THE STRUCTURE OF LYCOPODINE

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By

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SCOPE AND CONTENTS:

A study has been made of the chemistry of lycopodine, the major alkaloid of several species of Lycopodium. From the information obtained, most of the peripheral structure of the lycopodine molecule can be deduced. A complete structure is proposed for the alkaloid.

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GENERAL INTRODUCTION

The first isolation of an alkaloid from a species of Lycopodium (club-moss) was reported nearly eighty years ago (1). It has only been within the last twenty years, however, that an extensive study has been made of the alkaloid content of plants of this genus. Altogether, over fifty Lycopodium alkaloids have now been isolated and characterized. Most of these have sixteen or eighteen carbon atoms and a single nitrogen atom, but there are a few which have seventeen carbons and two nitrogens. Interest in the elucidation of the structures of Lycopodium alkaloids has increased in recent years and detailed studies of several of the more abundant alkaloids have been undertaken. The first and, until now, the only complete structural determination was achieved by Wiesner et al. (2), who were able in 1957 to propose a structure for annotinine, $C_{16}H_{21}O_3N$, the major alkaloid of L. annotinum L.

The object of the investigation which will be described in this thesis was the elucidation of the structure of lycopodine, $C_{16}H_{25}ON$, the most widely distributed of the known Lycopodium alkaloids. Lycopodine has been found in ten of the thirteen plant species which have been examined and is the major alkaloid in at least six species.

Although lycopodine was first isolated in 1881 (1), its correct formula was not established until 1938 (3), and it is only since 1950 that the alkaloid has been known to possess a carbonyl group (4). However, by the time the present study was initiated, a considerable

amount had been learned about the chemistry of lycopodine (4-8). It is true that the previous work had not yielded much definite information on the structure of the alkaloid, but a number of derivatives had been obtained which could be used in further degradation experiments.

The experiments which were carried out during the present investigation were primarily designed to clarify the nitrogen - carbonyl relationship and establish part of the peripheral structure of the molecule. It was hoped that enough information could be gathered in this way to enable the rest of the structure to be deduced from dehydrogenation data. As the work progressed, it became clear that the difference between the structures of lycopodine and annotinine was not restricted to their functional groups, but extended to their carbon skeletons as well. Since lycopodine is much more widely distributed than annotinine among the Lycopodium species, it will not be surprising if the lycopodine structure proves to be more typical of the $C_{16}N$ alkaloids than the annotinine structure.

HISTORICAL INTRODUCTION

In 1881 Bödeker (1) reported the first isolation of an alkaloid from a Lycopodium species. From L. complanatum L. he obtained a crystalline base that melted at 114 - 115°, to which he assigned the formula $C_{32}H_{52}O_3N_2$ and the name lycopodine. Several years later, Arata and Canzoneri (9) extracted from L. saururus Lam. a base, $C_{15}H_{20}ON_2$, which they called pillijanine. However, perhaps because extracts of Lycopodium have little medicinal value and possess only moderate physiological activity, it wasn't until almost fifty years later that extensive efforts began to be made to isolate, characterize, and determine the structures of Lycopodium alkaloids.

In 1938 Achmatowicz and Uzieblo (3) examined the alkaloid content of L. clavatum L. and succeeded in separating three crystalline bases, the most abundant of which melted at 115 - 116°. Although the formula $C_{16}H_{25}ON$ which they assigned to the major alkaloid differs from that which Bödeker gave for lycopodine, the alkaloid was undoubtedly the one which he had isolated. Their formula for lycopodine was later confirmed by Manske and Marion (10). The other two alkaloids were assigned the formulae $C_{16}H_{25}O_2N$ and $C_{17}H_{27}O_2N$ and were named clavatine and clavatoxine, respectively.

In 1942 Deulofeu and DeLanghe (11) reported that they had examined L. saururus Lam. for alkaloids and had been unable to find any compound with the properties of the alkaloid pillijanine isolated previously from

that species by Arata and Canzoneri. Instead, they obtained two new bases which they designated saururine, $C_{10}H_{19}N$, and sauroxine, $C_{17}H_{26}ON_2$. In the same year Manske and Marion published the first paper of a series (4, 10, 12-22) on the isolation and structural investigation of Lycopodium alkaloids. In the first paper (10) they reported the isolation of lycopodine, nicotine, and six new alkaloids from L. complanatum L. (re-identified later (14) as L. flabelliforme Fern.). One of the other species which they examined subsequently was L. clavatum L. (16) of Canadian origin, from which they obtained lycopodine, nicotine, and three other alkaloids. However, they were unable to find either clavatine or clavatoxine, which Achmatowicz and Uzieblo had reported to be present in plants of that species which had been collected in Europe.

Altogether, Manske and Marion have studied the alkaloid content of ten species of Lycopodium and have been able to isolate over thirty bases. Other workers who have examined plant material of European (23-25) and West Indian (26) origin have made further additions to the list of known Lycopodium alkaloids. The species which has been studied most thoroughly is L. annotinum L. (13, 19, 23-25, 27-30). Among the approximately twenty bases which have been isolated are lycopodine, nicotine, annotinine ($C_{16}H_{21}O_3N$), acrifoline ($C_{16}H_{23}O_2N$), α-obscurine ($C_{17}H_{26}ON_2$), β-obscurine ($C_{17}H_{24}ON_2$), and lycodine ($C_{17}H_{24}N_2$).

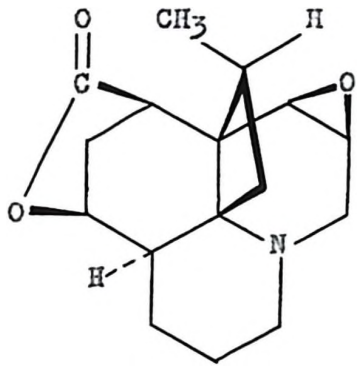
Of all the fifty or more alkaloids which have been characterized, lycopodine is the most widely distributed. It is the major alkaloid of at least six species and has been found in all the species of North American and European origin which have been examined. However, it has

been found in only one (22) of four species (11, 20, 22, 26) collected in the West Indies and the Southern Hemisphere.

Structural investigations have been carried out on six Lycopodium alkaloids: annotinine, lycopodine, acrifoline, lycodine, and α- and β-obscurine. Functional groups have been assigned to a number of minor alkaloids and some have been shown to be simple derivatives of lycopodine or other alkaloids. In the following sections there will be outlined the results of structural studies that were reported prior to or during the investigation which will be described in this thesis.

Annotinine

Annotinine is the only Lycopodium alkaloid whose structure has been definitely established. The correct structure, which is shown below, was first proposed in 1957 by Wiesner and co-workers (2) on the basis of chemical evidence. A few months later, Przybylska and Marion (31) reported the result of an X-ray crystallographic study on annotinine bromohydrin which confirmed the proposed structure. Their work also



verified the configurations previously assigned to most of the asymmetric centers and established the configuration at the methyl group, which was previously unknown. In 1958 Wiesner et al. (32) published a detailed

account of the chemistry of annotinine. A biogenetic scheme has been proposed for the alkaloid (33).

Lycopodine

Achmatowicz and Uzieblo (3) made the first attempt to determine the nature of the functional groups of lycopodine, $C_{16}H_{25}ON$. They found that the molecule contained no N-methyl or O-methyl groups and no active hydrogen. The alkaloid did not hydrogenate in the presence of palladium on charcoal and failed to react with reagents for the carbonyl group.

In 1942 Marion and Manske (12) reported the results of some degradation experiments on lycopodine. Dehydrogenation with selenium yielded a complex mixture from which they were able to isolate 7-methylquinoline, 5,7-dimethylquinoline, and three other bases. The unidentified bases analyzed for $C_{11}H_{15}N$, $C_{16}H_{19}ON$ and $C_{16}H_{21}N$. 7-Methylquinoline was also obtained when dehydrogenation was carried out with palladium - barium sulphate or phthalic anhydride. It was concluded that a reduced quinoline ring system was present in the lycopodine molecule. Since the alkaloid failed to react with phenyl magnesium bromide and was inert to high-pressure hydrogenation over Raney nickel, they suggested that the oxygen was in a cyclic ether function.

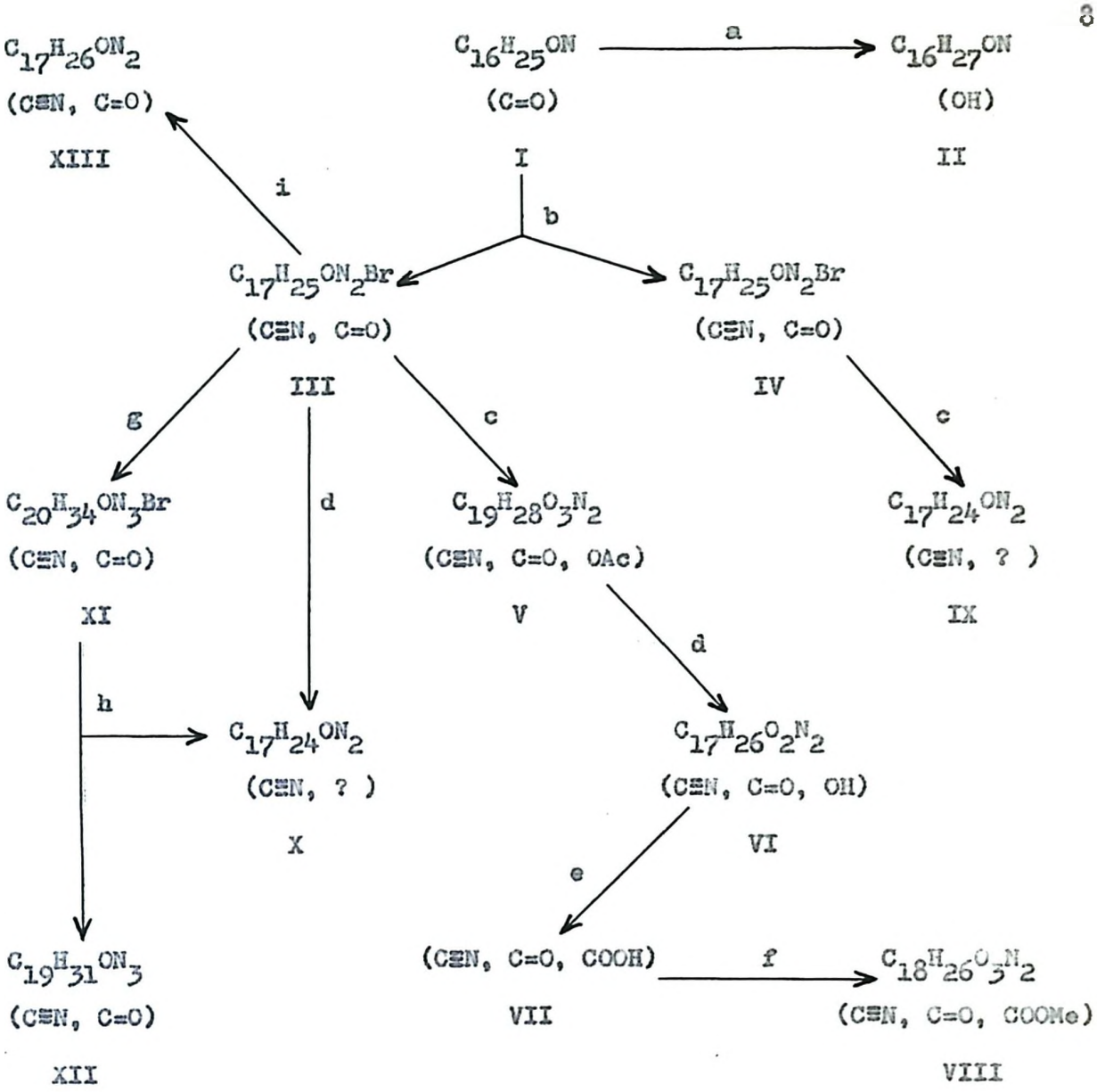
Eight years later MacLean, Manske and Marion (4) showed conclusively that the oxygen atom of lycopodine, I, was actually ketonic and not part of an ether ring. The first indication of the presence of a carbonyl group was provided by the alkaloid's infrared spectrum, which showed an absorption band near 1700 cm^{-1} in the region characteristic of the C=O stretching absorption. The presence of the carbonyl group

was confirmed by preparation of a hydrazone, reduction of lycopodine I to dihydrolycopodine II, and formation of a tertiary alcohol on treatment of I with phenyl lithium.

MacLean, Manske and Marion reported that attempts to degrade the alkaloid through the N-oxide or by the Hofmann and Emde degradations had been unsuccessful. However, they found that reaction of the alkaloid with cyanogen bromide yielded two isomeric ring scission products. These were designated α- and β-cyanobromolycopodine, III and IV, $C_{17}H_{25}ON_2Br$. They found that III, the more abundant isomer, could be converted to α-cyanoacetoxylycopodine V by treatment with potassium acetate in boiling ethanol. Alkaline hydrolysis of V gave α-cyanohydroxylycopodine VI, which on chromic acid oxidation yielded a non-crystalline carboxylic acid VII. Analysis of VII as its crystalline methyl ester VIII indicated that no loss of carbon had occurred in the oxidation. By this reaction sequence they showed that III was a primary bromide and therefore must have formed from lycopodine by cleavage between the nitrogen atom and a methylene group.

When β-cyanobromolycopodine IV was treated with boiling ethanolic potassium acetate, the bromine was not replaced by an acetoxyl group. Instead, elimination of the elements of hydrogen bromide occurred to give a product IX, $C_{17}H_{24}ON_2$, which was inert to both oxidation and hydrogenation. An isomeric product X was obtained when III was heated under reflux with potassium hydroxide in methanol. Since these compounds gave no evidence of unsaturation, it was concluded that the loss of hydrogen bromide had in both cases occurred in a cyclization reaction.

MacLean, Manske and Marion prepared a quaternary bromide XI by



a. $LiAlH_4$

b. BrCN

c. KOAc in boiling EtOH

d. KOH in boiling MeOH

e. CrO_3 in AcOH

f. CH_2N_2

g. $(CH_3)_3N$

h. 1. Ag_2O 2. pyrolysis

i. H_2, $Pd\text{-}CaCO_3$, KOH in MeOH

Figure 1: Reactions of α- and β-Cyanobromolycopodine (MacLean, Mansko and Marion, 1950)

treatment of α-cyanobromolycopodine III with trimethylamine and attempted to carry out a Hofmann elimination on it. However, instead of giving an olefin, the reaction yielded cyclized compound X and a base XII which formed from the quaternary hydroxide by loss of methanol.

It was found that reduction of III with hydrogen and palladium - calcium carbonate catalyst in alkaline medium yielded α-cyanolycopodine XIII. Although they reported that IV underwent a similar hydrogenolysis to β-cyanolycopodine, it has since been shown (6) that the product formed under the conditions described is actually cyclized compound IX.

The reactions which were studied by MacLean, Manske and Marion are summarized in Figure 1.

Douglas, Lewis and Marion (21) prepared anhydrodihydrolycopodine XIV, $C_{16}H_{25}N$, and acetyldihydrolycopodine XV, $C_{18}H_{29}O_2N$, and found that they were identical with Lycopodium alkaloids L.14 and L.2 respectively. XIV was prepared from dihydrolycopodine II by dehydration with phosphorus pentachloride, and XV was obtained by acetylation of II with acetic acid and trifluoroacetic anhydride. They were able to convert lycopodine to an oxime, but the oxime failed to undergo a Beckmann rearrangement under the usual conditions. An attempt to prepare a benzylidene derivative of lycopodine was unsuccessful.

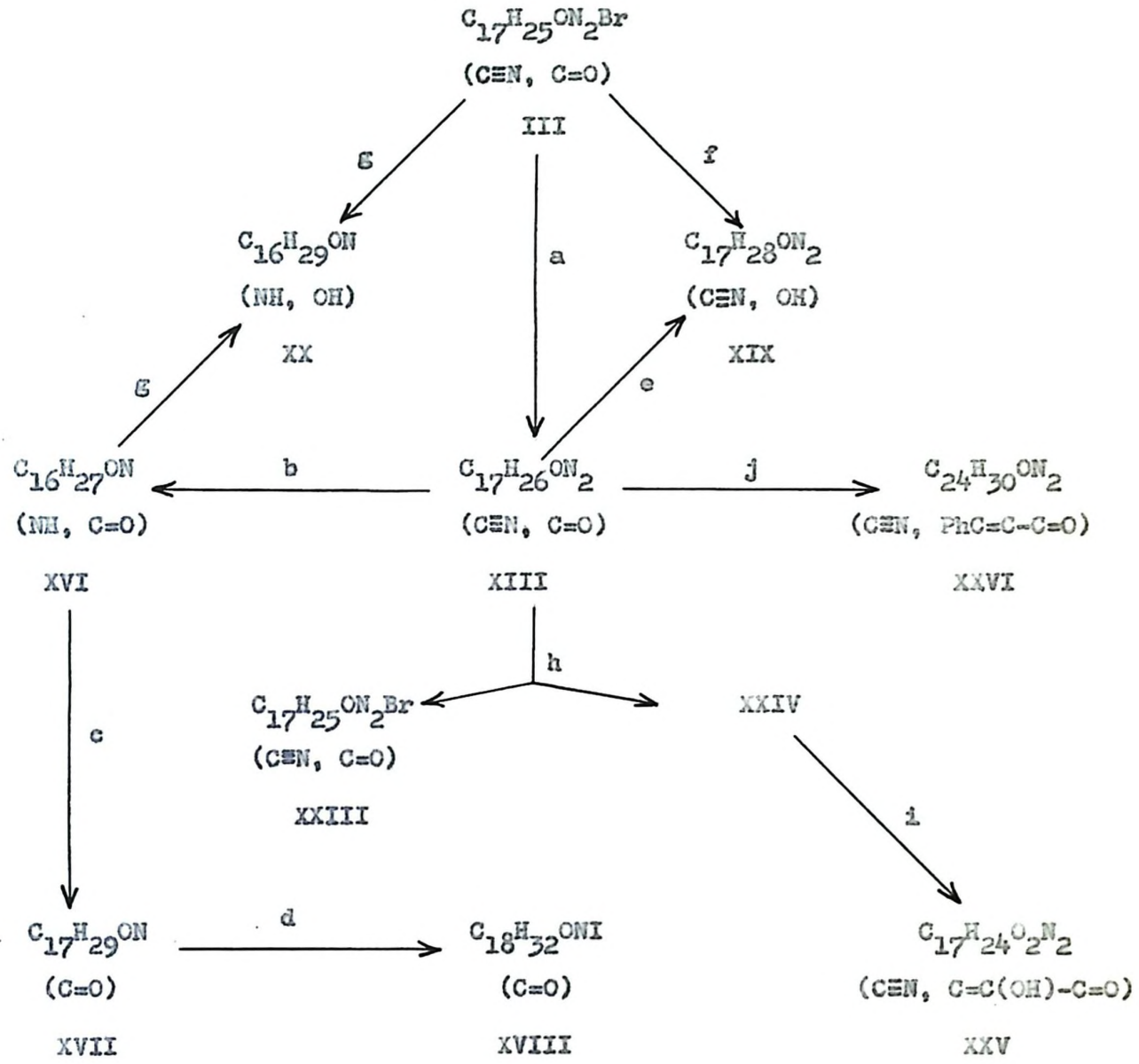
Barclay and MacLean (5) continued the investigation of the chemistry of α-cyanobromolycopodine which had been initiated by MacLean, Manske and Marion. A study of the reactions of α-cyanolycopodine XIII was made in an attempt to learn more about the ring structure of lycopodine. Acid hydrolysis of XIII yielded the secondary base XVI, $C_{16}H_{27}ON$, which could be methylated to the tertiary base XVII with formic

acid and formaldehyde. Conversion of XVII to its methiodide XVIII proceeded in such poor yield, however, that XVIII could not be used for extensive degradation experiments. A reductive fission of XVIII was attempted on a small scale, but only unreacted methiodide was recovered.

The Meerwein-Ponndorf reduction of XIII gave α-cyanodihydrolycopodine XIX, which was also obtained by the reduction of bromocyanamide III with sodium borohydride. Reduction of III with lithium aluminum hydride yielded a product XX which was identical with the hydride reduction product of the secondary base XVI.

Barclay and MacLean treated lycopodine I and the secondary base XVI with bromine in carbon tetrachloride and obtained the monobrominated bases, XXI and XXII, respectively. The pK_a values of XXI and XXII were found to be considerably lower than those of I and XVI. Since the bromine undoubtedly substituted at a position adjacent to the carbonyl group, the decreased basicity suggested that the carbonyl group was relatively close to the nitrogen atom. However, the accuracy of the pK_a measurements of XXI and XXII is open to question because of the rapidity with which these compounds decompose in alkali. On the basis of the similar pK_a values obtained for I and XVI it was suggested that the ring fission which takes place in the formation of α-cyanobromolycopodine III does not alter the carbonyl - nitrogen relationship.

Bromination of α-cyanolycopodine XIII gave some crystalline monobrominated derivative XXIII, but the bulk of the product was amorphous material XXIV which could not be induced to crystallize. Treatment of XXIV with aqueous alkali gave a crystalline alkali-soluble product XXV, $C_{17}H_{24}O_2N_2$, whose infrared and ultraviolet spectra were



a. H_2, Pd-$CaCO_3$, KOH in MeOH

b. 2N HCl under reflux

c. HCHO, HCOOH

d. CH_3I under reflux

e. Al(O-i-Pr)$_3$ in boiling i-PrOH

f. $NaBH_4$

g. $LiAlH_4$

h. Br_2 in CCl_4

i. 5% KOH in aq. dioxane

j. C_6H_5CHO, NaOMe in MeOH

Figure 2: Reactions of α-Cyanolycopodine XIII (Barclay and MacLean, 1956)

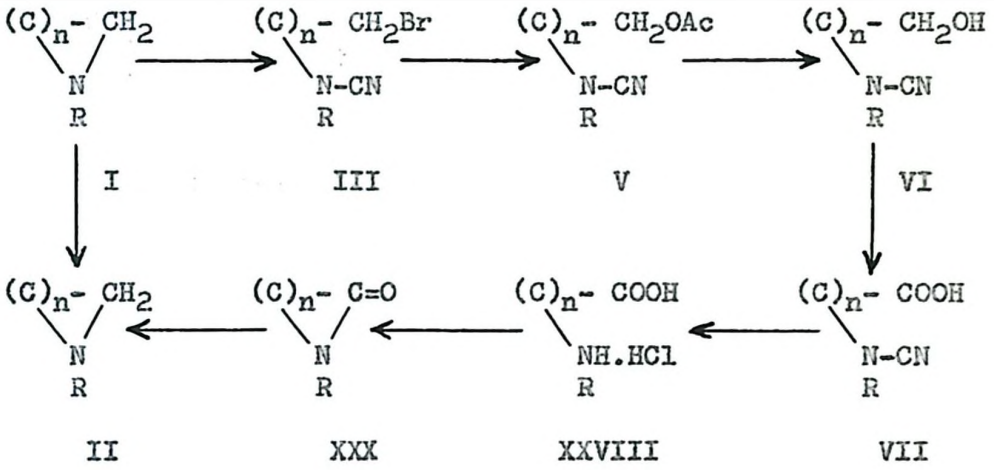
consistent with an α-diketone in the enol form. Isolation of the enol XXV indicated that XXIV contained a dibrominated product in which two hydrogens on a methylene group alpha to the carbonyl had been replaced by bromine. The presence of a methylene group adjacent to the carbonyl group was confirmed by conversion of α-cyanolycopodine XIII to its benzylidene derivative XXVI. Although Barclay and MacLean, like Douglas et al. (21), were unable to obtain a benzylidene derivative of lycopodine, it is nevertheless clear that the active methylene group of XIII must also be present in lycopodine. Barclay and MacLean suggested that the methylene group is in a hindered position in the alkaloid and that the hindrance is relieved by the ring fission which takes place in the formation of XIII.

The reactions which Barclay and MacLean reported are summarized in Figure 2.

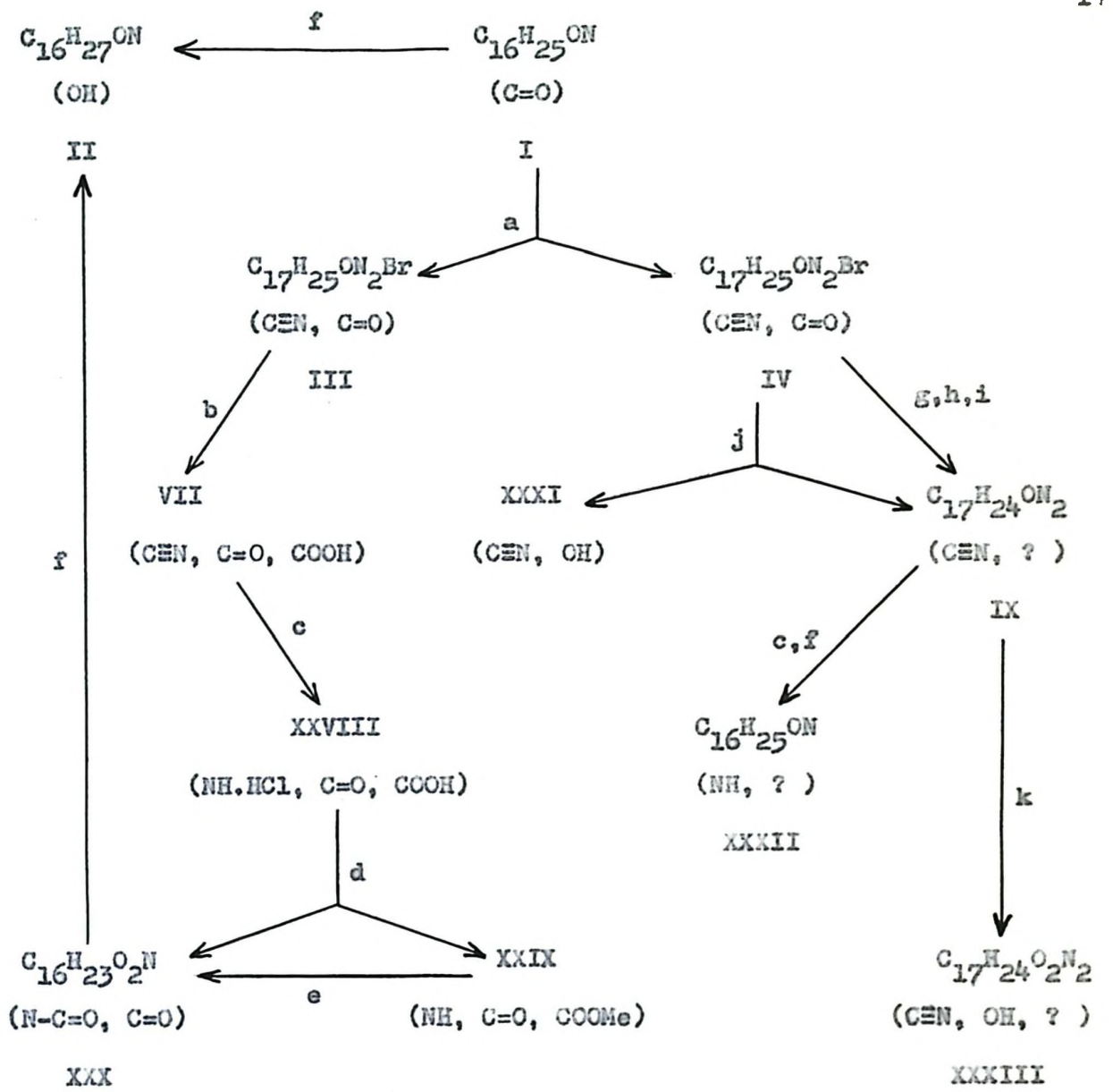
The reactions of the benzylidene derivative XXVI have been studied by Barclay (7). From the ozonolysis of XXVI he obtained an enolic product XXVII which was apparently not identical with the enol XXV obtained previously from the bromination of XIII. The formation of the two isomeric enols seemed to suggest that the carbonyl group of lycopodine was flanked on both sides by methylene groups.

The reaction sequence by which MacLean, Manske and Marion obtained the carboxylic acid VII from bromocyanamide III was extended by Harrison (6). Acid VII was hydrolyzed with hydrochloric acid to the amino acid hydrochloride XXVIII, which was converted to a mixture of an amino ester XXIX and a lactam XXX by treatment with diazomethane. The infrared spectrum of XXX showed strong absorption at 1635 cm^{-1}, indicating

that the lactam ring was six-membered or larger. Since XXX gave dihydrolycopodine II on reduction with lithium aluminum hydride, it can be concluded that the lactam ring corresponds to the ring opened in the formation of III and that the latter is also six-membered or larger. The reaction sequence can be formulated as follows:



An attempt was made by Harrison to convert bromocyanamide IV to β-cyanohydroxylycopodine. It was hoped that the oxidation of this alcohol, if it could be obtained, would reveal something about the structure of the ring opened in the formation of IV. MacLean, Manske and Marion had already found that the replacement of the bromine atom of IV by hydroxyl could not be accomplished by the method which had been successful in the case of bromocyanamide III. Treatment of IV with ethanolic potassium acetate in the first step of the procedure had resulted in the formation of cyclized compound IX instead of an acetate. Harrison found that treatment of IV with silver acetate in boiling benzene and alkaline hydrolysis of the crude product gave a low yield of a non-crystalline alcohol XXXI, which was apparently β-cyanohydroxy-



a. BrCN

b. 1. KOAc in boiling EtOH
2. KOH in boiling MeOH
3. CrO_3 in AcOH

c. 2N HCl under reflux

d. CH_2N_2

e. boiling xylene

f. $LiAlH_4$

g. KOAc in boiling EtOH

h. KOH in boiling MeOH

i. H_2, Pd-$CaCO_3$, KOH in MeOH

j. 1. AgOAc in boiling C_6H_6
2. KOH in boiling MeOH

k. NaOBr

Figure 3: Cyclized Compound IX and Lactam XXX (Harrison, 1957)

lycopodine, but the major product was still the cyclized compound IX. The amount of XXXI available at that time was considered to be insufficient for further study.

The hydrogenolysis of bromocyanamide IV was re-examined and it was found that the conditions described by MacLean, Manske and Marion gave the cyclized compound IX and not β-cyanolycopodine, as had been reported. Several attempts to alter the course of the reaction were unsuccessful.

Harrison made a study of compound IX in an attempt to discover the nature of the cyclization which had taken place, but was unable to decide whether IX was an enol ether or a ketone. The choice lay between a ketone which was inert to hydride reduction and an enol ether which was remarkably stable to acid hydrolysis. Both reduction with lithium aluminum hydride and prolonged heating with hydrochloric acid converted IX to the secondary base XXXII, $C_{16}H_{25}ON$. The infrared spectrum of XXXII showed that it contained the same oxygen function as IX. The only other derivative of IX which Harrison was able to obtain was a hydroxy compound XXXIII, which formed when IX was treated with sodium hypobromite.

The reactions studied by Harrison are summarized in Figure 3.

By this time considerable progress had been made toward the complete elucidation of the structure of annotinine. The relative positions of the functional groups had been determined and several complete structures had been proposed. Since both annotinine and lycopodine contain sixteen carbon atoms and a single nitrogen it is reasonable to expect that they will have a close structural relationship.

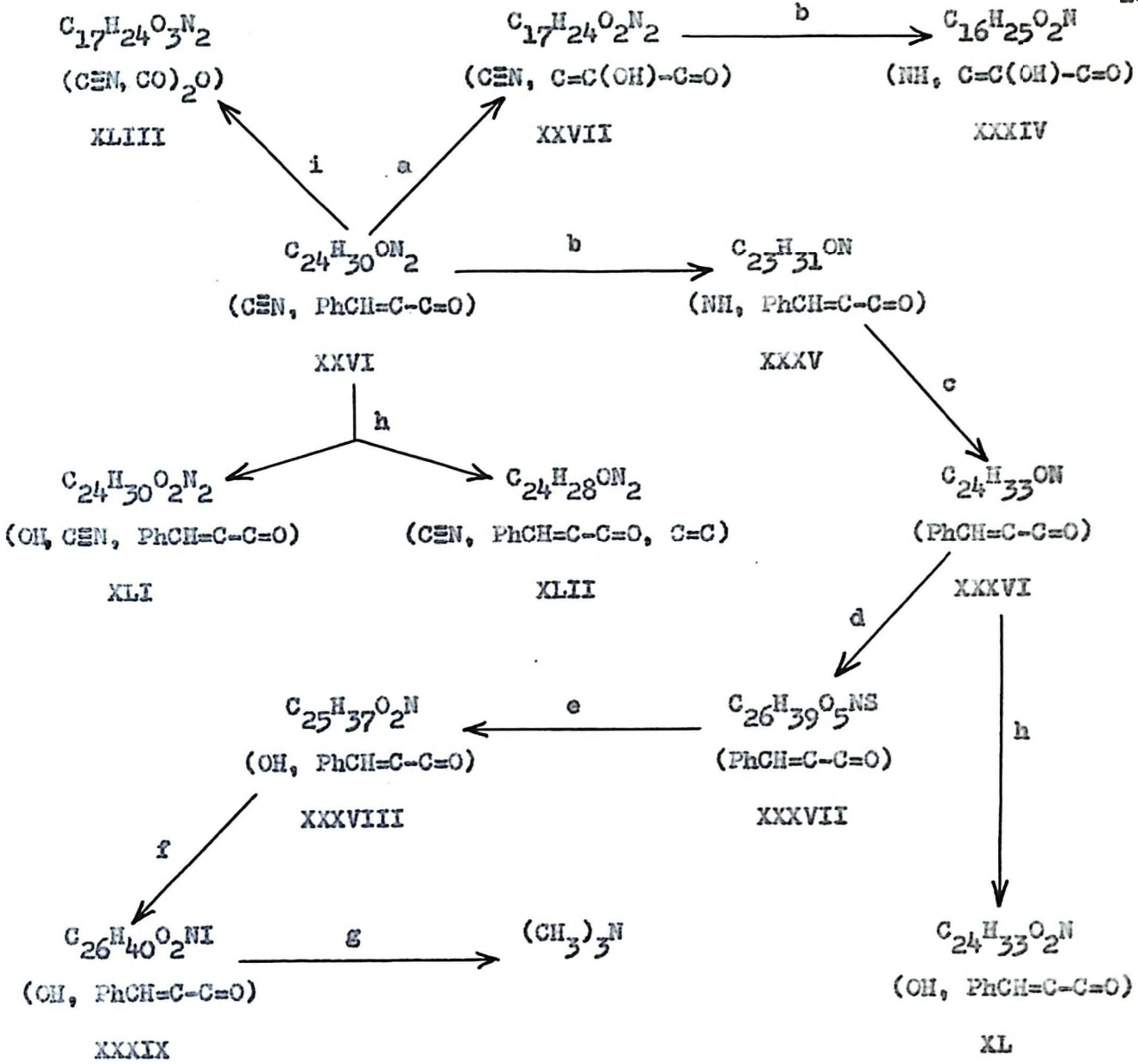
Harrison therefore reviewed the chemistry of lycopodine to see whether it might be accounted for by a structure which had an annotinine carbon skeleton and placed the carbonyl group in the position of one of the potential hydroxyl groups of annotinine. The isolation of the two enols XXV and XXVII by Barclay suggested that the carbonyl group was flanked on both sides by methylene groups, and therefore it seemed that a structure for I could be derived from the annotinine skeleton only if the carbonyl were placed at the second carbon from the nitrogen. Harrison felt that this β-piperidone model was worth considering and attempted to use it to formulate the reactions of lycopodine. However, although the structure is consistent with some of the reactions and accounts for the results of the basicity determinations of Barclay and MacLean, it requires an anomalous course for the reaction of the alkaloid with cyanogen bromide.

Barclay (7) soon presented spectral evidence that ruled out a β-piperidone structure of the type suggested by Harrison. This structure for I would require that the enol XXVII have either a carbonyl group or a double bond adjacent to the nitrogen, but the infrared and ultraviolet spectra of XXVII did not provide support for either arrangement. The spectra of enol XXV were very much like those of enol XXVII. Furthermore, if the β-piperidone structure were correct, removal of the cyanide function of XXVII by hydrolysis should give either a lactam or a vinyl amine. Barclay found that hydrolysis of XXVII gave a base XXXIV whose infrared spectrum was inconsistent with a vinyl amine structure.

Barclay investigated a number of reactions of compound XXVI, the

benzylidene derivative of α-cyanolycopodine XIII. Acid hydrolysis of XXVI gave the secondary base XXXV, $C_{24}H_{33}ON$, which yielded the N-methyl tertiary base XXXVI when treated with formic acid and formaldehyde. Base XXXVI failed to form a methiodide. However, by prolonged heating with dimethyl sulphate in benzene, XXXVI was converted to a crystalline compound XXXVII which analyzed for $C_{26}H_{39}O_5NS$. When an aqueous solution of XXXVII was treated with ammonia, an amorphous base XXXVIII was precipitated. The base XXXVIII, which seemed to be a mixture, analyzed poorly for $C_{25}H_{37}O_2N$ and gave some evidence of possessing a hydroxyl function. Base XXXVIII readily formed a methiodide XXXIX, $C_{26}H_{40}O_2NI$, which yielded trimethylamine on Hofmann degradation. The formation of trimethylamine indicated that two N-methylations and two carbon - nitrogen cleavages had taken place in the reaction sequence following the preparation of the tertiary base XXXVI. It was therefore concluded that the reaction of XXXVI with dimethyl sulphate had resulted in a bond fission rather than quaternary salt formation.

Oxidation of the base XXXVI with selenium dioxide gave a hydroxy compound XL. The infrared spectrum of XL favoured a structure with the hydroxyl group adjacent and axial to the carbonyl group. Reaction of the neutral compound XXVI with selenium dioxide yielded two oxidation products: a hydroxy compound XLI and an olefinic compound XLII. The infrared spectrum of the former suggested that the hydroxyl, like the hydroxyl of XL, was alpha and axial to the carbonyl group. The ultraviolet spectrum of XLII was consistent with a structure in which the new double bond was conjugated with the carbonyl group. The partial structures which were proposed for compounds XL, XLI and XLII can be



a. O_3 in MeOH

b. 2M HCl under reflux

c. HCHO, HCOOH

d. $(CH_3)_2SO_4$ in boiling C_6H_6

e. NH_4OH

f. CH_3I

g. 1. Ag_2O 2. pyrolysis

h. SeO_2

i. O_3 in EtOAc

Figure 4: Reactions of the Benzylidene Derivative XXVI (Barclay, 1957)

written as follows:

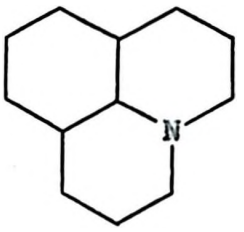
XL, XLI XLII

Failure of compound XL to undergo Oppenauer oxidation indicated that the hydroxyl function might be tertiary. This evidence for a branch at a carbon alpha to the carbonyl group conflicts with the explanation offered for the formation of enols XXV and XXVII, which requires that there be methylene groups on both sides of the carbonyl.

Barclay found that the product which was formed on ozonolysis of benzylidene derivative XXVI depended on the solvent in which the ozonization was carried out. When the solvent was methanol, the product was enol XXVII, as mentioned before, but when ethyl acetate was used, the product was a cyclic acid anhydride XLIII.

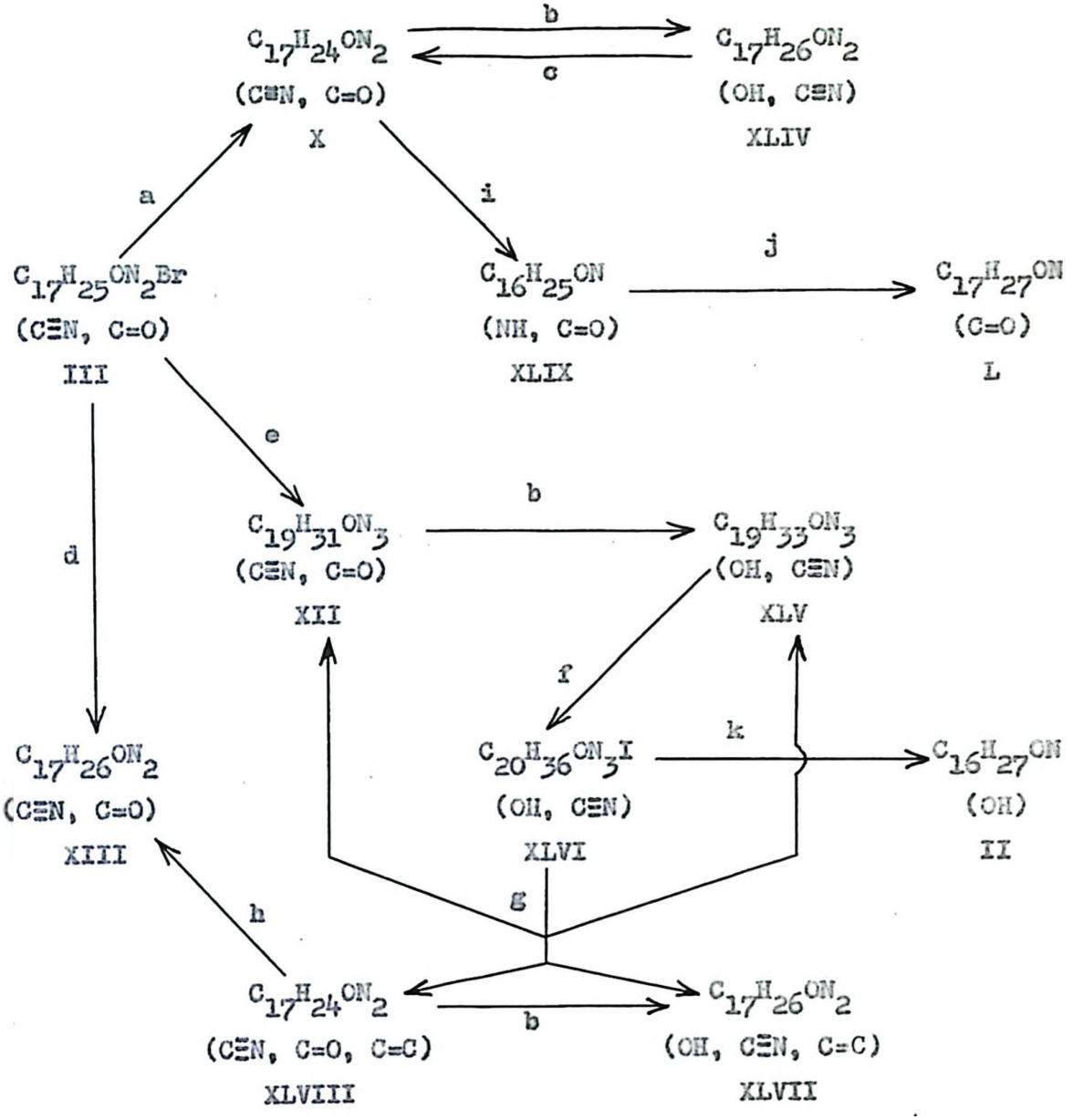
The reactions of compound XXVI are summarized in Figure 4.

Barclay dehydrogenated the secondary base XX (5) with palladium - charcoal and obtained a low yield of material whose ultraviolet spectrum resembled the spectra of quinoline and its homologues. This indicated that compound XX might contain a reduced quinoline nucleus. Since Harrison (6) had found that the ring which had been opened in the formation of XX was six-membered or possibly larger, Barclay suggested that lycopodine might contain a hexahydrojulolidine ring system (Structure A).



A

The formation of compound X by the action of boiling methanolic potassium hydroxide on α-cyanobromolycopodine III has already been described. Compound X is also one of the products obtained when a Hofmann degradation of the trimethylammonium bromide XI (Figure 1) is attempted. These reactions were first reported by MacLean, Manske and Marion (4), who suggested that compound X was the product of a cyclization reaction. Song (8) made a further study of compound X and its mode of formation. Compound X, unlike the isomeric compound IX studied by Harrison (6), readily reduced with sodium borohydride to a secondary alcohol XLIV, and the possibility that cyclization had occurred by O-alkylation was thus immediately ruled out. It was found that X, unlike lycopodine I, did not react with phenyl lithium and, unlike α-cyanolycopodine XIII, failed to yield a benzylidene derivative. These results indicate that the carbonyl group is more hindered in X than in I and that the reactive methylene group of XIII is either not present in compound X or is more hindered. Song concluded that in the formation of X cyclization had taken place at a position alpha to the carbonyl group. Although failure of X to form a benzylidene derivative suggested that the cyclization had occurred at the known methylene group, a definite assignment is not justified on the basis of this evidence alone because lycopodine, which obviously contains this methylene group,



a. KOH in boiling MeOH

b. $NaBH_4$

c. CrO_3-pyridine

d. H_2, Pd-$CaCO_3$, KOH in MeOH

e. Me_2NH

f. MeI

g. t-BuOK in boiling t-BuOH

h. H_2, PtO_2

i. 2M HCl under reflux

j. HCHO, HCOOH

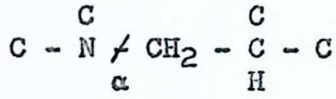
k. 30% NaOH

Figure 5: Dehydrobromination of Bromocyanamide III (Song, 1958)

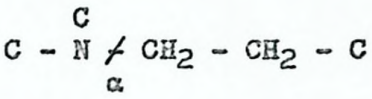
does not form a benzylidene derivative either (21).

Further evidence was provided for cyclization alpha to the carbonyl when it was shown that the Hofmann degradation, which had given X when attempted with the ketonic quaternary bromide XI, would proceed normally when the carbonyl group was absent. Song used the following method to obtain the olefin (XLVIII) expected from a normal dehydrobromination of III. Bromocyanamide III was first converted to the dimethylamino compound XII by treatment with dimethylamine. Reduction of the carbonyl group of XII with sodium borohydride gave the dimethylamino alcohol XLV, which readily reacted with methyl iodide to form the quaternary salt XLVI. Decomposition XLVI with potassium tertiary butoxide yielded a mixture from which two basic demethylation products, XII and XLV, and two neutral products, XLVII and XLVIII, were isolated. The infrared spectrum of XLVII, $C_{17}H_{26}ON_2$, showed olefin and hydroxyl absorption, while that of XLVIII, $C_{17}H_{24}ON_2$, showed olefin and carbonyl absorption. Compound XLVIII gave XLVII on reduction with sodium borohydride. Both compounds yielded formaldehyde on ozonolysis, indicating that the double bond was in a terminal position as expected. Catalytic hydrogenation of the keto-olefin XLVIII to α-cyanolycopodine XIII showed that no skeletal rearrangements had occurred during the elimination reaction. Song concluded that olefin XLVII had formed from XLVI by a normal Hofmann elimination. It was suggested that formation of the ketonic demethylation product XII and the keto-olefin XLVIII had resulted from oxidation catalyzed by potassium tertiary butoxide. Olefin XLVIII is the product which MacLean, Manske and Marion had tried unsuccessfully to obtain by Hofmann degradation of XI.

Song carried out oxidations of olefins XLVII and XLVIII in alkaline permanganate and was unable to isolate any significant quantity of acid product. This suggested that there might be a branch at the second carbon from the nitrogen in lycopodine (Structure B). However,



B



C

Song later found that oxidation of the olefins in acidic permanganate yielded a carboxylic acid (34). This acid was not fully characterized, but the yield of crude acid was sufficient to provide a strong indication of the presence of a methylene group beta to the nitrogen (Structure C).

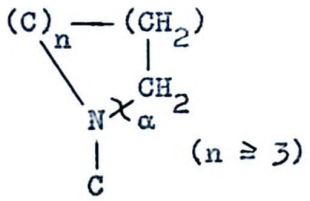
Song hydrolyzed cyclized compound X to the secondary base XLIX and carried out an N-methylation of XLIX with formic acid and formaldehyde. The resulting tertiary base L failed to form a methiodide. In this respect compound L resembled the analogous non-cyclized base XVII prepared by Barclay and MacLean (5), which formed a methiodide only in very low yield.

The reactions studied by Song are summarized in Figure 5.

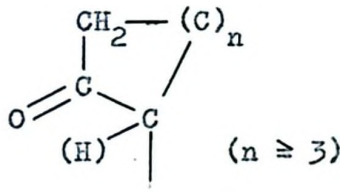
The investigations which have been reported so far allow only a few structural assignments to be made. The fact that the structure of annotinine is known, however, provides a basis for further speculation than would be justified by the chemical evidence alone.

It has been demonstrated (4) that the major product III from the reaction of lycopodine with cyanogen bromide is formed by cleavage between the nitrogen and a methylene group. The ring which is opened

in this way has been shown to be six-membered or perhaps larger (6) and it is probable that there is a methylene group beta to the nitrogen in the direction of cleavage (Structure D). The infrared spectrum of lycopodine indicates that the carbonyl group is in a six-membered ring or larger. The presence of a methylene group on one side of the carbonyl is known with certainty (5), and although evidence relating to the substituents on the carbon atom on the other side is conflicting, the presence of at least a third alpha hydrogen seems certain (Structure E).

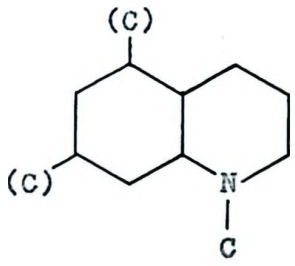


D



E

From the dehydrogenation experiments of Marion and Manske (12) it can be concluded that lycopodine contains a reduced quinoline ring system, possibly with substituents at the positions indicated (Structure F).



F

Barclay's dehydrogenation experiment on the 'α' base XX gave some evidence that a potential quinoline nucleus was still present, even though a nitrogen - carbon bond had been cleaved. Since it was known that

the ring which had opened was six-membered or larger, Barclay (7) suggested that the hexahydrojulolidine ring system could be used as a partial structure for the alkaloid (Structure A). The presence of the same ring system in annotinine makes this model particularly attractive. Analogy with the annotinine structure would suggest that the carbonyl group is most likely to be in the carbocyclic ring of A and gamma to the nitrogen.

α- and β-Obscurine

Moore and Marion (27) have carried out an investigation of the dinitrogen alkaloids α- and β-obscurine. The infrared spectrum of α-obscurine, $C_{17}H_{26}ON_2$, shows absorption in the NH region and carbonyl absorption at 1667 cm^{-1}. Since only one of the nitrogen atoms is basic, it can be assumed that the other is present in an amide function. Dehydrogenation of the alkaloid yielded 7-methylquinoline and 6-methyl-α-pyridone. Moore and Marion felt that the infrared and ultraviolet spectra of the alkaloid were most consistent with a five-membered, α,β-unsaturated lactam and suggested that the pyridone obtained on dehydrogenation resulted from a ring expansion. They noted that the two dehydrogenation products accounted for all but one of the carbon atoms in the molecule and concluded that the other carbon was probably present as part of the fourth ring demanded by the molecular formula.

The infrared and ultraviolet spectra of β-obscurine, $C_{17}H_{24}ON_2$, indicate the presence of an α-pyridone ring. The infrared spectrum also shows NH absorption. Since Anet and Eves (29) have found that β-obscurine cannot be acetylated, it can be concluded that the basic nitrogen is tertiary.

Lycodine

Lycodine, $C_{17}H_{24}N_2$, was first isolated from L. annotinum L. by Anet and Eves (29) in 1958. The nuclear magnetic resonance spectrum of the alkaloid indicates that it is a 2,3-disubstituted pyridine. The other nitrogen is basic and secondary. The molecule contains a single methyl group which appears to be attached to a quaternary carbon, since the NMR methyl peak is not split.

Acrifoline

Perry and MacLean (28) showed that acrifoline, $C_{16}H_{23}O_2N$, possesses a carbonyl group, a hydroxyl group, and one double bond. It follows that the alkaloid has a tetracyclic structure. Acetylation of the hydroxyl group was found to give another Lycopodium alkaloid, L.12, which is found in L. annotinum.

Acrifoline has been subjected to an extensive structural investigation by MacLean and French (35). They have found that the double bond is in a gamma-delta position relative to the nitrogen atom and that the hydroxyl group is either gamma or delta to the carbonyl group.

DISCUSSION OF RESULTS

When this study on the structure of lycopodine was undertaken a large amount of experimental data was already available and some tentative conclusions could be drawn about the ring structure and position of the functional groups. Previous investigators (3, 4) had shown that lycopodine, $C_{16}H_{25}ON$, contained a ketonic carbonyl group and a tertiary nitrogen which was shared by at least two rings. Since there were no other functional groups present, it followed that the molecule was tetracyclic. It had been found that there was a methylene group next to the nitrogen (4) and that the carbonyl group was flanked on at least one side by a methylene group (5). The presence of a reduced quinoline nucleus had been strongly suggested by dehydrogenation results (12) and there was some indication that lycopodine might, like annotinine (2, 31, 32), contain a reduced julolidine ring system (7). If the alkaloid did in fact contain a hexahydrojulolidine nucleus, there was little likelihood that the carbonyl group was in either of the nitrogen-containing rings (7).

In order to advance toward solution of the complete structure it was first necessary to obtain more detailed information on the structure of the rings containing the functional groups. It was believed that this could best be accomplished by extending the studies which had already been made (4-8) of α- and β-cyanobromolycopodine, III and IV, and their derivatives. Ultimately there would remain the problem of locating and elucidating the structure of the fourth ring

of lycopodine, but there was a possibility that establishment of the structure of three of the rings would leave only one structure for the fourth which would be consistent with dehydrogenation results and biogenetic considerations.

The reactions of α-cyanobromolycopodine III, $C_{17}H_{25}ON_2Br$, had been studied quite extensively and several compounds had been prepared which could be used in investigations on the structure of the ring opened in the formation of III. The benzylidene derivative XXVI of α-cyanolycopodine XIII (5) provided a convenient starting point for a study of structure in the vicinity of the carbonyl group. The ring opened in the formation of β-cyanobromolycopodine IV, however, presented a greater problem. Nothing definite had been learned so far about the structure of this ring. The methods which had been employed to convert III to useful derivatives such as α-cyanolycopodine XIII and α-cyano-acetoxylycopodine V (4) could not be applied to IV because of the ease with which it underwent cyclization to IX, $C_{17}H_{24}ON_2$, under the alkaline conditions of these reactions. As a result, in contrast to the thirty or more non-cyclized compounds which had been derived from bromocyanamide III, there had been only one non-cyclized compound prepared from IV. This was the non-crystalline alcohol XXXI, which the author had obtained in low yield by hydrolysis of the crude product of the reaction of IV with silver acetate (6). The ready formation of cyclized compound IX suggested a relatively close relationship of the nitrogen and carbonyl group in the direction of the 'β'-cleavage, and it was felt that very important information on this relationship could be gathered if non-cyclized 'β' derivatives could be prepared in sufficient quantity. It

did not appear that this information could be obtained from a study of cyclized compound IX alone. The author had previously worked with this remarkably unreactive compound and had been unable to obtain enough evidence even to show definitely whether it had a ketone or an enol ether structure (6). Another approach to the problem of the second nitrogen-containing ring would be to effect a 'β' cleavage by Hofmann degradation of the quaternary salt of a suitable base in the 'α' series. This, however, had been attempted previously by Barclay (5, 7) and Song (8) on both cyclized and non-cyclized derivatives of III, with little or no success. The major difficulty encountered here was in the preparation of the quaternary salts.

In the following discussion the rings opened in the formation of α- and β-cyanobromolycopodine will be referred to as rings A and B, respectively. The ring containing the carbonyl group will be called ring C and the fourth ring, D.

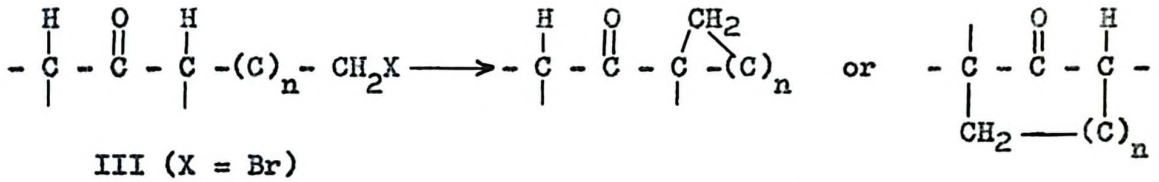
I. Position of the Carbonyl Group Relative to Nitrogen

It has been suspected for some time that the carbonyl group of lycopodine is situated fairly close to the nitrogen atom. For reasons which have already been discussed in the Historical Introduction it appears that this close relationship is unaffected by the carbon - nitrogen bond cleavages which occur in the formation of bromocyanamides III and IV. The fact that III and IV undergo cyclization reactions which involve the carbonyl group (4, 6, 8) puts some limitations on the distance of the carbonyl from nitrogen around the peripheries of rings A and B, but it does not allow a definite assignment to be made. The

experiments described in the following sections were performed in an attempt to learn more about the carbonyl - nitrogen relationship.

Carbonyl - Nitrogen Relationship Around Ring A

The cyclization of III (or XI) to X, which has been shown to occur by intramolecular alkylation at a carbon alpha to the carbonyl group (8), can be formulated in the following manner.

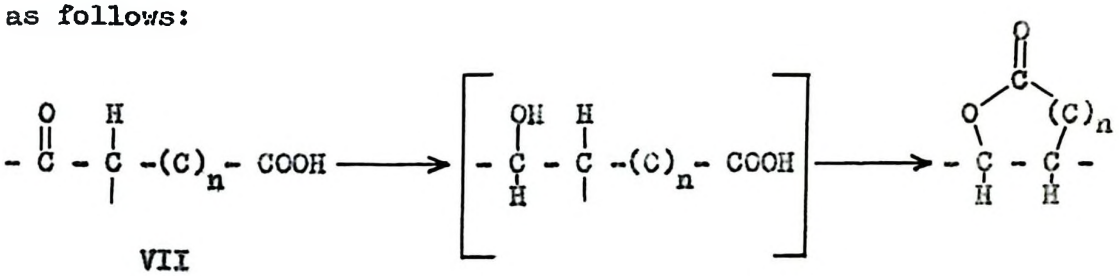


III (X = Br)

XI (X = $N(CH_3)_3Br$)

X

Structures for lycopodine which place the carbonyl group at the fourth carbon from nitrogen around the periphery of ring A (i.e., n = 1 in the formulae above) can be ruled out because the infrared spectrum of X is consistent with neither a cyclopropyl ketone nor a cyclopentanone structure. Therefore, the formation of X indicates that the carbonyl group is most likely to be at the fifth, sixth or seventh carbon. If the carbonyl group is at the fifth carbon, reduction of the keto acid VII derived from III should give a hydroxy acid which can readily cyclize to a six-membered lactone. The reaction which would occur can be depicted as follows:



VII

An attempt was made to carry out this lactonization. The sample of VII used was prepared from bromocyanamide III by the usual reaction sequence (4, 6). It was found that reduction of VII with sodium borohydride gave a hydroxy acid LI which could not be induced to lactonize. It is probable therefore that the carbonyl function of I is at least six carbon atoms away from the nitrogen in the direction of the 'α' cleavage.

Alcohol XXXI

Several years ago (6) a non-crystalline alcohol XXXI was prepared in a low yield by alkaline hydrolysis of the crude product of the reaction of bromocyanamide IV with silver acetate. The infrared spectrum of XXXI was distinctly different from that of α-cyanohydroxylycopodine VI in the 'fingerprint' region and it was concluded that XXXI was β-cyanohydroxylycopodine. In order to establish whether XXXI was a primary, secondary or tertiary alcohol, it was decided to subject it to a chromic acid oxidation. If the product turned out to be an acid, it would then be possible to obtain information on the carbonyl - nitrogen relationship around the periphery of ring B by reducing the keto acid and attempting a lactonization.

It was first necessary to obtain a supply of alcohol XXXI. The reaction of IV with silver acetate and hydrolysis of the resulting mixture of acetate LII and cyclized compound IX gave only about a 20% yield of the alcohol. Although a sufficient quantity of XXXI could have been prepared by this method, we were reluctant to use our small stockpile of IV for this purpose. Bromocyanamide IV is formed in only about 13% yield in the von Braun (cyanogen bromide) reaction and because of the

difficulty in separating it completely from III the actual yield of pure IV is not much more than 5%. It was hoped to reserve most of this material for attempts to prepare β-cyanolycopodine and other non-cyclized compounds which could not be derived from XXXI. Fortunately, an alternative source of alcohol XXXI was found.

The presence of alcohol VI in the crude product mixture from the von Braun reaction of lycopodine was demonstrated some time ago (5). Before an attempt is made to separate bromocyanamides III and IV by fractional crystallization, alcohol VI is removed from the mixture by chromatography. It was observed on using a longer column than usual to chromatograph the product of one reaction that the dark band which contained the alcohol split slightly as it neared the bottom of the column. The infrared spectrum of the more strongly absorbed portion of the band was almost identical with that of alcohol XXXI. It was found that reduction of the alcohol band with sodium borohydride gave a mixture which was separated more efficiently by chromatography. There was still a certain amount of band overlapping, but the reduction products, unlike VI and XXXI, crystallized readily and final purification was effected by recrystallization from acetone. The major product LIII, $C_{17}H_{28}O_2N_2$, was found to be identical with the diol obtained by sodium borohydride reduction of VI. The more strongly absorbed isomeric compound LIV proved to be identical with the borohydride reduction product of XXXI. Oppenauer oxidation of LIV gave a crystalline sample of alcohol XXXI, $C_{17}H_{26}O_2N_2$.

The yield of XXXI from the von Braun reaction was only about 1%, which was similar to the over-all yield obtained when it was prepared from the alkaloid by way of IV and the silver acetate reaction. However,

the alcohol fractions had been saved from most of the von Braun reactions which had been carried out previously, and these provided enough XXXI and LIV to perform the experiments described in the following section.

Carbonyl - Nitrogen Relationship Around Ring B

Chromic acid oxidation of either keto alcohol XXXI or diol LIV yielded the non-crystalline keto acid LV, which was characterized as its crystalline methyl ester LVI, $C_{18}H_{26}O_3N_2$. Formation of the acid showed that XXXI was a primary alcohol. It is evident that the 'β' cleavage of lycopodine, like the 'α', takes place between the nitrogen atom and a methylene group.

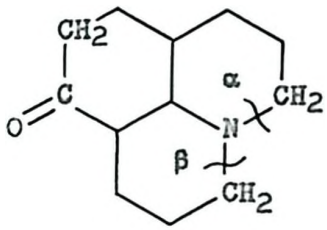
Reduction of acid LV with sodium borohydride yielded a lactone LVII, $C_{17}H_{24}O_2N_2$. No hydroxy acid was isolated. The infrared spectrum of a carbon tetrachloride solution of the lactone showed a strong band at 1761 cm^{-1}, which was attributed to the stretching vibration of the lactone carbonyl group. When the spectrum was determined in nujol the band appeared at 1743 cm^{-1}. The ranges which are usually quoted for the carbonyl absorption of five-membered and six-membered lactones are 1760 - 1780 cm^{-1} and 1735 - 1750 cm^{-1}, respectively. Although the band observed in the solution spectrum of LVII is within the five-membered lactone range, the fact that six-membered lactones have been known to absorb as high as 1790 cm^{-1} (36) forbids a definite assignment of ring size. One can conclude with certainty only that the carbonyl group of lycopodine is at either the fourth or fifth carbon from nitrogen around the periphery of ring B.

It should be mentioned that lactone LVII was actually encountered

for the first time in the product of an early attempt to convert acid VII to a lactone. The alcohol VI used to prepare the acid had been obtained by treating a mixture of III and IV with ethanolic potassium acetate and hydrolyzing the crude product with potassium hydroxide in methanol. The resulting mixture of VI and IX had been separated by chromatography. Reduction of crude acid VII with sodium borohydride gave, in addition to the hydroxy acid LI, a low yield of lactone LVII. It was thought at first that the lactone was an 'α' derivative. This erroneous conclusion has been cited by Song (8). It is not known whether the XXXI which contaminated the sample of VI arose from incomplete cyclization of IV to IX, or whether it was already present in the original bromocyanamide mixture.

A Partial Structure for Lycopodine

The results of the lactonization experiments show that the carbonyl group of lycopodine is at the fourth or fifth carbon from nitrogen in the direction of the 'β' cleavage and probably at the sixth or seventh carbon in the direction of the 'α' cleavage. The results can be accommodated by the partial structure G, which employs the hexahydrojulolidine ring system found in annotinine. Structure G shows the



G

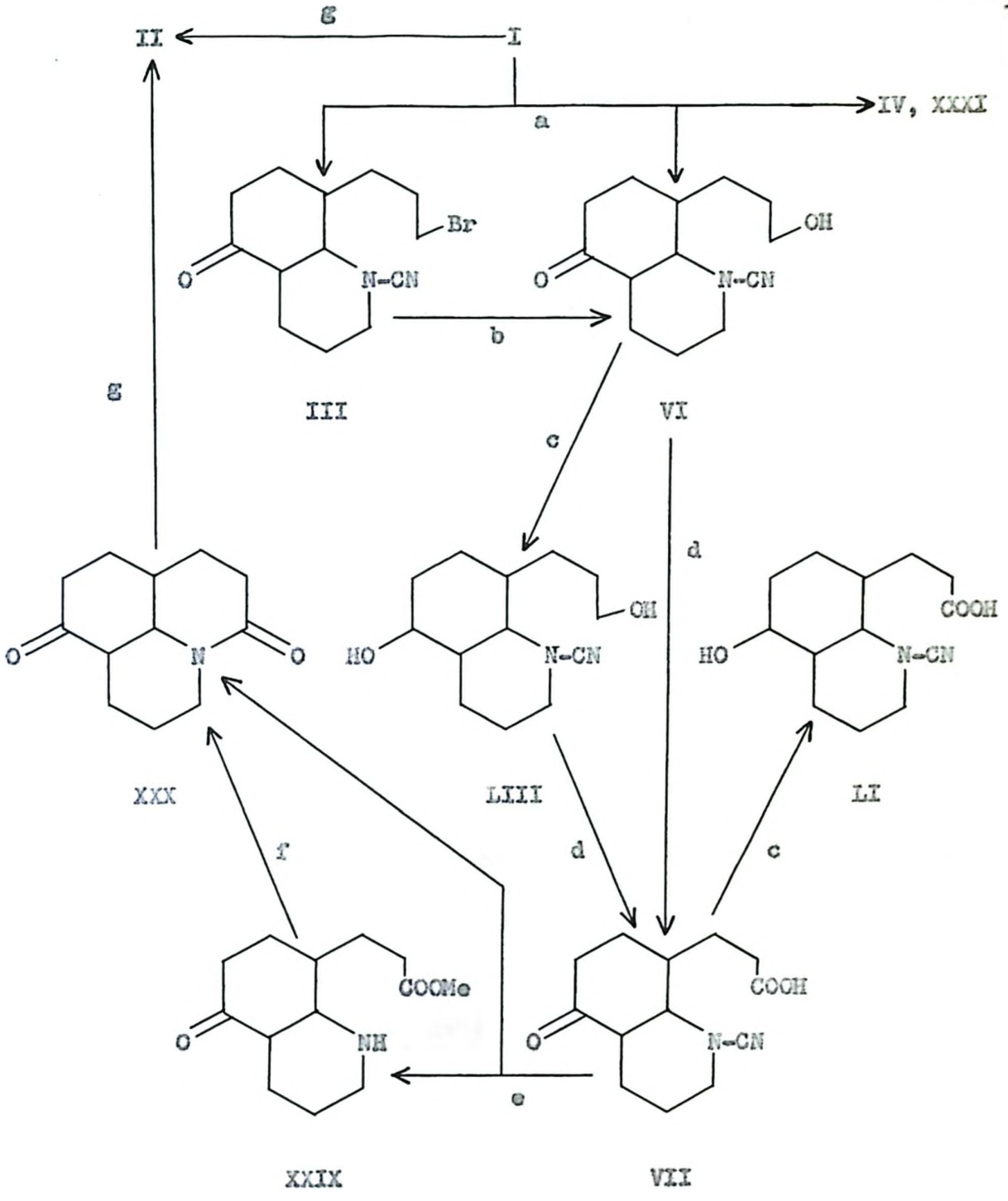
four carbon atoms which are known with certainty. It is probable that

there is a methylene group beta to the nitrogen in the direction of the 'α' cleavage (34).

The hexahydrojulolidine ring system has been suggested previously as a model for lycopodine (7), but because of the lack of chemical evidence, it was not possible to assign a position to the carbonyl group and relate it to the sites of 'α' and 'β' cleavage.

In order to accept structure G as a possible model for lycopodine it is necessary to discard the explanation offered previously for the formation of the two enols XXV and XXVII (6, 7), which required that the carbonyl group be flanked on either side by methylene groups. Some justification for doing this is provided by the failure of the hydroxyl group of compound XL to oxidize (7). (Compound XL is the selenium dioxide oxidation product of the tertiary base XXXVI obtained from the benzylidene derivative XXVI of α-cyanolycopodine XIII. It is believed that the hydroxyl group is alpha to the carbonyl.) However, the most direct and conclusive evidence on this matter has been furnished by MacLean and Carson (37), who have recently carried out deuterium exchange studies on lycopodine and several of its derivatives. They found that lycopodine exchanged three hydrogen atoms for deuterium when treated with sodium methoxide in deuteromethanol. Since the hydrogens which exchange are those which can take part in enolate ion formation, this result is in agreement with structure G and rules out the possibility of a second methylene group alpha to the carbonyl.

Although G is the structure which is suggested most readily by the results of the lactonization experiments and Barclay's dehydrogenations (7), it is not the only solution which is consistent with the chemical



a. BrCN

b. 1. KOAc in boiling EtOH
   2. KOH in boiling MeOH

c. $NaBH_4$

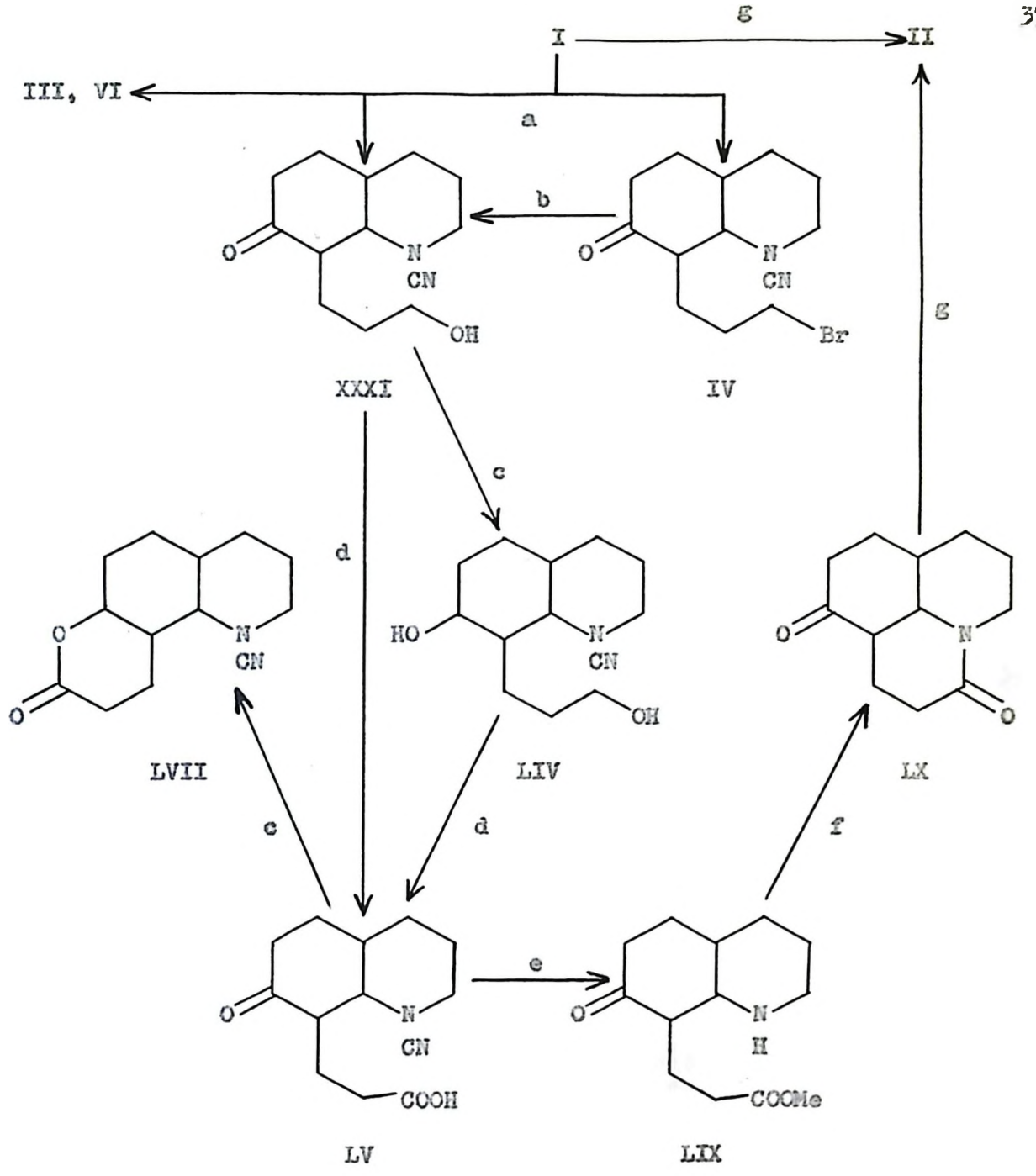
d. CrO_3 in AcOH

e. 1. 2M HCl under reflux
   2. CH_2N_2

f. xylene under reflux

g. $LiAlH_4$

Figure 6: Lactamization and Lactonization Experiments ('α' Series)



a. BrCN

b. 1. AgOAc in boiling C_6H_6
   2. KOH in boiling MeOH

c. $NaBH_4$

d. CrO_3 in AcOH

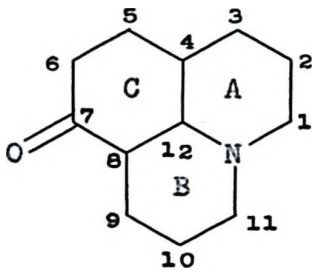
e. 1. 2M HCl under reflux
   2. CH_2N_2

f. xylene under reflux

g. $LiAlH_4$

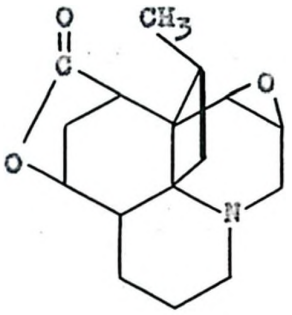
Figure 7: Lactamization and Lactonization Experiments ('β' Series)

data. However, G is promoted to a class by itself among the contenders by the fact that it has a ring structure which has already been shown to be present in a Lycopodium alkaloid. Another attractive feature of this structure is that it places the carbonyl group in a position corresponding to that of the lactonized hydroxyl function of annotinine. Structure G will therefore be adopted as a proposed partial structure for lycopodine and will be used frequently in discussing results and formulating reactions. The carbon atoms of G will be numbered as shown in formula H. The reactions which have already been discussed in connection with the lactonization experiments are formulated in Figures 6 and 7.

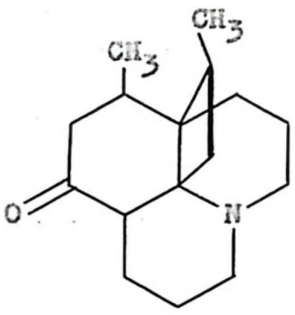


H

Since the experimental evidence which has been obtained is completely consistent with a structure closely related to part of the annotinine molecule, one might be tempted to predict that lycopodine has the same carbon skeleton as annotinine (J) and that the difference lies entirely in their functional groups. If this were true, lycopodine would have two C-methyl groups, as shown in formula K. However, it has been found that lycopodine analyzes for only one C-methyl group. It must be concluded that K does not represent the structure of lycopodine and that the two alkaloids differ not only in their functional groups,



J

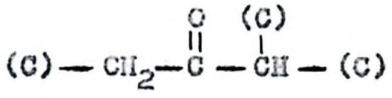


K

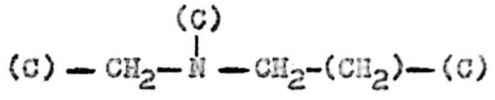
but in their carbon skeletons as well. It may be that the differences are limited to ring D and the methyl group, but it is possible that partial structure G should be revised.

II. The Peripheral Structure

So far in this discussion six of the sixteen carbon atoms of lycopodine have been accounted for, not counting the one in the methyl group. These are arranged in two groups centered at the carbonyl and nitrogen functions, as illustrated by partial structures M and N.



M



N

The lactonization experiments which were described in Part I gave enough information to allow the proposal of a partial structure (G) relating the two fragments. Further experiments have been performed in an attempt to determine the nature of additional carbon atoms and establish the carbonyl - nitrogen relationship with greater certainty. The investigations centered around the carbon atoms numbered 3, 5, 9

and 10 in the proposed model.

It was decided that the most convenient way to establish the nature of C-3 would be to carry out a modified Kuhn-Roth (chromic acid) oxidation on α-cyanolycopodine XIII or α-cyanodihydrolycopodine XIX and identify the monocarboxylic acids which formed. This method of determining the length of alkyl side-chains is much more rapid than carbon-by-carbon degradation and requires only a small amount of material. If there is no branch at C-3, then XIII (or XIX) will possess an n-propyl side-chain and should therefore give n-butyric acid as one of the oxidation products.

In order to complete a link between the functional groups, establishment of the structures of C-9 and C-10 was particularly desirable. Here again the best approach seemed to be the modified Kuhn-Roth oxidation procedure. However, the problem of obtaining a suitable non-cyclized derivative of IV had to be solved first. A considerable portion of the following discussion will therefore be devoted to the chemistry of IV. Before starting the investigation of the carbon atoms in ring B, it was decided to check the size of this ring by converting acid LV to a lactam by the procedure used previously with the corresponding acid VII of the 'α' series. The infrared spectrum of the lactam was expected to reveal whether the ring was five-membered or larger.

It was hoped that information could be gathered on the structure at C-5 from oxidation studies on the benzylidene compound XXVI and its derivatives.

Size of Ring B

The ultraviolet spectrum of the crude dehydrogenation product of base XX was reported by Barclay (7) to bear a strong resemblance to spectra of quinoline and its derivatives. On the basis of this observation he proposed that ring B was part of a potential quinoline nucleus. However, since none of the dehydrogenation products had been identified, and also because it was possible that their formation might have resulted from skeletal rearrangements, it was thought that some independent evidence on the size of ring B was desirable. It was decided to employ the method which had been used previously to obtain information on the size of ring A (6). This would require hydrolysis of acid LV to an amino acid and conversion of the latter to a lactam. The estimate of ring size would be based on the frequency at which the lactam carbonyl stretching absorption occurred in the infrared spectrum. The absorption normally occurs at 1700 - 1750 cm^{-1} for five-membered lactams and at 1630 - 1670 cm^{-1} for six-membered lactams or larger.

In order to perform the lactamization experiment it was once again necessary to obtain a supply of alcohol XXXI or diol LIV. The source used for the lactonization experiments, the alcohol fractions accumulated from a number of von Braun reactions of I, had been exhausted. It was found that a mixture of diols LIII and LIV could be obtained by carrying out a von Braun reaction on acetyldihydrolycopodine XV, treating the crude product with potassium acetate, and hydrolyzing the resulting mixture of diacetates with potassium hydroxide. The diols were separated by chromatography and fractional crystallization, as usual. The acetyldihydrolycopodine XV was prepared from dihydrolycopodine II by

the procedure described by Douglas, Lewis and Marion (21). The over-all yields of diols LIII and LIV from II were about 37% and 4%, respectively.

A sample of acid LV which had been prepared by the oxidation of diol LIV was converted to its methyl ester LVI and purified. Hydrolysis of LVI with hydrochloric acid gave a crude amino acid hydrochloride LVIII which yielded the amino ester LIX on treatment with diazomethane. When the crude amino ester was heated in boiling xylene, it was slowly converted to a lactam LX, $C_{16}H_{23}O_2N$. The infrared spectrum of a chloroform solution of LX showed strong bands at 1709 and 1622 cm^{-1}, which were attributed to ketone carbonyl and lactam carbonyl absorption, respectively. The position of the lactam band indicated that the ring was six-membered or larger. In order to be certain that ring B had been re-formed correctly by the lactamization procedure, lactam LX was reduced with lithium aluminum hydride. The product was found to be identical with dihydrolycopodine II prepared by the reduction of I. One can conclude that ring B of lycopodine is six-membered or possibly larger.

The reactions which were carried out in the lactamization experiment are formulated in Figure 7.

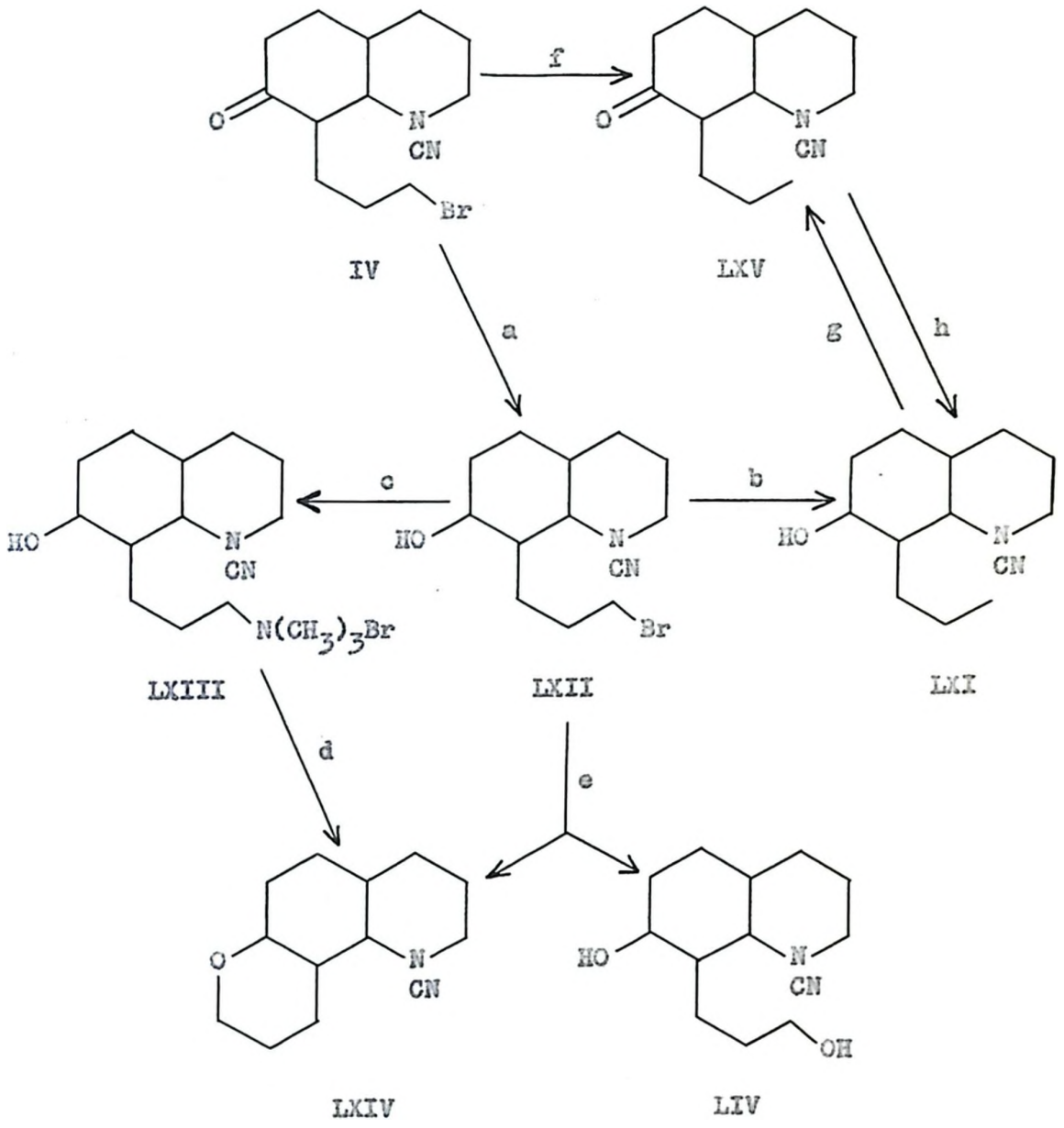
Non-cyclized Reduction Products of Bromocyanamide IV

It now became clear that before any more information could be gathered about the structure of ring B it would be necessary to prepare new non-cyclized derivatives of IV. Alcohol XXXI had been subjected to an investigation comparable to that made of alcohol VI and had yielded all the information that might be reasonably expected from it. Elucidation of the structure at C-9 and C-10 through studies based on the known derivatives of IV did not seem feasible. Certainly, the

amounts of XXXI and LIV which were available were too small to allow the possibility of a carbon-by-carbon degradation of the chain through acid LV to be seriously considered.

The studies that had been made of bromocyanamide III suggested two compounds which, if they were prepared, might be useful. One of these was the hydroxy olefin corresponding to XLVII. However, Song (8) had found that the method used to prepare XLVII from III could not be applied to IV because the first step in the procedure, treatment of the bromocyanamide with dimethylamine, resulted in cyclization. The second as yet unknown compound was β-cyanolycopodine. Although α-cyanolycopodine XIII had been used up to this time almost exclusively as a starting material for studies of ring C, it was also an obvious candidate for a modified Kuhn-Roth oxidation, as was pointed out earlier. It was felt that information about C-9 and C-10 might be obtained from a similar oxidation of β-cyanolycopodine. Several years before, MacLean, Manske and Marion (4) had reported that β-cyanolycopodine could be prepared by treatment of an alkaline methanol solution of IV with hydrogen and palladium - calcium carbonate catalyst at room temperature. However, a subsequent investigation showed that the conditions described actually result in almost complete conversion of IV to the cyclized compound IX (6).

Sodium borohydride reduction is one of several reactions that has been used to convert mixtures of III and IV to useful products which can be separated easily. These 'chemical separations' are all based on the fact that IV is cyclized in alkaline media much more rapidly than III. In the case of borohydride reduction, the chief



a. $NaBH_4$ (0°)

b. H_2, Pd-$CaCO_3$, KOH in MeOH (20°)

c. $(CH_3)_3N$ in MeOH

d. KO-t-Bu in boiling t-BuOH

e. 1. KOAc in boiling EtOH
   2. KOH in boiling MeOH

f. H_2, Pd-$CaCO_3$, KOH in MeOH (-60°)

g. $Al(O\text{-}i\text{-}Pr)_3$, acetone in boiling C_6H_6

h. $NaBH_4$

Figure 8: Non-cyclized Derivatives of Bromocyanamide IV

products are α-cyanodihydrolycopodine XIX and cyclized compound IX, which can be separated readily by chromatography (6, 7). During the present investigation the author had occasion to use this reaction as a source of XIX. When the product mixture was chromatographed, it was observed that the band containing alcohol XIX was followed very closely down the column by another, weaker band. The second band yielded a crystalline alcohol LXI which had never been isolated before. It was thought that LXI might be β-cyanodihydrolycopodine.

A sample of pure bromocyanamide IV was reduced with sodium borohydride to see whether any alcohol LXI would be formed. The reaction was carried out by slowly adding an ethanol solution of borohydride to an ice-cooled ethanol solution of IV. It was hoped that the low temperature and inverse addition would keep cyclization to a minimum. The infrared spectrum of the crude product showed that indeed very little cyclized compound IX had formed, and that reduction of the carbonyl group had been almost complete. The product which was isolated was not LXI, however, but the hydroxy bromide LXII, $C_{17}H_{27}ON_2Br$. Hydrogenolysis of LXII gave a virtually quantitative yield of LXI, $C_{17}H_{28}ON_2$, which could now be confidently designated β-cyanodihydrolycopodine.

Treatment with trimethylamine converted the hydroxy bromide LXII to a quaternary salt LXIII, $C_{20}H_{36}ON_3Br$. This compound, it will be noted, is analogous to quaternary salt XLVI derived from III, from whose Hofmann degradation Song obtained the hydroxy olefin XLVII. However, the product which was isolated after decomposition of quaternary salt LXIII with potassium tertiary butoxide was not an olefin, but

a cyclic ether LXIV, $C_{17}H_{26}ON_2$.

In another experiment, hydroxy bromide LXII was treated with potassium acetate in boiling ethanol. It was expected that hydrolysis of the crude product of this reaction would give diol LIV. However, although some diol was obtained, the yield was only about 22%. The major product, formed in 65% yield, was cyclic ether LXIV.

The results which had been obtained by altering the conditions used for the borohydride reduction of IV encouraged another attempt to perform the hydrogenolysis of IV. It was found that the formation of cyclized compound IX could be prevented by cooling the hydrogenolysis mixture with dry ice during the first hour or two of reduction and avoiding a large excess of alkali in the mixture. In this manner a virtually quantitative yield of β-cyanolycopodine LXV, $C_{17}H_{26}ON_2$, was obtained. Compound LXV was also prepared by the Oppenauer oxidation of LXI. Sodium borohydride reduction of LXV gave alcohol LXI.

The reactions of bromocyanamide IV which have been described in this section are formulated in Figure 8.

Chromic Acid Oxidation of I, XIX and LXI

The Kuhn-Roth procedure has been used for many years for the quantitative determination of terminal methyl groups in organic molecules (38). The analysis is carried out by oxidizing a sample of compound under reflux with a mixture of chromic acid and sulphuric acid. After the alkyl groups have been oxidized as completely as possible to acetic acid, the acetic acid is distilled from the mixture and titrated with standard alkali. Actually, if there are alkyl groups

larger than methyl present, the intermediate oxidation products will include volatile fatty acids other than acetic and, presumably, certain amounts of these will survive further oxidation. However, whether they are completely oxidized to acetic acid or not, the titration result will not be affected.

A very convenient method of determining the length of alkyl side-chains is provided by a modification of the Kuhn-Roth procedure. In this case the primary object is not to determine the total yield of volatile acid, but to identify the acids other than acetic that may be present in the distillate. To provide the other acids with a better opportunity to escape, the chromic acid oxidation mixture is distilled continuously throughout the reaction time. The water lost by distillation is replaced periodically to prevent the mixture from going to dryness. The acids which are obtained in the distillate can be separated and identified by paper, column or gas chromatography. When paper chromatography is employed, the determination can be carried out on less than a milligram of compound (39). Since the distillate may contain volatile neutral oxidation products as well as acids, titration with alkali would not be expected to give as accurate an estimate of the number of C-methyl groups as that provided by the normal procedure. In certain cases, such as when the compound contains branched alkyl groups, identification of the neutral ketonic products can be useful as well.

The acids which will be present in the distillate are determined by the structure of the alkyl groups in the molecule oxidized. For example, an n-butyl group attached to carbon can give rise to acetic, propionic, n-butyric and n-valeric acid, depending on the site of cleavage. The

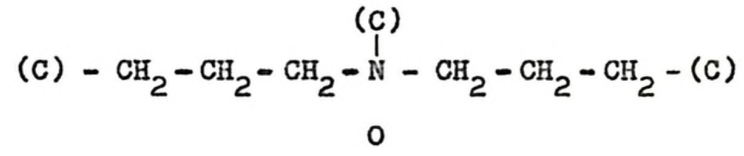
distillate was then evaporated to dryness. The acids were liberated from the residue by the action of liquid hydrogen chloride, distilled, and converted to their methyl esters by treatment with an ether solution of diazomethane. The methyl esters were separated by liquid-vapor partition chromatography and identified by comparison of their retention times with values determined by running known mixtures under the same conditions. The relative yields of the acids were estimated by measuring the areas under the recorded peaks.

Both α- and β-cyanodihydrolycopodine, XIX and LXI, were found to yield mixtures of acetic, propionic and n-butyric acid on oxidation, and it can be concluded that both compounds contain n-propyl groups attached to carbon. Oxidation of lycopodine under the same conditions gave only acetic acid. Therefore we can now write methylene groups at C-3, C-9 and C-10 in partial structure G. The results confirm the presence of the methylene group at C-2.

It was found that the acid mixture obtained from the oxidation of XIX consisted of roughly 62% acetic, 14% propionic and 24% butyric acid. The total yield of acid was only 50% of the quantity expected from a molecule with two alkyl groups. The proportions of acetic, propionic and butyric acid in the distillate from the oxidation of LXI were about 69%, 23% and 8%, respectively. In this case, the total acid yield was 70%. The difference in the percentage compositions of the acid mixtures is worthy of notice, but the difference in the total acid yields may have no significance, since the ratio of organic compound to oxidant was not the same in both cases. The relatively high yield of butyric acid from XIX suggests that C-4 is not quaternary. If this

interpretation is correct, a departure from the annotinine skeleton is indicated.

Although the oxidation results are in complete agreement with partial structure G, one cannot infer that the establishment of the nature of C-9 and C-10 has verified the proposed peripheral structure between the nitrogen and carbonyl group around ring B. Earlier in the discussion, the portions of the lycopodine molecule which were definitely known were formulated by the two partial structures M and N. The oxidation experiments have enabled fragment N to be enlarged to O, but



they have not provided the evidence necessary to connect O to M. The third methylene group in the direction of the 'β' cleavage might conceivably be the methylene group which is next to the carbonyl in fragment M. This arrangement would be incompatible with a hexahydrojulolidine ring system, but no indisputable chemical evidence against it has been presented so far.

It should also be pointed out that the identification of butyric acid among the oxidation products of XIX and XLI allows one only to assign a <u>minimum</u> length to the side-chains in these compounds. It is probable that a yield of 1% or less of valeric acid would not have been detected.

<u>Enols XXV and XXVII</u>

The problem of establishing the structure at C-5 was attacked by studying oxidation products of benzylidene derivative XXVI. A key compound in the investigation was the enolic diketone XXVII which is

obtained by ozonolysis of XXVI. Some observations were made on the properties of this enol which shed new light on the reported derivation of two enols from α-cyanolycopodine XIII.

The first enol, XXV (m.p. 158°), was reported by Barclay and MacLean (5), who obtained it by treating a crude bromination product of XIII with alkali. Barclay (7) later described the preparation of a second enol XXVII (m.p. 143°) by ozonolysis of the benzylidene derivative XXVI of XIII. He reported that enol XXVII was also formed when XIII was oxidized with selenium dioxide or sodium hypobromite. The isolation of two enols seemed to suggest that the carbonyl group of XIII was flanked by two methylene groups, and that the various reagents preferentially attacked either one or the other. However, this interpretation came into conflict with other evidence almost immediately and was finally ruled out unequivocally by the deuterium exchange studies of MacLean and Carson (37), which were mentioned previously.

During investigation of the structure of ring C, an ozonolysis of XXVI was performed in order to prepare a sample of enol XXVII for some oxidation experiments. The enolic product which was isolated was found to melt at 158°, not at 143° as had been expected. Although the ozonization had been carried out under somewhat different conditions from those used by Barclay, it did not appear reasonable that these changes could have been responsible for altering the product. The most plausible explanation seemed to be that Barclay's two enols were actually two crystalline forms of the same enol. On reviewing the earlier reports it was noted that Barclay had recrystallized the enolic

product of the ozonolysis reaction from ether. The author, on the other hand, had used ether - petroleum ether, the same solvent mixture which Barclay and MacLean had used to recrystallize the enol XXV obtained from the bromination reaction.

When a sample of the enolic ozonolysis product (m.p. 158°) was recrystallized from ether alone, crystals were obtained which melted at 143°. The liquid from the melting-point determination crystallized immediately when seeded with a crystal of the higher-melting material, and the resulting crystalline mass did not melt again until the temperature reached 158°. The enolic ozonolysis product was thus shown to be dimorphic. There can be no doubt that what have hitherto been designated enols XXV and XXVII are actually the higher-melting and the lower-melting crystalline forms, respectively, of the same compound. Unfortunately, samples from Barclay's original preparations were not available for direct comparison.

Barclay has reported that the infrared spectra of enols XXV and XXVII are different. However, since the spectra were of crystalline samples suspended in nujol, they cannot be presented as evidence that the molecules are structurally different. The dissimilarities observed can be attributed to the different crystal structures.

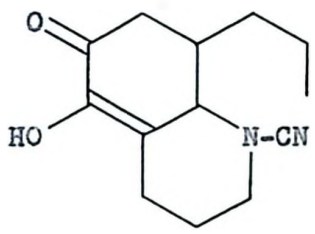
Studies on the Structure of Ring C

Several years ago, Barclay (7) carried out oxidations of benzylidene derivative XXVI with ozone and selenium dioxide. From ozonolysis he obtained the enolic compound XXVII (or XXV) which was discussed in the preceding section. Selenium dioxide oxidation of XXVI

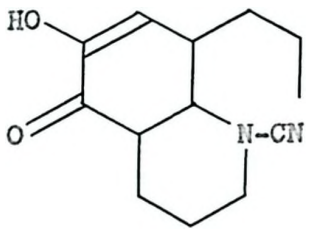
gave two products: a hydroxy compound XLI, $C_{24}H_{30}O_2N_2$, and an olefinic compound XLII, $C_{24}H_{28}ON_2$. After examining the infrared spectrum of XLI and the ultraviolet spectrum of XLII, he proposed that the hydroxyl group and the double bond had entered positions adjacent to the carbonyl group. It was decided to extend Barclay's work by studying the further oxidation of XXVII and XLI. It was hoped to learn enough from the products to establish the structure at C-5.

It was expected from the outset that a branch of some sort would be found at C-5, since this carbon would presumably correspond to the one of annotinine to which the lactonized carboxyl group is attached. It seemed possible that the methyl group of lycopodine was at this position. The original object of the oxidation experiments was to cleave the bond between C-5 and C-6 and introduce a carbonyl group at C-5.

Enol XXVII was the first compound selected for oxidative study. This perhaps was a questionable choice, because it was not known which of the following two structures for XXVII was the correct one. It was



XXVII(a)



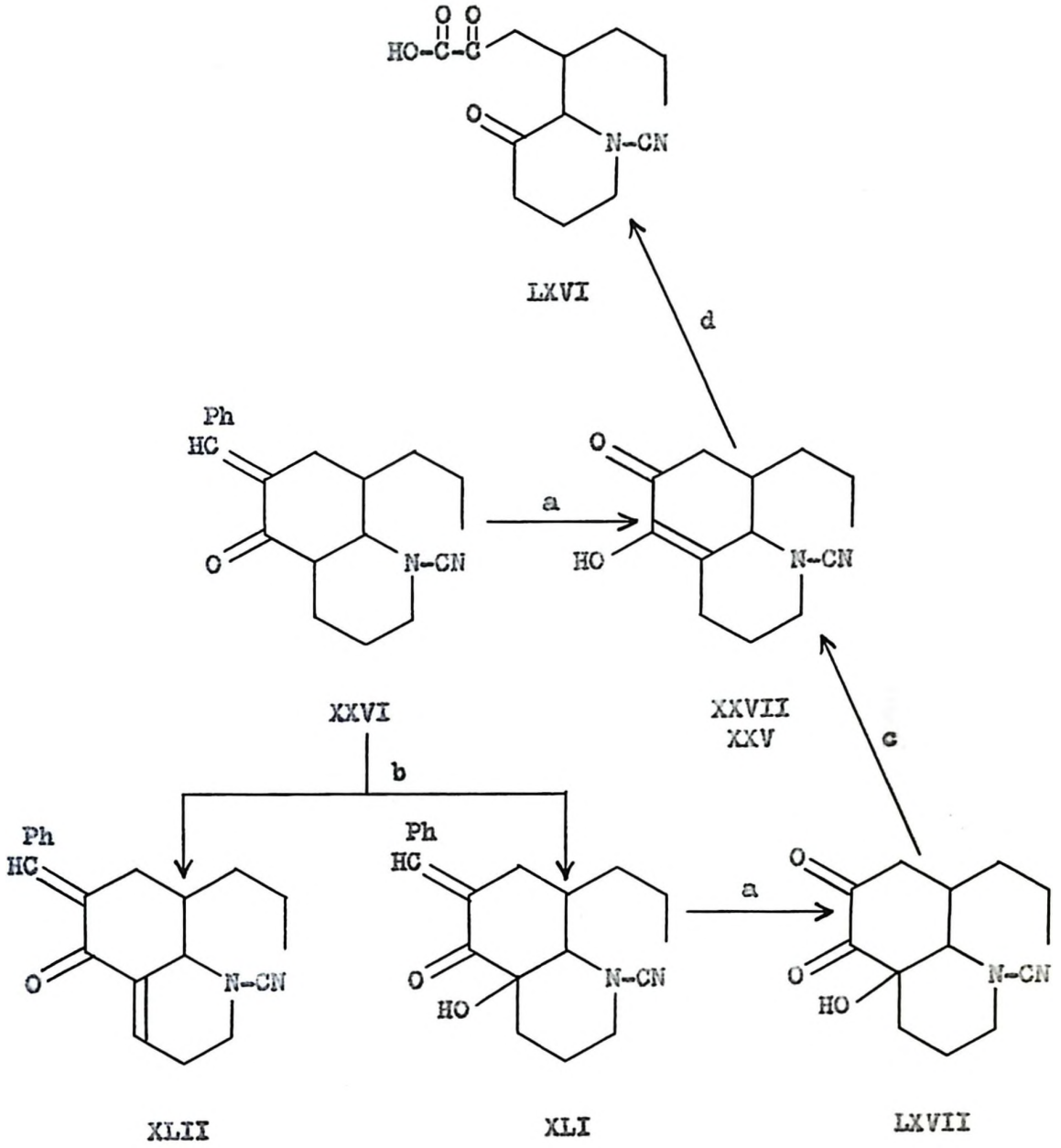
XXVII(b)

thought, however, that it might be possible to deduce the structure of XXVII from the nature of the oxidation products. For example, if the enol had structure (b) and had a methyl group at C-5, the products might give a positive iodoform test. A low yield of a crystalline

diketo acid LXVI, $C_{17}H_{24}O_4N_2$, was obtained by oxidizing XXVII with potassium permanganate in aqueous dioxane. This compound, however, did not yield any useful information. No iodoform was detected when LXVI was treated with hypoiodite, and attempts to decarbonylate the compound failed to give any product that could be characterized. Oxidations of enol XXVII were also performed with ozone and with chromic acid, but no crystalline products were isolated.

An attempt was then made to prepare an enolic compound which would be certain to have a double bond in the C-5 - C-6 position. Since it was believed that the hydroxyl group of XLI was at C-8, it was expected that ozonolysis of this compound would give a diketone which could only have an enol form corresponding to XXVII(b). The first attempt to prepare the diketone was unsuccessful. In this experiment, performed jointly with M. D. Curcumelli, the procedure which was followed was the same as that used previously to prepare enol XXVII from the benzylidene derivative XXVI. In this procedure, catalytic hydrogenation is used to decompose the ozonide. It was found that under these reducing conditions the hydroxyl group was lost and the only product isolated was enol XXVII.

Curcumelli (42) then repeated the ozonization of XLI and decomposed the ozonide by steam distillation of the reaction mixture. The major non-volatile product was a yellow crystalline compound LXVII which gave analytical results in agreement with a diketo alcohol structure. This compound was found to possess no enolic properties. It was apparent that the hydroxyl group had replaced the hydrogen atom which is involved in the enolization of XXVII. This interpretation of the results was



a. O_3

b. SeO_2

c. H_2, PtO_2

d. $KMnO_4$

Figure 9: Oxidation of Benzylidene Derivative XXVI

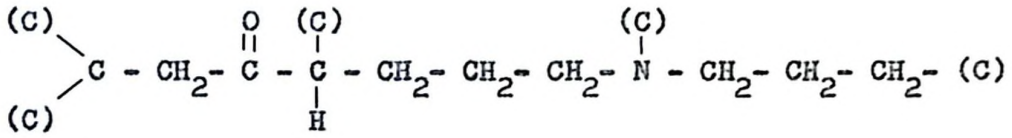
confirmed when it was found that reduction of LXVII with hydrogen and platinum oxide gave enol XXVII. It can be concluded therefore that the carbon next to the non-enolized carbonyl group of XXVII is either quaternary or at a bridgehead where double bond formation would be in violation of Bredt's rule. If the original assumption that the hydroxyl group of XLI is at C-8 is correct, then enol XXVII must have structure (a).

Other experiments have furnished ample support for this formulation. The deuterium exchange studies of MacLean and Carson (37) have shown that C-8 is tertiary and that the hydrogen on this carbon can take part in enolization. Support is also provided by the existence of two lycopodine derivatives which are believed, on the basis of independent evidence, to possess double bonds at the C-7 - C-8 position. One of these derivatives is anhydrodihydrolycopodine XIV. The position of its double bond can be assigned because the nuclear magnetic resonance spectrum of the compound shows the presence of only one hydrogen substituted on the double bond. The second derivative is cyclized compound IX, whose structure and chemistry will be discussed in detail in Part IV.

It can be concluded that C-5 is either a tertiary carbon at a bridgehead position or a quaternary carbon. In either case, a departure from the annotinine structure is indicated. Although our knowledge of the structure at C-5 is still incomplete, sufficient information has been obtained to allow partial structures to be assigned to all the compounds discussed in this section (Figure 9).

Since it is now known that the carbon next to the methylene group in partial structure M is either tertiary or quaternary, fragments M and O can be combined unambiguously to give a large part of the

peripheral structure of the lycopodine molecule (structure P). This is



P

the only arrangement left which is in agreement with the results of the lactonization experiments described in Part I. This structure is completely consistent with the proposed partial structure G.

III. The Complete Structure

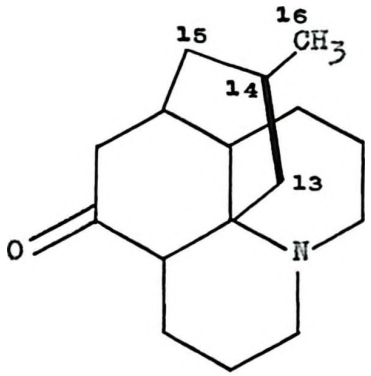
The chemical evidence supporting partial structure G is quite substantial, but not unequivocal. Most of the periphery of the lycopodine molecule has been established and it has been shown to agree with the proposed model. It is now also virtually certain that rings A and B together make up a reduced quinolazine ring system. If these rings are six-membered, as the lactamization and dehydrogenation experiments indicate, there can be no other possibility. There is still room for reasonable doubt, however, about the structure of ring C. The dehydrogenation experiments of Barclay (7) suggest that rings B and C form a reduced quinoline nucleus, but this evidence does not necessarily indicate that rings A, B and C make up a hexahydrojulolidine ring system. The main weakness in the argument for structure G is that there is no chemical evidence to show that C-5 is, in fact, attached to C-4. The presence of a reduced julolidine nucleus in annotinine remains the strongest reason for proposing the C-4 - C-5 link.

Structure of Ring D

Up to this point in the discussion no attempt has been made to propose a complete structure for lycopodine. It has been established, however, that the carbon skeleton of the alkaloid is not identical with that of annotinine. Since there is evidence to show which carbon atoms are most affected by the difference between the lycopodine and annotinine skeletons, it is now possible to attack the problem of assigning a structure to ring D of lycopodine.

If we accept partial structure G as the starting point, we find that there are only three carbon atoms to which ring D can be attached: C-4, C-5 and C-12. Since it has been demonstrated that C-5 is either quaternary or at a bridgehead, and because there is evidence from the chromic acid oxidation experiments that C-4 is not quaternary, it appears very likely that ring D is closed by a bridge between C-5 and C-12. The information which allows a structure to be proposed for this bridge was obtained eighteen years ago by Marion and Manske (12). They studied the dehydrogenation of lycopodine and, of the five basic products which they isolated and characterized, they were able to identify two: 7-methylquinoline and 5,7-dimethylquinoline. It was noted that the tendency to form 7-methylquinoline was particularly strong. With a branch at C-5, the portion of the lycopodine molecule represented by structure G could conceivably give rise to 7-methylquinoline, but it is difficult to imagine why this should be the preferred product. It is even harder to account for the formation of 5,7-dimethylquinoline. This suggests that it may be ring D, rather than ring C, which is involved in the formation of the principal dehydrogenation products. By arranging the four previously

unassigned carbon atoms in a bridge from C-5 to C-12, as shown in structure Q, one can obtain a complete structure for lycopodine which is entirely consistent with the dehydrogenation results and the rest of the chemical evidence. The four carbon atoms will be numbered as shown.



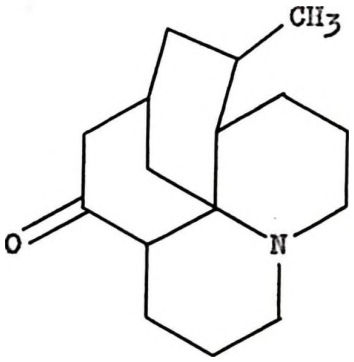
Q

Since the nuclear magnetic resonance spectrum of lycopodine reveals that the single C-methyl group present in the molecule is attached to a tertiary carbon, there are actually only four other complete structures that can be derived from G. Two of these can be obtained by moving the methyl group to C-13 or C-15, the other two, by joining C-13 to C-5 instead of to C-12. These structures are not compatible with the dehydrogenation results.

The fact that Q bears a simple structural relationship to annotinine makes it a particularly attractive solution. The only difference between the carbon skeletons of annotinine and Q is that in the former C-14 is attached to C-4, while in the latter it is attached to C-15. Several biogenetic schemes can be postulated which can account for such a difference. For example, we might have a process in which, at some stage before ring C has formed, a carbonyl group at C-14 is able

to react either with the methyl group that becomes C-15 or with C-4. The carbon skeleton of Q, like that of annotinine, can be constructed from acetate units.

Since the evidence for partial structure G is still not complete, the possibility of a structure for lycopodine which does not contain a hexahydrojulolidine nucleus cannot be ignored. If we abandon the proposal that C-4 is attached to C-5, it becomes possible to draw six additional structures. These can be derived either by placing a one-carbon bridge between C-4 and C-5 and a two-carbon bridge between C-5 and C-12 or vice versa. By moving the methyl group one obtains the six structures. Of these, only structure R would be expected to give 7-methylquinoline and 5,7-dimethylquinoline on dehydrogenation. It would also be expected to yield a considerable quantity of 5-methylquinoline, but the failure of Marion and Manske to isolate this base does not provide a very sound argument against this formulation.



R

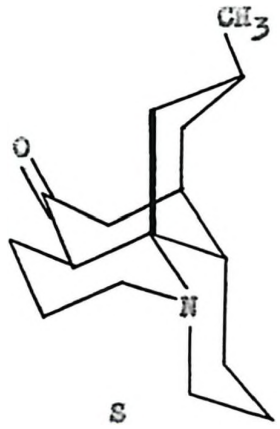
Structure R is not excluded by the chemical evidence. However, since it represents a much more radical departure from the annotinine skeleton than Q, it seems to be a far less likely solution. It is

proposed, therefore, that Q correctly depicts the structure of lycopodine.

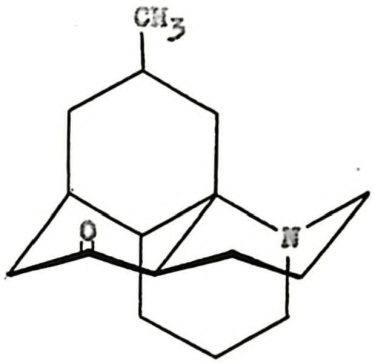
Configuration at C-4, C-8 and C-14

The proposed structure Q contains three carbon atoms whose configurations are not determined unambiguously by the nature of the ring system. These are C-4, C-8 and C-14. Examination of models reveals that the configuration at C-4 must be the one with the hydrogen on the same side of the molecule as the ring D bridge. The opposite configuration would not allow bromocyanamide III to form cyclized compound X on dehydrobromination. This reaction is known to result in bond formation between C-1 and a carbon alpha to the carbonyl group (8). The configuration at C-8 is less certain, but it seems likely that the hydrogen is on the side of the molecule opposite to the ring D bridge. The presence of the carbonyl group at C-7 allows this center to be in its most stable configuration, and it would be expected that the structure with the trans fusion of rings B and C would be the one preferred. In annotinine, or at least in certain derivatives of annotinine, it has been established that cis fusion of rings B and C is actually more stable than trans. However, this may be due in large part to the presence of the cyclobutane ring, which prevents ring C from assuming a full chair form. The resulting effect on the hydrogen - hydrogen interactions would make cis fusion of rings B and C more favorable in annotinine than in lycopodine.

The following two perspective formulas S and T give an idea of the spatial arrangement which results when C-4 and C-8 have the configurations proposed for them.



S



T

It is not yet possible to assign a configuration to C-14. The configuration which is shown in formula S has been chosen because it corresponds to that in annotinine. Since the methyl group would encounter great steric hindrance in the axial position, ring D is shown in the boat form. It can be seen from the illustrations that rings A and D are arranged particularly well for the production of 7-methylquinoline on dehydrogenation. Since the fusion of ring A with ring C is cis and rings A and B are both shown in the chair form, the axial hydrogens on C-1, C-3, C-6, C-8 and C-10 occupy sterically hindered positions. The axial hydrogens on C-9 and C-11 are also quite close to the hydrogens of C-13. Three of the strongest interactions (C-1 - C-8, C-1 - C-10, C-11 - C-13) are eliminated when rings A and B are both in the boat form, and it is therefore quite possible that this conformation is actually favored in the alkaloid and some derivatives.

IV. Chemistry of Lycopodine

Most of the reactions of lycopodine which have been discussed in the preceding sections are quite consistent with partial structure

G and require no further comment. However, a few reactions and reaction failures were encountered during these and earlier investigations which could not be fully understood without knowledge of the complete structure. The proposed structure Q is able to account for all the apparent anomalies.

In a previous study (6), the author made an unsuccessful attempt to determine whether the cyclized compound IX derived from bromocyanamide IV was a ketone or an enol ether. Although there seemed to be only these two possibilities, the compound did not undergo reactions characteristic of either structure. It is now believed that the enol ether structure is the correct solution. Actually, it became possible to write a partial structure for IX by the time the carbonyl - nitrogen relationship had been clarified, but the partial structure did not provide an explanation for the unusual stability of the compound. It was felt that the chemistry of IX could best be discussed after a complete structure had been proposed for lycopodine. Other investigators in this laboratory (8, 37) have established the structure of the isomeric cyclized compound X derived from bromocyanamide III.

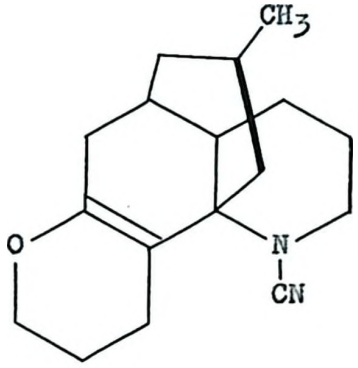
Cyclized Compounds IX and X

Bromocyanamides III and IV both undergo cyclization reactions in alkaline media. The conversion of III to the cyclized product X requires the action of boiling methanolic potassium hydroxide, but the conversion of IV to IX has been found to occur in the presence of such reagents as potassium acetate (4), dimethylamine (8) and sodium borohydride (7), which yield normal, non-cyclized reaction products with III.

Although compounds IX and X are isomeric, their properties differ in several ways. The infrared spectrum of X, like the spectra of III and IV, has a strong band near 1700 cm^{-1} in the carbonyl region. The presence of a carbonyl group in X is confirmed by the fact that X can be easily reduced with sodium borohydride to the alcohol XLIV (8). Compound IX, on the other hand, shows somewhat weaker absorption near 1675 cm^{-1} in the infrared and gives no chemical indication of possessing a carbonyl group. It has been thought for some time that IX might be a cyclic enol ether.

Compound IX is unusually stable to acid for an enol ether. It was noted, however, in an earlier study (6) that when IX was converted to the cyclized base XXXIII by acid hydrolysis of the cyanamide group, the infrared spectrum of the crude product showed some absorption at 1700 cm^{-1} in addition to the absorption at 1675 cm^{-1} characteristic of IX and XXXII. The 1700 cm^{-1} band might have been caused by the presence of some ketonic non-cyclized products formed by acid hydrolysis of the enol ether linkage. Further investigations were carried out on the behaviour of IX in hydrochloric acid, methanolic hydrogen bromide, acetic anhydride - zinc chloride mixture, and various other acidic media. These experiments were performed at a time when the only non-cyclized 'β' derivatives known were bromocyanamide IV and the still incompletely characterized alcohol XXXI. Although it was expected that there would be considerable attack on the cyanamide function, it was hoped that some IX would be converted to IV, XXXI or a simply related compound, such as the acetate of XXXI. The spectra of the crude products all showed weak to medium absorption at 1700 cm^{-1} and several showed

absorption in the hydroxyl region, but attempts to purify and characterize non-cyclized compounds were unsuccessful. MacLean (43), however, was able to convert IX back to lycopodine in low yield by treating it with a phosphoric acid - hydrobromic acid mixture and heating the crude product in ethanol under reflux. The observed behaviour is more in keeping with a cyclic enol ether than with either of the cyclized ketonic structures which can be derived from the known peripheral structure P. Since the infrared spectrum of IX does not exhibit any maxima or inflections in the region 3000 - 3120 cm^{-1} characteristic of the =C-H stretching absorption, it is proposed that the compound has the following structure.



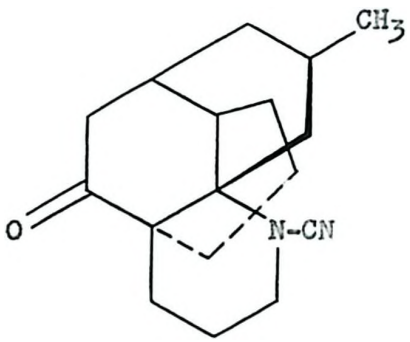
IX

The nuclear magnetic resonance spectrum of IX was found to be consistent with this structure. As was anticipated, the spectrum showed two groups of peaks which were distinctly separate from the bulk of the absorption. The areas under the curves corresponded in both cases to two protons. One group of peaks (displacement (δ) = 0.86 parts per million from water) was in the region characteristic of the protons in a CH_2-O group (44), while the other (δ = 1.64 ppm) was very probably associated with the hydrogens of C-1, which is adjacent to the cyanamide function. No band was observed which could be assigned to a

proton in a C=C-H group. The NMR spectrum of cyclized compound X was run for comparison and it showed one unresolved band (δ = 1.59 ppm, area = two protons) slightly displaced from the rest of the absorption. This band was attributed to the protons of C-11.

Some additional support for an enol ether structure for IX has been provided by MacLean and Carson (37), who have found that the compound does not undergo deuterium exchange. This evidence rules out the cyclobutyl ketone structure for IX which would be formed by cyclization at C-8, but does not eliminate the cyclohexanone structure which would result from cyclization at C-6. The latter structure would not exchange because enolization would be sterically forbidden.

MacLean and Carson have found that cyclized compound X exchanges two hydrogens for deuterium. This result confirms Song's conclusion (8) that X is formed from bromocyanamide III by a cyclization occurring alpha to the carbonyl group. Since the infrared spectrum of X has a weak absorption band at 1420 cm^{-1} which is not present in the spectrum of the deuterated compound, MacLean and Carson have concluded that X has a methylene group alpha to the carbonyl. The following structure can therefore be assigned to this compound.

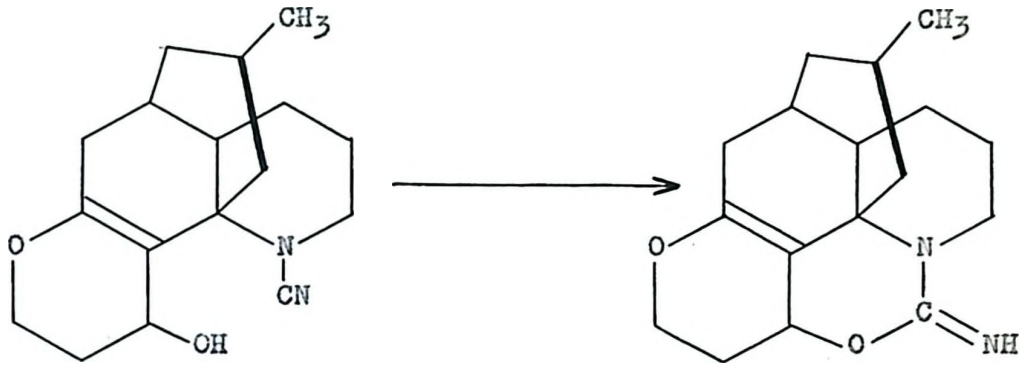


X

Attempted Ozonolysis of Cyclized Compound IX

About ten years ago, MacLean, Manske and Marion (4) reported that cyclized compound IX, $C_{17}H_{24}ON_2$, was inert to ozone. When the evidence began to point strongly to an enol ether structure for IX, it was decided to re-investigate the ozonolysis of this compound. It was found that IX was oxidized when ozone was passed through a methanol - acetic acid solution of the compound at about -40°. However, it did not appear that the double bond was the major point of attack in the oxidation. While it is true that the infrared spectrum of the crude product from the attempted ozonolysis showed a considerable reduction in the intensity of the 1675 cm^{-1} double bond peak, there was very little new absorption in the carbonyl region. The presence of a strong band at 3450 cm^{-1} suggested that the major product was an alcohol. A good yield of a crystalline compound LXVIII, $C_{17}H_{24}O_2N_2$, was obtained from the crude product. The infrared spectrum of LXVIII showed strong cyanamide and hydroxyl absorption and medium double bond absorption (1683 cm^{-1}).

When compound LXVIII was chromatographed on alumina it underwent an isomerization to a compound, LXIX, whose infrared spectrum showed no hydroxyl or cyanamide absorption, but which had a strong peak at 1680 cm^{-1} and a weak peak at 3320 cm^{-1}. The isomerization was also found to occur when LXVIII was treated with methanolic hydrochloric acid or when the compound was heated to its melting point. This behavior suggests that the oxidation of cyclized compound IX has introduced a hydroxyl group at C-9. The resulting relationship of the hydroxyl and cyanamide functions would then be favorable for the following cyclization reaction.



LXVIII LXIX

The weakened double bond absorption which is observed in the infrared spectrum of LXVIII can be attributed to the increased symmetry of the bond which results from the inductive effect of the hydroxyl partially counteracting that of the ether oxygen.* The bands at 1680 and 3320 cm^{-1} in the spectrum of LXIX can be assigned to the C=N and N-H stretching absorptions, respectively.

The configuration of C-9 in compound LXVIII is not known. The reaction between the hydroxyl and cyanamide functions should be possible for both epimers.

It seems probable that the isomeric alcohol XXXIII, which was obtained when cyclized compound IX was treated with sodium hypobromite (6), has its hydroxyl group at C-6. This compound exhibits no weakening of the infrared double bond absorption and is not noticeably affected by chromatography on alumina.

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* A similar effect has been observed for some derivatives of IX which have a positive charge on the nitrogen. The best example is the methiodide of the N-methyl base derived from secondary base XXXII (43), which shows only weak double bond absorption.

The reasons for the unusual stability of the enol ether function of IX become clear when an examination is made of a molecular model. As can be seen from the perspective formulas of lycopodine, S and T, C-8 is particularly subject to steric hindrance. The approach of bulky reagents to the vicinity of C-8 will therefore be opposed. This will be generally true, not only of the alkaloid, but also of all the known derivatives. In addition, the nature of the structure is such that the C-7 - C-8 position is a particularly favorable one for a double bond. The introduction of unsaturation at C-7 - C-8 (or C-8 - C-9) will relieve steric hindrance by eliminating the interaction of the C-8 hydrogen with the axial hydrogens of C-1 and C-3 as well as the interaction between the hydrogens of C-9 and C-13. It is to be expected that any attempt to destroy the planarity of C-8 by attack on the double bond will be resisted.

Cyclized compound IX is not the only known example of a lycopodine derivative with a very stable C-7 - C-8 double bond. Anhydrodihydrolycopodine XV has also been found to resist hydrogenation and ozonolysis (45).

Formation of Methiodides

Several years ago, while investigating the possibility of opening ring B in compounds of the 'α' series, Barclay and MacLean (5) attempted to prepare the methiodide of N-methyl base XVII. They found that the methiodide was very reluctant to form and were unable to obtain enough of it to perform any large-scale degradation experiments. Song (8) later encountered a similar obstacle when he tried to find a way to open ring

B of cyclized compound X. In this case, the N-methyl base (L) failed to give any methiodide at all. The N-methyl base derived from cyclized compound IX, on the other hand, has been found to yield a methiodide without much difficulty (43).

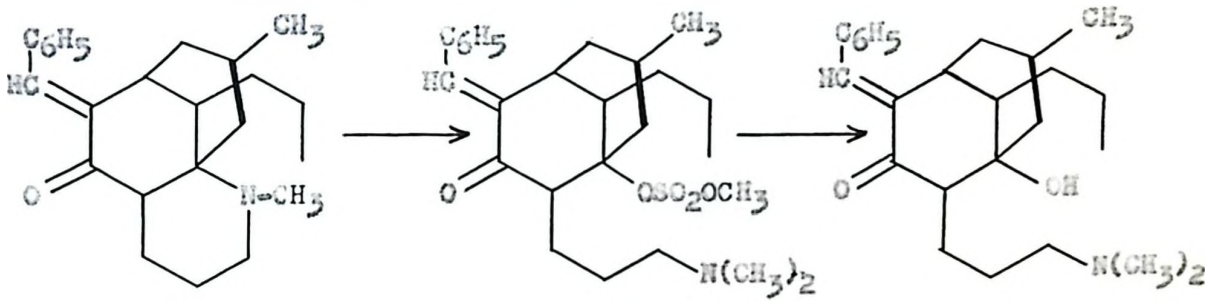
These results are consistent with the proposed structure for lycopodine. One can see this by referring to the perspective formulas S and T, and imagining that the N-methyl group of each of the three bases mentioned is in the relatively unhindered site which would be occupied by the N-methyl group in lycopodine methiodide. The second methyl group will then have to be placed in the position formerly occupied by C-1 or C-11, depending on which ring is open. However, these positions will now be hindered by the hydrogens of C-3 or C-9, as the case may be. It is to be expected therefore that an N-methyl base will react with methyl iodide less readily than lycopodine does. Since the axial hydrogen of C-1 is more hindered in lycopodine than the axial hydrogen of C-11, one would also expect that an N-methyl base of the 'β' series will accommodate a second methyl group somewhat more readily than a corresponding 'α' base. The 'β' base derived from cyclized compound IX has the additional advantage of having the C-9 - C-11 chain tied back. It is obvious that the 'α' cyclized base L, which has C-1 attached to C-8, will resist introduction of the second methyl group much more strongly than base XVII.

Elimination of the Nitrogen Atom from Lycopodine

Barclay (7) found that the N-methyl base XXXVI derived from benzylidene compound XXVI would not form a methiodide. However, the base did react slowly with methyl sulphate to give a product XXXVII which analyzed for $C_{26}H_{39}O_5NS$. This compound did not behave like a

methosulphate. On treatment with dilute ammonium hydroxide XXXVII was converted to an ether-soluble base XXXVIII which, unlike XXXVI, formed a methiodide readily. When subjected to a Hofmann degradation, the methiodide yielded trimethylamine. This result indicated that the reaction of XXXVI with methyl sulphate had resulted in a bond fission rather than formation of a quaternary salt. Since the pK_a of XXXVIII is higher than that of XXXVI, Barclay suggested that the fission had occurred between the nitrogen and the carbonyl group. The spectrum and chemical properties of XXXVIII indicate that the reaction results in the introduction of a hydroxyl group, rather than a double bond.

At first glance the postulated bond fission might seem impossible in the ring system proposed for lycopodine, since it requires that a carbonium ion be placed at C-12, a bridgehead carbon. However, it is found on examination of molecular models that the bicyclo-(3.3.1)-nonane ring system, which is involved here, can accommodate a bridgehead carbonium ion without too much difficulty. A double bond in a bridgehead position, on the other hand, puts a severe strain on the system. Therefore the observed behavior, the occurrence of cleavage without elimination, is wholly consistent with the proposed structure for the alkaloid. The reactions can be formulated as follows:



XXXVI XXXVII XXXVIII

Since there is reason to believe that the sample of XXXVIII obtained by Barclay contained at least one impurity in significant quantity, it may be that these reactions are not the only ones taking place.

Formation of Benzylidene Derivatives

When Barclay and MacLean (5) first reported the preparation of the benzylidene derivative XXVI of α-cyanolycopodine XIII, they concluded that the failure of lycopodine to react similarly with benzaldehyde was due to steric hindrance. It appeared that the opening of ring A must have left the active methylene group in an environment which was less hindered than before. It now seems probable that the steric effect involved is not hindrance in the usual sense of the term. When one examines models of the proposed structures, one can see no reason why approach of a benzaldehyde molecule to the vicinity of C-6 should be more difficult in the case of lycopodine than it is with α-cyanolycopodine XIII. Therefore, the failure of the alkaloid to react seems to be connected with the influence of the over-all structure on the conformation of ring C, rather than direct blocking by a particular part of the molecule. Some support for this interpretation is provided by the fact that β-cyanolycopodine LXV can be converted to a benzylidene derivative LXXI.

Barton et al. (46) have compared the rates of benzylidene derivative formation for a large number of steroid and triterpenoid ketones and have shown that the reaction is quite sensitive to conformational effects. The rate-determining step in the reaction is presumably in the elimination which introduces the double bond between

the phenyl group and the carbonyl. Apparently, the rate reflects the amount of difficulty which the ring containing the carbonyl group encounters in assuming a semi-chair form. Since the conformational change will require changes in the exocyclic bond angles, the overall ring structure will be expected to influence the rate of benzylidene derivative formation. It would appear that while the tetracyclic ring systems of lycopodine and cyclized compound X resist the required change in the conformation of ring C, the tricyclic structures of compounds XIII and LXV are flexible enough to accommodate it.

EXPERIMENTAL

Infrared spectra were determined with a Perkin-Elmer Model 21B recording spectrophotometer equipped with a sodium chloride prism. Unless otherwise stated, the spectra were of samples in Nujol mull. Ultraviolet spectra were measured in methanol solution on a Perkin-Elmer Spectracord 4000. Gas chromatography was carried out with a Perkin-Elmer Model 154 Vapor Fractometer using helium as the carrier gas.

Nuclear magnetic resonance spectra were measured in concentrated chloroform solution using a Varian V-4300B spectrometer equipped with a field stabilizer at a fixed frequency of 56.4 Mc/sec. Chemical shifts were measured by the side-band technique using chloroform as the reference material and corrected to give the displacement (δ) in parts per million from water.

Microanalyses were performed by Drs. G. Weiler and F. B. Strauss, Oxford, England and E. Thommen, Basel, Switzerland.

Lycopodine

The lycopodine used in these experiments was isolated from Lycopodium flabelliforme var. ambiguum Victorin collected in the Wentworth Valley of Nova Scotia. The dry, ground plant material was extracted by the procedure of Manske and Marion (10). Lycopodine was separated from the crude alkaloid extract by chromatography on alumina with either chloroform or benzene as the eluant. Lycopodine passes

through the column more rapidly than the other alkaloids in the mixture. Further purification was effected by recrystallization from petroleum ether or by conversion to the perchlorate.

The nuclear magnetic resonance spectrum of the alkaloid showed a strong doublet, $\delta = 4.10$ ppm, whose area corresponded to three protons. This was attributed to the presence of a methyl group attached to a tertiary carbon.

Calc. for $C_{16}H_{25}ON$: one C-methyl, 6.1%.

Found: 5.0%.

Reaction of Lycopodine with Cyanogen Bromide

The reaction was carried out and bromocyanamides III and IV (α- and β-cyanobromolycopodine) isolated according to the procedure described previously (5-8). The strongly adsorbed alcoholic fraction which was obtained on chromatographing the crude neutral product was saved and later reduced with sodium borohydride (vide infra).

Preparation of Acid VII

The preparation of acid VII from bromocyanamide III was first reported by MacLean, Manske and Marion (4). The purified bromocyanamide was converted to α-cyanoacetoxylycopodine V by treating it with potassium acetate in boiling methanol. The crude acetate was then hydrolyzed to the alcohol VI, which was oxidized to VII with chromium trioxide in 90% acetic acid at about -5 to -10° (6). Acid VII has also been prepared by oxidation of α-cyanohydroxydihydrolycopodine LIII under the same conditions.

Reduction of Acid VII with Sodium Borohydride

Acid VII (0.3 g) was dissolved in aqueous sodium carbonate and treated with an excess of sodium borohydride. After the mixture had stood for 12 hours at room temperature, the excess hydride was destroyed with acetone and the mixture evaporated. The residue was taken up in water, hydrochloric acid was added, and the acidified mixture extracted with chloroform. The chloroform extract was dried and evaporated to give a crystalline reduction product LI in virtually quantitative yield. Recrystallization from ether, in which the compound is only sparingly soluble, gave an analytical sample which melted at 193 - 194°. The compound analyzed for a monohydrate.

Calc. for $C_{17}H_{26}O_3N_2 \cdot H_2O$: C, 62.94; H, 8.70; N, 8.64%.

Found: C, 62.97, 63.28; H, 8.35, 8.54; N, 8.71, 8.68%.

Loss of weight on drying: calc., 5.5; found, 5.8%.

Reduction of VI with Sodium Borohydride

α-Cyanohydroxylycopodine VI (0.2 g) was dissolved in ethanol and treated with an excess of sodium borohydride. Three hours later, the unreacted hydride was destroyed by the addition of acetone and the mixture evaporated to dryness. Water was added to the residue and the mixture was extracted with chloroform. The chloroform extract was dried and evaporated to give a virtually quantitative yield of a crystalline product LIII. Recrystallization from acetone yielded an analytical sample which melted at 195 - 197°.

Calc. for $C_{17}H_{28}O_2N_2$: C, 69.82; H, 9.65; N, 9.58%.

Found: C, 70.13, 70.17; H, 9.97, 9.85; N, 9.11, 9.20%.

Reaction of IV with Silver Acetate (6)

Silver acetate (1.0 g) was added to a solution of 0.82 g of β-cyanobromolycopodine IV in benzene (80 ml) and the mixture heated under reflux for 5 hours. After the insoluble salts had been removed by filtration, the benzene solution was evaporated to give 0.67 g of non-crystalline material. The infrared spectrum of the crude product (film) showed bands at 1700 and 1735 cm^{-1}, attributed to ketone and acetate carbonyl groups respectively, as well as a strong band at 1675 cm^{-1}, characteristic of cyclized compound IX.

To facilitate separation of the cyclized and non-cyclized products, the acetate LII which had been formed was hydrolyzed to the alcohol by treating the mixture for 2.5 hours with potassium hydroxide (0.8 g) in boiling methanol (20 ml). The methanol was evaporated, water added to the residue, and the mixture extracted with chloroform. The oil obtained on evaporation of the extract was chromatographed on alumina with chloroform as the eluant. The first fractions yielded 0.52 g (82%) of crystalline cyclized compound IX, identified by comparison with a sample prepared according to the procedure of MacLean, Manske and Marion (4). A yellow band which moved more slowly down the column yielded 0.12 g (18%) of an almost colorless oil, the infrared spectrum of which had absorption bands in the hydroxyl, cyanamide and carbonyl regions. Reduction of this crude keto alcohol XXXI is described below.

A second silver acetate reaction was carried out using 0.47 g of IV. In this run the cyclized compound - acetate mixture was reduced with sodium borohydride and the resulting mixture separated by chromatography on alumina with chloroform as the eluant. This procedure yielded

0.26 g (72%) of cyclized compound IX and 0.09 g (23%) of compound LIV described below.

Reduction of XXXI with Sodium Borohydride

The non-crystalline alcohol XXXI (0.12 g) obtained by the foregoing procedure was dissolved in 95% ethanol, sodium borohydride (0.1 g) added, and the mixture left overnight at room temperature. When the reaction mixture was worked up in the usual manner there was obtained a nearly quantitative yield of a crystalline product LIV, which melted at 181 - 182° after recrystallization from acetone.

Calc. for $C_{17}H_{28}O_2N_2$: C, 69.82; H, 9.65; N, 9.58%.

Found: C, 70.09, 69.92; H, 9.36, 9.43; N, 8.8, 8.9%.

The infrared spectrum of the compound showed a band at 3360 cm^{-1} in the hydroxyl region, cyanamide absorption at 2195 cm^{-1}, and no absorption in the carbonyl region.

Reduction of the Alcohol Fraction from the Cyanogen Bromide Reaction

An ethanol solution containing the alcohol fractions (3.6 g) from a number of reactions of lycopodine with cyanogen bromide was added to a solution of 1.0 g of sodium borohydride in 95% ethanol. The reaction mixture was allowed to stand overnight at room temperature. The excess hydride was destroyed by the addition of formalin and the solution taken to dryness. Water was added to the residue, the mixture extracted with chloroform, and the chloroform extract washed with dilute hydrochloric acid. The non-crystalline basic material which was recovered from the acid washings weighed 0.22 g. The neutral fraction obtained on evaporation of the chloroform solution was dissolved

in acetone. Concentration of the acetone solution yielded 0.31 g of a crystalline compound, m.p. 193 - 196°, which was identical with compound LIII obtained by borohydride reduction of VI.

The material recovered by evaporation of the mother liquors from the crystallization of LIII was dissolved in chloroform and adsorbed on an alumina column. Elution with 99:1 chloroform - methanol first yielded a fraction containing 0.43 g of cyclized compound IX, followed by a brown band (0.43 g) from which no crystalline compounds could be isolated. Evaporation of the next fractions yielded an additional 0.78 g of crystalline compound LIII. This diol was closely followed down the column by another compound (0.22 g, m.p. 179 - 181°) which was found by a mixed melting point determination and comparison of infrared spectra to be identical with LIV obtained by borohydride reduction of XXXI. Further elution of the column yielded only non-crystalline mixtures of unknown composition. The yields of cyclized compound IX, α-cyanohydroxydihydrolycopodine LIII, and β-cyanohydroxydihydrolycopodine LIV were 12%, 30%, and 6%, respectively.

Oppenauer Oxidation of LIV

To 0.60 g of β-cyanohydroxydihydrolycopodine LIV partially dissolved in anhydrous benzene (70 ml) were added 5 ml of acetone and 1.5 g of aluminum isopropoxide. The mixture was heated under reflux for 11 hours and then poured into water. The benzene layer was separated and the aqueous phase extracted with more benzene. The crude product obtained by evaporation of the combined benzene extract was dissolved in chloroform and adsorbed on an alumina column. Evaporation of the

fractions collected on elution with chloroform yielded 0.38 g of β-cyanohydroxylycopodine which melted at 123 - 124° after crystallization from ether - petroleum ether.

Calc. for $C_{17}H_{26}O_2N_2$: C, 70.30; H, 9.03; N, 9.65%.

Found: C, 69.77, 69.63; H, 8.82, 8.89; N, 9.95, 10.10%.

The infrared spectrum showed absorption bands at 3475 cm^{-1} in the hydroxyl region, 2195 cm^{-1} in the cyanamide region, and 1685 cm^{-1} in the carbonyl region. The spectrum of a non-crystalline film of the compound was found to be identical with that of the non-crystalline alcohol XXXI obtained by hydrolysis of the product of the reaction of IV with silver acetate.

Elution of the column with 19:1 chloroform - methanol gave a fraction containing 0.09 g of unreacted diol.

Oxidation of XXXI with Chromic Acid

A solution of 0.15 g of β-cyanohydroxylycopodine XXXI (m.p. 123 - 124°) in 90% acetic acid was added dropwise over 1.5 hours to a stirred solution of chromium trioxide (0.3 g) in 90% acetic acid maintained at -5 to -10°. The reaction mixture was stirred 3 hours longer, during which time the temperature rose to 0°. After the excess chromium trioxide had been destroyed by the addition of methanol, the mixture was evaporated under reduced pressure. Water was added to the residue and the mixture extracted repeatedly with chloroform. The chloroform solution was extracted with aqueous sodium bicarbonate and the bicarbonate solution acidified and extracted in turn with chloroform. Evaporation of the chloroform yielded 0.11 g (70%) of a non-crystalline acid LV, whose infrared spectrum (film) showed absorption maxima at 1695

(ketone carbonyl), 1710 (acid carbonyl), and 2200 cm^{-1} (cyanamide), and absorption in the hydroxyl region.

A sample of the acid (35 mg) was converted to the methyl ester by dissolving it in methanol and treating it with an excess of an ether solution of diazomethane. After it had stood for 2 hours the solution was evaporated and the residue dissolved in chloroform. The chloroform solution was washed with aqueous sodium bicarbonate and then evaporated. The product, LVI, crystallized readily and melted at 127 - 129° after recrystallization from ether - petroleum ether.

Calc. for $C_{18}H_{26}O_3N_2$: C, 67.90; H, 8.23; N, 8.80%.

Found: C, 67.79, 67.57; H, 8.41, 8.23; N, 8.92%.

The infrared spectrum of the ester showed absorption at 1700 (ketone carbonyl), 1725 (ester carbonyl), and 2210 cm^{-1} (cyanamide).

The esterification of acid LV was carried out several times, and in subsequent runs, when ether was the solvent used for recrystallization, the product was usually found to melt at 160 - 162°. The melting point was not depressed by the addition of ester LVI melting at 127 - 129°. When the melting point was determined on a hot stage under a microscope, it was observed that liquid from the lower melting crystals quickly recrystallized on contact with crystals of the higher melting form. The infrared spectrum of the nujol mull of the high-melting form of LVI was distinctly different from that of the low-melting form, but the spectra of the carbon tetrachloride solutions were identical.

Acid LV has also been prepared by the oxidation of the diol LIV with chromic acid. The same conditions were used as for the oxidation of XXXI.

Reduction of the Acid LV with Sodium Borohydride

A solution of the non-crystalline acid LV (70 mg) in aqueous sodium bicarbonate was added to an ethanolic solution of sodium borohydride (300 mg). After the reaction mixture had stood at room temperature for 2 hours, the excess hydride was destroyed by adding formalin and the mixture evaporated. The residue was acidified with dilute hydrochloric acid and extracted with chloroform. The chloroform extract was washed with aqueous sodium bicarbonate and then evaporated to give 45 mg (68%) of a crystalline neutral compound. A small amount of starting material was recovered when the bicarbonate washings were acidified and extracted with chloroform, but no hydroxy acid was detected. Recrystallization of the neutral compound, LVII, from ether yielded colorless needles, m.p. 201 - 203°.

Calc. for $C_{17}H_{24}O_2N_2$: C, 70.79; H, 8.39; N, 9.72%.

Found: C, 70.71, 70.47; H, 8.52, 8.48; N, 9.80, 9.95%.

The infrared spectrum of compound LVII exhibited cyanamide absorption and a single band in the carbonyl region, which appeared at 1761 cm^{-1} when the spectrum was determined in carbon tetrachloride solution, and at 1743 cm^{-1} when determined in nujol mull. There was no absorption in the hydroxyl region.

Preparation of Diols LIII and LIV (Reaction of Acetyldihydrolycopodine XV with Cyanogen Bromide)

A sample of dihydrolycopodine II (11 g), prepared by reduction of lycopodine I with lithium aluminum hydride (4), was acetylated by treatment with a mixture of freshly prepared trifluoroacetic anhydride (10 ml) and glacial acetic acid (6 ml). The reaction was carried out

and the product isolated according to the procedure given by Douglas, Lewis and Marion (21). The crude acetate XV was dissolved in benzene and added slowly to a stirred solution of cyanogen bromide (30 g) in benzene. The reaction mixture was allowed to stand overnight at room temperature. The benzene and excess cyanogen bromide were then evaporated under reduced pressure, water was added to the residue, and the mixture worked up into acid-soluble and neutral fractions. The crude neutral product was treated for 12 hours with potassium acetate (20 g) in boiling ethanol. The crude product from this reaction was hydrolyzed with potassium hydroxide (10 g) in boiling methanol. The mixture of diols LIII and LIV obtained by this procedure was separated in the usual manner by chromatography on alumina and fractional crystallization. The over-all yields of α- and β-cyanohydroxydihydrolycopodine, LIII and LIV, from dihydrolycopodine were about 37% and 4% respectively.

Preparation of Lactam LX

To a solution of 216 mg of ester LVI (m.p. 160 - 162°) in n-propyl alcohol (2.5 ml) was added 15 ml of 2N hydrochloric acid. After the mixture had been heated for 15 hours on a steam bath it was evaporated to dryness and the residue was washed with ether to remove unhydrolyzed material. The residue, crude amino acid hydrochloride LVIII, was dissolved in methanol and the ice-cooled solution was treated with an excess of freshly prepared diazomethane solution. After 20 minutes the solution was evaporated and the residue worked up to give the basic product (150 mg). The infrared spectrum of the crude product, LIX, showed strong ketone and ester carbonyl bands and weak absorption in the N-H region.

A solution of the crude amino ester LIX (150 mg) in xylene (30 ml) was distilled very slowly at atmospheric pressure. After 10 hours, when the solution had been reduced to about one-third of its original volume, the rest of the solvent was removed under reduced pressure. The infrared spectrum of the residual oil showed a strong band at 1625 cm^{-1} and weakened ester absorption. The oil was worked up to give basic and neutral fractions. Further purification of the neutral fraction was effected by chromatography on alumina with benzene - methanol as the eluant. A band which passed down the column yielded 58 mg of crystalline material. Recrystallization from ether - petroleum ether gave long, colorless needles which melted at 177 - 179°.

Calc. for $C_{16}H_{23}O_2N$: C, 73.52; H, 8.87; N, 5.36%.

Found: C, 73.25, 73.52; H, 8.78, 8.71; N, 5.55, 5.47%.

The infrared spectrum of this compound, LX, in Nujol had strong maxima at 1700 cm^{-1} (ketone) and 1626 cm^{-1} (lactam carbonyl). When the spectrum was determined in chloroform solution the bands appeared at 1709 and 1622 cm^{-1}. There was no absorption in the cyanamide and carbonyl regions.

A mixed melting-point determination showed that lactams LX and XXX were not the same.

Reduction of Lactam LX with Lithium Aluminum Hydride

About 30 mg of lactam LX was reduced for 20 hours with lithium aluminum hydride (0.1 g) in boiling tetrahydrofuran. The excess hydride was destroyed by the addition of wet tetrahydrofuran and the mixture evaporated. Sodium hydroxide solution was added to the residue and the

organic material extracted with chloroform. Evaporation of the chloroform solution yielded an oil which partially crystallized, but attempts to obtain a sharp-melting product by recrystallization were unsuccessful. The crude product was therefore dissolved in acetone and treated with methyl iodide. The crystalline methiodide which formed melted with decomposition at 287 - 289°. Its melting point was not depressed by the addition of dihydrolycopodine methiodide, m.p. 293 - 295° (decomp.). The infrared spectra of the methiodides were identical.

Reduction of IV with Sodium Borohydride

A solution of 0.45 g of sodium borohydride in 95% ethanol was added dropwise over 0.5 hour to a stirred, ice-cooled solution of 0.50 g of β-cyanobromolycopodine IV in 95% ethanol. The reaction mixture was stirred for 7 hours at 0°, then made slightly acid with dilute hydrochloric acid and evaporated to dryness under reduced pressure. Water was added to the residue and the resulting mixture extracted with ether. The ether extract was washed with dilute hydrochloric acid and dried over anhydrous sodium sulphate. Evaporation of the ether solution yielded a crystalline residue whose infrared spectrum exhibited strong hydroxyl and cyanamide absorption and very weak absorption in the carbonyl region. The presence of only a very small peak at 1675 cm^{-1} showed that little cyclization of IV to IX had occurred. Recrystallization of the product from ether gave 0.25 g of a compound, LXII, which melted at 139 - 141° with decomposition. The compound gave a positive Beilstein test for halogen.

Calc. for $C_{17}H_{27}ON_2Br$: C, 57.45; H, 7.66; N, 7.89%.

Found: C, 57.47, 57.14; H, 7.66, 7.90; N, 8.04, 8.10%.

Preparation of Quaternary Salt LXIII

An ice-cold methanolic solution of 200 mg of hydroxy bromide LXII was treated with an excess of a methanolic solution of trimethylamine. The solution was left overnight in the refrigerator. The solvent and excess amine were removed by evaporation under reduced pressure and the crystalline residue was washed with warm ether and then dissolved in acetone. Concentration of the acetone solution yielded a crop of crystals (135 mg) which melted at 271 - 272° with decomposition. The infrared spectrum showed strong cyanamide absorption at 2200 cm^{-1}, a band of medium strength at 1640 cm^{-1}, and three bands in the hydroxyl region: a strong band at 3315 cm^{-1}, and medium bands at 3490 and 3550 cm^{-1}. When the sample was dried under vacuum at 56° for 4 hours a loss of weight of about 8% was observed, which corresponds to a loss of two moles of water. On exposure to the atmosphere the sample recovered about half of the lost weight in less than five minutes, but further gain in weight took place very much more slowly. The infrared spectrum of material which had been dried and then exposed to the air showed cyanamide absorption as before, but only two bands in the hydroxyl region: a strong one at 3370 cm^{-1} and a very weak one at 3600 cm^{-1}. The band at 1640 cm^{-1} was no longer present, but several weak peaks were observed in the region 1600 - 1660 cm^{-1}. The analytical sample, which melted at 271 - 272° (decomp.), was taken from material which had been dried and then exposed to the atmosphere.

Calc. for $C_{20}H_{36}ON_3Br.H_2O$: C, 55.54; H, 8.86; N, 9.72%.

Found: C, 55.75, 55.60; H, 8.62, 8.62; N, 9.40, 9.53%.

Decomposition of LXIII with Potassium Tertiary Butoxide

A solution of potassium tertiary butoxide was prepared by dissolving 0.35 g of potassium metal in 10 ml of tert-butyl alcohol. To this solution was added 75 mg of the quaternary salt LXIII. The reaction mixture was heated under reflux for 3 hours and then the solvent was removed under vacuum. The residue was treated with water and extracted with ether. The ether solution was washed with dilute hydrochloric acid and dried over anhydrous sodium sulphate. Evaporation of the ether yielded a crystalline product (48 mg) which, after recrystallization from petroleum ether and a second recrystallization from ether, melted at 130 - 136°. An analytical sample, m.p. 135.5 - 137.5°, was prepared by sublimation of this material at 75 - 110° under a pressure of about 0.05 mm of mercury.

Calc. for $C_{17}H_{26}ON_2$: C, 74.40; H, 9.55%.

Found: C, 74.10, 74.28; H, 9.47, 9.31%.

The infrared spectrum of the product, LXIV, showed strong cyanamide absorption, but no hydroxyl or olefin absorption. A strong band was observed at 1070 cm^{-1}, which is in the region where the ether C-O stretching absorption has been found to occur.

Reaction of LXII with Potassium Acetate

A sample of the hydroxy bromide LXII (100 mg) was heated under reflux for 5.5 hours with 0.5 g of potassium acetate in 15 ml of ethanol. The solvent was evaporated in vacuo, water was added to the residue, and the organic material extracted with chloroform. The residue obtained on evaporation of the chloroform extract was dissolved in methanol along with several pellets of potassium hydroxide. The solution

was heated under reflux for 2.5 hours and then evaporated. The residue was worked up to give 78 mg of crystalline neutral material. The crude product was chromatographed on alumina with chloroform as eluant. From the first fraction of eluate was obtained 50 mg of a crystalline compound which melted at 136 - 138° after recrystallization from ether. A mixed melting-point determination and comparison of infrared spectra showed that this compound was identical with the ether LXIV obtained by decomposition of LXIII. Elution of the column with chloroform - methanol gave a fraction which yielded 18 mg of crystals, m.p. 179 - 181°. The melting point of the crystals was not depressed when they were mixed with crystals of diol LIV. The yields of ether LXIV and diol LIV were 65% and 22% respectively.

Hydrogenolysis of LXII

A pellet (ca. 50 mg) of potassium hydroxide and 30 mg of 2% palladium - calcium carbonate catalyst were added to hydrogenation bottle containing a methanolic solution of 30 mg of the hydroxy bromide LXII. The mixture was shaken under hydrogen at a pressure of 24 lb./sq. in. for 4.5 hours and then filtered. The residue from evaporation of the filtrate was treated with water and extracted several times with ether. The ether solution was dried over anhydrous sodium sulphate and filtered. Evaporation of the solvent yielded a crystalline product which melted at 183 - 185.5° after recrystallization from ether. The product, LXI, gave a negative Beilstein halogen test.

Calc. for $C_{17}H_{28}ON_2$: C, 73.86; H, 10.21; N, 10.14%.

Found: C, 73.92, 74.18; H, 10.36, 10.25; N, 9.47, 9.76%.

Oppenauer Oxidation of LXI

To a solution of 0.20 g of β-cyanodihydrolycopodine LXI in 25 ml of anhydrous benzene were added 2.5 ml of acetone and 0.5 g of aluminum isopropoxide. The mixture was refluxed for 24 hours and then poured into water. The benzene layer was separated and dried over anhydrous sodium sulphate. Evaporation of the benzene solution yielded a residue (0.20 g) which partially crystallized on standing. Recrystallization of the residue from ether - petroleum ether yielded 54 mg of unreacted LXI. The material recovered from evaporation of the mother liquor was chromatographed on an alumina column using benzene as eluant. The first fractions yielded 84 mg of crystals whose infrared spectrum showed carbonyl (1705 cm^{-1}) and cyanamide absorption (2210 cm^{-1}), and no hydroxyl absorption. Recrystallization from ether - petroleum ether gave an analytical sample, m.p. 82.5 - 84°.

Calc. for $C_{17}H_{26}ON_2$: C, 74.40; H, 9.55; N, 10.21%.

Found: C, 74.65, 74.90; H, 9.60, 9.30; N, 10.52%.

After the band containing the oxidation product, LXV, had been collected, elution of the column was continued with 99:1 benzene - methanol and a fraction was obtained which yielded a further 40 mg of starting material.

Hydrogenolysis of IV

β-Cyanobromolycopodine IV (0.20 g) was dissolved in 25 ml of methanol containing 1 g of potassium hydroxide and 0.2 g of 2% palladium - calcium carbonate catalyst. The mixture was shaken with hydrogen at 40 lb./sq. in. for 6 hours and then worked up in the manner described

previously in the account of the hydrogenolysis of LXII. The reaction conditions are those which are given by MacLean, Manske and Marion (4). The crude crystalline product gave a negative Beilstein test for halogen. The infrared spectrum of the product had a strong absorption band near 1675 cm^{-1} and only weak absorption at 1700 cm^{-1}. After recrystallization from petroleum ether the product melted at 98 - 100° and proved to be identical with cyclized compound IX obtained by treatment of IV with methanolic potassium hydroxide. No β-cyanolycopodine LXV was isolated.

The reaction was carried out again under the same conditions and this time the crude product was treated with sodium borohydride to reduce any LXV present to the alcohol LXI and thus facilitate chromatographic separation. The hydride reduction mixture was worked up in the usual way and the crude product was absorbed on alumina and eluted with benzene - methanol. After the cyclized compound IX had passed through the column a fraction was collected which contained 15 mg of material whose infrared spectrum showed absorption in the hydroxyl region and at 1630 - 1650 cm^{-1}. It could not be ascertained whether or not any LXI was present in this fraction. Further elution of the column failed to yield any more material.

A methanol solution of 0.25 g of IV was poured into a hydrogenation bottle containing a solution of 0.05 g of potassium hydroxide in methanol which had been cooled to about -60° by fitting the bottle with a jacket packed with dry ice. Palladium - calcium carbonate catalyst (0.2 g) was added to the solution and the mixture was shaken under hydrogen at 40 lb./sq. in. for 6 hours., During the first 1 or 2 hours of shaking a low temperature was maintained by keeping the jacket filled with dry

ice, but after this period the mixture was allowed to come to room temperature. The mixture was worked up by the usual procedure. The infrared spectrum of the crude product showed strong cyanamide absorption at 2210 cm^{-1} and a strong band at 1705 cm^{-1} in the carbonyl region. The 1675 cm^{-1} absorption characteristic of the cyclized compound was absent. The material was purified by chromatography on alumina with benzene - methanol as the eluant. The product (0.15 g) melted at 82 - 84° after recrystallization from ether - petroleum ether and was found to be identical with β-cyanolycopodine LXV prepared by Oppenauer oxidation of compound LXI.

Reduction of LXV with Sodium Borohydride

About 0.3 g of crude LXV, obtained by the hydrogenolysis of 0.39 g of β-cyanobromolycopodine IV (m.p. 107 - 109°), was dissolved in ethanol and reduced with sodium borohydride (0.25 g). After the mixture had stood for two days at room temperature the product was isolated by the usual procedure. The crude product was recrystallized from ether. The first crop of crystals was recrystallized a second time to give 70 mg of compound LXI, which melted at m.p. 184 - 186°. This material was used in the chromic acid (modified Kuhn-Roth) oxidation experiment described below.

Preparation of XIII

α-Cyanolycopodine XIII was obtained by hydrogenolysis of bromocyanamide III under the conditions given by MacLean, Manske and Marion (4). The procedure is the same as that used for the conversion of LXII to LXI.

Preparation of XIX

α-Cyanodihydrolycopodine XIX can be prepared by sodium borohydride reduction of either α-cyanolycopodine XIII or bromocyanamide III (5). The sample used in the chromic acid oxidation experiment (vide infra) was prepared by reduction of a mixture of bromocyanamides III and IV with sodium borohydride in warm ethanol. Barclay (7) has reported that this procedure converts III to XIX and IV to cyclized compound IX. It was found that the conversion of IV to IX was incomplete and that the product contained some β-cyanodihydrolycopodine LXI. Chromatography of the product on alumina removed the cyclized compound IX, but it did not entirely free compound XIX of LXI. Final purification was effected by recrystallization from ether. The sample melted at 190 - 191°. The formation of an appreciable quantity of LXI suggests that the temperature at which the reduction was carried out may have been lower than that used by Barclay.

Chromic Acid Oxidation of I, XIX and LXI

To a sample of the organic compound (10 - 25 mg) in a 50 ml pear-shaped flask was added 10 ml of a 30% aqueous solution of chromium trioxide. The flask was attached to a distillation apparatus and two-thirds immersed in an oil-bath maintained at 140 - 160°. A slow stream of nitrogen was bubbled through the oxidation mixture during the distillation. A graduated cylinder was used as the receiver and after each 5 ml of distillate was collected, 5 ml of distilled water was added to the reaction mixture from a dropping funnel. The distillation was continued until the volume of distillate reached 50 ml. This

oxidation procedure was originally developed by Lemieux and Purves (41) for the quantitative estimation of acetyl, ethylidene, ethoxy and α-hydroxyethyl groups.

The distillate, which was pale yellow, was redistilled and the colorless second distillate was titrated (pH meter) with 0.1 N sodium hydroxide. A small excess of alkali was added and the solution was evaporated almost to dryness. The residue was transferred to a test-tube fitted with a ground glass joint and the evaporation completed.

Recovery of the acids was carried out by treating the residue of sodium salts with liquid hydrogen chloride. For this reaction a vacuum line was employed which had a manifold with three side-tubes, each equipped with a stopcock and a ground glass joint. To the first side-tube was attached a trap, which served as the hydrogen chloride reservoir. The tube containing the salts of the acids (tube A) was connected to the second side-tube, and a smaller tube (tube B), into which the liberated acids were to be distilled, was attached to the third. The vacuum line was equipped with a simple vertical mercury manometer, which served as a safety valve during the hydrogen chloride distillations. The conversion of the salts to the free acids was accomplished in the following manner. About 5 g of hydrogen chloride, prepared by dropping concentrated hydrochloric acid into concentrated sulphuric acid, was collected in the trap, which was cooled with liquid air. The pressure in the vacuum line was reduced to about one-third of an atmosphere, the flask of liquid air was moved from the trap to tube A, and the trap was immersed in a dry ice - acetone bath. This allowed the hydrogen chloride to distil into the tube. The hydrogen

chloride was then distilled back into the trap by reversing the positions of the dry ice and liquid air flasks. During the distillations the pressure in the line rose to about one atmosphere. The stopcock between the manifold and the hydrogen chloride reservoir was closed and the rest of the vacuum line including tube A, which was still cooled by dry ice - acetone, was evacuated. After the line had been pumped out for about 10 minutes, the stopcock leading to the pumping system was closed and the liberated acids were distilled under vacuum from tube A to tube B. This distillation was carried out by removing the dry ice - acetone bath from tube A and immersing tube B in liquid air. After about 30 minutes air was let into the vacuum line and tube B was disconnected. The acids were recovered from their salts in about 90 - 95% yield by this procedure. The liberated acids contained a considerable amount of hydrogen chloride.

The recovered acids were treated with a slight excess of a freshly prepared solution of diazomethane in ether and the resulting solution of methyl esters was dried with a few crystals of anhydrous sodium sulphate. The solution was then separated into its components by gas chromatography. The methyl esters were identified by comparing the retention times of the bands with values obtained for known mixtures. The separation was carried out on a Perkin-Elmer column 'A' (didecyl phthalate on Celite) at 75° and at a flow-rate of 150 ml/min. Under these conditions the following retention times were obtained:

ether (used as reference)	0.00 min.
methyl acetate	1.2
methyl propionate	4.3

methyl isobutyrate	6.8
methyl butyrate	10.7
methyl isovalerate	17.2
methyl valerate	27

The actual retention time for ether was about 2 minutes. The relative yields of the acids formed in the oxidation were estimated by comparing the areas under the recorded ester peaks. The factors needed to correct the measured band areas for differences in the thermal conductivities of the esters were calculated from graphs obtained on chromatography of solutions of known composition. The over-all yield of acid was estimated from the amount of standard alkali which was required to neutralize the distillate from the oxidation. Since no titration blanks were run, however, the figures obtained for the over-all yields are not considered to be very reliable.

When oxidized by this procedure, both α- and β-cyanodihydrolycopodine, XIX and LXI, gave a mixture of acetic, propionic and butyric acid. Lycopodine gave only acetic acid. The yields of the acids were as follows:

	Relative Yields (%)			Total Yield of Acid (%)
	acetic	propionic	butyric	
I	100	-	-	(50)
XIX	62	14	24	(50)
LXI	69	23	8	(70)

The yields listed in the table for I and XIX were obtained by the oxidation of 100 mg samples with 10 ml of 30% aqueous chromium trioxide. Oxidation of this much material was found to be unnecessary

and a sample size of 10 - 25 mg is now recommended.

Preparation of Benzylidene Derivative XXVI

The benzylidene derivative of α-cyanolycopodine XIII was prepared by the procedure given by Barclay and MacLean (5).

Preparation of Enol XXVII

A slightly modified version of the procedure described by Barclay (7) was used for the preparation of enol XXVII. A solution of benzylidene derivative XXVI (0.40 g) in 15 ml of glacial acetic acid and 15 ml of methanol was cooled to -40° in a dry ice - acetone bath. A stream of oxygen containing 5% ozone was passed through the solution for 15 minutes at a rate of 50 ml/min. The solution was transferred to a hydrogenation bottle, platinum oxide catalyst was added, and the mixture was shaken for about 2 hours under hydrogen at a pressure of 30 lb./sq. in. The solution was then filtered and evaporated, dilute aqueous bicarbonate and ether were added to the residue, and the ether layer was separated. The ether solution was washed successively with dilute hydrochloric acid, sodium bicarbonate solution and water, and then extracted with a solution of sodium hydroxide. The alkaline extract was acidified with hydrochloric acid and extracted with chloroform. Evaporation of the chloroform solution gave a crystalline product which melted at 157 - 158° after recrystallization from ether - petroleum ether. The melting point is the same as that reported by MacLean and Barclay (5) for enol XXV, which was obtained by treating a crude bromination product of XIII with alkali. The melting point which Barclay gave for enol XXVII was 141 - 143°. When a sample of the enol obtained in this

experiment was recrystallized from ether, the solvent used by Barclay for the recrystallization of XXVII, crystals were obtained which melted at 143 - 144°. These did not depress the melting point of the higher-melting crystals obtained previously from ether - petroleum ether. It is clear that the enolic compound is dimorphic. The two enols XXV and XXVII reported previously (7) are undoubtedly two crystalline forms of the same compound.

Oxidation of Enol XXVII

A solution of 0.56 g of potassium permanganate in 25 ml of pyridine and 25 ml of water was added over 15 minutes to an ice-cooled solution of 0.50 g of enol XXVII (or XXV) in 10 ml of pyridine and 10 ml of water. Oxidation occurred rapidly and no permanganate color remained after addition was complete. Sulphur dioxide was passed into the reaction mixture until all the manganese dioxide dissolved. The solution was evaporated under vacuum, dilute hydrochloric acid was added to the residue, and the mixture was extracted with ether. The acidic products were then extracted from the ether solution with dilute aqueous carbonate. The carbonate solution was acidified and extracted in turn with ether. Evaporation of the ether extract yielded 0.25 g of crude acid product. Concentration of an acetone - petroleum ether solution of the acid product gave crystals (70 mg) which melted with decomposition at about 145°.

Calc. for $C_{17}H_{24}O_4N_2.H_2O$: C, 60.33; H, 7.75; N, 8.28%.

Found: C, 60.91, 60.84; H, 7.53, 7.55; N, 8.41, 8.2%.

Determination of the neutralization equivalent showed that the acid, LXVI, was monocarboxylic. The infrared spectrum showed hydroxyl

and cyanamide absorption and peaks at 1715 (ketone carbonyl) and 1740 cm^{-1} (acid carbonyl). The acid did not give an iodoform test. Attempts to obtain a decarbonylation product were unsuccessful.

Oxidation of enol XXVII with acidic potassium permanganate, chromic acid and ozone all gave acidic products but no crystalline compounds were isolated.

Cyclized Compounds IX and X

Bromocyanamide IV is readily converted to cyclized compound IX in basic media (4, 6-8). Most of the IX used in these investigations was obtained by treatment of mixtures of bromocyanamides III and IV with sodium borohydride (6, 7), dimethylamine (8), or potassium acetate (4, 6). The usual procedures were followed.

Cyclized compound X was prepared by treating bromocyanamide III with potassium hydroxide in boiling methanol. The reaction was carried out and the product isolated according to the procedure given by MacLean, Manske and Marion (4).

The nuclear magnetic resonance spectrum of cyclized compound IX showed bands at δ = 0.86 and 1.64 ppm, whose areas corresponded to two protons each. These bands were attributed to the protons in a $-CH_2O-$ group and a $-CH_2N-$ group respectively. The spectrum of X had a band at 1.59 ppm (area = 2 protons), which was assigned to a $-CH_2N-$ group, but no absorption in the $-CH_2O-$ region. The spectra of both IX and X showed a split methyl peak at 3.88 ppm.

Attempted Ozonolysis of Cyclized Compound IX

A solution of 0.30 g of compound IX in 20 ml of 1:1 methanol - acetic acid was ozonized for 40 minutes at -35 to -40°. During the

reaction time about two liters of oxygen containing 5% ozone was bubbled through the solution. After ozonization the solution was reduced with hydrogen (35 lb./sq. in.) and platinum oxide catalyst for 1.5 hours. The mixture was then filtered and the filtrate evaporated under reduced pressure. Sodium bicarbonate solution was added to the residue, the resulting mixture extracted with chloroform, and the chloroform solution filtered through anhydrous sodium sulphate and evaporated. The infrared spectrum of the residual oil showed strong hydroxyl and cyanamide absorption, weak absorption at 1710 - 1730 cm^{-1}, and a band of medium strength at 1680 cm^{-1}. Concentration of a solution of the oil in ether - petroleum ether yielded crystals (0.15 g) which melted at 158 - 162°.

Calc. for $C_{17}H_{24}O_2N_2$: C, 70.79; H, 8.39; N, 9.72%.

Found: C, 70.76, 70.76; H, 8.48, 8.62; N, 9.99, 10.02%.

The spectrum of this compound, LVIII, showed strong hydroxyl (3450 cm^{-1}) and cyanamide bands, and absorption at 1683 cm^{-1} which was considerably weaker than that exhibited by the starting material. Although LXVIII melted with no visible signs of decomposition, it was found that the infrared spectrum of a carbon tetrachloride solution of material recovered from the melting point tubes exhibited weakened cyanamide and hydroxyl absorption and new absorption near 1670 cm^{-1}. The infrared spectrum of LXVIII in carbon tetrachloride showed hydroxyl and cyanamide absorption at 3580 and 2210 cm^{-1} respectively, and a medium band at 1684 cm^{-1}.

In one experiment an attempt was made to purify a sample of compound LXVIII by chromatography on alumina with benzene - chloroform -

methanol as eluant. The compound, however, reacted on the column and the earlier fractions of eluate, which contained the bulk of the material, yielded a new compound, LXIX. A small amount of unchanged LXVIII was recovered from one of the later fractions. Compound LXIX melted at 148 - 150° after recrystallization from ether - petroleum ether.

Calc. for $C_{17}H_{24}O_2N_2$: C, 70.79; H, 8.39; N, 9.72%.

Found: C, 70.51, 70.55; H, 8.51, 8.48; N, 9.26, 9.39%.

The infrared spectrum of LXIX showed a weak band at 3320 cm^{-1} and a strong band at 1680 cm^{-1}. There was no cyanamide absorption. Compound LXIX was a base and it formed a hydrochloride LXX which melted with decomposition at about 205°.

Treatment of LXVIII with Hydrochloric Acid

Two or three drops of hydrochloric acid were added to a solution of 50 mg of compound LXVIII in methanol. The solution was heated for 15 minutes on a steam cone and stored overnight in the refrigerator. The residue obtained from evaporation of the solution was washed with warm ether and then dissolved in acetone - chloroform. Concentration of this solution yielded a solid precipitate which melted with decomposition at about 207°. This product was identical with the hydrochloride LXX prepared previously. When the hydrochloride was dried under vacuum at 110° a weight loss of 2.3% was observed, corresponding to a loss of about 0.4 mole of water. The sample rapidly recovered part of the lost weight on exposure to the atmosphere, and its weight became stable only after about 0.3 mole of water had been adsorbed. An analytical sample was taken from material which had been dried and then exposed to the atmosphere.

Calc. for $C_{17}H_{24}O_2N_2 \cdot HCl \cdot (0.3)H_2O$: C, 61.81; H, 7.81; N, 8.49%.

Found: C, 62.19, 62.25; H, 7.75, 7.82; N, 8.71, 8.80%.

The infrared spectrum of the hydrochloride showed strong absorption at 1685 cm^{-1} and medium absorption at 1610 cm^{-1}.

Reaction of LXV with Benzaldehyde

To a boiling solution of β-cyanolycopodine LXV (100 mg) and benzaldehyde (0.1 ml) in 2 - 3 ml of anhydrous methanol was added 0.5 ml of sodium methoxide solution (4 g of sodium dissolved in 50 ml of methanol). The reaction mixture was heated under reflux for 45 minutes and then allowed to stand overnight at room temperature. The procedure is the same as that used previously to prepare the benzylidene derivative of α-cyanolycopodine XIII (5). After the solvent had been removed by evaporation under vacuum, the residue was treated with water and worked up to give the neutral product. The neutral product was chromatographed on alumina using benzene as eluant. One of the fractions which was collected yielded 23 mg of crystals on evaporation. The infrared and ultraviolet spectra of this material showed that it was a benzylidene derivative. The product was recrystallized, but since it did not give a very good melting point, it was not analyzed.

SUMMARY

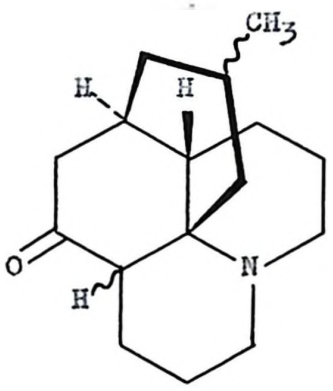
A study has been made of the chemistry of β-cyanobromolycopodine. Among the new non-cyclized 'β' derivatives which have been prepared are β-cyanobromodihydrolycopodine, β-cyanohydroxydihydrolycopodine, β-cyanolycopodine and β-cyanodihydrolycopodine. Since β-cyanohydroxydihydrolycopodine was found to yield a keto acid on oxidation, the ring cleavage which occurs in the formation of β-cyanobromolycopodine must take place between the nitrogen atom and a methylene group. Reduction of the keto acid with sodium borohydride gave a lactone which was either five- or six-membered. The carbonyl group of lycopodine is therefore at either the fourth or fifth carbon from nitrogen in the direction of the 'β' cleavage. Reduction of the corresponding keto acid derived from α-cyanobromolycopodine gave a hydroxy acid which could not be lactonized. Evidence was obtained which shows that the 'β' ring of lycopodine is six-membered or possibly larger.

Both α- and β-cyanodihydrolycopodine yielded a mixture of acetic, propionic and butyric acid on drastic chromic acid oxidation, whereas lycopodine yielded only acetic acid. Therefore α- and β-cyanodihydrolycopodine must both contain n-propyl groups.

The results of the lactonization and oxidation experiments, when added to evidence gathered by other investigators, allow most of the peripheral structure of the lycopodine molecule to be deduced. The nuclear magnetic resonance spectrum of lycopodine indicates that the

single C-methyl group present in the molecule is attached to a tertiary carbon. The fact that lycopodine possesses only one C-methyl group rules out the possibility that the alkaloid has the same carbon skeleton as annotinine.

The information which has been obtained in this investigation has enabled the following structure to be proposed for lycopodine:



The proposed structure accounts for all the available chemical evidence, including the formation of 7-methylquinoline and 5,7-dimethylquinoline on dehydrogenation of the alkaloid (12). The configuration at the asymmetric center adjacent to the carbonyl group cannot be definitely assigned, but it is probable that the hydrogen is cis to the nitrogen. The configuration at the methyl group is unknown.

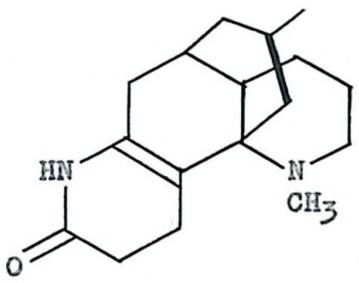
Further studies have been carried out on the properties of the cyclized compound derived from β-cyanobromolycopodine and it is now clear that the compound is a cyclic enol ether.

ADDENDUM

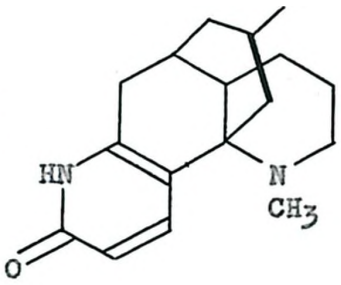
Recent Developments

A brief communication outlining part of the investigation described in this thesis and proposing structure Q for lycopodine was published several months ago (47). Since then structures have been proposed for five other Lycopodium alkaloids: acrifoline (48), α-obscurine, β-obscurine, lycodine (49) and selagine (50). The information obtained from earlier studies on the structures of the first four of these alkaloids has been summarized in the Historical Introduction.

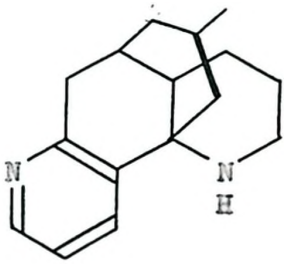
Ayer and Iverach (49) have proposed the closely related structures (a), (b) and (c) for α-obscurine, β-obscurine and lycodine, respectively.



(a)



(b)

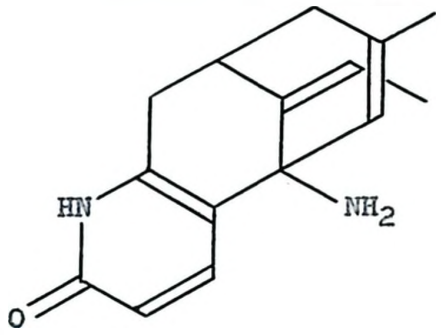


(c)

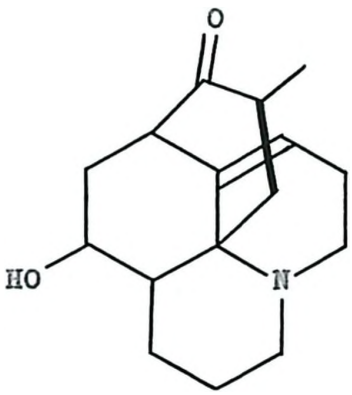
The formula $C_{16}H_{22}N_2$ which they assign to lycodine agrees with the analytical results at least as well as the formula $C_{17}H_{24}N_2$ given by

Anet and Eves (29).

Valenta, Yoshimura, Rogers, Terbah and Wiesner (30) have proposed that selagine, $C_{15}H_{18}ON_2$, has the following structure:



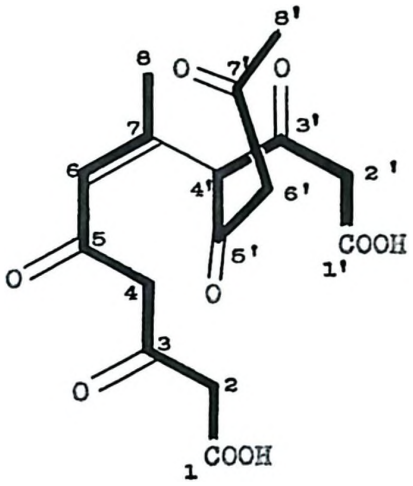
French and MacLean (48) have recently elucidated the structure of acrifoline. The ring system is identical with that proposed for lycopodine.



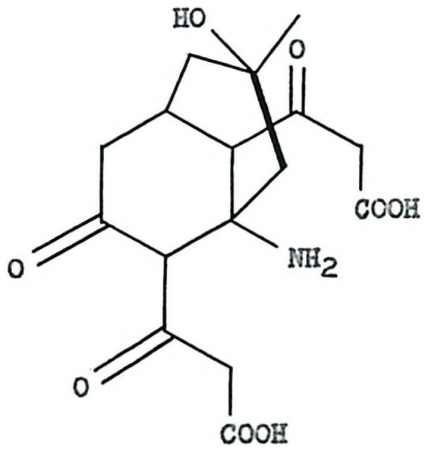
The evidence for this structure is more complete at present than the evidence for the structure of lycopodine. The carbonyl group of acrifoline provides an entry to a portion of the carbon skeleton which so far has been inaccessible in lycopodine.

Conroy (51) has proposed that the Lycopodium alkaloids are formed in the plant by condensation of two unbranched eight-carbon

polyacetate precursors. He suggests that the first step in the biosynthesis is condensation of two molecules of 3,5,7-triketo-octanoic acid, or a biogenetic equivalent, to give a structure of the following type:



Aldol condensation between C-8 and C-7', reduction of the double bond, and a Mannich condensation with ammonia would then yield the following intermediate:



Two lactamizations and the appropriate reductions would give lycopodine. Addition of ammonia to the carbonyl group at C-5 and subsequent lactamization at both amino groups would lead to the formation of

lycodine and the obscurines. Loss of C-1' by decarboxylation would yield a precursor of selagine. The annotinine skeleton would be formed as a result of condensation taking place between C-7' and C-4' instead of between C-7' and C-8.

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