STUDIES ON THE STRUCTURE OF LYCOPODINE

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OF
LYCOPODINE

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A Thesis

Submitted to the Faculty of Arts and Science

in Partial Fulfilment of the Requirements

for the Degree

Master of Science

Hamilton College

McMaster University

April 1957

MASTER OF SCIENCE (1957)
(Chemistry)

HAMILTON COLLEGE
McMASTER UNIVERSITY
Hamilton, Ontario.

TITLE: Studies on the Structure of Lycopodine

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SUPERVISOR: Professor D. B. MacLean

NUMBER OF PAGES: vii, 51

SCOPE AND CONTENTS:

The ring opened in the formation of α-cyanobromolycopodine has been found to be six-membered or larger. The product of hydrogenation of β-cyanobromolycopodine in alkaline medium has been shown to be the cyclized compound, $C_{17}H_{24}ON_2$, not β-cyanolycopodine, $C_{17}H_{26}ON_2$, as thought previously. A study was made of the β-cyclized compound but it could not be ascertained whether it has a ketone or an enol-ether structure. The possibility of a β-piperidone structure for lycopodine is discussed.

ACKNOWLEDGEMENTS

The author wishes to express his appreciation to Professor D. B. MacLean for his kind advice and encouragement during the course of this research.

The author also gratefully acknowledges the receipt of a Bursary (1955-56) from the National Research Council.

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GENERAL INTRODUCTION

The first Lycopodium alkaloid, lycopodine ($C_{16}H_{25}ON$), was isolated in 1881 by Boedeker (10), but it is only since 1938 that it has been subjected to structural investigation. Lycopodine is the most widely distributed of the Lycopodium alkaloids, having been found in all but two of the species examined. However in spite of its availability, progress in determining its structure has been very slow. This is due in large part to the scarcity of functional groups in the molecule, which contains, in addition to the tertiary nitrogen, only a single carbonyl group. To the present time only three of the carbon atoms have been definitely assigned. One of these is, of course, in the carbonyl group and the other two are in methylene groups, one α to the nitrogen, the other α to the carbonyl.

Another of the Lycopodium alkaloids, annotinine, $C_{16}H_{21}O_3N$, has been investigated more extensively and with more success. In the last few years several total structures have been proposed, and since the members of a family of alkaloids are generally related quite simply, these have always been considered carefully in the hope that they might shed some light on the lycopodine problem.

The reaction of lycopodine with cyanogen bromide yields two isomeric products, α- and β-cyanobromolycopodine (21). The main object of the work described here was to investigate the reactions of β-cyanobromolycopodine (the isomer which is formed in lower yield) and its derivatives, particularly the so-called cyclized compound formed by treatment with alkali. Owing to the ready formation of the cyclized compound the reactions used in the α series could not be applied to the β. All attempts

to obtain a useful yield of a non-cyclized derivative from β-cyanobromolycopodine were unsuccessful. Of necessity attention became centred on the readily available β-cyclized compound. Although spectral evidence suggested the presence of a carbonyl group, it failed to undergo any characteristic carbonyl reactions, including hydride reduction. The cyclized compound reacted very readily with bromine in carbon tetrachloride. Bromination in alkaline medium introduced a hydroxyl group into the molecule, but this derivative did not show much promise for further work, since it could not be cleaved with periodic acid or lead tetra-acetate nor dehydrated.

While the above work was under way an investigation was made in the α series to obtain some information on the size of the ring opened in the von Braun (cyanogen bromide) reaction. α-Cyanohydroxylycopodine was oxidized to an acid, the nitrile group hydrolyzed off and the amino acid converted to the lactam. The position of the infrared lactam carbonyl absorption indicated that the lactam ring was six-membered or larger. Reduction of the lactam to dihydrolycopodine showed that no rearrangements or loss of carbon had taken place.

HISTORICAL INTRODUCTION

In 1881, Boedeker (10) isolated an alkaloid from Lycopodium complanatum L., to which he assigned the formula $C_{32}H_{52}O_3N_2$ and the name lycopodine. Over fifty years later, Orekhov (29) and Muszynski (28) again called attention to the genus Lycopodium as a source of alkaloids. In 1938, Achmatowicz and Uzieblo (1) isolated three alkaloids from L. clavatum L., the major one of which was apparently Boedeker's lycopodine. They changed the formula to $C_{16}H_{25}ON$ and showed that there was no methoxyl or N-methyl group present and no active hydrogen (Zerewetinoff). They also found that lycopodine was indifferent to reagents for the carbonyl group and could not be hydrogenated with palladium-charcoal catalyst.

In 1942, Manske and Marion (23), in the first of a series of papers on the alkaloids of Lycopodium species, reported the isolation of lycopodine, nicotine and six other alkaloids from L. complanatum L. (later reidentified as L. flabelliforme Fernald), and confirmed the formula $C_{16}H_{25}ON$ for lycopodine. In the second paper (26), they reported the results of some degradation experiments with lycopodine. From the selenium dehydrogenation they isolated five bases, of which two were identified as 7-methylquinoline and 5,7-dimethylquinoline. Heating lycopodine with palladium-barium sulphate or with phthalic anhydride also yielded 7-methylquinoline. The alkaloid failed to react with phenyl magnesium bromide and could not be hydrogenated over Raney nickel at 200° C. and 2000 p.s.i. It was concluded therefore that the molecule contained a completely hydrogenated quinoline nucleus and that the oxygen was probably present as a cyclic ether.

Manske and Marion have investigated other American species of Lycopodium and isolated a total of over thirty alkaloids (19). Lycopodine is the most widely distributed of the alkaloids, having been found in all but two species so far examined. The occurrence of nicotine in several species is interesting, and should be a clue, if not to the structure of the alkaloids, at least to their biogenesis in the plants.

MacLean, Manske and Marion (21) found that the oxygen atom of lycopodine was present in a carbonyl group, and not in an ether linkage as had been suspected previously. This was first indicated by the infrared absorption spectrum, which showed a band at 1693 cm.$^{-1}$ in the carbonyl region, and was confirmed by the formation of a hydrazone, by reduction to an alcohol (dihydrolycopodine) with lithium aluminum hydride, and by formation of a tertiary carbinol with phenyl lithium.

After unsuccessful attempts to degrade lycopodine through the N-oxide, and by the Emde and Hofmann degradations, they found that treatment with cyanogen bromide yielded two isomeric bromocyanamides ($C_{17}H_{25}ON_2Br$), α and β-cyanobromolycopodine. By treating α-cyanobromolycopodine with potassium acetate in ethanol, they obtained α-cyanoacetoxylycopodine ($C_{19}H_{28}O_3N_2$), which yielded an alcohol ($C_{17}H_{26}O_2N_2$) on alkaline hydrolysis. Oxidation of the alcohol to an acid showed that the bromine in α-cyanobromolycopodine was primary. When β-cyanobromolycopodine was reacted with ethanolic potassium acetate, a non-alcoholic halogen-free neutral compound was obtained ($C_{17}H_{24}ON_2$), which was resistant to oxidation and catalytic hydrogenation. An isomeric compound with similar properties was obtained

from α-cyanobromolycopodine by boiling it with methanolic potassium hydroxide. It was suggested that, in the formation of these compounds, the removal of hydrogen bromide had been accompanied by cyclization. On catalytic hydrogenation of α and β-cyanobromolycopodine, they obtained two apparently isomeric non-cyclized cyanolycopodines ($C_{17}H_{26}ON_2$). (It was found in the course of the present investigation that the β product was actually the cyclized compound, $C_{17}H_{24}ON_2$.) They were able to convert α-cyanobromolycopodine to α-cyanotrimethylammoniumlycopodine bromide with trimethylamine, but on converting it to the quaternary base with silver oxide and pyrolysis, the only products were the α-cyclized compound and α-cyanodimethylaminolycopodine.

Lycopodine was related to two of the minor Lycopodium alkaloids by Douglas, Lewis and Marion (13). Dihydrolycopodine was dehydrated by the action of phosphorus pentachloride to give anhydrodihydrolycopodine, $C_{16}H_{25}N$, which was shown to be identical with alkaloid L 14, obtained from L. tristachyum. The O-acetate of dihydrolycopodine was found to be identical with alkaloid L 2, which has been isolated from L. flabelliforme. On distillation of alkaloid L 2 at atmospheric pressure, some decomposition took place, yielding 7-methylquinoline. These workers also prepared the oxime of lycopodine and tried to carry out a Beckmann rearrangement, but without success. An attempt to condense lycopodine with benzaldehyde to obtain a benzal derivative was unsuccessful.

Barclay and MacLean of this laboratory have recently published a paper on the reactions of α-cyanobromolycopodine and its derivatives (2). The work was undertaken in the expectation that it would yield

further information on the ring structure of lycopodine, but to date has not been particularly rewarding. α-Cyanobromolycopodine was converted to α-cyanolycopodine by the method of MacLean, Manske and Marion, and this compound hydrolyzed to the secondary base, α-des-dihydrolycopodine ($C_{16}H_{27}ON$). Heating the base with acetic anhydride in the presence of anhydrous sodium acetate resulted in the formation of an amorphous acetate. Although the product could not be crystallized, it showed strong infrared absorption at 1640 $cm.^{-1}$ in the amide region, which, along with the absence of NH absorption, showed it to be the N-acetyl derivative. This gives some hope that it may be possible to introduce an n-butyl group by butyrylation followed by reduction. This could then be followed by treatment with cyanogen bromide, which should open a second ring. Smaller N-alkyl groups are cleaved in preference to ring fission.

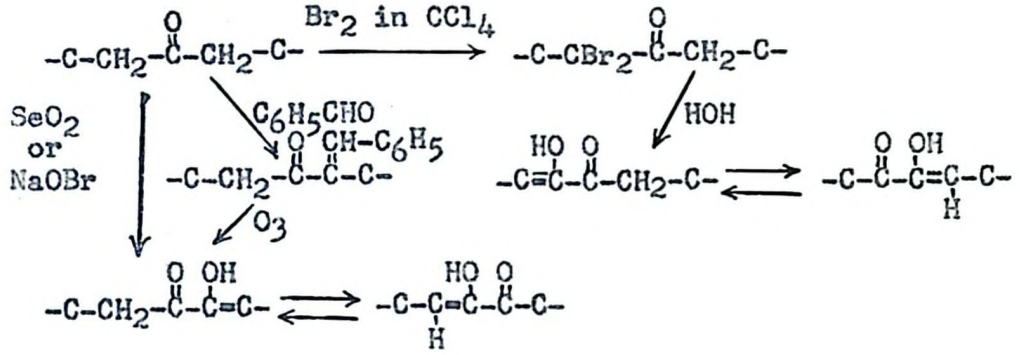
α-Des-dihydrolycopodine was methylated by formaldehyde and formic acid to a tertiary base which analyzed for a single N-methyl group. Refluxing the tertiary base with methyl iodide gave only a very poor yield of the quaternary salt, making it of little use for degradative studies. A reductive fission (Emde) was attempted, but only the unreacted methiodide was recovered.

Barclay and MacLean reported several hydride reductions which were carried out on α-cyanobromolycopodine and its derivatives. The secondary base, $C_{16}H_{27}ON$, was converted to the carbinol, $C_{16}H_{29}ON$, by lithium aluminum hydride. This carbinol could also be obtained directly from α-cyanobromolycopodine by reduction with lithium aluminum hydride. Poor results were obtained from the Meerwein-Ponndorf reduction of

α-cyanolycopodine, but sodium borohydride reduction of the bromocyanamide gave a good yield of the desired compound, α-cyanodihydrolycopodine ($C_{17}H_{28}ON_2$).

The bromination of α-cyanolycopodine was studied in an attempt to prepare derivatives suitable for oxidative degradation. α-Cyanolycopodine was reacted readily with bromine in carbon tetrachloride to form an amorphous precipitate, from which a small amount of monobromo derivative was isolated. The spectrum of this compound showed a shift of the carbonyl absorption from its position at 1700 $cm.^{-1}$ in the starting material to 1710 $cm.^{-1}$, a displacement consistent with the formation of an α-bromoketone. The rest of the material yielded a crystalline compound, $C_{17}H_{24}O_2N_2$, when treated with alkaline aqueous dioxane. This compound had enolic properties and the infrared and ultraviolet spectra indicated a structure of the type $-\overset{|}{C}=\overset{OH}{\overset{|}{C}}-\overset{O}{\overset{\|}{C}}-$. Such a structure would arise from an α, α-dibrominated ketone, leading to the conclusion that α-cyanolycopodine and lycopodine contain a methylene group α to the carbonyl. This was confirmed by the formation of a benzal derivative of α-cyanolycopodine. However, like Douglas et al., Barclay and MacLean were not able to prepare a benzal derivative of lycopodine, which suggests that the position of the carbonyl is more hindered in the alkaloid and that the ring opening in the formation of the α compounds has made it more accessible. Evidence was also reported that suggests that the carbonyl group is relatively close to the nitrogen in lycopodine. It was found that monobromination of lycopodine and α-des-dihydrolycopodine greatly reduced the basicity of these bases.

Barclay (3) has also investigated the reactions of the benzal derivative of α-cyanolycopodine. It has been found that oxidation with ozone yields an enol isomeric with that obtained by bromination of α-cyanolycopodine. This isomeric enol was also obtained by reaction of α-cyanolycopodine with selenium dioxide or sodium hypobromite. These results suggest that the carbonyl group of lycopodine is flanked by two methylene groups.



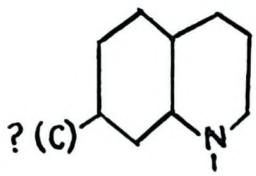
If such is the case, the proximity of the nitrogen atom might explain why different products are obtained under different conditions. This will be discussed further in connection with results of reactions in the β series of compounds.

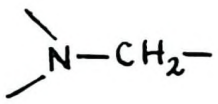
Barclay has recently succeeded in obtaining a nitrogen-free derivative of lycopodine. The benzal derivative of α-cyanolycopodine was hydrolyzed to the secondary base by the usual method, and then methylated to a tertiary base. Although this compound would not react with methyl iodide, it did react with methyl sulphate to give a product which on treatment with ammonium hydroxide gave a new tertiary base. (Apparently this was a dimethyl base with a second ring opened.) This tertiary base, in contrast with the first one, formed a methiodide, which on Hofmann

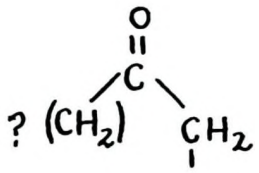
degradation yielded a crude nitrogen-free compound and trimethylamine. The sequence was carried out on rather a small scale, so further purification and examination of the products was not possible. The reactions are being repeated, with the initial aim of verifying the formation of trimethylamine, which was identified only by the melting point of its picrate in the first run.

Work has been done in this laboratory on the dehydrogenation of some derivatives of lycopodine, but this was temporarily suspended in favor of more promising lines of investigation, without any pertinent information having been obtained.

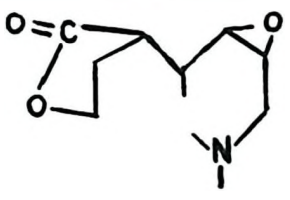
In summary, lycopodine has been found to have the following structural features:



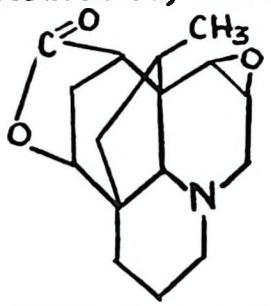




Considerably more is known of the structure of another of the Lycopodium alkaloids, annotinine ($C_{16}H_{21}O_3N$), the major alkaloid of L. annotinum L. It has been shown that annotinine is a tertiary base containing no N-methyl, methoxyl or hydroxyl groups. Two of the oxygen atoms are present in a lactone ring (24), while the third is in an epoxide ring (22). The functional groups have been related (18) and from these studies part of the annotinine molecule has been established as:



From dehydrogenation studies, Wiesner and his co-workers have proposed several total structures, the most recent of which is (33):



One would expect the structure of lycopodine to be related in some fairly simple way to that of annotinine, as is usual for alkaloids occuring in the same plant. At the present stage it is difficult to see what the relationship is, and the results of the work to be discussed here do not do much to clarify the picture.

DISCUSSION OF RESULTS

Preparation of the α-Lactam

The reaction of lycopodine with cyanogen bromide was first investigated by MacLean, Manske and Marion (21), who isolated two isomeric products, α- and β-cyanobromolycopodine ($C_{17}H_{25}ON_2Br$). In the course of their studies on these compounds, they converted α-cyanobromolycopodine to α-cyanoacetoxylycopodine by the action of potassium acetate in ethanol. The acetate was then hydrolyzed to α-cyanohydroxylycopodine, which was in turn oxidized to an acid (methyl ester: $C_{18}H_{26}O_3N_2$), showing that the bromine in the α-bromocyanamide is primary.

This sequence of reactions has been extended to obtain information on the size of the ring opened in the formation of α-cyanobromolycopodine. The isomeric bromocyanamides were prepared by the improved procedure reported in the paper by Barclay and MacLean (2). α-Cyanobromolycopodine, the major product of the reaction (about 59% yield, compared to 13% for the β-isomer), was converted to α-cyanohydroxylycopodine by the method of MacLean, Manske and Marion. A variation of their procedure was used for the oxidation of α-cyanohydroxylycopodine to α-cyanolycopodine carboxylic acid. The acid was then hydrolyzed with hydrochloric acid to the amino acid hydrochloride, which was converted by the action of diazomethane to a mixture of the α-lactam ($C_{16}H_{23}O_2N$) and the methyl ester. The products were separated by chromatographing the crude mixture on an alumina column. The infrared spectrum of the lactam

showed a strong absorption band at 1635 cm.$^{-1}$, indicating that a lactam ring of six or more members has been formed. It has been found that the amide carbonyl group of six- and seven-membered lactams absorbs in about the same region as that for normal amides, 1630-1670 cm.$^{-1}$, whereas in five-membered lactams the band is shifted to 1700-1750 cm.$^{-1}$ (4). Refluxing the methyl ester in xylene for two hours partially converted the ester to the lactam, as shown by the infrared spectrum of the crude product, which exhibited a weakened ester carbonyl band at 1730 cm.$^{-1}$ and a strong band at 1635 cm.$^{-1}$, which was not present in the spectrum of the starting material. To be certain that no rearrangements had taken place, a sample of the lactam was reduced with lithium aluminum hydride in boiling tetrahydrofuran to a product which was identified as dihydrolycopodine by mixed melting point with an authentic sample and comparison of spectra. Therefore, the ring of lycopodine which is opened in forming α-cyanobromolycopodine is six-membered or larger.

Studies in the β Series

An investigation of the reactions of β-cyanobromolycopodine was undertaken to see whether some of the reactions which had been successful with the α series could be applied to obtain information on the structure of the β compounds. The work has not been particularly rewarding as far as providing structural evidence is concerned. To some extent this is due to the small yield of β-cyanobromolycopodine in the von Braun reaction and the difficulty in separating it from the α isomer. The number and scale of the reactions which could be carried out on the β-bromocyanamide were therefore limited. Some of the first experiments

in the β series had to be discarded as inconclusive, as it soon became apparent that the early samples of β-cyanobromolycopodine, which had been thought quite pure, actually contained large proportions of the α isomer. Although, in working up the von Braun product, a few crops of pure α-cyanobromolycopodine can be obtained initially by recrystallization of the mixture from ether, a point is reached where further attempts at separation by this method are impractical. Occasionally a small crop of β-cyanobromolycopodine may be obtained by recrystallization, but usually one must resort to mechanical separation if a pure sample is desired. Fortunately the α and β crystals differ sufficiently to be distinguished by eye.

The β-Cyano Cyclized Compound

MacLean, Manske and Marion found that refluxing β-cyanobromolycopodine with ethanolic potassium acetate yielded a compound, $C_{17}H_{24}ON_2$, which was resistant to oxidation by potassium permanganate, chromic acid or ozone, and also to catalytic hydrogenation. An isomeric compound with similar properties was obtained by refluxing α-cyanobromolycopodine with methanolic potassium hydroxide. They found that α-cyanobromolycopodine could be hydrogenated catalytically to α-cyanolycopodine, $C_{17}H_{26}ON_2$, and reported that the β-bromocyanamide could similarly be reduced to an isomeric compound. However, it has been found that under the conditions used (palladium- calcium carbonate catalyst in methanolic potassium hydroxide), the β product is actually identical with the compound formed by treatment with potassium acetate. Heating β-cyanobromolycopodine for a few minutes with potassium hydroxide in methanol

also yields this compound. It was suggested by MacLean et al. that the properties of these products indicated that the removal of hydrogen bromide had been accompanied by cyclization rather than unsaturation.

Two procedures have been devised to take advantage of the difference in the ease of cyclization of the two bromocyanamides to obtain useful and easily separable compounds of the α and β series from the von Braun product. The mixture of α- and β-cyanobromolycopodine may be treated with ethanolic potassium acetate, followed by methanolic potassium hydroxide, to yield α-cyanohydroxylycopodine and the β-cyano cyclized compound, or it may be reacted with sodium borohydride in ethanol to yield α-cyanodihydrolycopodine (2) and the β-cyano cyclized compound. In both cases the products may be separated readily by chromatographing on an alumina column.

Spectral and Chemical Investigation of β-$C_{17}H_{24}ON_2$

The infrared spectrum of the β-cyano cyclized compound differs quite markedly from that of the α isomer, the most prominent feature being the presence of a band at 1675 $cm.^{-1}$ replacing the carbonyl band found at 1700 $cm.^{-1}$ in the bromocyanamide. The α-cyano cyclized compound and α-cyanobromolycopodine both absorb strongly at 1695 $cm.^{-1}$. The 1675 $cm.^{-1}$ band of the β-cyano cyclized compound is at the upper (high wave number) end of the region characteristic of carbon-carbon double bond absorption and in the lower part of the range of ketonic carbonyl absorption. Other double bonds which absorb in this region, such as the C=O bond in some amides or C=N bonds are excluded as possibilities by the analytical results and the spectral evidence that the cyanamide group

has not been affected. The band therefore must arise either from a ketonic structure with some feature causing a lowering of the carbonyl absorption frequency, or a non-ketonic structure in which an unusually strongly absorbing ethylenic bond has been formed. The possibility that the band has arisen from a shift of the carbonyl absorption band of the bromocyanamide will be discussed first.

Although the shift is less than that usually observed for $\alpha\beta$-unsaturated carbonyl compounds, which generally have their carbonyl stretching absorption displaced to lower wave numbers by about 40 cm.$^{-1}$ (5), it suggested that the loss of hydrogen bromide might have taken place with double bond formation rather than cyclization in the case of this compound. However, the spectrum does not show a band near 1600 cm.$^{-1}$, the region characteristic of the stretching vibration of a conjugated carbon-carbon double bond (6). Attempted reductions with hydrogen and platinic oxide or Raney nickel catalysts had no observable effect on the position or strength of the infrared carbonyl absorption. (The extent of reduction of the cyanamide group varied.) Finally, the ultraviolet spectrum of the β-cyano cyclized compound (Fig. I) is not of the type characteristic of $\alpha\beta$-unsaturated ketones, which have an intense band in the region 220-250mμ ($\epsilon_{max.}$ ca. 10,000) and a weak band near 310 mμ ($\epsilon_{max.}$ 10-100) (16). The spectrum of over the range 211-400 mμ shows a maximum only at 270 mμ ($\epsilon_{max.}$ 70). This is much more like the spectra of non-conjugated ketones, which absorb near 275 mμ ($\epsilon_{max.}$ ca. 15). Therefore, since the compound is not an $\alpha\beta$-unsaturated ketone and since its inertness to oxidation and reduction suggests that it does not contain

a carbon-carbon double bond in any other position, one must return to the suggestion of MacLean, Manske and Marion that a new ring has been formed. The reason for the shift of infrared carbonyl frequency in the β-cyano cyclized compound must lie then in the nature of the cyclization.

One way in which a cyclization could lower the carbonyl frequency is by the formation of a cyclopropane ring α to the carbonyl group. The cyclopropane ring possesses many properties similar to those of double bonds, including the ability to conjugate with double bonds (30). It has been observed that cyclopropyl ketones (e.g. in i-steroids) show displacement of the carbonyl absorption band about 30 $cm.^{-1}$ toward lower wave numbers from its normal position (15). The β-cyano cyclized compound also shows several characteristic bands of medium strength which are not observed in β-cyanobromolycopodine. Three of these, at 1000 $cm.^{-1}$, 890 $cm.^{-1}$, and 865 $cm.^{-1}$, correspond to bands observed in many cyclopropane derivatives (7, 20). Absence of a weak band near 3040 $cm.^{-1}$ shows that if a cyclopropane ring is present, it does not contain an unsubstituted $-CH_2-$ group (12). (The region is also characteristic of the C-H stretching absorption of ethylenic double bonds. This has been cited as evidence for the sp^2 character of the bonds of a $-CH_2-$ group in a cyclopropane ring.)

The ultraviolet spectra of cyclopropyl ketones are found to differ from those of simple ketones, but not as sharply as the spectra of αβ-unsaturated carbonyl compounds. The band near 280 mμ is increased about twofold and the strong band found near 190 mμ in simple ketones is shifted to about 210-215 mμ (14). The ultraviolet spectrum of the β-cyano cyclized compound could not be examined below 211 mμ, and is

therefore inconclusive. The chemical investigations yielded no evidence for or definitely against a cyclopropyl ketone structure. Although the cyclopropane ring ordinarily exhibits many properties of a double bond, such as the ability to add bromine, hydrogen bromide or hydrogen (catalytically), with ring opening in each case (30), the presence of a carbonyl group has a stabilizing effect, as does usually a high degree of alkyl substitution.

Attempted Reaction of Hydrides and Other Carbonyl Reagents with β-$C_{17}H_{24}ON_2$

If a carbonyl group is present in the β-cyano cyclized compound, it shows a remarkable resistance to reduction. The strength of the band at 1675 $cm.^{-1}$ was unaffected by treatment of the compound with sodium borohydride in alcohol or pyridine, or lithium aluminum hydride in ether or tetrahydrofuran. The β-cyclized base, prepared by acid hydrolysis of the cyano compound or reduction with lithium aluminum hydride, also failed to react appreciably with lithium aluminum hydride in boiling tetrahydrofuran. A very small amount of alcoholic material was formed which did not show absorption at 1675 $cm.^{-1}$, but the quantity was too small to identify. Longer treatment did not seem to increase the yield. An unsuccessful attempt was also made to hydrogenate the β-cyclized base with copper-chromite catalyst, a method which has been used to reduce the carbonyl in methyl cyclopropyl ketone in preference to ring opening (31). It has been found (32) that the α-cyano cyclized compound, in contrast to the β isomer, may be reduced without difficulty to an alcohol with sodium borohydride in ethanol.

The β-cyano cyclized compound failed to form an oxime under the conditions successful for the preparation of lycopodine oxime, and did not react with phenyl lithium.

The inertness of the β-cyclized compounds to carbonyl reagents - to hydride reduction in particular - is almost incompatible with the presence of a carbonyl group. The stabilizing effect of a cyclopropane ring alone would not be sufficient to explain its failure to react. If the compound has a ketonic structure, one must conclude that the inertness of the carbonyl is largely due to steric hindrance. Indeed, it might be quite unnecessary to have a cyclopropyl ketone structure to account for the shift of the infrared carbonyl absorption. The displacement could perhaps be explained by steric factors distorting the sp^2 configuration of the carbonyl group and giving the C=O bond more p character. The resulting weakened C=O bond would have its stretching absorption shifted to lower wave numbers. (The opposite effect is observed in four- and five-membered cyclic ketones, where ring strain causes changes in the sp hybridization ratios which strengthen the C=O bond and thereby raise the stretching frequency (8).) A lowering of carbonyl frequency has been observed for increased alkyl substitution α to the carbonyl (15), as for example in the series (11): di-n-butyl ketone, 1716 $cm.^{-1}$; di-sec-butyl ketone, 1710 $cm.^{-1}$; di-tert-butyl ketone, 1684 $cm.^{-1}$; but this is not an analagous case of hindrance, since both the α- and β-cyclized compounds would supposedly have the same degree of α substitution. A theoretical interpretation of the frequency shift with increased α substitution was not encountered in the literature,

but it is likely due to the forced spreading of the sp^2 $C\overset{(Q)}{-}C-C$ bonds giving them more sp character and the C=O bond more p character.

Non-ketonic Structures for β-$C_{17}H_{24}ON_2$

The failure of the β-cyanocyclized compound to react with carbonyl reagents strongly suggests that any possible non-ketonic structures for the compound should be considered carefully. The most reasonable alternative to a ketonic structure is one in which the oxygen is contained in a cyclic enol ether link. This would be compatible with the infrared spectrum since, for example, some 2,3-dihydrofuran derivatives have a strong band near 1675 $cm.^{-1}$ (27), but would not account for the maximum at 270 $m\mu$ in the ultraviolet.

No reactions designed specifically to prove or disprove an enol ether structure have been carried out on the compound, but in a number of the reactions tried the conditions were such that an enol ether would be expected to react. Although an enol ether structure would explain the inertness of the β-cyclized compounds to hydrides and carbonyl reagents, the resistance observed to oxidation and catalytic reduction would not be expected for such a structure. Furthermore, an enol ether should be quite susceptible to cleavage by acids, but the β-cyclized compounds did not show any marked instability in acid media. When the β-cyano cyclized compound was converted to the base by prolonged acid hydrolysis, the infrared spectrum of the crude product showed weak absorption at 1700 $cm.^{-1}$, which suggests that some non-cyclized material may have been formed. Even if this is the case, however, it would not favor an enol ether over a cyclopropyl ketone structure since cleavage of a cyclopropane

ring might be expected to take place under those conditions. Further heating of crude β-base in hydrochloric acid did not increase the intensity of the 1700 cm.$^{-1}$ band.

In discussing the cyclopropyl ketone structure, mention was made of the possible significance of the absence of a weak C-H absorption band near 3040 cm.$^{-1}$. If the cyclized compounds have an enol ether structure, lack of absorption in this region indicates that the double bond is fully substituted. This is at variance with the findings in the α series, which suggest that the carbonyl group of lycopodine may be flanked by two methylene groups.

Any non-ketonic structure in which the carbon-carbon double bond is not in an enol ether system is ruled out by both spectral and chemical evidence. An isolated double bond cannot explain the strength of the band at 1675 cm.$^{-1}$ or the presence of an R band in the ultraviolet spectrum.

It is apparent that the evidence does not permit a conclusion to be drawn at this stage as to the nature of the β-cyclized compounds. Both the ketonic and enol ether structures account for the infrared spectrum. Although the former is favored by the ultraviolet spectrum and analogy with the α-cyano cyclized compound, which is known to contain a carbonyl group (32), it is difficult to reconcile it with the inertness of the β-cyclized compounds to carbonyl reagents. The fact that a search of the literature did not reveal a single case of a hindered carbonyl group which could not be reduced fairly readily with hydrides is the strongest point in favor of an enol ether structure.

Bromination and Hypobromination of β-$C_{17}H_{24}OH_2$

The β-cyano cyclized compound reacted readily with bromine in carbon tetrachloride to give an amorphous mixture, which when chromatographed yielded a small quantity of material which was halogen-free (Beilstein) and had infrared absorption bands at 1645 cm.$^{-1}$ (conjugated carbonyl?) and 1610 cm.$^{-1}$ (double bond?), as well as C≡N and OH absorption. Other brominations yielded small amounts of halogen-containing material with bands near 1645 cm.$^{-1}$. However, no one product was obtained in sufficient quantity to be useful for further study. The spectra are similar to that of the enol obtained by Barclay from bromination of α-cyanolycopodine.

Hypobromination, on the other hand, gave a good yield of an amorphous solid, m. 130-139°C., which analyzed well for $C_{17}H_{24}O_2N_2$ and showed strong hydroxyl absorption at 3610 cm.$^{-1}$. The "cyclized" absorption band was shifted to 1680 cm.$^{-1}$, an increase of 5 cm.$^{-1}$. This suggests that if the cyclized compound has a ketonic structure, the hydroxyl group has entered α to the carbonyl, but in an axial configuration so that it affects the carbonyl stretching frequency only through its inductive effect. If the OH were equatorial, hydrogen bonding would be expected to lower the frequency by 10-20 cm.$^{-1}$ (9).

The β-cyanohydroxy cyclized compound was not oxidized by periodic acid, but gave a product with lead tetraacetate whose infrared spectrum showed "cyclized", hydroxyl, nitrile and O-acetate absorption. Apparently, lead tetraacetate oxidizes the compound by substituting acetoxyl for a hydrogen α to the carbonyl or double bond, as the case may be. The

β-cyanohydroxy cyclized compound did not dehydrate when refluxed in toluene in the presence of toluene sulphonic acid, and when refluxed with acetic anhydride gave a product with both O-acetate and N-acetate absorption and no OH or C≡N absorption in the infrared.

Unlike α-cyanolycopodine, the β-cyano cyclized compound did not form a benzal derivative. However, this cannot be given as strong evidence for cyclization α to the carbonyl, since lycopodine itself, which undoubtedly has an unsubstituted methylene group α to the carbonyl, does not form a benzal derivative.

Attempts to Obtain Non-cyclized β-Compounds

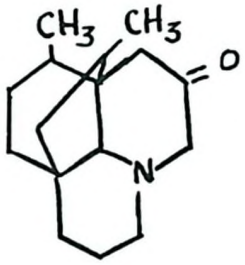
Several attempts were made to reduce β-cyanobromolycopodine to a non-cyclized compound corresponding to α-cyanolycopodine. When hydrogenation was attempted with palladium-calcium carbonate catalyst using a few drops of concentrated ammonium hydroxide instead of potassium hydroxide, cyclization took place as before. When the reduction was carried out without base, there was no reaction. Hydrogenation in acetic acid solution with palladium-barium sulphate catalyst yielded only unchanged starting material.

When β-cyanobromolycopodine was treated with silver acetate in benzene or acetonitrile, the main product was the β-cyano cyclized compound, but the infrared spectrum showed some acetate absorption. Refluxing the mixture in methanolic potassium hydroxide converted the acetate to a hydroxy compound, which was separated from the cyclized compound by chromatographing in chloroform on alumina. The spectrum of the alcohol was distinctly different from that of α-cyanohydroxylycopodine

in the region below 1500 cm.$^{-1}$, and it is therefore probable that it is the corresponding β-alcohol. The yields of cyclized compound and alcohol were 82% and 16% respectively. The low yield and the difficulty in obtaining sufficient quantities of pure β-cyanobromolycopodine prevented an investigation of this compound such as has been carried out on the α-alcohol.

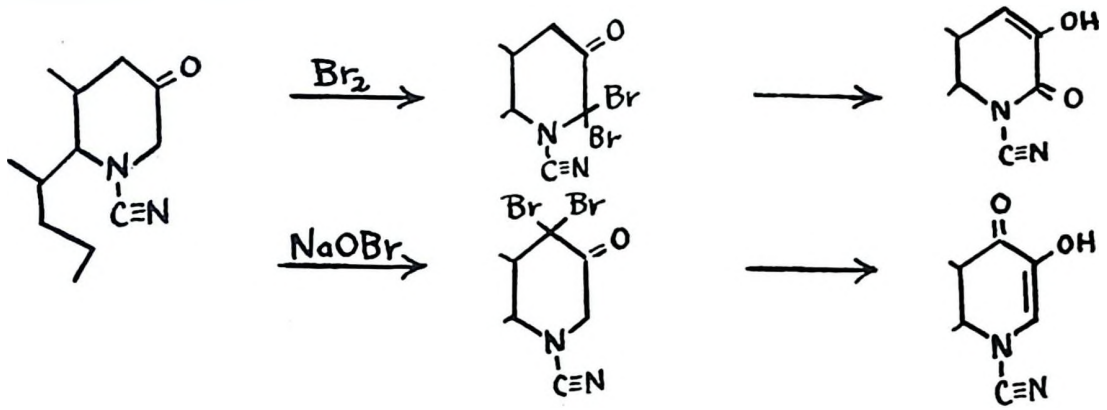
Lycopodine and the Proposed Structure of Annotinine

Since lycopodine and annotinine are both C_{16} alkaloids containing a single nitrogen, one would expect their structures to be related in some simple way. Usually alkaloids which occur together in plants have the same carbon skeleton and vary only in their functional groups. The simplest possible relationship between the two alkaloids would be to have the carbonyl group of lycopodine in the position of one of the potential hydroxyl groups of annotinine. Since the formation of isomeric enolic compounds from α-cyanolycopodine (3) suggests that there are two methylene groups flanking the carbonyl group in the alkaloid, the only structure of this type that fits the annotinine structure proposed by Wiesner is:



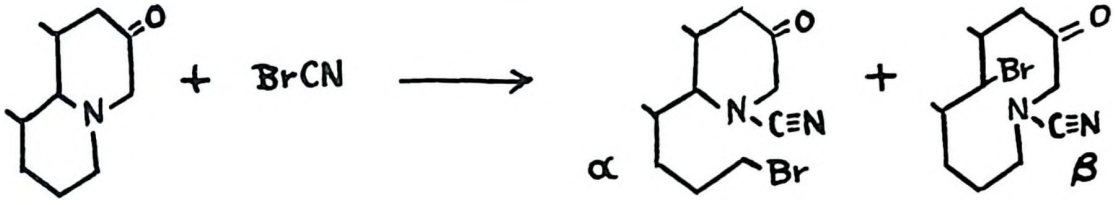
Although arguments can be raised against this structure, it does have some interesting features, and it may be useful to see how well or how poorly it explains the reactions of lycopodine. In the first place it fits in with the basicity determinations carried out by Barclay on

lycopodine and some of its derivatives (2), which indicate that the carbonyl group is relatively close to the nitrogen. Secondly, a β-piperidone relationship of carbonyl and nitrogen could explain the different enols obtained from α-cyanolycopodine under different conditions. Bromination with bromine in carbon tetrachloride would be expected to take place most readily at the methylene group between the nitrogen and carbonyl, since enolization which places the double bond vinyl to the nitrogen should be favored by conjugation with the unshared electron pair of the nitrogen. By analogy with the haloform reaction, base-catalyzed bromination would be expected to attack the other methylene group more readily, as would benzalation, which also takes place by a carbanion mechanism. Thus:



On the basis of a β-piperidone structure, other reactions of lycopodine may be formulated as follows.

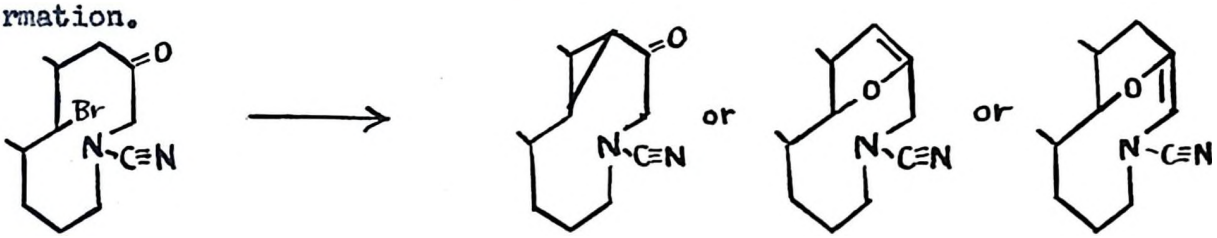
Cyanogen bromide reaction:



An argument against this formulation is that the presence of the carbonyl should facilitate opening of the piperidone ring, although

possibly not as greatly as a double bond in the corresponding allyl position. The reaction is believed to proceed by displacement of bromine in cyanogen bromide by the nitrogen of the alkaloid to form a quaternary ammonium intermediate, followed by displacement of the nitrogen from the α carbon of one of the rings by negative bromine (17). The carbonyl group should, by its electron-withdrawing power, make the methylene group of the piperidone ring more liable to nucleophilic attack, and therefore activate opening of that ring. However, there may be other factors coming into play, because, surprisingly, the allyl amine derivative of annotinine (Unsaturated Lactone A) does not react with cyanogen bromide.

The above structure for β-cyanobromolycopodine provides for the formation of a cyclopropane ring on cyclization, and also for enol ether formation.



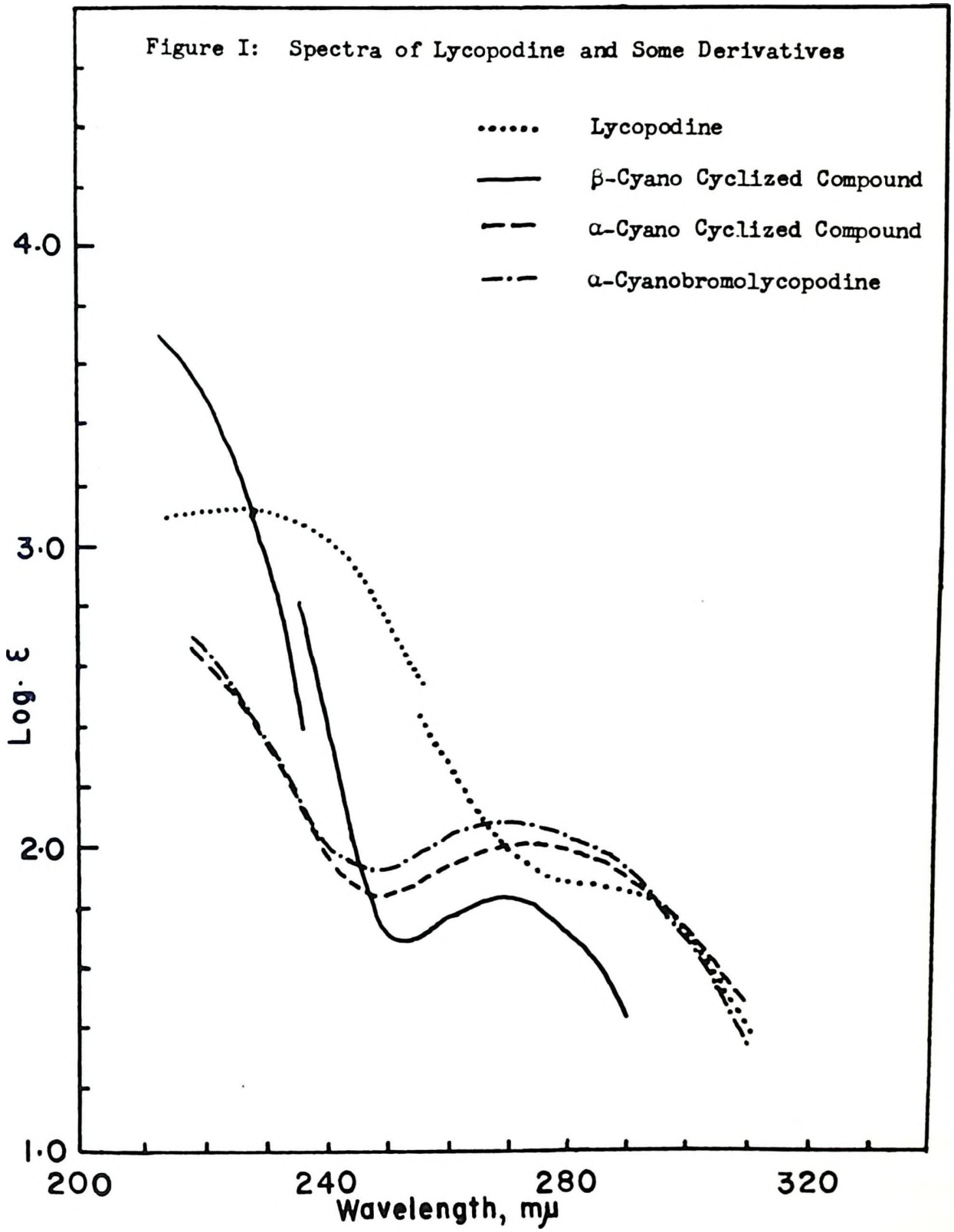
(Note that in the last structure there is the possibility of interaction among the electrons of the unsaturated bonds and the unshared electrons of oxygen and nitrogen, which might explain why, if the β-cyano cyclized compound is an enol ether, it does not exhibit the reactivity expected of such a structure.)

More difficulty is encountered when one attempts to explain the formation of the α-cyclized compound. With the annotinine skeleton, α-cyanobromolycopodine is sterically prevented from cyclizing to either methylene group α to the carbonyl. The remaining, somewhat dubious,

possibility is cyclization α to the nitrogen. It is also difficult to see from an examination of models any steric factor which would allow α-cyanolycopodine and not lycopodine to form a benzal derivative.

There is always the possibility that the two alkaloids have quite different carbon skeletons, which however are closely related biogenetically and represent alternate developments from a common precursor.

Figure I: Spectra of Lycopodine and Some Derivatives



EXPERIMENTAL

Infrared spectra were determined with a Perkin-Elmer Model 21 recording spectrophotometer, ultraviolet spectra, with a Beckmann Model DU spectrophotometer.

Microanalyses were carried out in the laboratories of Drs. G. Weiler and F. B. Strauss, Oxford, England.

All melting points are corrected.

Isolation of Lycopodine

The lycopodine used in these experiments was isolated from Lycopodium flabelliforme var. ambiguum* and Lycopodium clavatum collected in the Wentworth Valley of Nova Scotia. The dried, ground plant material was extracted by the method of Manske and Marion (23), and the lycopodine separated by chromatographing the crude alkaloid mixture in chloroform on an alumina column. Evaporation of the first fractions yielded lycopodine which was pure enough to be used in the cyanogen bromide reaction. When necessary, the alkaloid was purified further by converting it to the perchlorate. Lycopodine which was not used immediately was stored as the perchlorate. The yields of the alkaloids from L. flabelliforme and L. clavatum were 2.3 gm. and 0.5 gm. per kilo. respectively. The pure base melts at 116°C.

The ultraviolet spectrum of lycopodine (Fig. I) was determined in absolute ethanol for comparison with the spectra of some derivatives.

* Kindly identified by Dr. A. E. Roland, Nova Scotia Agricultural College, Truro, N. S.

Reaction of Lycopodine with Cyanogen Bromide

An anhydrous solution of lycopodine (15.3 gm.) in benzene (150 ml.) was added dropwise over 10 hr. to a stirred solution of cyanogen bromide (51 gm.) in anhydrous benzene (150 ml.) at room temperature. Stirring was continued for about 20 hr. after addition was complete. The benzene and excess cyanogen bromide were distilled off in vacuo, keeping the temperature below 60°C. The residue was dissolved in chloroform and washed in succession with water, dilute hydrochloric acid, dilute sodium bicarbonate solution and water. Working up the first water washings and the acid washings yielded 1.1 gm. of unreacted lycopodine. The chloroform solution was dried, concentrated and chromatographed on an alumina column. The first material to come through the column was the bromocyanamide mixture which was followed by an intense pink band consisting largely of α-cyanohydroxylycopodine (1.0 gm., 6.0 %), identified by the mixed melting point of the acetate, m. 111-113°C. The bromocyanamide mixture was recrystallized from ether, the first crops yielding 8.7 gm. of α-cyanobromolycopodine, m. 140°C., followed by a crop of 0.8 gm. of the β isomer, m. 108-109°C. Further attempts at crystallization gave only mixtures of the two. The rest of the mixture was recovered from the ether solution by evaporation and treated with ethanolic potassium acetate, followed by methanolic potassium hydroxide (detailed procedure given below). This practically quantitative procedure yielded 1.5 gm. of β-cyano cyclized compound and 2.7 gm. of α-cyanohydroxylycopodine, and therefore, calculating back, the yields of the α- and β-bromocyanamides were 59% and 13% respectively.

The ultraviolet spectrum of α-cyanobromolycopodine was determined (Fig. I).

Preparation of the α-Lactam

α-Cyanohydroxylycopodine

The α-cyanohydroxylycopodine used in the preparation of the α-lactam was obtained from α-cyanobromolycopodine by the method of MacLean, Manske and Marion (21), from the mixed bromocyanamides by the procedure described below, and from the lycopodine-cyanogen bromide reaction, which gives a yield of about 6% as a by-product.

Oxidation of α-Cyanohydroxylycopodine

The oxidation method used by MacLean et al. was sensitive to small changes in reaction conditions and often gave low yields. The following procedure was found to be more satisfactory.

α-Cyanohydroxylycopodine (1.9 gm.) was dissolved in 90% acetic acid and added slowly to a solution of chromium trioxide (4 gm.) in 90% acetic acid at -10 to -15°C. Formaldehyde solution was added to reduce the excess chromium trioxide and the mixture evaporated to dryness in vacuo. Water was added and the product extracted continuously with chloroform for two days. The chloroform solution was extracted with sodium carbonate solution, the carbonate extracts acidified and extracted with chloroform. Evaporation of the chloroform solution yielded 0.9 gm. of crude non-crystalline acid. Reworking the solutions yielded an additional 0.2-0.3 gm. Yield, 55-60%.

Hydrolysis of α-Cyanolycopodine Carboxylic Acid

Two molar hydrochloric acid (60 ml.) was added to the crude α-cyanolycopodine carboxylic acid (1.2 gm.) dissolved in n-propanol (12 ml.), and the solution heated overnight on the steam cone. The mixture was evaporated to dryness in vacuo and the residue washed several times with warm ether to remove unreacted material. 1.1 gm. (95% yield) of crude amino acid hydrochloride was obtained.

Treatment of the Amino Acid Hydrochloride with Diazomethane

The crude amino acid hydrochloride (1.1 gm.) was dissolved in methanol and an excess of diazomethane in ether added over about 15 min. After allowing the mixture to stand for an hour, the methanol, ether and excess diazomethane were distilled off under reduced pressure. The residue was dissolved in chloroform, washed with dilute hydrochloric acid, dried and evaporated. The crude product (0.5 gm.) was chromatographed in chloroform on an alumina column. Practically all the material came off the column quite rapidly as a single band. After removing the chloroform in vacuo, the residue was recrystallized from petroleum ether (b.30-60°C.), which gave colorless needle-like crystals, m. 159-161°C. Calculated for $C_{16}H_{23}O_2N$: C, 73.56; H, 8.88; N, 5.37%. Found: C, 73.87, 73.92; H, 9.29, 9.36; N, 5.14, 5.21%. The infrared spectrum showed a strong band at 1635 $cm.^{-1}$, indicating a six-membered lactam ring or larger. The overall yield of the lactam from α-cyanohydroxylycopodine was about 30%.

The acid washings from the purification of the lactam were worked up and yielded 0.2-0.3 gm. of amino ester. The ester was slowly converted

to the lactam by refluxing in xylene. This was shown by the infrared spectrum of the crude product, which exhibited a decrease in the intensity of the ester carbonyl absorption at 1730 $cm.^{-1}$ and the appearance of the lactam carbonyl band at 1635 $cm.^{-1}$.

Reduction of the Lactam with Lithium Aluminum Hydride

A sample of the lactam (0.15 gm.) was dissolved in anhydrous tetrahydrofuran and added with stirring to a solution of lithium aluminum hydride (0.2 gm.) in tetrahydrofuran. The mixture was refluxed for 4 hr. and then allowed to stand overnight. After adding wet tetrahydrofuran to decompose excess hydride, the solution was filtered off and the precipitated hydroxides extracted thoroughly with chloroform. The combined chloroform and tetrahydrofuran solutions were evaporated in vacuo. The residue was chromatographed in chloroform solution on an alumina column and then recrystallized several times from petroleum ether. The product melted at 161-165° and at 162-166°C. in admixture with a sample of dihydrolycopodine (m. 165-167° C.) prepared by reducing lycopodine with hydride. The infrared spectra of carbon tetrachloride solutions of the two samples were identical.

Reactions of β-Cyanobromolycopodine and its Derivatives

The β-Cyano Cyclized Compound

A sample of β-cyanobromolycopodine (0.5 gm.) was refluxed with potassium hydroxide (1 gm.) in methanol (40 ml.) for about 12 minutes. The solution was made slightly acid and evaporated to dryness in vacuo.

The residue was treated with water and extracted with chloroform. The chloroform solution was dried and evaporated to yield 0.38 gm. (quantitative yield) of semi-crystalline β-cyano cyclized compound. The infrared spectrum of the product showed a complete disappearance of the bromocyanamide carbonyl absorption band at 1700 $cm.^{-1}$ and appearance of a new band at 1675 $cm.^{-1}$. The material was recrystallized from petroleum ether, m. 98-100°C. The identity of the product was confirmed by mixed melting point with a sample prepared by the method of MacLean, Manske and Marion (by refluxing the bromocyanamide with ethanolic potassium acetate).

Reduction of β-Cyanobromolycopodine using Palladium-Calcium Carbonate Catalyst (21)

β-Cyanobromolycopodine (1 gm.) was dissolved in methanol (100 ml.), potassium hydroxide (0.8 gm.) and 2% palladium-calcium carbonate catalyst (0.2 gm.) added, and the mixture shaken with hydrogen at 45 p.s.i. for 6 hr. After standing overnight under pressure, the methanol solution was decanted from the catalyst, the hydrogenation flask and catalyst washed several times with methanol, and the combined methanol solutions made slightly acid with hydrochloric acid. After distilling off the methanol in vacuo, the residue was treated with water and extracted with ether. The ether solution was dried over anhydrous sodium sulphate and evaporated. The residue was chromatographed in chloroform on alumina and recrystallized from petroleum ether to yield a product (m. 98-100°C.) which, by infrared spectrum and mixed melting point, was found to be identical with the β-cyano cyclized compound obtained by treatment of the bromocyanamide with methanolic potassium hydroxide or ethanolic potassium

acetate. Therefore reduction does not take place as was suggested previously by MacLean et al.

Preparation of β-Cyano Cyclized Compound and α-Cyanohydroxylycopodine from Bromocyanamide Mixtures

When recrystallization of the mixed bromocyanamides from the reaction of cyanogen bromide with lycopodine ceased to bring about sufficient separation to be worthwhile (see above), the material recovered on evaporation of the ether solutions (5.2 gm.) was dissolved in ethanol (200 ml.), potassium acetate (15 gm.) added, and the solution refluxed for about 20 hr. The ethanol was evaporated off, water added and the crude mixture of β-cyano cyclized compound and α-cyanoacetoxylycopodine extracted with chloroform. The residue from evaporation of the chloroform was dissolved in methanol (100 ml.), potassium hydroxide (5 gm.) added, and the solution refluxed 2 hr. The organic material was separated as before and chromatographed in chloroform on an alumina column. The β-cyano cyclized compound (1.5 gm.) came down the column first, followed by α-cyanohydroxylycopodine (2.7 gm.). The yield is almost quantitative.

Borohydride Reduction of Bromocyanamide Mixtures

In addition to the preceding procedure, β-cyano cyclized compound was obtained from bromocyanamide mixtures by the following method. The bromocyanamide mixture (5 gm.) was dissolved in ethanol and the solution added to a warm ethanolic solution of sodium borohydride (5 gm.). After standing overnight, the excess hydride was destroyed with formaldehyde, and the mixture made just acid with acetic acid and evaporated to

dryness in vacuo. The residue was extracted with chloroform and the chloroform solution washed successively with dilute hydrochloric acid, sodium bicarbonate solution and water. The chloroform solution was dried, concentrated and chromatographed on alumina. β-Cyano cyclized compound passed through the column first, followed by α-cyanodihydrolycopodine (2). The latter compound is useful in investigations in the α series. The yield is practically quantitative.

Attempted Hydrogenation of β-Cyano Cyclized Compound with Platinic Oxide Catalyst

A mixture of α-cyanolycopodine and the β-cyano cyclized compound (0.1 gm.), obtained from the reduction of impure β-cyanobromolycopodine with palladium-calcium carbonate catalyst, was placed in a hydrogenation flask and methanol (15 ml.) and platinic oxide catalyst (0.02 gm.). The mixture was shaken with hydrogen at 53 p.s.i. for over 5 hr. and allowed to stand overnight under pressure. The methanol solution was decanted off and evaporated to dryness. The infrared spectrum of the product was almost identical with that of the starting material. The 1675 $cm.^{-1}$ band of the β-cyano cyclized compound was unaffected.

Attempted Hydrogenation of β-Cyano Cyclized Compound with Raney Nickel Catalyst

β-Cyano cyclized compound (0.3 gm.), Raney nickel catalyst (about 0.5 gm.) and absolute ethanol (10 ml.) were shaken with hydrogen at 1200 p.s.i. for 4 hr. The temperature was raised to about 100°C. for the first 2 hr., then the heater turned off. After cooling for 2 more

hours the bomb was opened. The ethanol solution was filtered to remove the catalyst and the filter paper washed several times with methanol. Evaporation of the combined alcohol solutions yielded a residue (0.3 gm.) whose infrared spectrum showed absorption bands at 1675, 1650, 1610, and 1560 cm.$^{-1}$.

The crude product was dissolved in chloroform and extracted with dilute hydrochloric acid. Basifying the acid solution and extracting with chloroform yielded a small amount of basic material. The non-basic fraction recovered from the first chloroform solution was chromatographed in chloroform on an alumina column. All the chromatograph fractions except the first, which consisted of less than 10 mg. of material, showed a band at 1675 cm.$^{-1}$ in the infrared. A band came down the column which, surprisingly, yielded a hydrochloride (0.14 gm.), which melted about 260°C. with decomposition, and which absorbed at 1675 (medium), 1650 (strong) and 1560 cm.$^{-1}$ (very strong) in the infrared. The spectrum suggests that this may be an imine hydrochloride, formed by partial hydrogenation of the cyanamide group. The other fractions contained less material and had spectra suggesting that they were a succession of mixtures of several compounds. The significant feature is the persistance of the peak at 1675 cm.$^{-1}$.

Attempted Reduction of β-Cyano Cyclized Compound with Sodium and Alcohol

A sample of the β-cyano cyclized compound (0.25 gm.) was dissolved in ethanol (4.5 ml.) and some water (1.5 ml.) added. Sodium metal (1 gm.) was added piecewise, keeping the temperature at 10-15°C. About 4 or 5 volumes of water were added and the solution extracted with chloroform.

The chloroform solution was dried and evaporated. The infrared spectrum showed that the residue was almost entirely the starting material. There were weak bands at 1610 and 1560 cm.$^{-1}$, but these were probably due to attack on the cyanamide group. The 1675 cm.$^{-1}$ band was not affected.

Attempted Reductions of β-Cyano Cyclized Compound with Sodium Borohydride

β-Cyano cyclized compound (0.8 gm.) in 95% ethanol was added to a stirred solution of sodium borohydride (1 gm.) in ethanol and allowed to stand overnight. The excess hydride was decomposed with formaldehyde and the solution made slightly acid with acetic acid and evaporated to dryness in vacuo. Water and a few drops of hydrochloric acid were added and the mixture extracted several times with chloroform. The chloroform solution was washed with dilute hydrochloric acid, sodium bicarbonate solution and water, dried and evaporated. The residue (0.8 gm.) was found to be unchanged starting material.

The recovered cyclized compound was dissolved in pyridine and added to sodium borohydride (1 gm.) in pyridine with stirring. The solution was heated for 2 hr. on the steam cone and allowed to stand overnight. The product was worked up as before and again only the starting material (0.8 gm.) was obtained.

β-Cyano Cyclized Compound with Lithium Aluminum Hydride

A solution of the β-cyano cyclized compound (0.60 gm.) in anhydrous tetrahydrofuran (20 ml.) was added to lithium aluminum hydride (1 gm.) in tetrahydrofuran (25 ml.) with stirring. The mixture was

refluxed for 4 hr. and allowed to stand overnight. The excess hydride was destroyed with wet tetrahydrofuran and the tetrahydrofuran solution filtered off. The precipitated hydroxides were washed several times with warm chloroform and the combined chloroform-tetrahydrofuran solution evaporated. The residue was dissolved in ether and extracted with dilute hydrochloric acid. Evaporation of the ether solution yielded 0.08 gm. of non-basic material (not the starting material - no C≡N band at 2200 $cm.^{-1}$ in the infrared). The acid extract was made basic, extracted with ether, and the ether solution dried and evaporated. The residue (0.21 gm.) was purified by chromatographing the chloroform solution on alumina. The semi-crystalline base was then converted to the hydrochloride and recrystallized from acetone-ether. The infrared spectra of the bases and the hydrochlorides and the mixed melting point of the hydrochlorides, 255-257°C., showed that the product was identical with the β-cyclized base obtained by acid hydrolysis (see following procedure).

Hydrolysis of the β-Cyano Cyclized Compound

A sample of the β-cyano cyclized compound (0.60 gm.) was dissolved in n-propanol (6 ml.), 2 M hydrochloric acid (30 ml.) added, and the mixture heated for 20 hr. on the steam cone. After evaporating the reaction mixture to dryness in vacuo, the residue was extracted with water containing a few drops of hydrochloric acid, the aqueous solution made basic and extracted several times with ether. The ether solution was dried and evaporated, and the residue (0.33 gm.) chromatographed in chloroform on an alumina column. The material (0.24 gm.) which preceded a dark band down the column had strong infrared absorption at 1675 $cm.^{-1}$

and no C≡N band. This semi-crystalline fraction was treated with hydrochloric acid in acetone and the hydrochloride which formed was recrystallized from acetone-ether, m. 256-257°C. The product is apparently the cyclized base. Unfortunately, although the analytical results for the hydrochloride fit the formula $C_{16}H_{26}ONCl$ somewhat better than any alternative, the difference from the calculated percentage for carbon is considerably greater than it should be. Calculated: C, 67.70; H, 9.24; N, 4.94%. Found: C, 67.08, 66.80, 66.58, 66.76; H, 8.92, 8.90, 9.08, 8.98; N, 4.70, 4.86, 4.80, 4.90%.

It should be noted that the crude hydrolysis product showed weak infrared absorption at 1700 $cm.^{-1}$, perhaps due to the formation of some uncyclized material. However, longer heating with concentrated hydrochloric acid in acetone solution did not seem to increase the intensity of the 1700 $cm.^{-1}$ band.

β-Cyclized Base with Lithium Aluminum Hydride

A sample of β-cyclized base (0.1 gm.) was added to lithium aluminum hydride (0.4 gm.) in tetrahydrofuran and refluxed 2.5 hr. After standing overnight, more lithium aluminum hydride (0.2 gm.) was added and the mixture refluxed for 2.5 hr. longer. The excess hydride was destroyed by adding wet tetrahydrofuran. The tetrahydrofuran solution was filtered and the precipitated hydroxides washed several times with chloroform. The chloroform and tetrahydrofuran solutions were combined and evaporated. The residue (0.9 gm.) showed weak infrared absorption at 3640 $cm.^{-1}$ (spectrum done in carbon tetrachloride) in the hydroxyl region, but the absorption at 1675 $cm.^{-1}$ was still very strong. When the reaction

was repeated with another sample of the base, this time refluxing for 70 hr., the product again showed only very weak hydroxyl absorption in the infrared. Chromatographing the product yielded a trace of crude material with no 1675 $cm.^{-1}$ absorption.

Attempted Hydrogenation of the β-Cyclized Base with Copper-Chromite Catalyst

The following procedure is similar to one which has been used to reduce the carbonyl in methyl cyclopropyl ketone without ring fissure (31).

β-Cyclized base (0.075 gm.) was dissolved in absolute ethanol (7 ml.), copper-chromite catalyst (0.03 gm.) added and the mixture shaken for 7 hr. with hydrogen at 1125 p.s.i. (initial pressure) at 110-120°C. The temperature was raised to 135°C. (maximum pressure: 1500 p.s.i.), the heater turned off and the mixture left overnight under pressure. The bomb was opened, the alcohol solution filtered and the glass liner and catalyst washed thoroughly with alcohol. The residue on evaporation of the alcohol solution showed strong carbonyl absorption in the infrared. Considerable decomposition had taken place. The crude product was dissolved in chloroform and the solution extracted with dilute hydrochloric acid. Working up the acid extract yielded 15 mg. of basic material, which had a strong band at 1675 $cm.^{-1}$. The spectrum of the non-basic product (53 mg.) obtained from evaporation of the chloroform solution showed a strong band at 1675 $cm.^{-1}$ and absorption at 2400-2800 $cm.^{-1}$, suggesting that it was largely the β-cyclized base hydrochloride. The significant features are the presence of the

absorption at 1675 cm.$^{-1}$ and lack of hydroxyl absorption in the spectra of the products.

Attempt to Prepare Oxime of β-Cyano Cyclized Compound

A sample of the β-cyano cyclized compound (0.3 gm.) was refluxed in ethanol with hydroxylamine hydrochloride (0.3 gm.) and potassium hydroxide (1.2 gm.) for 2.5 hr. The ethanol was distilled off, water added and the mixture made almost neutral. The insoluble material was filtered off and was found to be the starting compound. The infrared spectrum showed no oxime absorption.

The same procedure using lycopodine (0.5 gm.) gave lycopodine oxime (0.4 gm.), m. 263-265°C.

β-Cyclized Base with Phenyl Lithium

Phenyl lithium was prepared by adding bromobenzene (1.3 ml., 2 gm.) to dry ether (10 ml.) containing fine slices of lithium metal (0.15 gm.) and refluxing for 0.5 hr. Then a solution of β-cyclized base (0.09 gm.) in anhydrous ether (15 ml.) was added and the solution refluxed 1 hr. The reaction mixture was poured into concentrated hydrochloric acid (5 ml.) and ice (50 gm.). The aqueous solution was washed with ether, then made basic and extracted with ether. The ether extract was dried and evaporated. The infrared spectrum of the product showed strong absorption at 1675 cm.$^{-1}$ and was similar to that of the starting material, with the exception of phenyl absorption probably due to the presence of some biphenyl. (The product had the odor of biphenyl.)

β-Cyano Cyclized Compound with Bromine

β-Cyano cyclized compound (0.41 gm.) was dissolved in carbon tetrachloride and a solution of bromine in carbon tetrachloride (concentration: 8 gm./50 ml.) was added dropwise until the bromine color persisted. The amount required was slightly over 3 ml., which corresponds roughly to the theoretical amount for substitution of two bromine atoms. The precipitate was filtered off and the filtrate evaporated *in vacuo*. The residue from the filtrate was dissolved in chloroform and washed with sodium bisulphite solution, dilute hydrochloric acid and water. The chloroform solution was dried and evaporated to yield 0.21 gm. of crude material. Chromatographing in chloroform on alumina gave a fraction (0.05 gm.) which yielded an amorphous solid when reprecipitated from petroleum ether. The infrared spectrum of the material showed bands at 1645 $cm.^{-1}$, 1610 $cm.^{-1}$ and in the hydroxyl region. It gave a negative Beilstein test for halogen. The precipitates of two bromination runs yielded small amounts of amorphous solid material when extracted with petroleum ether. These contained halogen and showed bands near 1645 $cm.^{-1}$. One sample melted with much decomposition at about 150°C.

Treatment of β-Cyano Cyclized Compound with Hypobromite

β-Cyano cyclized compound (0.7 gm.) was dissolved in dioxane and 10% sodium hydroxide solution (10 ml.) added. The solution was warmed and bromine (0.5 ml.) added slowly with stirring. The reaction mixture was heated on the steam cone for 1.5 hr. and then evaporated to dryness *in vacuo*. Water was added to the residue and the organic material

extracted with chloroform. The chloroform solution was dried, concentrated and chromatographed on alumina. The first band (about 0.1 gm.) was largely the starting compound. The material from the second band (0.5 gm.) showed hydroxyl absorption at 3610 $cm.^{-1}$ and carbonyl absorption at 1680 $cm.^{-1}$ (spectrum determined in CCl_4 solution). The product was recrystallized several times from petroleum ether, m. 130-139°C. Calculated for $C_{17}H_{24}O_2N_2$: C, 70.78; H, 8.40; N, 9.72%. Found: C, 70.50, 70.46; H, 8.50, 8.30; N, 9.70, 9.65%.

β-Cyanohydroxy Cyclized Compound with Periodic Acid

β-Cyanohydroxy cyclized compound (0.058 gm.) was dissolved in ethanol (40 ml.) and potassium periodate (0.060 gm. in 20 ml. water) and 2 N sulphuric acid (2.0 ml.) added. An equivalent sample of benzoin (0.042 gm.) and a blank were treated similarly at the same time. After standing overnight, 10 ml. aliquots were taken, 5 ml. of 20% potassium iodide solution added to each, and titrated with approximately 0.02 N sodium thiosulphate, using starch indicator. 14.17, 10.73, and 13.68 ml. of thiosulphate were required to destroy the starch-iodine color for the hydroxy-cyclized compound, benzoin and blank respectively. After a week the volumes required were 9.90, 7.73 and 9.13 ml. Therefore there was no reaction.

β-Cyanohydroxy Cyclized Compound with Lead Tetraacetate

A semi-crystalline sample of the β-cyanohydroxy cyclized compound (0.17 gm.) was dissolved in 90% acetic acid (5 ml.) and lead tetraacetate (0.30 gm.) added. The mixture was warmed to 50°C. for

1½ hr., then allowed to stand overnight. The equivalent amount of sulphuric acid was added, the precipitated lead sulphate filtered off, and the filtrate evaporated *in vacuo*. The residue was extracted with ether and the ether solution evaporated. The crude product (0.16 gm.) was dissolved in chloroform and washed with dilute hydrochloric acid, sodium bicarbonate solution and water. The chloroform solution was dried, concentrated and chromatographed on alumina. A band came through the column which yielded 0.06 gm. of material showing strong infrared absorption bands at 3610 (hydroxyl), 2210 (nitrile), 1740 (acetoxyl) and 1680 $cm.^{-1}$, and weak bands at 1625 and 1585 $cm.^{-1}$ (spectrum done in chloroform solution). The reaction apparently resulted in the substitution of an acetoxyl group for a hydrogen, α to the carbonyl group or double bond.

Attempts to Dehydrate β-Cyanohydroxy Cyclized Compound

A sample of crude β-cyanohydroxy cyclized compound (0.15 gm.) was dissolved in toluene and refluxed overnight with a little toluene sulphonic acid (0.01 gm.). The toluene solution was washed with aqueous sodium carbonate solution, dried and evaporated under reduced pressure. The infrared spectrum of the residue showed no change from that of the starting material.

The recovered β-cyanohydroxy cyclized compound was then dissolved in acetic anhydride and refluxed overnight. There was considerable darkening and tar formation. The acetic anhydride was distilled off *in vacuo* and the residue dissolved in chloroform. The chloroform solution was washed with sodium carbonate solution, dried

and evaporated. The residue was chromatographed in benzene solution on an alumina column. The first fraction (0.03 gm.) showed acetate absorption at 1740 cm.$^{-1}$ and a broad peak at 1660 cm.$^{-1}$, probably due to combined "cyclized" and N-acetate absorption. There was no cyanamide or hydroxyl absorption.

β-Cyano Cyclized Compound with Benzaldehyde

β-Cyano cyclized compound (0.5 gm.) and benzaldehyde (0.5 gm.) were dissolved in methanol (15 ml.), the solution heated to reflux, and sodium methylate solution (2 ml. of concentration 12 gm./150 ml.) added over 20 min. When no crystals had formed after standing in the refrigerator for several days, the solution was worked up. Only the starting material was obtained.

Attempted Hydrogenation of β-Cyanobromolycooodine with Palladium-Calcium Carbonate Catalyst in the Presence of Ammonium Hydroxide

β-Cyanobromolycopodine (0.4 gm.), palladium-calcium carbonate catalyst (0.1 gm.) and concentrated ammonium hydroxide (about 5 drops) were added to methanol (50 ml.) in a hydrogenation flask and the mixture shaken for 5 hr. with hydrogen at 39 p.s.i. and allowed to stand overnight. Since the catalyst had not darkened, the shaking was resumed for 9 hr. and again allowed to stand overnight. The methanol solution was decanted off and evaporated to dryness. The residue was dissolved in chloroform and washed with dilute hydrochloric acid, sodium bicarbonate solution and water, dried and evaporated. The infrared spectrum (film) of the residue showed bands at 1675 and 1700 cm.$^{-1}$ (the latter probably due to an α compound - the purity of the starting material was doubtful)

and some absorption in the hydroxyl region at 3300-3400 cm.$^{-1}$ of unknown origin.

Only unreacted starting material was obtained when the hydrogenation was attempted in the absence of base.

Attempted Hydrogenation of β-Cyanobromolycopodine with Palladium-Barium Sulphate Catalyst

β-Cyanobromolycopodine (0.6 gm.), palladium-barium sulphate catalyst (1 gm.), and fused sodium acetate (0.1 gm.) were added to acetic acid (35 ml.) and shaken with hydrogen at 41 p.s.i. for 5 hr. After standing overnight under pressure, the mixture was filtered and the acetic acid solution evaporated in vacuo. The residue was treated with water and extracted with ether. The ether extract was washed with dilute hydrochloric acid, sodium bicarbonate solution and water, dried and evaporated. The residue (0.55 gm.) was recrystallized from ether and was found, by mixed melting point and spectrum, to be the starting material.

β-Cyanobromolycopodine with Silver Acetate

A sample of pure β-cyanobromolycopodine (0.82 gm.) was dissolved in benzene (80 ml.), silver acetate (1 gm.) added, and the mixture refluxed for 5 hr. (In another run the reaction was carried out in acetonitrile solution, with much the same results.) Evaporation of the benzene and extraction of the residue with ether yielded 0.67 gm. of material which showed infrared absorption bands at 1675 ("cyclized"), 1700 (carbonyl) and 1740 cm.$^{-1}$ (acetate). This crude product was refluxed with potassium hydroxide (0.8 gm.) in methanol (20 ml.) for

2.5 hr. The methanol solution was neutralized and evaporated, and the residue treated with water and extracted with chloroform. The chloroform solution was dried, concentrated and chromatographed on alumina. The first material (0.52 gm.) to come off the column was found to be the β-cyano cyclized compound. When chloroform containing some methanol was run through the column, a band of alcoholic material (0.10 gm.) was obtained. The infrared spectrum showed hydroxyl and carbonyl absorption, but was quite different from α-cyanohydroxylycopodine in the region 850-1500 $cm.^{-1}$. The yield of alcohol and cyclized compound were 16% and 82% respectively.

SUMMARY

It has been found that the ring opened in the formation of α-cyanobromolycopodine, the major product of the reaction of lycopodine with cyanogen bromide, is six-membered or larger.

A study of the reactions of β-cyanobromolycopodine has been made. In alkaline media it readily undergoes a cyclization, preventing an investigation by the methods used with the α isomer. The product of hydrogenation in alkaline medium is the cyclized compound, $C_{17}H_{24}ON_2$, not β-cyanolycopodine, $C_{17}H_{26}ON_2$, as had been suspected previously. Attempts to reduce β-cyanobromolycopodine in acid media to a compound corresponding to α-cyanolycopodine were unsuccessful. Reaction with silver acetate in benzene gave a low yield of an alcohol, apparently β-cyanohydroxylycopodine.

The β-cyano cyclized compound was investigated in an attempt to determine the nature of the cyclization which had taken place. Although infrared and ultraviolet spectra suggest that the carbonyl group is still present, this could not be confirmed by chemical means. The possibility of an enol ether structure cannot be excluded. The cyclized compound did not react with benzaldehyde, but reacted readily with bromine in carbon tetrachloride. On hypobromination it yielded a hydroxy derivative which did not react with periodic acid, but gave a product with lead tetraacetate in which a hydrogen had apparently been replaced by an acetoxyl group.

The reactions of lycopodine have been reviewed in the light of the structure proposed for annotinine (33) and the possibility of

relating the structures of the two alkaloids discussed. A β-piperidone relationship of nitrogen and carbonyl is suggested to explain some of the reactions of lycopodine.

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