	0.56

INFRARED

	($cm.^{-1}$)	
Nujol	3300 (w)	-OH
	1712)	
	1725) (s)	C=O
CS_2	3550 (s)	-OH
$CHCl_3$	1720-1730	C=O
	1644	unsaturation

SOLUBILITIES at 19°

$CHCl_3$	1:3
benzene	1:7
MeOH	1:10
acetone	1:16
ligroin	1:1338

ULTRAVIOLET
290mμ, weak
325mμ, weak

REFERENCES
(16) (19) (20)

Table No. 1(i)

NAME	FORMULA
O-ACETYLACRIFOLINE (L-12)	$C_{18}H_{25}O_3N$

CRYSTALS.
colourless stout prisms m.p. 120° from hexane

DERIVATIVES
perchlorate m.p. 244° from acetone-EtOAc.

pKa

STRUCTURE
See Acrifoline Fig. 9.

ROTATION

PAPER CHROMATOGRAPHY n-butanol — water

Buffer pH	3	4	5	6	7
Rf	0.28	0.33	0.38	0.60	0.86

INFRARED (cm.$^{-1}$)
No hydroxyl absorption

1740)
1230) attributable to acetoxy

1715 C=O group

SOLUBILITIES

ULTRAVIOLET

REFERENCES
(16) (41)

Table No. 1(j)

NAME	FORMULA
ANHYDRODIHYDROLYCOPODINE (L-14)	$C_{16}H_{25}N$
CRYSTALS	
DERIVATIVES perchlorate m.p. 238° from MeOH-EtOAc	
pK_a	STRUCTURE See lycopodine
ROTATION perchlorate, $[\alpha]_D^{26} = -107°$ (c, 1.1% MeOH)	Fig. 8.
PAPER CHROMATOGRAPHY n-butanol — water Buffer pH 3 4 5 6 7 Rf	
INFRARED	SOLUBILITIES
ULTRAVIOLET	
REFERENCES (28) (30)	

Table No. 1(k)

NAME
ACRIFOLINE (L-27)

FORMULA
$C_{16}H_{23}O_2N$

CRYSTALS
fine colourless needles m.p. 97° from ether (other (m.p. (99-104°

DERIVATIVES

perchlorate	stout prisms	266° from acetone-EtOAc
nitrate	small prisms	236 (d)
hydriodide	clusters	259
hydrochloride		275
methiodide		267-268°

pKa
8.34 (50% aqueous MeOH)

ROTATION
$[\alpha]_D^{19}$ = -266.2° (c, 1% $CHCl_3$)
$[\alpha]_D$ = -250° (c, 1% EtOAc)

STRUCTURE
See Fig. 9.

PAPER CHROMATOGRAPHY n-butanol — water

Buffer pH	3	4	5	6	7
Rf	0.23	0.23	0.23	0.30	0.60

INFRARED ($cm.^{-1}$)

Nujol	3310	-OH
	1700 (w)	C=O
	1670	unsaturation
$CHCl_3$	1700	C=O
	1675	unsaturation

SOLUBILITIES at 19°
$CHCl_3$ very sol.

MeOH	1:3
acetone	1:5
benzene	1:7
ether	1:54
ligroin	1:116

ULTRAVIOLET
See reference (19)

REFERENCES
(18) (19) (20) (22) (41)

Table No. 1(1)

NAME	FORMULA
ANNOTOXINE	$C_{32}H_{44}O_5N_2$

CRYSTALS

rhombs. m.p. 197° from acetone

DERIVATIVES

dinitrate	217°
diperchlorate	228
methiodide	261

pKa

ROTATION

STRUCTURE

Molecular complex of acrifoline and annotine.

PAPER CHROMATOGRAPHY n-butanol — water

Buffer pH	3	4	5	6	7
Rf	(0.13 (0.23	0.13 0.23	0.13 0.23	0.20 0.30	0.56 0.60

INFRARED

ULTRAVIOLET

SOLUBILITIES

REFERENCES

(19) (20)

Table No. 1(m)

NAME	FORMULA
ISOLYCOPODINE (may be L-13)	$C_{16}H_{25}ON$

CRYSTALS

m.p. 136°

DERIVATIVES

hydriodide	284°
hydrochloride	273
methiodide	321
perchlorate	274
picrate	201
methochloride	270

pKa

STRUCTURE

ROTATION

$[\alpha]_D^{14} = -158°$ (c, 1% in acetone

PAPER CHROMATOGRAPHY n-butanol — water

Buffer pH	3	4	5	6	7
Rf					

INFRARED

SOLUBILITIES at 16°

acetone	1:8
ether	1:9
ligroin (60-80°)	1:33

ULTRAVIOLET

REFERENCES

(20) (22)

Table No. 1(n)

NAME	FORMULA
PSEUDOSELAGINE (may be L-23)	$C_{16}H_{25}O_2N$

CRYSTALS
rectangular platelets m.p. 163° from acetone

DERIVATIVES

hydrochloride	307°
hydriodide	314
perchlorate	295
methiodide	279
methochloride	268

pKa

STRUCTURE

ROTATION
$[\alpha]_D^{19} = -42.7°$ (c, 1% in acetone)

PAPER CHROMATOGRAPHY n-butanol — water

Buffer pH	3	4	5	6	7
Rf					

INFRARED

SOLUBILITIES at 15°

benzene	very sol.
$CHCl_3$	very sol.
MeOH	1:15
acetone	1:34
ether	1:41
ligroin	1:190

ULTRAVIOLET

REFERENCES
(35)

Table No. 1(o)

NAME	FORMULA
LYCODINE	$C_{17}H_{24}N_2$

CRYSTALS m.p. 118° from n-pentane
(distills at 100-110° (air-bath)(0.18 mm Hg))

DERIVATIVES

dipicrate	radiating clusters of prisms	229-233°(d) MeOH
N-acetyllycodine monopicrate		180-182.5°(d) from H_2O.

pKa 1) 8.08 (50% aqueous MeOH)
2) 3.97

STRUCTURE

See Fig. 22

ROTATION
$[\alpha]_D = -10 \pm 2°$ (c, 1.01% in EtOH)

PAPER CHROMATOGRAPHY n-butanol — water

Buffer pH	3	4	5	6	7
Rf	0.41	0.53	0.60	0.73	0.87

INFRARED (See Fig. 20) (cm.$^{-1}$)

3270	N-H
1580	pyridine
1382	C-Me

SOLUBILITIES

ULTRAVIOLET (See Fig. 19)
Free base λ_{max} 268.2mμ, log ε, 3.728
275.7mμ, log ε, 3.615
Salt λ_{max} 272 mμ, log ε, 3.926

NMR

See Fig. 21

REFERENCES

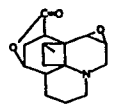
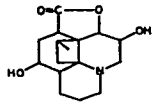
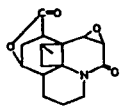
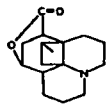
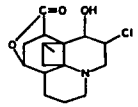
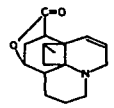
(21)

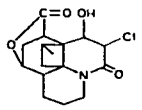
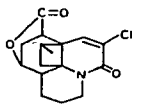
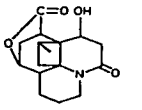
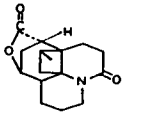
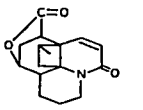
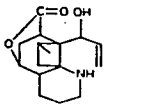
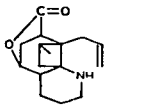
TABLE NO. 2

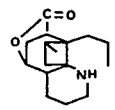
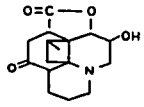
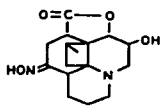
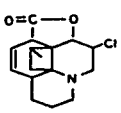
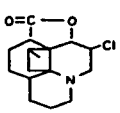
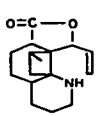
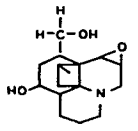
Physical Data

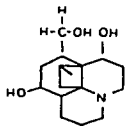
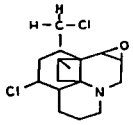
Of The Reaction Products

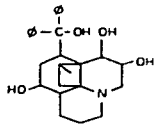
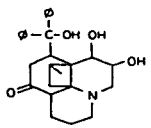
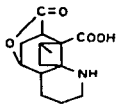
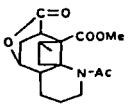
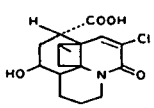
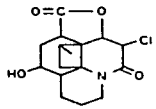
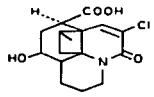
In The Chemistry of Annotinine

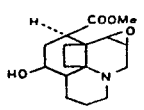
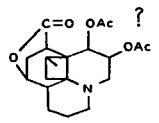
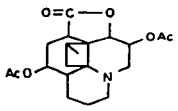
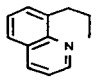
NO.	STRUCTURE	FORMULA	NAME	M.P. °C	INFRARED cm⁻¹	ULTRAVIOLET λ LOG ε	$[\alpha]_D$ TEMP.	pK_a	REF.
I		$C_{16}H_{21}O_3N$	ANNOTININE	232	1776 γ-LACTONE C=O	325mμ 1.25	−27.5 C, 1.0 $CHCl_3$		16 19 37 42 59
II		$C_{16}H_{23}O_4N$	HYDRATE	228	NUJOL: 1762 γ-LACTONE C=O; 3500 -OH; CH_2Cl_2: 3625 S. -OH; 3465 B. -OH				18 43
III		$C_{16}H_{21}O_4N$		290					18
IV		$C_{16}H_{19}O_4N$	LACTAM	234	1768 γ-LACTONE C=O; 1640 LACTAM C=O				18 43 55
V		$C_{16}H_{23}O_2N$	DIHYDRO LACTONE A; SATURATED LACTONE; DESOXODIHYDRO-	109	1768 γ-LACTONE C=O; -OH, >NH NONE		−35 C, 1.4 EtOH 24°	8.48	18 44 47 62
			PERCHLORATE	277					
VI		$C_{16}H_{22}O_3NCl$	CHLOROHYDRIN	190					18
			PERCHLORATE	278					
VII		$C_{16}H_{21}O_2N$	UNS. LACTONE A	132	1762 γ-LACTONE C=O		−70 C, 2.6 EtOH 24°	7.06	18 44 47

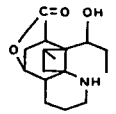
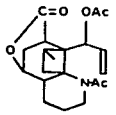
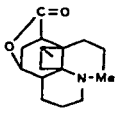
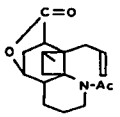
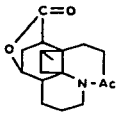
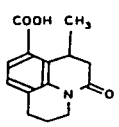
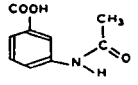
NO.	STRUCTURE	FORMULA	NAME	M.P. °C	INFRARED cm⁻¹	ULTRAVIOLET λ LOGε	$[\alpha]_D$ TEMP	pK_A	REF.
VIII & IX		$C_{16}H_{20}O_4NCl$	LACTAM CHLORO-HYDRIN	265	1760 γ-LACTONE C=O 1624 LACTAM C=O 3330 S. -OH				18 43 46
X		$C_{16}H_{18}O_3NCl$	ANHYDROCHLORO CPD.	187	1657 } CONJ. AMIDE 1618 }	227mμ 3.9 } CONJ. 275mμ 2.9 } LACTAM			46 49 55
XI		$C_{16}H_{21}O_4N$	HYDROXY ANNOTININE LACTAM	303					46
XII		$C_{16}H_{21}O_3N$		177					46
XIII		$C_{16}H_{19}O_3N$		175					46
XIV		$C_{16}H_{23}O_3N$	HYDROXYLACTONE	174	1767 γ-LACTONE C=O 3100 B. -OH		-30 C, 1.42 EtOH 22°	8.21	43 44 47
XV		$C_{16}H_{23}O_2N$	UNS. LACTONE "B"	135	1752 γ-LACTONE C=O			8.44	43 44 47

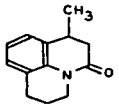
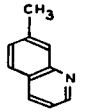
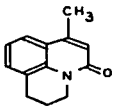
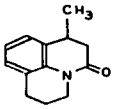
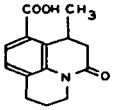
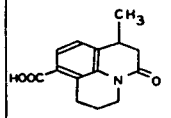
NO.	STRUCTURE	FORMULA	NAME	M.P. °C	INFRARED cm^{-1}	ULTRAVIOLET λ LOG ε	$[\alpha]_D$ TEMP.	pK_A	REF.
XVI		$C_{16}H_{25}O_2N$	DIHYDROLACTONE "B"	135 (WITH XV DEPRESSED 30°)	1755 γ-LACTONE C=O 3325 s. (>N-H)			8.44	43 44 47
XVII		$C_{16}H_{21}O_4N$	HYDROXYKETONE	160	1768 γ-LACTONE C=O 1712 KETONE (6-RING) 3390 -OH				43
XVIII		$C_{16}H_{22}O_4N_2$	HYDROXYKETONE OXIME	262					43
XIX	 ?	$C_{16}H_{20}O_2NCl$	UNS. CHLOROLACTONE	136	1758 γ-LACTONE C=O				43
XX	 ?	$C_{16}H_{22}O_2NCl$	DIHYDROCHLORO-LACTONE	147	1756 γ-LACTONE C=O				43
XXI	 ?	$C_{16}H_{23}O_2N$	DIHYDROLACTONE "C"	120	1772 γ-LACTONE C=O 3355 >N-H (?)				43
XXII	 ?	$C_{16}H_{25}O_3N$	-DIHYDROXY ETHER -HYDROCHLORIDE	220 214	3455 } 2-OH's 3375 } (NO CARBONYL)				48

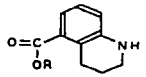
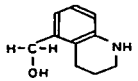
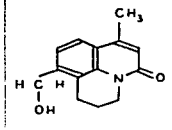
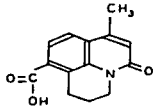
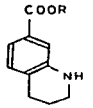
NO.	STRUCTURE	FORMULA	NAME	M.P. °C	INFRARED cm^{-1}	ULTRAVIOLET λ LOG ε	$[\alpha]_D$ TEMP.	pK_A	REF.
XXIII	 ?	$C_{16}H_{27}O_3N$	TRIHYDROXY CPD.	215 (AFTER SUBL.) 170 (AFTER DRYING AT 100°)	SINGLE BEAM MULL - 3384, 3298, 3173 (3 OH'S) DOUBLE BEAM 3305, 3230				48
XXIV	 ?	$C_{16}H_{23}ONCl_2$	DICHLORO ETHER HYDROCHLORIDE	292					48
XXV		$C_{16}H_{23}O_4NS$	CYCLIC SULFITE ESTER	147	1206 S-O				48
XXVI		UNCHARACTERIZED	SULFITE ESTER GROUPING BELIEVE Cl PRESENT						48
XXVII		$C_{16}H_{25}O_2N$	UNSAT'D DIHYDROXY CPD. "A"	169	3615 (FREE OH) 3379 (BONDED OH)				48
XXVIII		UNCHARACTERIZED	DIHYDRO-DIHYDROXY CPD. "A"						48
XXIX		$C_{16}H_{25}O_2N$	ISOMERIC UNSAT'D DIHYDROXY CPD. "B"	147	3360, 3315 DOUBLE PEAK 3180 3060				48

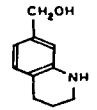
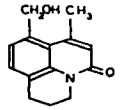
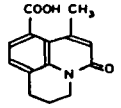
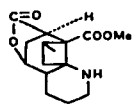
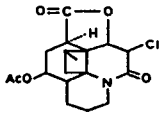
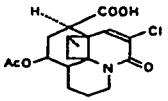
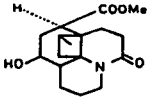
NO.	STRUCTURE	FORMULA	NAME	M.P. °C	INFRARED cm⁻¹	ULTRAVIOLET λ LOG ε	$[\alpha]_D$ TEMP.	pK_A	REF.
XXX		$C_{28}H_{35}O_4N$	DIPHENYL ANNOTININE	239	•HYDROXYL ABSORPTION BANDS •NO C=O ABSORPTION				48
XXXI		$C_{28}H_{33}O_4N$	BASIC OXIDATION PRODUCT OF XXX	251	•HYDROXYL ABSORPTION BANDS •1720 S. •C=O				48
XXXII		$C_{14}H_{19}O_4N$	AMINO ACID •SULPHATE (B·½H_2SO_4) •HYDROCHLORIDE (B·HCl) •METHYL ESTER	 233 (d) 220 122		210mμ 2.58 (END ABSORPTION)	−31 C, 1.7 H_2O 25°	4.03 9.3	44 47 49 55
XXXIII		UNCHARACTERIZED	N-ACETYL ESTER DERIV. OF XXXII −NEUTRAL & OILY	OIL					49
XXXIV		$C_{16}H_{20}O_4NCl$	HYDROXY ACID "A"	184	3280 •OH BAND 1728 CARBOXYLIC C=O 1645, 1617 CONJ. LACTAM C=O	230 mμ 4.02 CONJ. AMIDE 280 mμ 3.1			37 49 55
XXXV		$C_{16}H_{20}O_4NCl$	HYDROXY LACTONE (NEUTRAL)	220	3400 •OH BAND 1760 γ-LACTONE C=O 1660 LACTAM C=O	END ABSORPTION			37 49 55
XXXVI		$C_{16}H_{20}O_4NCl$	HYDROXY ACID "B"	187	3400 •OH BAND 1700 CARBOXYLIC C=O 1641, 1615 CONJ. LACTAM C=O	230 mμ 4.02 CONJ. AMIDE 280 mμ 3.1			37 49 55

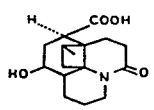
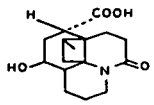
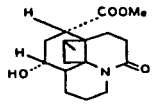
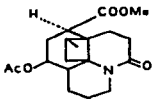
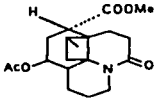
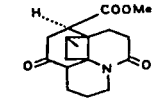
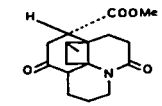
NO.	STRUCTURE	FORMULA	NAME	M.P. °C	INFRARED cm⁻¹	ULTRAVIOLET λ LOGε	$[\alpha]_D$ TEMP.	pK_A	REF.
XXXVII		$C_{21}H_{25}O_4N$	METHYL DIHYDROISOCINCHOTINATE	184	NUJOL 3510 OH BANDS 1725 ESTER C=O CS_2 3580 DIMINISHED INTENSITY 1738, 1733 } ESTER C=O				49 51
XXXVIII		$C_{18}H_{23}O_4N$	ISOMERIC ANNOTININE HYDRATE	75 PROB. MIXTURE SEE C	OH BANDS 1774 γ-LACTONE C=O				50
XXXIX		$C_{20}H_{27}O_6N$	DIACETYL DERIV. OF XXXVIII	168					50
XL		$C_{20}H_{27}O_6N$	DIACETYL ANNOTININE HYDRATE	187					50
XLI		$C_{12}H_{13}N$	8-n-PROPYL-QUINOLINE PICRATE CHLOROPLATINATE	 140 193					52
XLII		$C_{15}H_{13}N$	BASIC CPD. PICRATE	 255		SPECTRUM INDICATES α or β-NAPHTHOQUINOLINE			52
XLIII		$C_{15}H_{11-15}N$	NEUTRAL CPD. TRINITROBENZOATE	 175	NO OH OR NH BANDS	SPECTRUM SHOWN IN LITERATURE REFERENCE			52

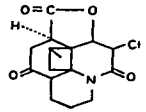
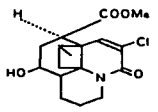
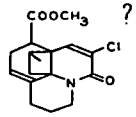
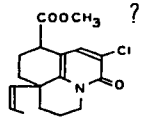
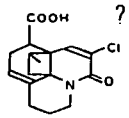
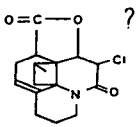
NO.	STRUCTURE	FORMULA	NAME	M.P. °C	INFRARED cm⁻¹	ULTRAVIOLET λ LOG ε	$[\alpha]_D$ TEMP.	pK_A	REF.
XLIV		$C_{18}H_{25}O_3N$	•DIHYDROHYDROXY-LACTONE	142			-65 C, 1.47 EtOH 22°	8.00	44 47
XLV		$C_{20}H_{27}O_5N$	O,N-DIACETYL DERIV.	133	NUJOL: 1740 -O-Ac 1678 >N-Ac				44 47
XLVI		$C_{18}H_{21}O_2N\cdot(CH_3)_2SO_4$	METHOSULFATE OF VII	235					44 47
XLVII		$C_{17}H_{27}O_2N$	TERTIARY BASE	91					44 47
XLVIII		$C_{18}H_{25}O_3N$	•N-ACETYL UN-SAT'D LACTONE B	138 (WITH XV DEPRESSED TO 125°)	1762 1780 γ-LACTONE				44 47
XLIX		$C_{18}H_{27}O_3N$	•N-ACETYL DIHYDROLACTONE B	142	1755 1780 γ-LACTONE				44 47
L		$C_{14}H_{15}O_3N$	•DEHYDROGENATED ACID •LACTAM CARBOXYLIC ACID •METHYL ESTER	250(d) 90	NUJOL: 1715 $-\overset{OH}{C}=O$ 1620 AMIDE 1575 BENZENE (NO γ-LACTONE)	SPECTRUM IN REFERENCE SIMILAR TO m-ACETYL-AMINOBENZOIC ACID 	+47 C, 0.9 EtOH 25°		44 47

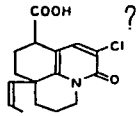
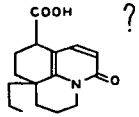
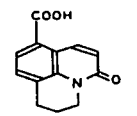
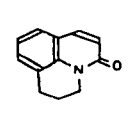
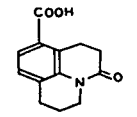
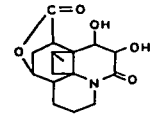
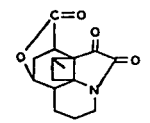
NO.	STRUCTURE	FORMULA	NAME	M.P. °C	INFRARED cm⁻¹	ULTRAVIOLET λ LOG ε	$[\alpha]_D$ TEMP.	pK_A	REF.
LI		$C_{13}H_{15}ON$	DECARBOXYLATED ACID	70	NUJOL: 1685 & 1660 -AMIDE 1660 -BENZENE $CHCl_3$ 1655 1600	SPECTRUM IN REF.			44 47
LII			WEAK BASE PICRATE	172(d)					44 47
LIII		$C_{10}H_9N$	7-METHYLQUINOLINE PICRATE	240					44 47
LIV		$C_{13}H_{13}ON$		130					53
LV		$C_{13}H_{15}ON$	SYNTHETIC RACEMATE OF LI	45	SAME IR AS LI				53
LVI		$C_{14}H_{15}O_3N$	SYNTHETIC RACEMATE OF L		IR OF METHYL ESTER SAME AS IR OF METHYL ESTER OF L	U.V. OF METHYL ESTER SAME AS U.V. OF METHYL ESTER OF L			53
LVII		$C_{14}H_{15}O_3N$	ISOMERIC WITH LVI			SLIGHTLY BUT DISTINCTLY DIFFERENT FROM LVI METHYL ESTER			53

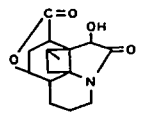
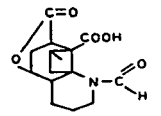
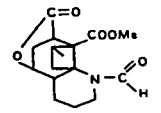
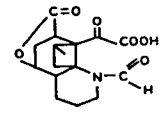
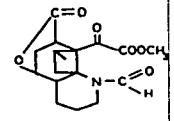
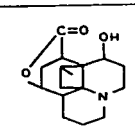
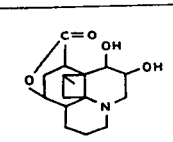
NO.	STRUCTURE	FORMULA	NAME	M.P. °C	INFRARED cm⁻¹	ULTRAVIOLET λ LOG ε	$[\alpha]_D$ TEMP.	pK_A	REF.
LVIII TO LXVIII	*(See below)*		STEPS IN THE SYNTHESIS OF LIV LVI LV-I						53
LVIII		$C_9H_{11}N$							53
LIX & LX	 LIX (R=H) LX ($R=CH_3$)								53
LXI									53
LXII									53
LXIII									53
LXIV & LXV	 LXIV (R=H) LXV ($R=CH_3$)								53

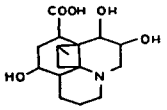
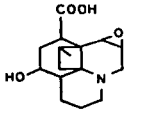
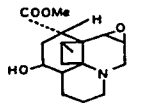
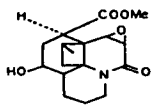
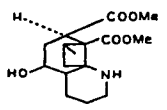
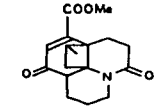
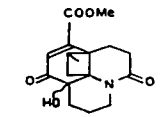
NO.	STRUCTURE	FORMULA	NAME	M.P. °C	INFRARED cm⁻¹	ULTRAVIOLET λ LOG ε	$[\alpha]_D$ TEMP.	pK_A	REF.
LXVI									53
LXVII									53
LXVIII									53
LXIX		$C_{15}H_{21}O_4N$	METHYL ESTER	122				6.8	55
LXX		$C_{18}H_{22}O_5NCl$	ACETOXY γ-LACTONE	229	1780 γ-LACTONE C=O 1740 ACETOXY C=O 1670 LACTAM C=O	END ABSORPTION ONLY			55
LXXI		$C_{18}H_{22}O_5NCl$	ACETOXY ACID	205	1740 ACETOXY C=O 1710 CARBOXYL C=O 1653, 1625 CONJ. LACTAM	230mμ 4.0 CONJ. LACTAM 280mμ 3.04			55
LXXII		$C_{17}H_{25}O_4N$	HYDROXY METHYL ESTER	210	3220 OH 1730 CARBO-METHOXYL C=O 1600 LACTAM C=O		-14.1 EtOH 24°		55

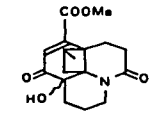
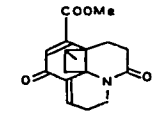
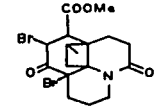
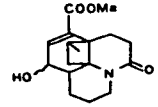
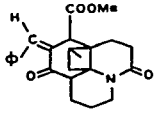
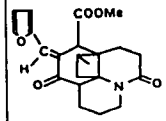
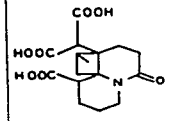
NO.	STRUCTURE	FORMULA	NAME	M.P. °C	INFRARED cm⁻¹	ULTRAVIOLET λ LOG ε	$[\alpha]_D$ TEMP.	pK_A	REF.
LXXIII		$C_{16}H_{23}O_4N$	HYDROXY ACID						55
LXXIV		UNCHARACTERIZED	HYDROXY ACID						55
LXXV		$C_{17}H_{25}O_4N$	HYDROXY METHYL ESTER	166	3430 OH 1725 CARBOMETHOXY C=O 1607 LACTAM C=O		-45.6 EtOH 24°		55
LXXVI		$C_{19}H_{27}O_5N$	ACETOXY METHYL ESTER	142	1750 ACETOXY C=O 1730 CARBOMETHOXY C=O 1620 LACTAM C=O		-17.1 EtOH 24°		55
LXXVII		$C_{19}H_{27}O_5N$	ACETOXY METHYL ESTER	138	1750 ACETOXY C=O 1730 CARBOMETHOXY C=O 1628 LACTAM C=O		-49.1 EtOH 24°		55
LXXVIII		$C_{17}H_{23}O_4N$	LACTAM KETO ESTER	200	1725 KETONE & CARBOMETHOXYL $-\overset{\vert}{C}=O$ 1640 LACTAM C=O				55
LXXIX		$C_{17}H_{23}O_4N$	KETO ESTER	170	1735 CARBOMETHOXYL C=O 1720 KETONE C=O 1640 LACTAM C=O				55

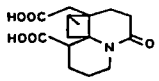
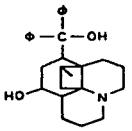
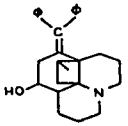
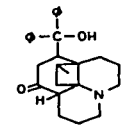
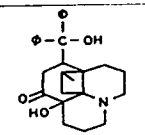
NO.	STRUCTURE	FORMULA	NAME	M.P. °C	INFRARED cm⁻¹	ULTRAVIOLET λ log ε	$[\alpha]_D$ TEMP.	pK_A	REF.
LXXX		$C_{16}H_{18}O_4NCl$	KETO LACTONE	234	1790 γ-LACTONE C=O 1725 KETONE 1665 LACTAM	END ABSORPTION ONLY			55
LXXXI		UNCHARACTERIZED	KETO ACID						55
LXXXII		$C_{17}H_{22}NO_4Cl$	HYDROXY ESTER						56 57
LXXXIII		$C_{17}H_{20}NO_3Cl$	ANHYDRO ESTER	181					56 57
LXXXIV		$C_{17}H_{20}NO_3Cl$	α-PYRIDONE ESTER						56 57
LXXXV		$C_{16}H_{18}NO_3Cl$		295					56 57
LXXXVI		$C_{16}H_{18}NO_3Cl$	γ-LACTONE	310	1792 γ-LACTONE C=O 1668	END ABSORPTION			56 57

NO.	STRUCTURE	FORMULA	NAME	M.P. °C	INFRARED cm⁻¹	ULTRAVIOLET λ LOG ε	$[\alpha]_D$ TEMP.	pK_A	REF.
LXXXVII		$C_{16}H_{18}NO_3Cl$	PYRIDONE ACID						56 57
LXXXVIII		$C_{16}H_{21}NO_3$	PYRIDONE ACID	235	1716 1655 1570 1520	237 mμ 3.91 315 mμ 3.89			56 57
LXXXIX		$C_{13}H_{11}O_3N$	QUINOLONE ACID	286					56 57
XC		$C_{12}H_{11}ON$	QUINOLONE						56 57
XCI		$C_{13}H_{13}O_3N$	DIHYDROQUINOLONE ACID	267	VERY SIMILAR TO LVI	IDENTICAL WITH LVI			56 57
XCII		$C_{16}H_{21}O_5N$	ANNOTININE LACTAM DIOL	239	3530 (S) -OH 3300 (B) -OH 1770 γ-LACTONE C=O 1620 LACTAM C=O				58
XCIII		$C_{15}H_{17}O_4N$		199	1780 γ-LACTONE C=O 1760 5-NUMBERED CYCLIC KETONE C=O 1708 LACTAM C=O				58

NO.	STRUCTURE	FORMULA	NAME	M.P. °C	INFRARED cm⁻¹	ULTRAVIOLET λ LOG ε	$[\alpha]_D$ TEMP.	pK_A	REF.
XCIV		$C_{15}H_{19}O_4N$	NEUTRAL	255	1780 γ-LACTONE C=O 1682 γ-LACTAM C=O 3335 OH REGION				58
XCV		$C_{15}H_{19}O_5N$	ACIDIC N-FORMYL DERIV. OF AMINO ACID XXXII	205	1775 γ-LACTONE C=O 1705 CARBOXYLIC ACID C=O 1610 AMIDE C=O				58
XCVI		$C_{16}H_{21}O_5N$	METHYL ESTER OF THE N-FORMYL DERIV. OF AMINO ACID XXXII	123	1788 γ-LACTONE C=O 1730 CARBOMETHOXY C=O 1653, 1665 DOUBLE PEAK -AMIDE REGION				58
XCVII		UNCHARACTERIZED							58
XCVIII		$C_{17}H_{21}O_6N$	METHYL ESTER OF XCVII	195	1780 γ-LACTONE C=O 1743 CARBOMETHOXY C=O 1710 KETO C=O 1645, 1633 DOUBLE PEAK -AMIDE REGION				58
XCIX		$C_{16}H_{23}O_3N$		205	3515 OH REGION 1752 LACTONE C=O				58
C		$C_{16}H_{23}O_4N$	ANNOTININE DIOL HYDROCHLORIDE (ANHYD) HYDROCHLORIDE (HYDRATED)	192 296 233	3440 OH 1750 LACTONE C=O				51

NO.	STRUCTURE	FORMULA	NAME	M.P. °C	INFRARED cm^{-1}	ULTRAVIOLET λ LOG ε	$[\alpha]_D$ TEMP	pK_A	REF.
CI		$C_{16}H_{25}O_5N$ AS SULPHATE							51
CII		$C_{16}H_{23}O_4N$	ANNOTININIC ACID	259	3330 OH 1575 } DIFFUSE – 1568 } AMINO ACID ZWITTERION				51
CIII		$C_{17}H_{25}O_4N$	(METHYL ANNOTININATE)	134	NUJOL 3460 OH 1732 ESTER C=O CS_2 3580 LOWER INTENSITY OH 1732 (BROADENED)				51
CIV		$C_{17}H_{23}O_5N$	METHYL EPIANNOTININATE LACTAM	193	3375 OH 1723 ESTER C=O 1636 LACTAM C=O				51
CV		$C_{16}H_{25}O_5N$	DIESTER	B.R 115–122 (10^{-4} m m Hg)					51
CVI		$C_{17}H_{21}O_4N$		145	1725 ESTER C=O 1698 1640	244 mμ 3.8			59
CVII		$C_{17}H_{21}O_5N$		247	1732 1701 1628	242 mμ 3.8			59

NO.	STRUCTURE	FORMULA	N.M.	M.P. °C	INFRARED cm⁻¹	ULTRAVIOLET λ LOG ε	$[\alpha]_D$ TEMP	pK_A	REF.
CVIII		$C_{17}H_{21}O_5N$		213	1726 1709 1640	244mμ 3.9			59
CIX		$C_{17}H_{19}O_4N$		128	1731 1672 1625	251mμ 4.0			59
CX		$C_{17}H_{21}O_4NBr_2$		163.5	1741 ESTER & KETONE C=O 1635 LACTAM C=O				59
CXI		$C_{17}H_{23}O_4N$		236	1718 ESTER C=O 1624 LACTAM C=O	222 mμ 4.0			59
CXII		$C_{24}H_{27}O_4N$	BENZYLIDENE DERIV. OF LXXVII	213	1736 ESTER C=O 1696 KETONE C=O 1623 LACTAM C=O 1575 (BENZENE)	295 mμ 4.28			59
CXIII		UNCHARACTERIZED	FURFURILIDENE DERIV. OF LXXVIII AMORPHOUS						59
CXIV		UNCHARACTERIZED	AMORPHOUS ACID						59

NO.	STRUCTURE	FORMULA	NAME	M.P. °C	INFRARED cm⁻¹	ULTRAVIOLET λ LOG ε	$[\alpha]_D$ TEMP.	pK_A	REF.
CXV		$C_{15}H_{21}O_5N$	DICARBOXYLIC ACID	304					59
CXVI		$C_{28}H_{33}O_2N$	DIPHENYLDESOXO-DIHYDRO ANNO-TININE	220	3600 OH 3150 (BROAD) OH 1590 (WEAK) PHENYL	258mμ 2.8	-108 (C, 1% $CHCl_3$) 21°		62
CXVII		$C_{28}H_{33}ON$	ANHYDRO DERIV. OF CXVI	190	3630 OH 1600 PHENYL 1580 CONJ'D PHENYL	-STRONG BUT BROAD ABSORPTION 220-280mμ (LOG ε 3.8-4.0) -U.V. SPECTRUM CHARACTERISTIC OF 1,1-DIPHENYLETHYLENE			62
			-HYDROCHLORIDE	295(d)					
CXVIII		$C_{28}H_{33}O_2N$	HYDROXY KETONE	201	3500 OH 1700 KETO C=O 1595 PHENYL		+100.7 (C, 1% $CHCl_3$) 21°		62
CXIX		$C_{28}H_{33}O_3N$	DIHYDROXY KETONE	208	3500-3550 OH 3450 OH 1720 (SHOULDER 1728) KETO C=O 1590 PHENYL	260mμ 2.9	+43 (C, 1% $CHCl_3$) 21°		62
			HYDROCHLORIDE MONOHYDRATE OF CXIX	276	3350-3420 (BROAD) OH 1720 KETO C=O 1595 PHENYL	260mμ 2.69			62
			METHIODIDE	245	3250 OH 3430 OH 1720 KETO C=O 1600 PHENYL				62

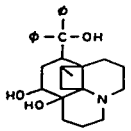
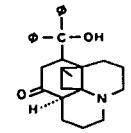
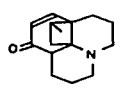
NO.	STRUCTURE	FORMULA	NAME	M.P. °C	INFRARED cm^{-1}	ULTRAVIOLET λ	LOG ε	$[\alpha]_D$	TEMP.	pK_A	REF.
CXX		$C_{28}H_{35}O_3N$	TRIHYDROXY COMPOUND	209	3620 (SLIGHT) OH 3430 (BROAD) OH 1695 PHENYL						62
CXXI		$C_{28}H_{33}O_2N$	EPIMER OF CXVIII	223	3420 OH 1710 KETO C=O 1595 PHENYL	258 mμ	2.75	-60.2 (C,1%CHCl₃)	21°		62
CXXII		$C_{15}H_{21}ON$	CONJ'D KETONE		1680 CONJ'D KETO C=O						62
			HYDROCHLORIDE	262	1625 UNSATURATION	318 mμ (WEAK) C=O 228mμ	3.85 CONJ'D C=O				
			HYDROBROMIDE	268							

TABLE NO. 3

Partition Data -- Chloroform-Buffer System †

	Lycopodine	Annotinine	Acrifoline	Annotine	Lycodine
✕ True Partition Coefficient (k)	37	120	19	38	540
✕ Apparent Partition Coeff. (k') at pH5.2	0.21	6.0	0.014	0.19	0.71
(% in $CHCl_3$).....	18%	86%	1%	16%	42%
✕ Apparent Partition Coeff. (k') at pH6.0	1.3	38	0.093	1.2	4.5
(% in $CHCl_3$).....	57%	97%	9%	55%	82%
$pH_{\frac{1}{2}}$ (i.e. where k'=1).	5.9	4.4	7.1	5.9	5.4

Note: † - See Appendix "C" for calculations.

✕ - Partition coefficients expressed as chloroform / buffer.

TABLE NO. 4

Chemical Shifts and Coupling Constants (in c/sec.)

In NMR Spectra for Lycodine and Some Pyridine Derivatives

	Lycodine†		2,3-lutidine ✱(76)		Pyridine✱ (102)
	Assign't 1	Assign't 2	Assign't 1	Assign't 2	
Chemical Shifts					
$H_\beta - H_\alpha$	54.4	54.4	55.1	54.9	60.6
$H_\gamma - H_\alpha$	20.2	20.2	43.4	43.4	45.6
$H_\gamma - H_\beta$	34.2	34.2	11.7	11.5	15.0
Coupling Constants					
$H_\alpha \cdot H_\beta$	5.8	5.3	5.0	5.2	5.5
$H_\beta \cdot H_\gamma$	8.6	8.7	7.35	7.35	7.5
$H_\alpha \cdot H_\gamma$	1.2	-1.9	1.3	-2.0	1.9

Note: † As conc. sol'n in CCl_4. (See Appendix "F" for cal'n of lycodine values.)

✱ As pure liquids. (The effect of CCl_4 on the chemical shift of these spectra is small, even at infinite dilution and is mainly on the α-proton (102). Calculations (✱) from Bernstein et al (76) and (102).

TABLE NO. 5

Observed and Calculated NMR Spectra for Lycodine

Line	Origin of Transition	Energy (c/sec.)		Rel. Intensity	
		Calc'd	Obs'd	Calc'd	Obs'd
1.	β	0	0	0.77	0.65
2.	β	5.2	5.1	0.74	0.76
3.	β	8.7	8.8	1.23	1.17
4.	β	13.9	14.0	1.26	1.41
5.	γ	37.0	37.2	1.23	1.24
6.	γ	38.8	38.8	1.26	1.54
7.	γ	45.7	45.6	0.77	0.69
8.	γ	47.5	47.5	0.74	0.89
9.	α	58.4	58.5	1	1.85
10.	α	60.2	60.2	1	
11.	α	63.6	63.5	1	0.83
12.	α	65.4	65.2	1	0.96

Note: See Appendix "F" for calculation of the above values (1st assignment). Calculated values of energy are the same for both assignments. Calculated values of relative intensities are within ±0.01 for the 1st and 2nd assignments (see Appendix "F").

Figure 1 - Flow chart to consolidate the reactions of annotinine, as described in the literature, with reference to its known structure.

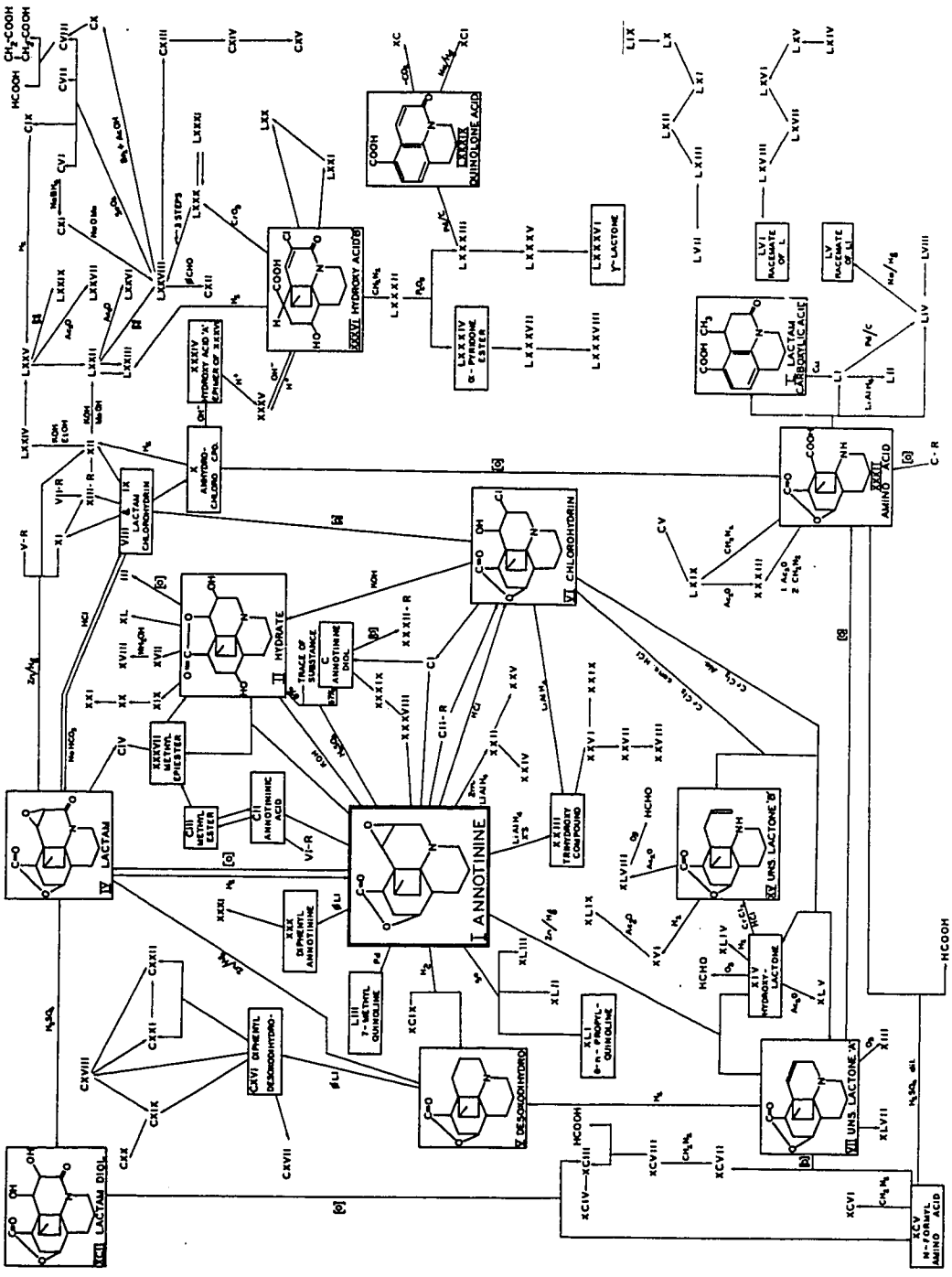

I ANNOTININE
II HYDRATE
III METHYL ESTER
IV LACTAM
V CHLOROHYDRIN
VI AMINO ACID
VII UNS LACTONE 'A'
VIII LACTAM DIOL
IX LACTAM CHLOROHYDRIN
X AMYDRO-CHLORO COMPOUND
XI METHYL ESTER
XII ANNOTININIC ACID
XIII TRIHYDROXY COMPOUND
XIV HYDROXY-LACTONE
XV UNS LACTONE 'B'
XVI LACTAM CARBOXYLIC ACID
XVII RACEMATE OF L
XVIII RACEMATE OF LI
XIX N-FORMYL AMINO ACID
XX ANNOTININE DIOL
XXI TRACE OF SUBSTANCE
XXII METHYL EPIESTER
XXIII HYDROXY ACID 'A' LACTONE
XXIV HYDROXY ACID 'A' EPIMER OF XXIII
XXV HYDROXY ACIDS
XXVI QUINOLONE ACID
XXVII α-PYRONE ESTER
XXVIII γ-LACTONE
XXIX DIHYDRO-ANNOTININE
XXX 7-METHYL QUINOLINE
XXXI 8-PROPYL-QUINOLINE
XXXII DIHYDRO-DESOXOHYDRO
XXXIII DESOXOHYDRO
HCOOH
CH2-COOH
CH2-COOH

Figure 2 - Suggested mechanism (46) of the dehydration of the amide chlorohydrin (see Table No. 2 (IX)) in annotinine chemistry.

Figure 3 - Suggested mechanism (58) of the conversion of annotinine lactam diol (Table No. 2 (XCII)) to the neutral product (Table No. 2 (XCIII)) and the acidic product (Table No. 2 (XCV)).

Figure 4 - Probable mechanism of isomerization of the alcohol (Table No. 2 (CXI)) back to the lactam keto ester (Table No. 2 (LXXVIII)).

Fig.2

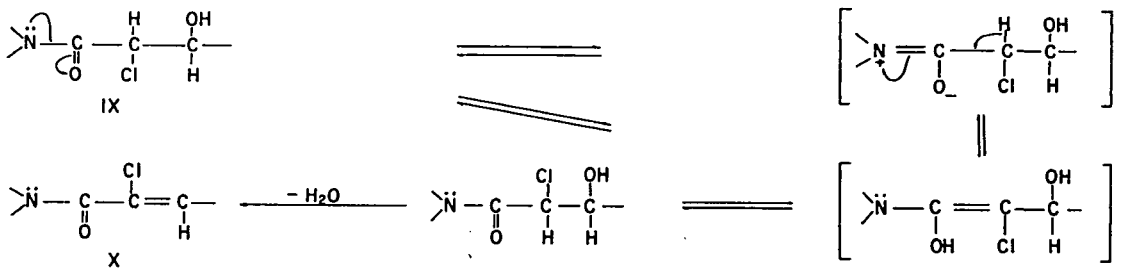


Fig.3

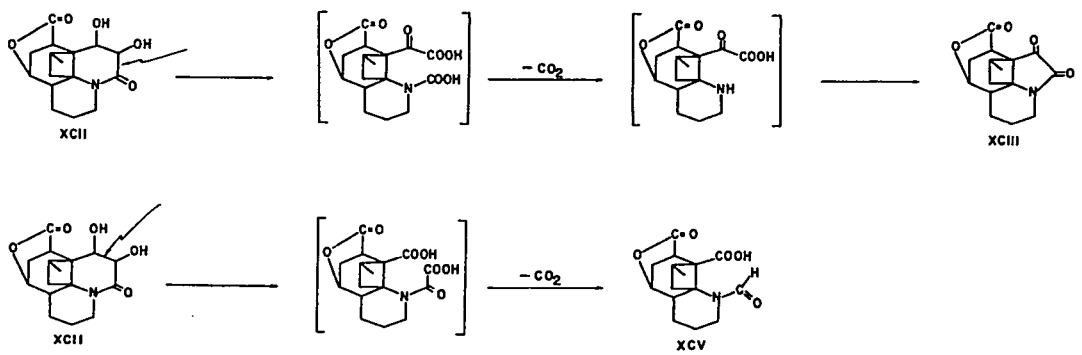


Fig.4

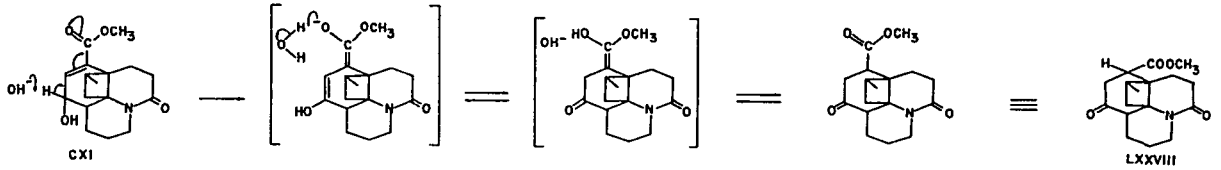
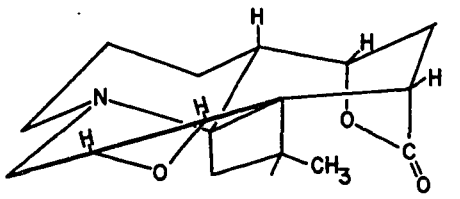
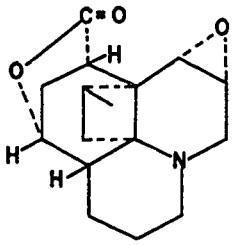


Figure 5 - Drawings showing the conversion of annotinine to annotinine bromohydrin from the standpoint of their configurations (61) (42) and possible conformations.

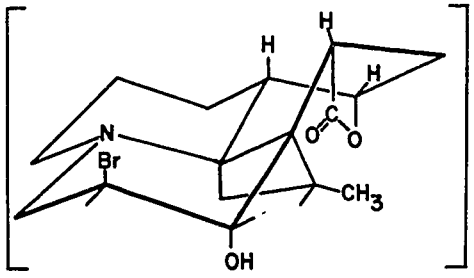




ANNOTININE

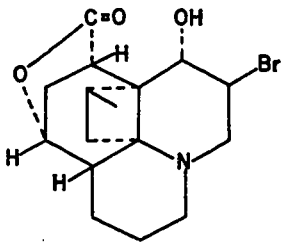
configuration

H Br



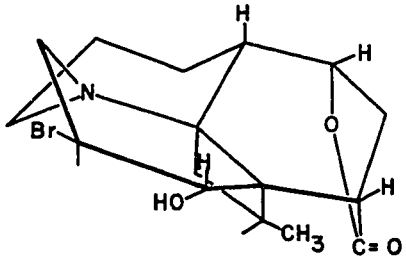
Br & OH "trans - diaxial" conformation

H Br



Annotinine bromohydrin

configuration



Br & OH "trans - diequatorial" conform'n

Figure 6 - Probable mechanism ("reverse-Michael" reaction) (62) of the elimination of the diphenyl carbinol group from the ketone (Table No. 2 (CXXI)) to the conjugated ketone (Table No. 2 (CXXII)).

Figure 7 - Possible mechanism for the conversion of the amino acid (Table No. 2 (XXXII)) to the lactam carboxylic acid (Table No. 2 (L)).

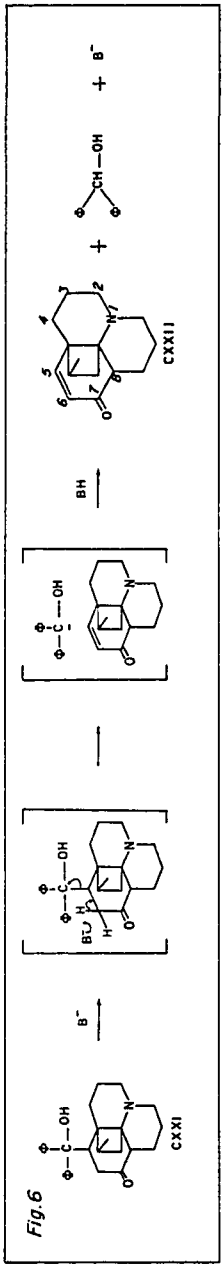


Fig.6

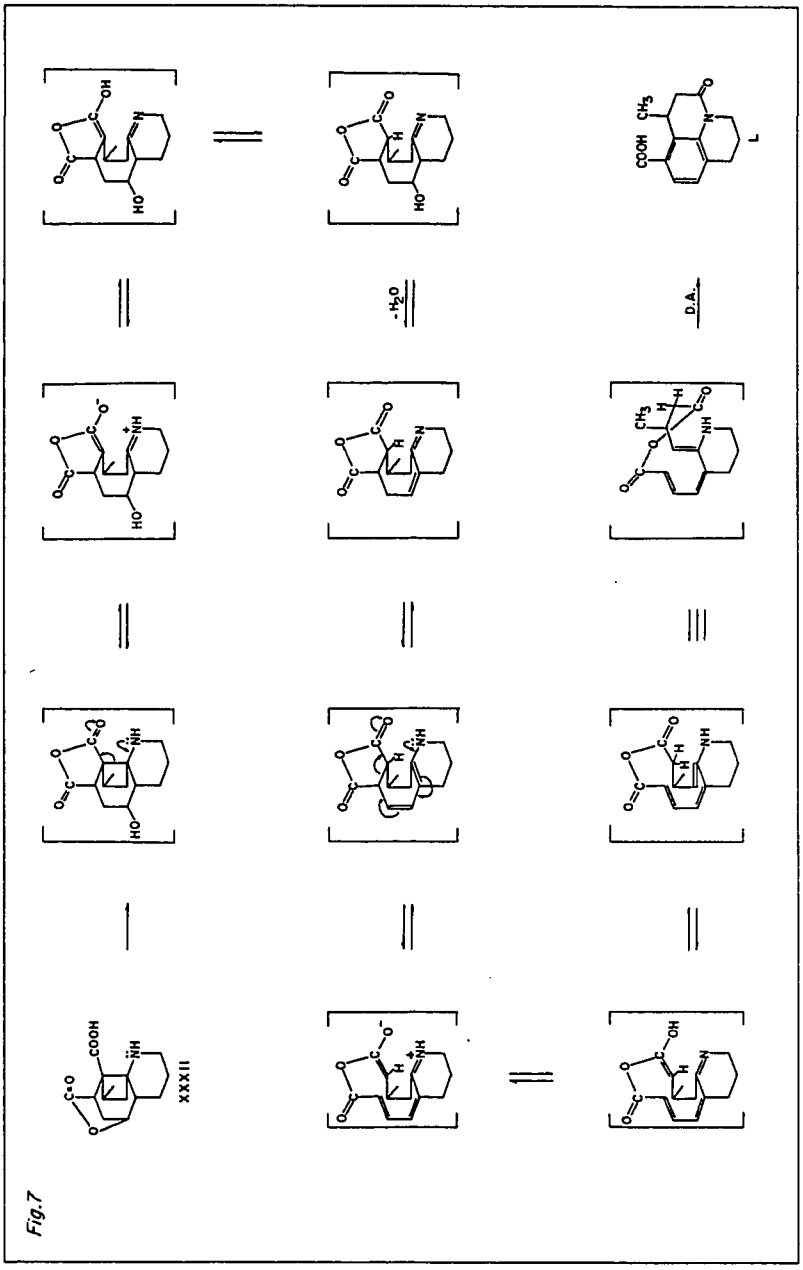


Fig.7

Figure 8 - Flow chart to consolidate the reactions of lycopodine, as described in the literature, with reference to its partial structure.

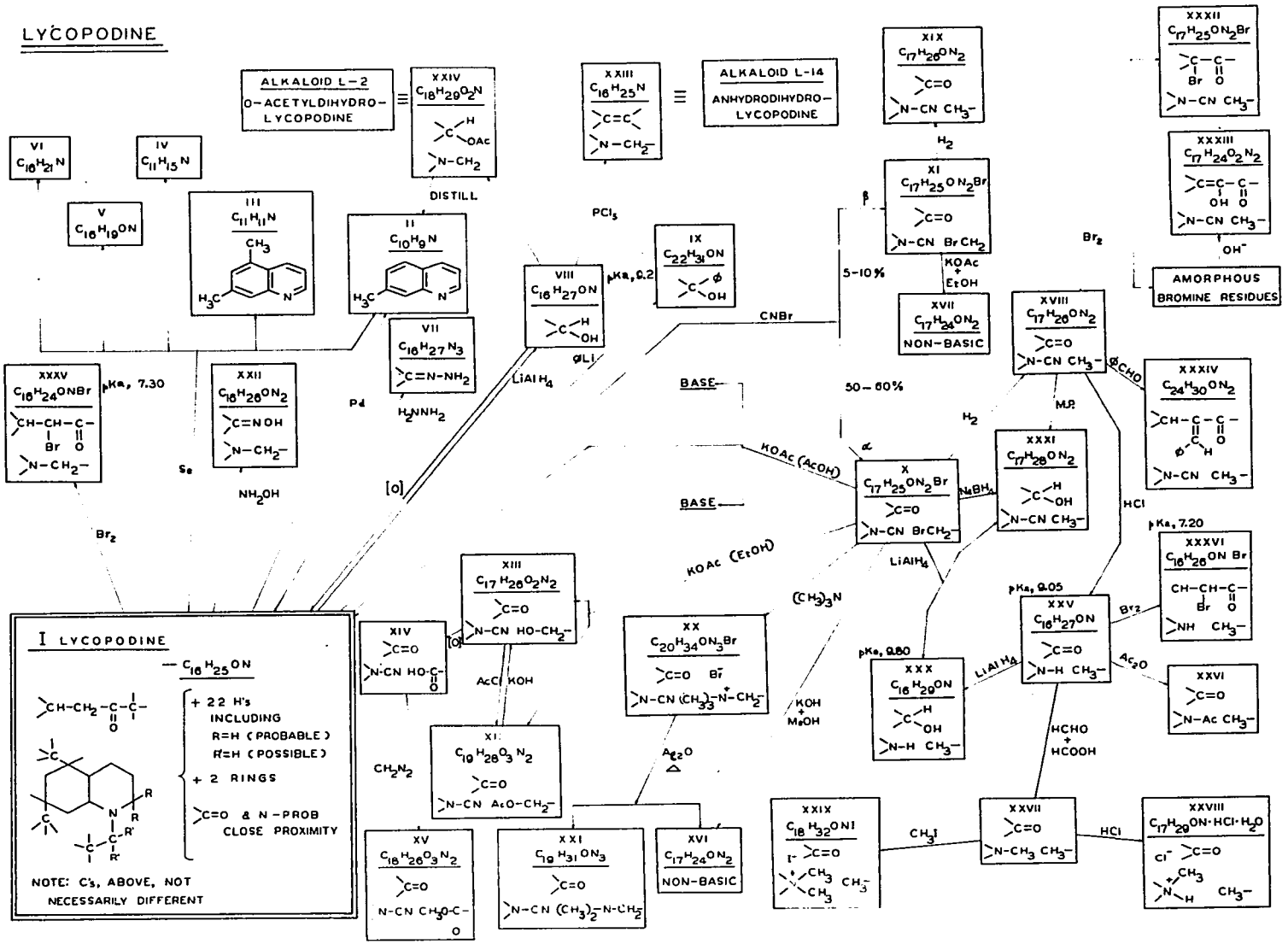

LYCOPODINE
ALKALOID L-2
O-ACETYLDIHYDRO-LYCOPODINE
XXIV C18H29O2N
DISTILL
ALKALOID L-14
ANHYDRODIHYDRO-LYCOPODINE
XXIII C16H25N
VI C16H21N
IV C11H15N
V C16H19ON
III C11H11N
II C10H9N
VII C16H27N3
VIII C16H27ON
IX C22H31ON
PCl5
pKa, 9.2
CNBr
LiAlH4
9Li
P4
H2NNH2
XXXV C16H24ONBr
pKa, 7.30
XXII C16H26ON2
NH2OH
Se
Br2
[O]
I LYCOPODINE
— C16H25ON
+ 22 H's
INCLUDING
R=H (PROBABLE)
R=H (POSSIBLE)
+ 2 RINGS
C=O & N-PROB
CLOSE PROXIMITY
NOTE: C's, ABOVE, NOT
NECESSARILY DIFFERENT
XIII C17H26O2N2
XIV
AcCl KOH
XII C19H28O3N2
CH2N2
XV C18H26O3N2
XXI C19H31ON3
XVI C17H24ON2
NON-BASIC
XX C20H34ON3Br
Ac2O
KOH
MeOH
(CH3)3N
BASE
KOAc (AcOH)
BASE
KOAc (EtOH)
X C17H25ON2Br
XIX C17H26ON2
H2
XI C17H25ON2Br
β
5-10%
KOAc
EtOH
XVII C17H24ON2
NON-BASIC
50-60%
α
NaBH4
XXXII C17H25ON2Br
XXXIII C17H24O2N2
OH⁻
Br2
AMORPHOUS
BROMINE RESIDUES
XVIII C17H26ON2
ØCHO
M.P.
H2
XXXIV C24H30ON2
XXXI C17H28ON2
HCl
pKa, 7.20
XXXVI C16H26ONBr
pKa, 9.05
XXV C16H27ON
Br2
pKa, 9.80
XXX C16H29ON
LiAlH4
Ac2O
XXVI
HCHO
HCOOH
XXIX C18H32ONI
CH3I
XXVII
HCl
XXVIII C17H29ON·HCl·H2O

Figure 9 - Flow chart to consolidate the reactions of acrifoline, as described in the literature, with reference to its partial structure.

Figure 10 - Partial structure of the obscurines, as described in the literature.

Figure 11 - Flow chart to consolidate the reactions of annotine (alkaloid L-11), as described in the literature, with reference to its partial structure.

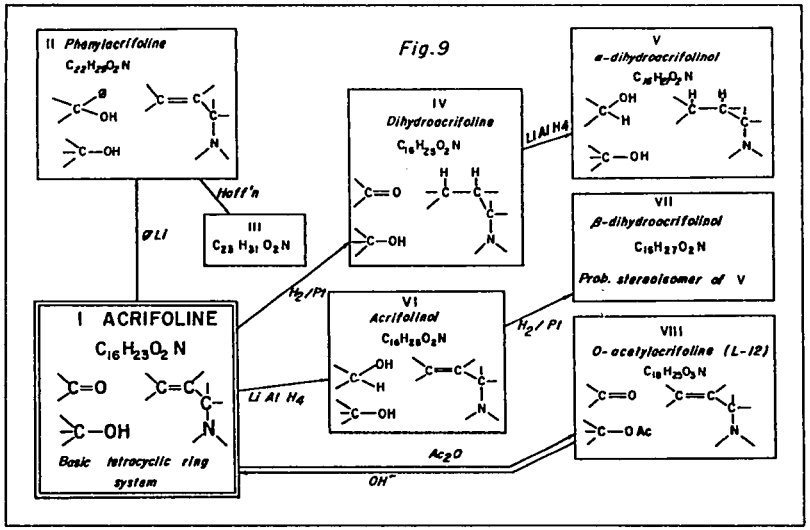


Fig.9

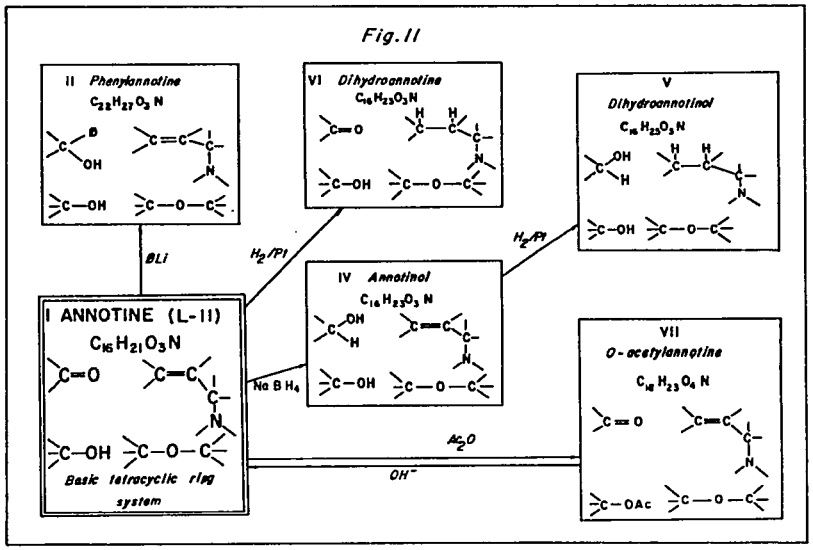


Fig.11

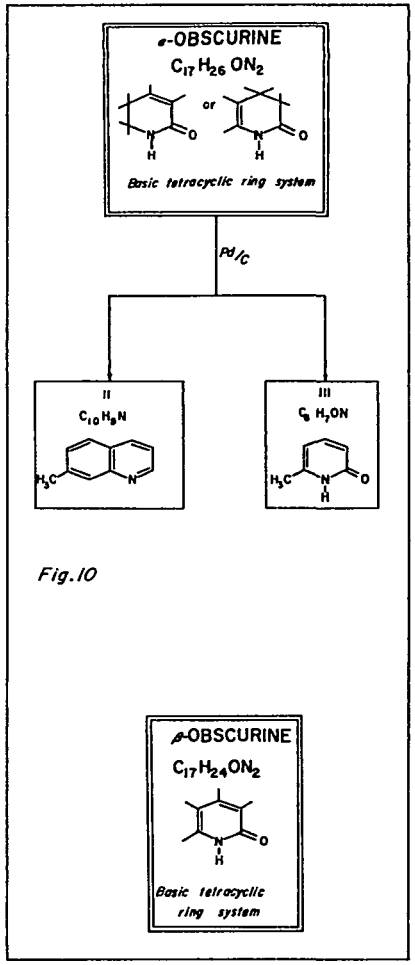


Fig.10

Figure 12 - Photographs of developed paper chromatograms of alkaloids of Lycopodium annotinum L. on buffered paper, the alkaloid spots having been made visible.

"A" - descending chromatograms of annotinine and lycopodine on Whatman No. 1 paper buffered at pH values of 2.28, 5.70 and 8.05 using n-butanol / water as the developer.

"B" - descending two-dimensional chromatograms of the "crude" alkaloid mixture on Whatman No. 3 paper (18 1/4" x 22 1/2") buffered at pH 5.85, using n-butanol / water in one direction and methyl ethyl ketone / water in the direction at right angles to the first direction.

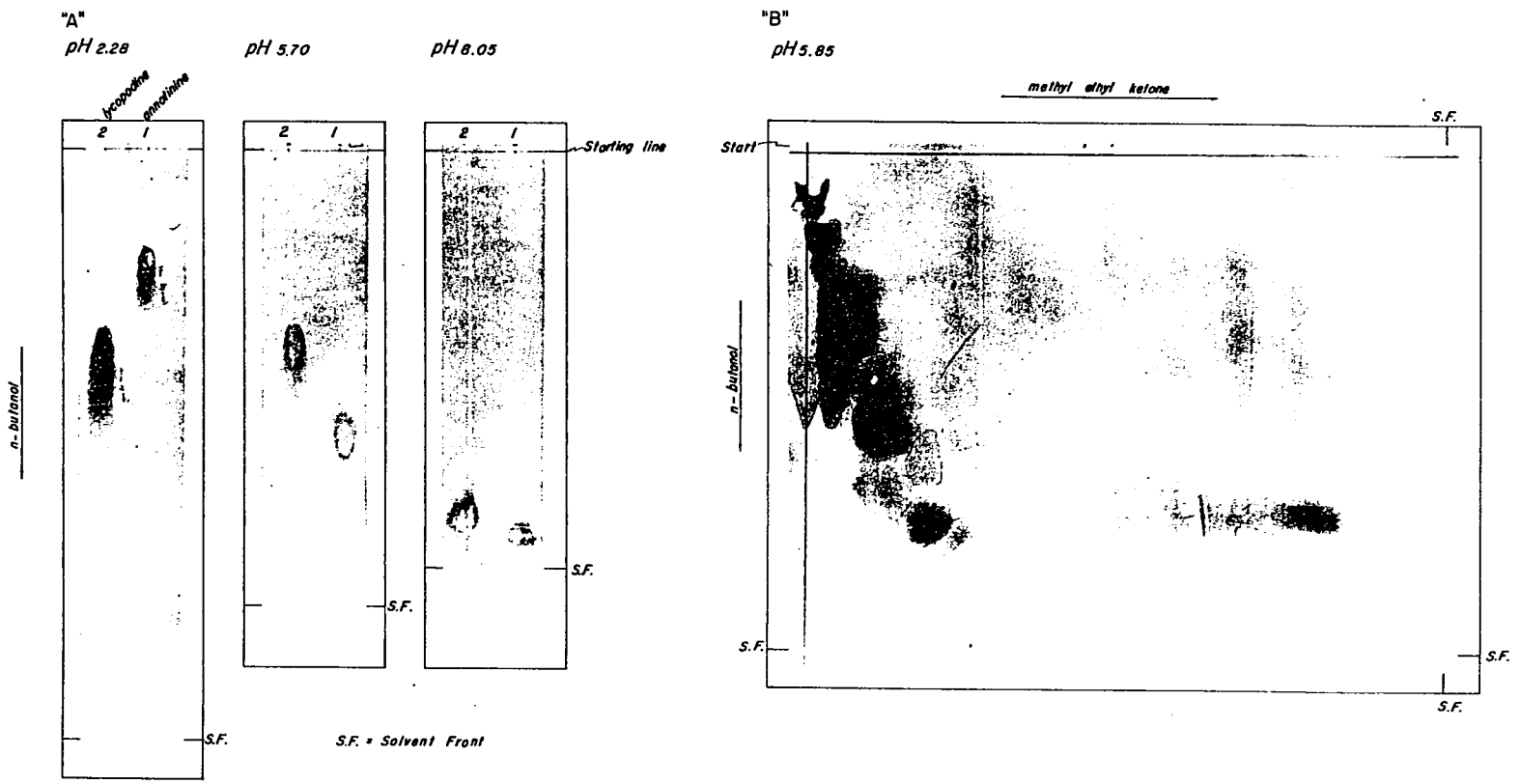

"A"
pH 2.28
pH 5.70
pH 8.05
lycopodine
annotinine
2
1
Starting line
S.F.
n-butanol
S.F. = Solvent Front
"B"
pH 5.85
methyl ethyl ketone
Start
S.F.
n-butanol

Figure 13 - Plot showing Rf values versus pH of buffered paper in the descending paper chromatography of alkaloids of Lycopodium annotinum L. using n-butanol / water.

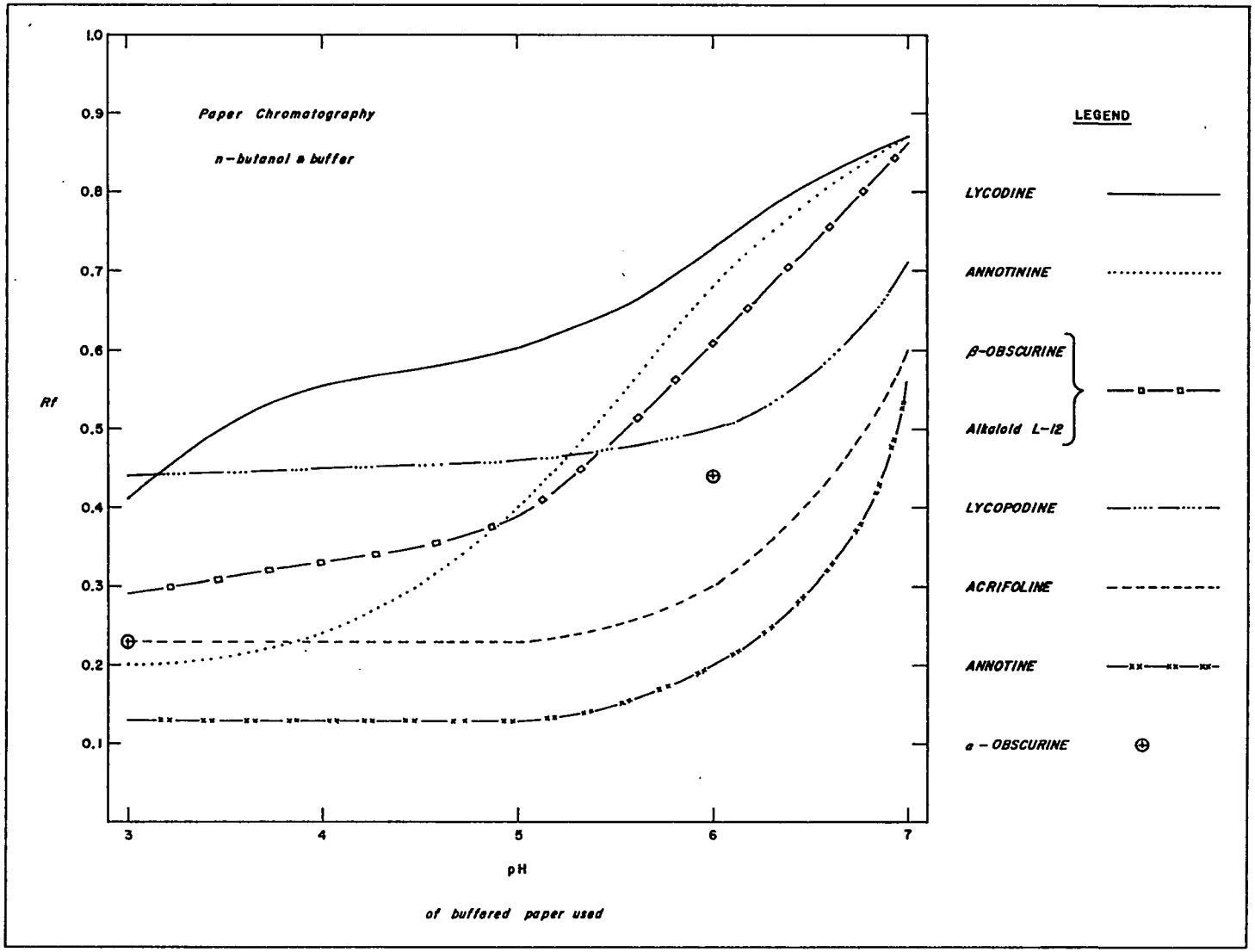

Paper Chromatography
n-butanol a buffer
Rf
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
3
4
5
6
7
pH
of buffered paper used
LEGEND
LYCODINE
ANNOTININE
β-OBSCURINE
Alkaloid L-12
LYCOPODINE
ACRIFOLINE
ANNOTINE
a - OBSCURINE

Figure 14 - Chart of Rf values (on paper buffered at pH 5.65) of alkaloids of Lycopodium annotinum L. plotted against volume (ml.) of effluent from the partition chromatography of the "crude" alkaloid mixture on a Celite column buffered at pH 5.65, using n-butanol / water. The four major fractions (Groups A, B, C and D) are indicated.

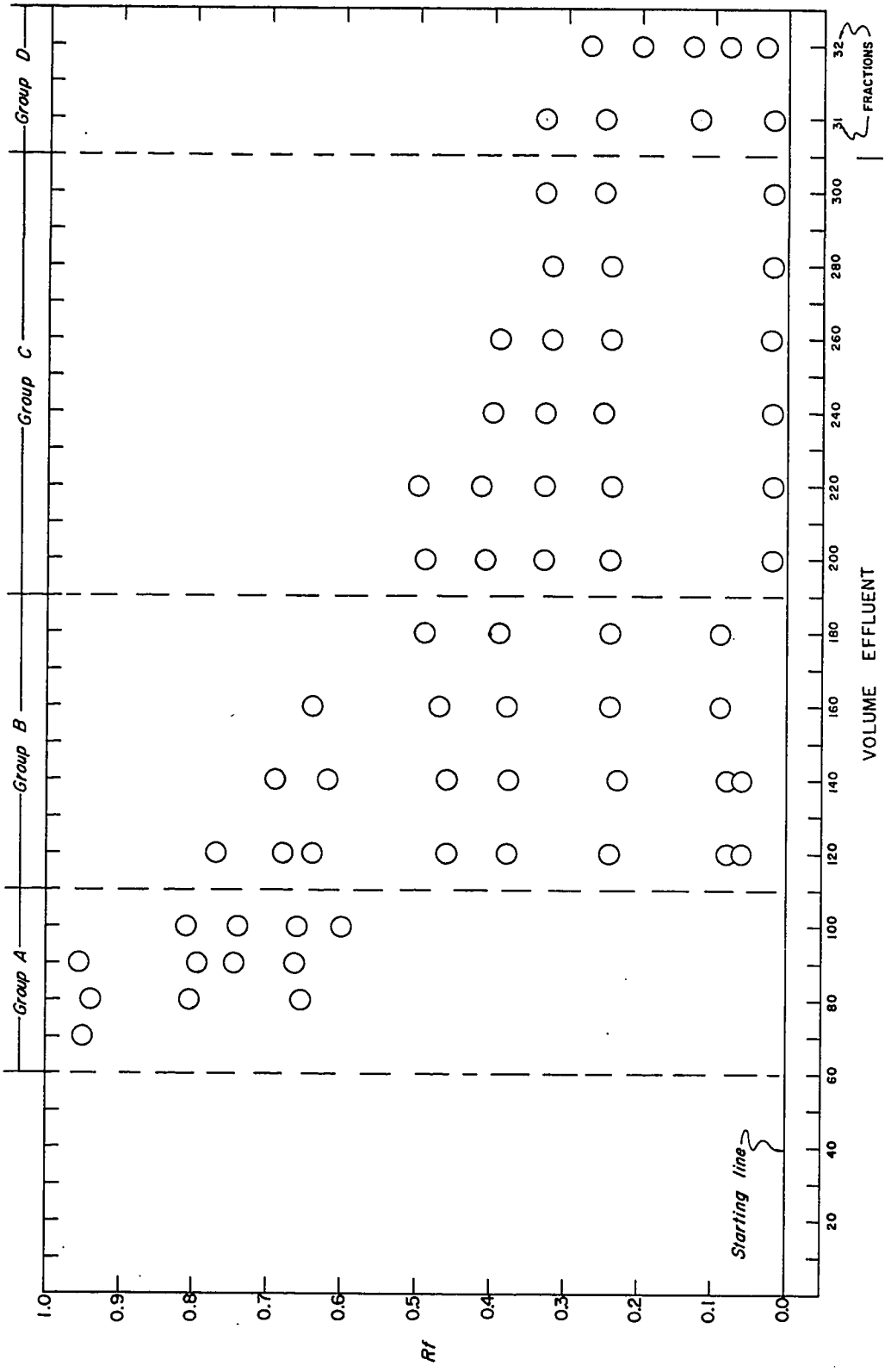
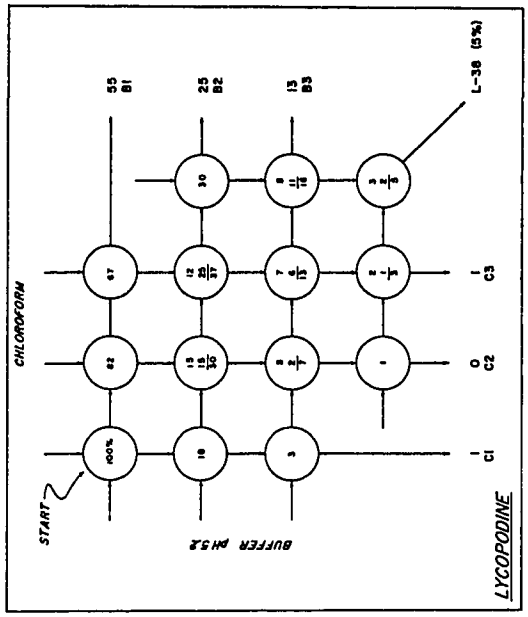
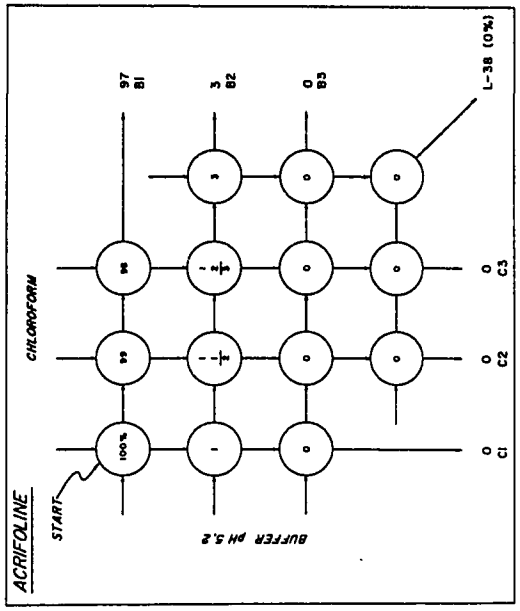
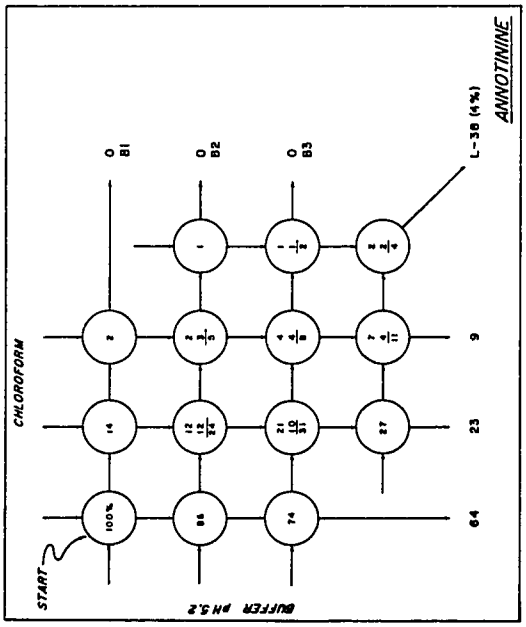

Group A
Group B
Group C
Group D
Starting line
Rf
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
31
32
FRACTIONS
VOLUME EFFLUENT
ml/s

Figure 15 - Diagram of countercurrent distribution scheme carried out on "crude" alkaloid mixture (68 g.) from Lycopodium annotinum L. between chloroform and concentrated buffer pH 5.2 (500 ml. in each phase). The calculations of the theoretical distribution of the alkaloids, annotinine, lycopodine, acrifoline and lycodine are shown within the circles representing the partitions.


CHLOROFORM
START
BUFFER pH 5.2
LYCOPODINE
L-36 (5%)


CHLOROFORM
START
BUFFER pH 5.2
ACRIFOLINE
L-36 (0%)


CHLOROFORM
START
BUFFER pH 5.2
ANNOTININE
L-36 (4%)

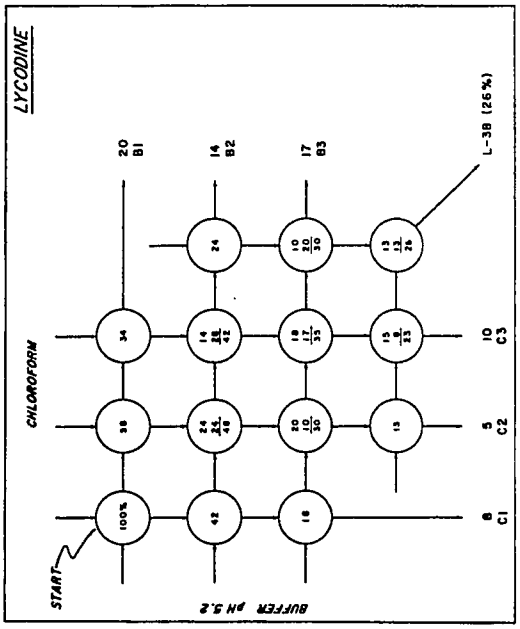
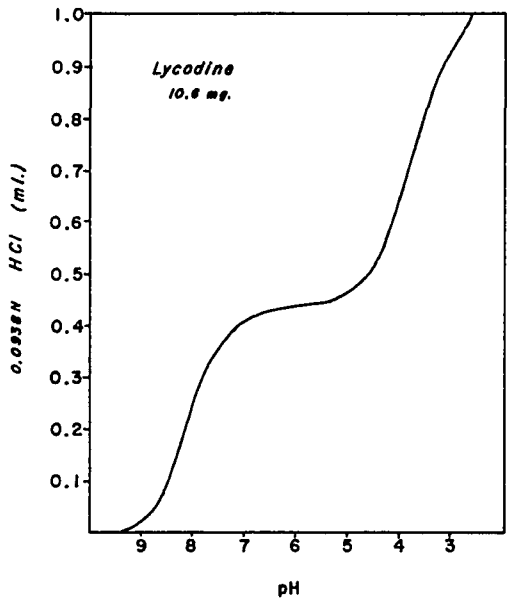
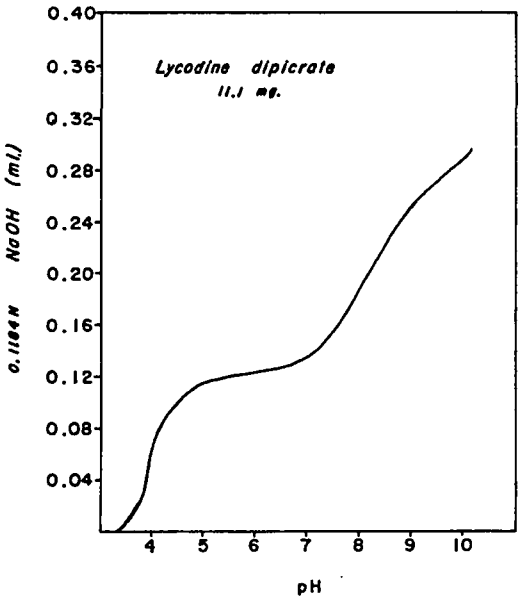
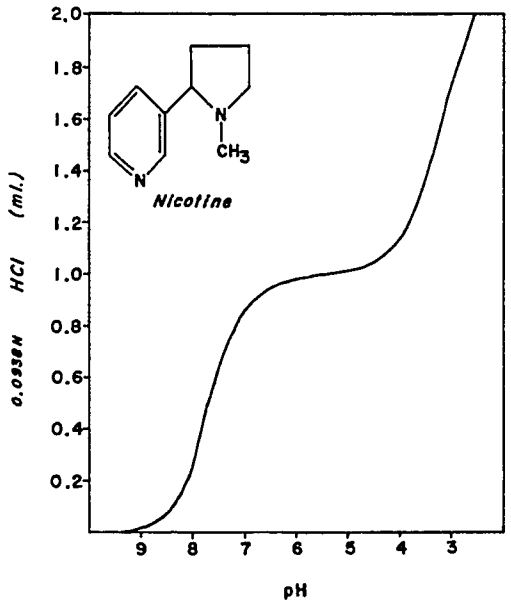

CHLOROFORM
START
BUFFER pH 5.2
LYCODINE
L-36 (26%)

Figure 16 - Plots showing microtitrations (electrometric) of lycodine (10.6 mg.), lycodine dipicrate (11.1 mg.), N-acetyl lycodine picrate (12 mg.) and nicotine in 50% aqueous methanol.


Lycodine
10.6 mg.
0.0538N HCl (ml.)
pH


Lycodine dipicrate
11.1 mg.
0.1184N NaOH (ml.)
pH


Nicotine
0.0538N HCl (ml.)
pH

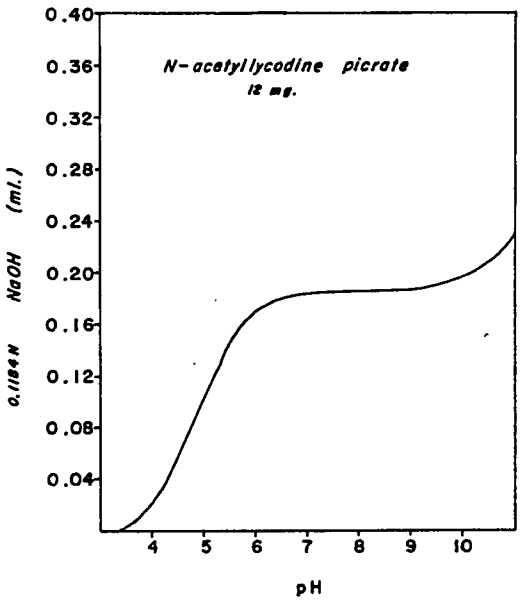

N-acetyllycodine picrate
12 mg.
0.1184N NaOH (ml.)
pH

Figure 17 - Chart illustrating, by paper chromatography as well as by infrared and ultraviolet absorption spectroscopy, the distribution of the "crude" alkaloid mixture (2.6 g.) of Lycopodium annotinum L. by the Craig countercurrent machine (60-tube) using chloroform / buffer pH 6 (40 ml. in each phase).

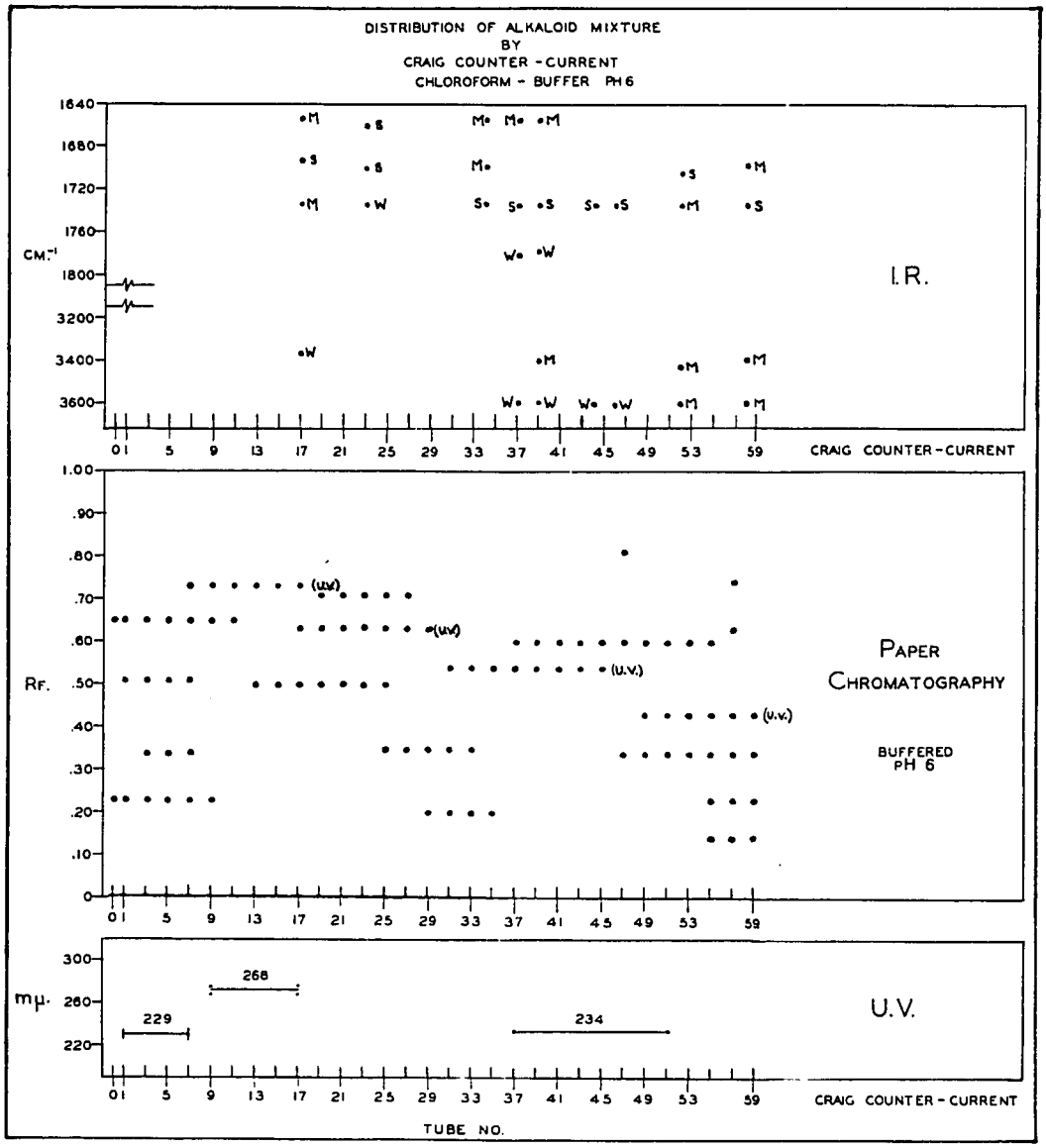

DISTRIBUTION OF ALKALOID MIXTURE
BY
CRAIG COUNTER - CURRENT
CHLOROFORM - BUFFER PH 6
CM.⁻¹
I.R.
CRAIG COUNTER-CURRENT
Rf.
PAPER
CHROMATOGRAPHY
BUFFERED
PH 6
mμ.
U.V.
268
229
234
CRAIG COUNTER - CURRENT
TUBE NO.

Figure 18 - Diagram of the scheme used in the isolation of lycodine from the "crude" alkaloid mixture (ca 40 g.) of Lycopodium annotinum L. in the successive stages:

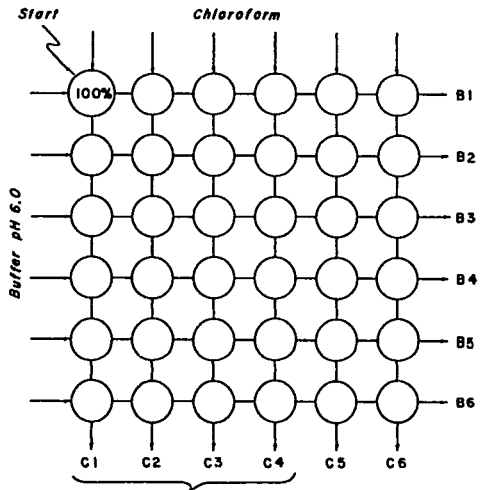
(a) countercurrent distribution between chloroform-buffer pH 6,0 (600 ml. in each phase);

(b) distributions by Craig countercurrent machine (60-tube) using chloroform-buffer pH 5.2 (40 ml. in each phase); and,

(c) adsorption chromatography on activated alumina.

ISOLATION OF LYCODINE

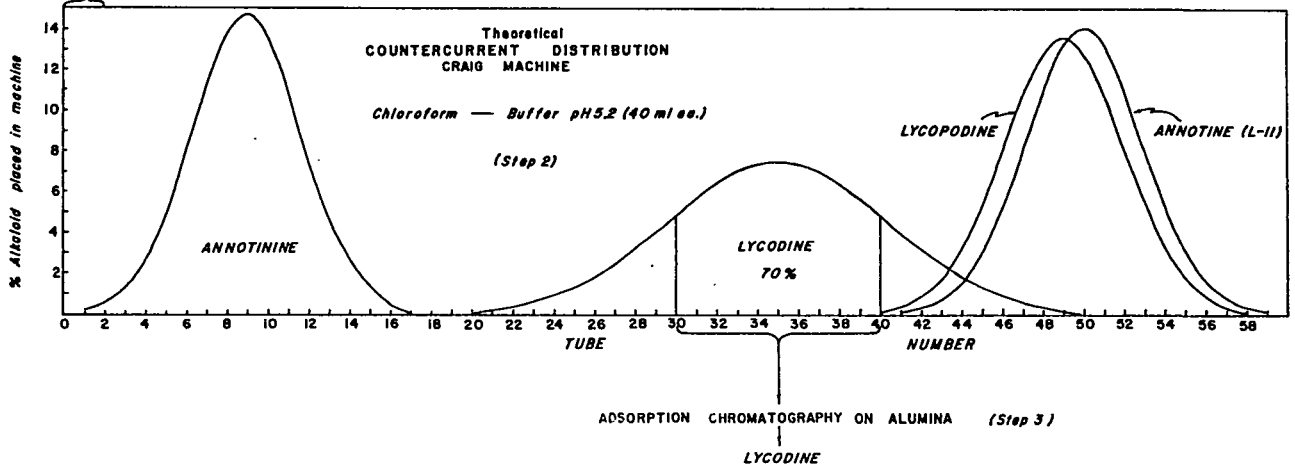
COUNTERCURRENT DISTRIBUTION — Large Scale *(Step 1)*



Theoretical Percentage Distribution of Alkaloids
Countercurrent Distribution — Large scale
chloroform — buffer pH 6.0
600 ml in each phase

	C1	C2	C3	C4	C5	C6	B6	B5	B4	B3	B2	B1	TOTAL
LYCOPODINE	3	9	14	15	15	13	9	9	7	4	2	0	100
ANNOTININE	82	17	1	0	0	0	0	0	0	0	0	0	100
ACRIFOLINE	0	0	0	0	0	0	0	0	3	9	31	57	100
ANNOTINE	3	7	12	14	14	13	11	9	8	5	3	1	100
LYCODINE	30	34	20	10	4	2	0	0	0	0	0	0	100

Lycodine — 94%



ADSORPTION CHROMATOGRAPHY ON ALUMINA *(Step 3)*

LYCODINE

Figure 19 - Comparison of the ultraviolet absorption spectra of lycodine and of 2,3-disubstituted pyridines (51). "A" - free bases. "B" - salts.

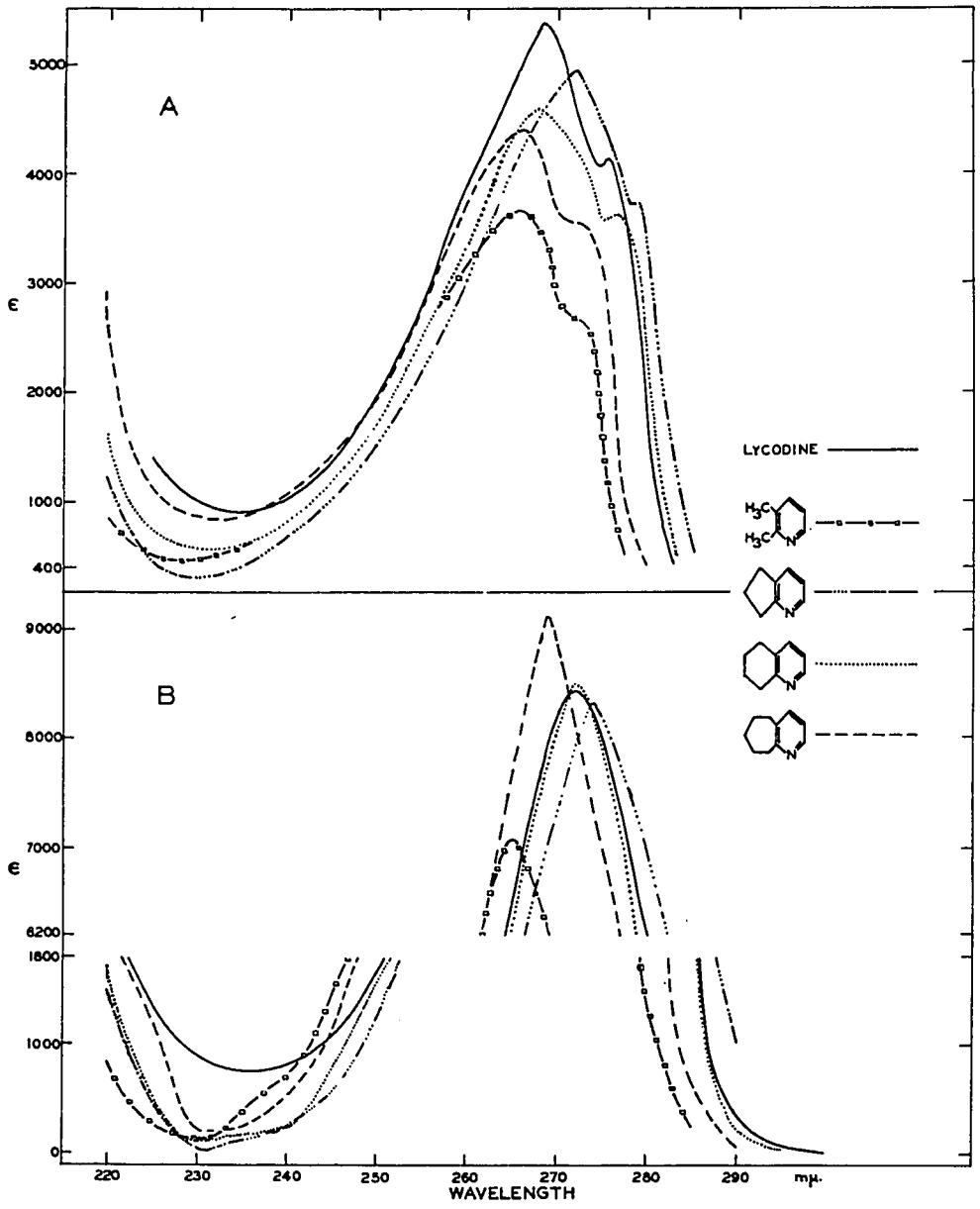

A
B
ε
5000
4000
3000
2000
1000
400
9000
8000
7000
6200
1800
1000
0
220
230
240
250
260
270
280
290
mμ.
WAVELENGTH
LYCODINE

Figure 20 - Infrared absorption spectra of lycodine.
"A" - Nujol mull. "B" - CCl_4 solution.
(1 mm. path length, 31 mg. per ml.)

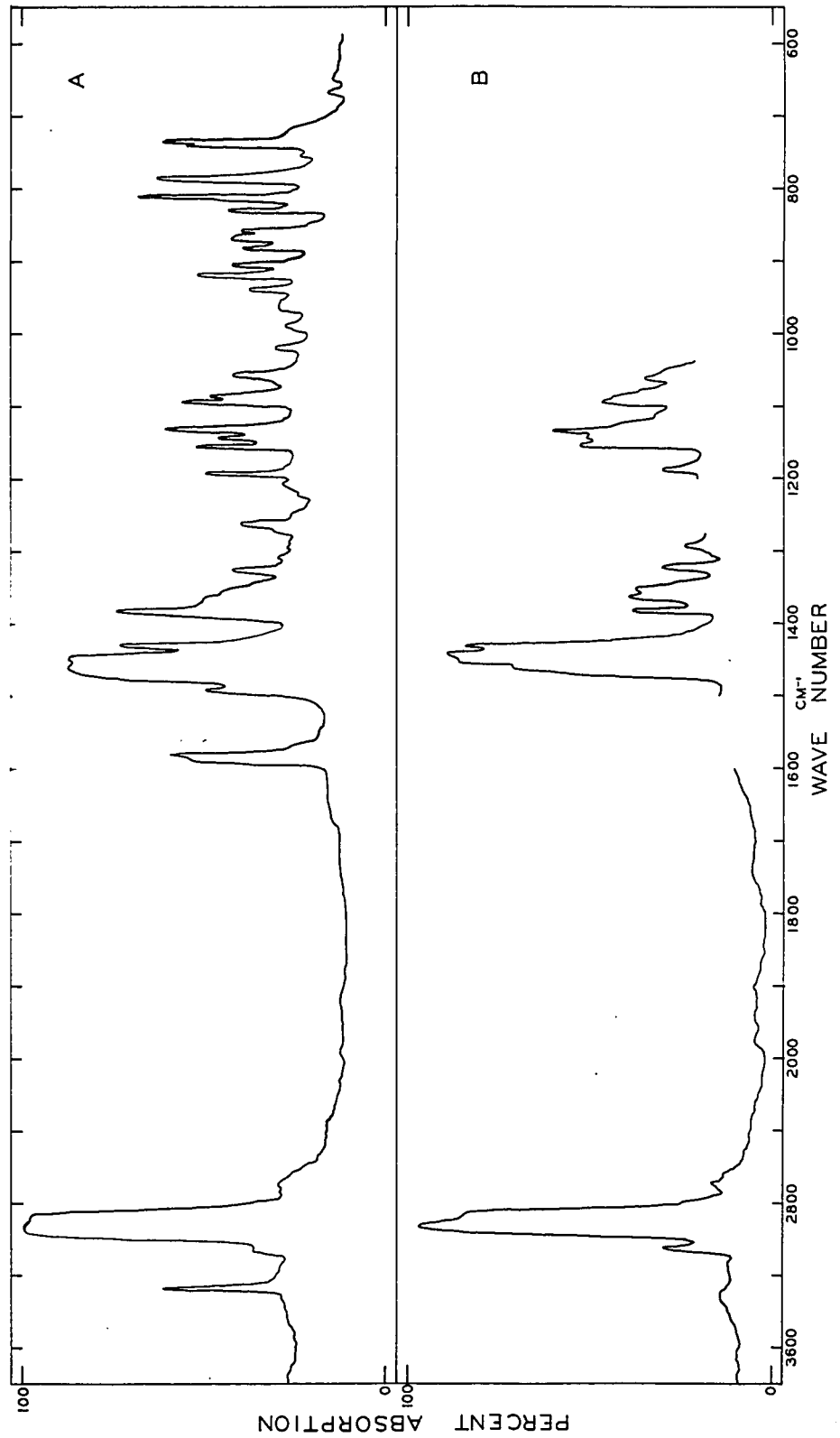

A
B
100
0
100
0
3600
2800
2000
1800
1600
1400
1200
1000
800
600
CM⁻¹
WAVE NUMBER
PERCENT ABSORPTION

Figure 21 - Nuclear magnetic resonance spectrum of lycodine. The upper curve represents the peaks at left of lower curve at higher resolution.

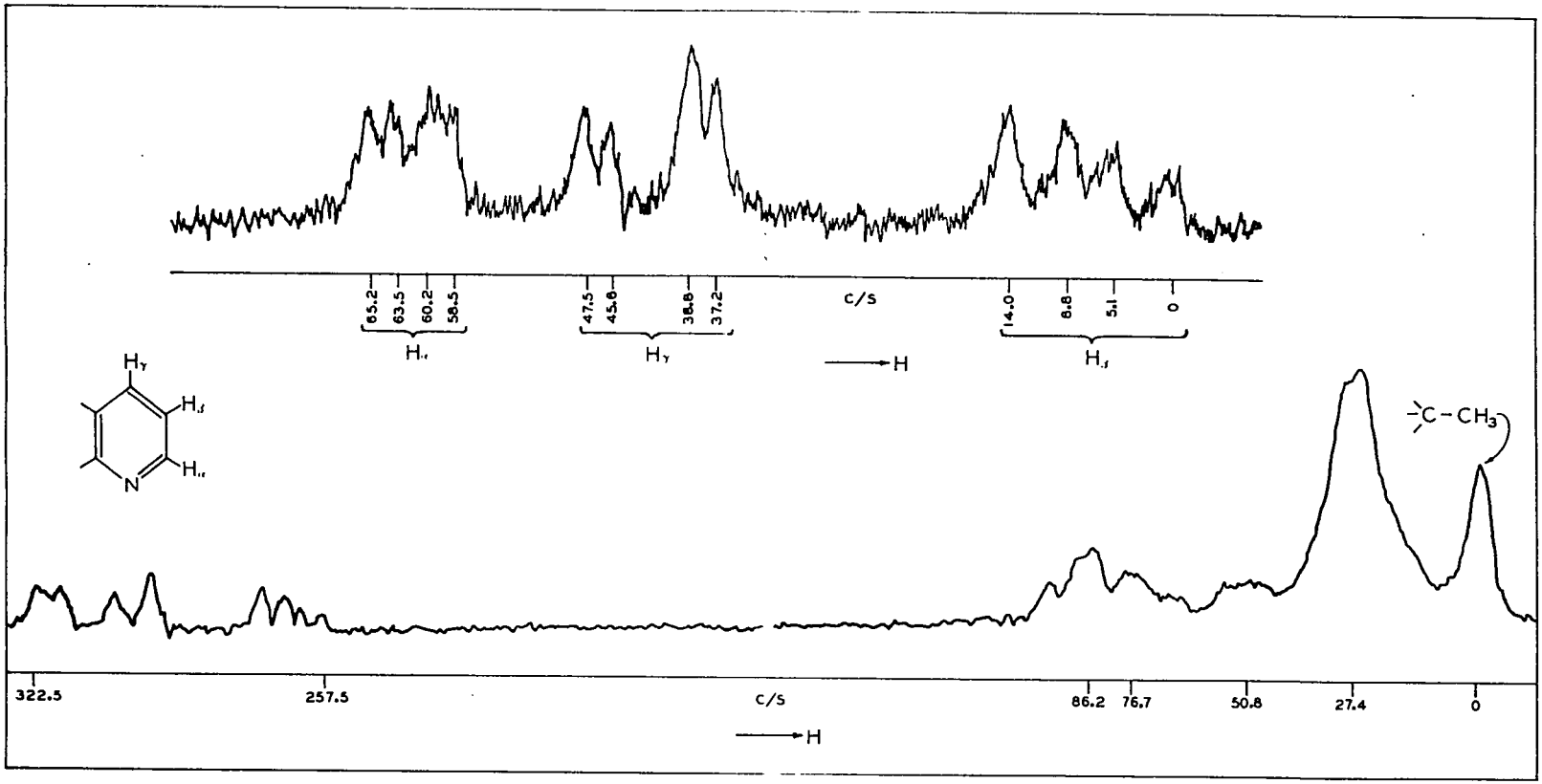
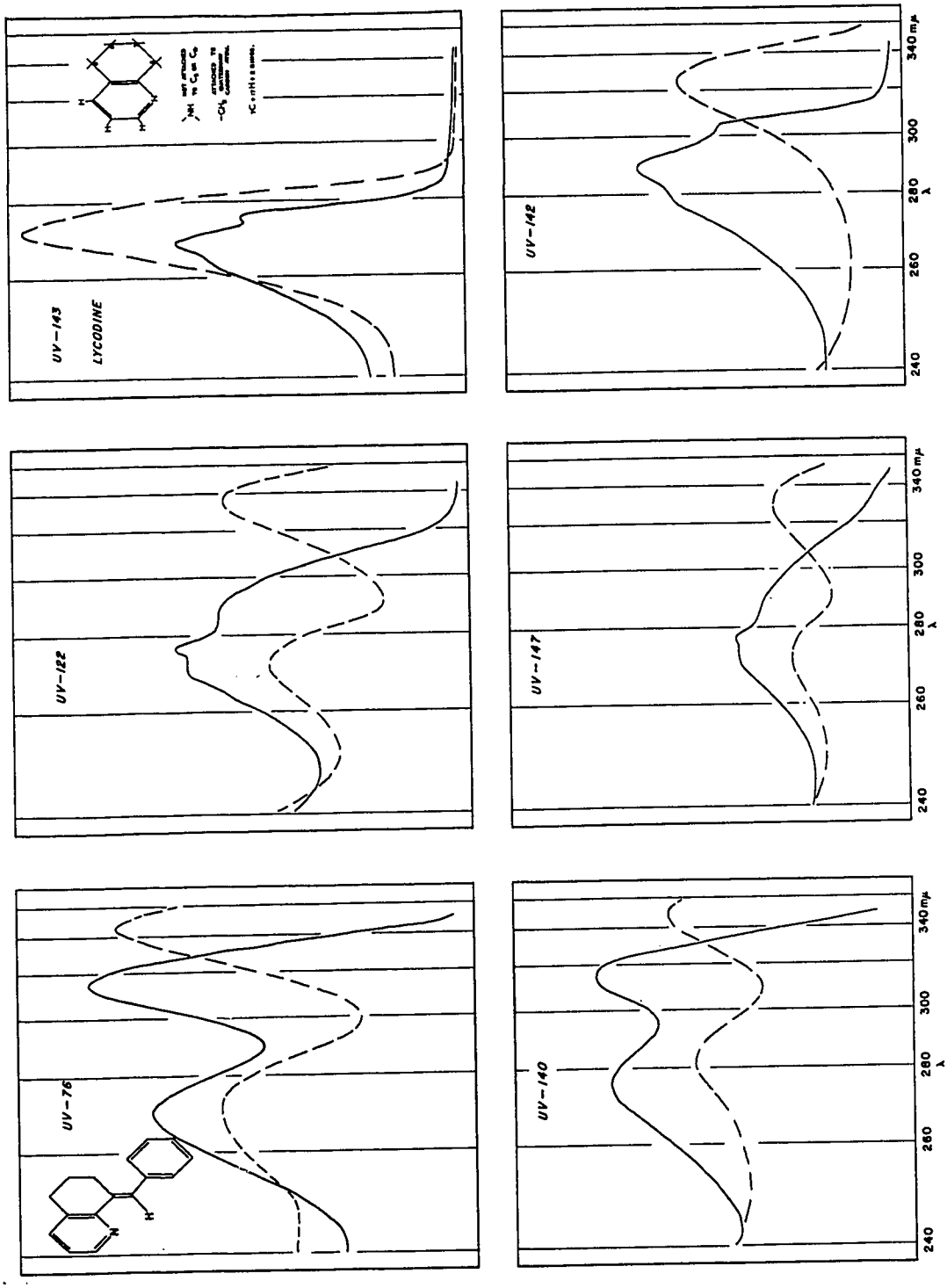
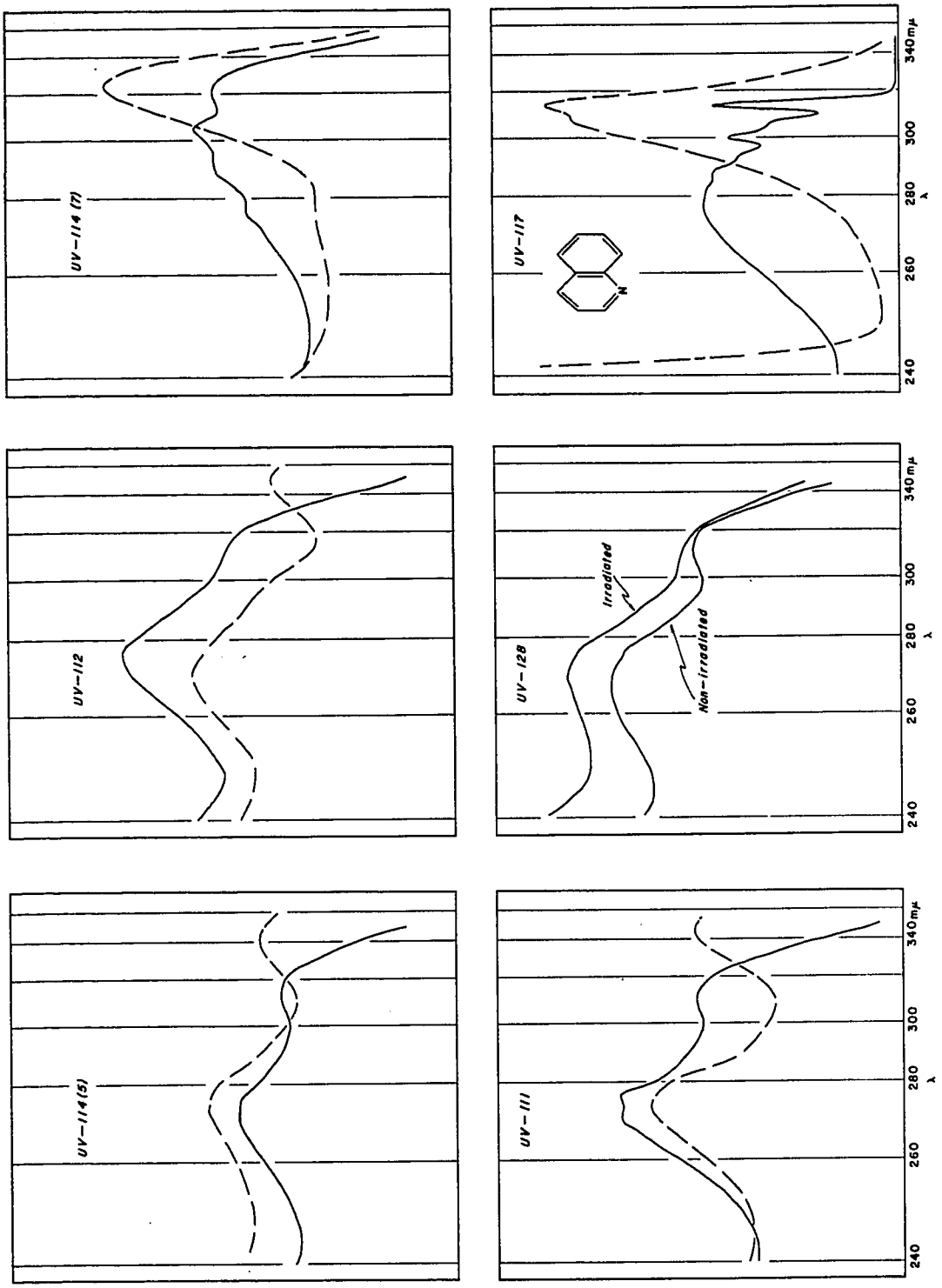

65.2
63.5
60.2
58.5
47.5
45.6
38.8
37.2
c/s
14.0
8.8
5.1
0
H
H
H
H
H
H
C-CH3
322.5
257.5
c/s
86.2
76.7
50.8
27.4
0
H

Figure 22 - Comparison of ultraviolet absorption spectra of lycodine, benzylidene derivative of 5,6,7,8-tetrahydroquinoline, quinoline and the reaction products in the attempted formation of a benzylidene derivative of lycodine. Solid lines indicate free bases. Dotted lines indicate salts.


UV-143
LYCODINE
UV-142
UV-122
UV-147
UV-76
UV-140
340mμ
300
280
λ
260
240


UV-114 (7)
UV-117
N
UV-112
UV-128
Irradiated
Non-irradiated
UV-114 (5)
UV-111
240
260
280
300
340mμ
λ