THE STRUCTURE AND REACTIONS OF ANNOTININE

THE STRUCTURE AND REACTIONS OF AMMONIUMINE

By

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SCOPE AND CONTENTS:

A study of the oxidation reactions of annotinine lactamdiol provided chemical evidence that ring A of annotinine was six-membered and confirmed that the ether ring in annotinine was an epoxide ring. The detailed structure of ring B of annotinine was elucidated through a study of the reactions of diphenyldesoxo-dihydroannotinine. Unsuccessful attempts were made to correlate the structure of annotinine with the structures of lycopodine and other Lycopodium alkaloids.

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The author wishes to express her sincere appreciation to Professor D. D. MacLean for his patient direction and encouragement during the course of the investigation.

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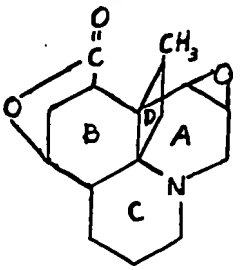
GENERAL INTRODUCTION

Annotinine, $C_{10}H_{21}O_3N$, the major alkaloid of *L. annotinum* L. was isolated in 1943 by Manske and Marion (1). In the intervening years and particularly in the last five years the structure of the alkaloid has been studied by several groups of chemists. This work culminated in 1957 when Wiesner, Ayer, Fowler and Valenta (2) proposed, on the basis of chemical evidence, structure I for annotinine. This structure was confirmed a short time later by Przybylska and Marion (3) who carried out an X-ray analysis on annotinine bromohydrin.



I

The work reported in this thesis was begun in 1955 when many features of the structure of annotinine were still unknown. While the work was in progress, two schools, Wiesner and his co-workers at the University of New Brunswick and Marion and his co-workers at the National Research Council, were also carrying out structural studies on annotinine. They used a different approach but arrived at the same conclusions that are reported in this thesis. In this investigation the structures of rings

A and B were studied. The oxidation of annotinine produced a lactam which, when treated with dilute sulphuric acid, gave a lactamdiol. A study of the oxidation reactions of annotinine lactamdiol provided chemical evidence that ring A was six-membered and confirmed that the ether ring in annotinine was an epoxide ring. The detailed structure of ring B was elucidated through a study of the reactions of diphenyldesoxodihydroannotinine, produced by the reaction of desoxodihydroannotinine with phenyllithium.

When the structure of annotinine was confirmed by X-ray analysis further structural studies on the alkaloid were discontinued and efforts were made to correlate the structure of annotinine with the structure of lycopodine, the most widely distributed Lycopodium alkaloid. However, neither lycopodine nor any derivative of it was obtained when annotinine was transformed into compounds having corresponding formulae and functional groups. In the course of this work a number of compounds were prepared which had molecular formulae corresponding to those of other Lycopodium alkaloids. No definite correlations between these compounds and the alkaloids were observed.

HISTORICAL INTRODUCTION

The presence of alkaloids in a species of the genus Lycopodium was first reported by Boedeker (4) in 1881. He isolated an alkaloid which he called lycopodine from Lycopodium complanatum L.. The same alkaloid, and two others were found in L. clavatum L. by Achmatowicz and Uzieblo (5) fifty-seven years later. In 1942 Manske and Marion (6) published the first of a series of papers in which they described the isolation of more than thirty alkaloids from eight species of the genus Lycopodium. Lycopodine occurred in all except two of the species, L. cornuum L. and L. saururus L. (7, 8). Most of the alkaloids had sixteen carbon atoms and a single nitrogen atom. Several have been shown to be simple derivatives of others (9, 10).

In 1943 Manske and Marion (1) isolated eight alkaloids from L. annotinum L.. The chief alkaloid was named annotinine and the formula $C_{16}H_{21}O_3N$ was assigned to the new base which melted at 232°C. Four years later Manske and Marion (11) found that annotinine was also the major alkaloid of L. annotinum var. acrifolium Fern.. Annotinine was isolated by Bertho and Stoll (12) from L. Annotinum L. of German origin and by Achmatowicz and Rodewald (13) from L. annotinum L. of Polish origin.

The Functional Groups of Annotinine

The characterization of the functional groups of annotinine, I, was begun in 1943 by Manske and Marion (1). They concluded that a lactone ring was present in annotinine because the alkaloid formed a water soluble salt, without loss of carbon, when it was heated with alcoholic alkali. Treatment of the salt with acid yielded a base, $C_{16}H_{23}O_4N$, II, called annotinine hydrate, which contained an added molecule of water relative to annotinine. Later, it was shown that the infrared spectrum of annotinine contained a band at 1776 cm.$^{-1}$ which is in the region of absorption of γ-lactones (12).

Manske and Marion (11) observed that hydrochloric acid added to annotinine to form a chlorohydrin, $C_{16}H_{22}O_3NCl$, III, and concluded that annotinine contained an ether bridge. Treatment of the chlorohydrin, III, with alcoholic alkali also yielded annotinine hydrate, II. They concluded that annotinine hydrate was a dihydroxy compound formed by hydrolysis of the oxide ring.

In their preliminary study of the structure of annotinine, Manske and Marion (11) treated annotinine with potassium permanganate and isolated the compound, $C_{16}H_{19}O_4N$, IV. They thought that compound IV was a base containing two carbonyl groups because it yielded a saturated base, $C_{16}H_{23}O_2N$, V, on Clemmensen reduction. They concluded that the two carbonyl groups of compound IV were formed by oxidation at both ends of the oxide ring of annotinine. Owing to the fact that the oxidation product IV, was colourless and quite stable Manske and Marion (11) thought

it was not a 1,2 or 1, 3-diketone, and they proposed that the ether bridge in annotinine was five or six-membered. This was supported by the fact that annotinine hydrate was not oxidized with periodic acid, which suggested that it was not a 1,2-diol. In another sequence of experiments they treated annotinine chlorohydrin, III, with chromous chloride and isolated an unsaturated base, $C_{16}H_{21}O_2N$, VI, which yielded the saturated base $C_{16}H_{23}O_2N$, V, on catalytic reduction.

Bertho and Stoll (12) reported that annotinine was a tertiary base because it would undergo neither N-acetylation nor N-methylation and found that there was no N-methyl group in annotinine. Bankiewicz, Henderson, Stonner, Valenta and Wiesner (14) found that a Kuhn-Roth oxidation of annotinine was in agreement with one, or less likely, two C-methyl groups.

The work reported above indicated that an ether ring, a γ-lactone function, a tertiary nitrogen atom and a C-methyl group were present in annotinine. From this information and the formula of annotinine, $C_{16}H_{21}O_3N$, it was apparent that there was a combination of four carbocyclic and heterocyclic rings in the alkaloid.

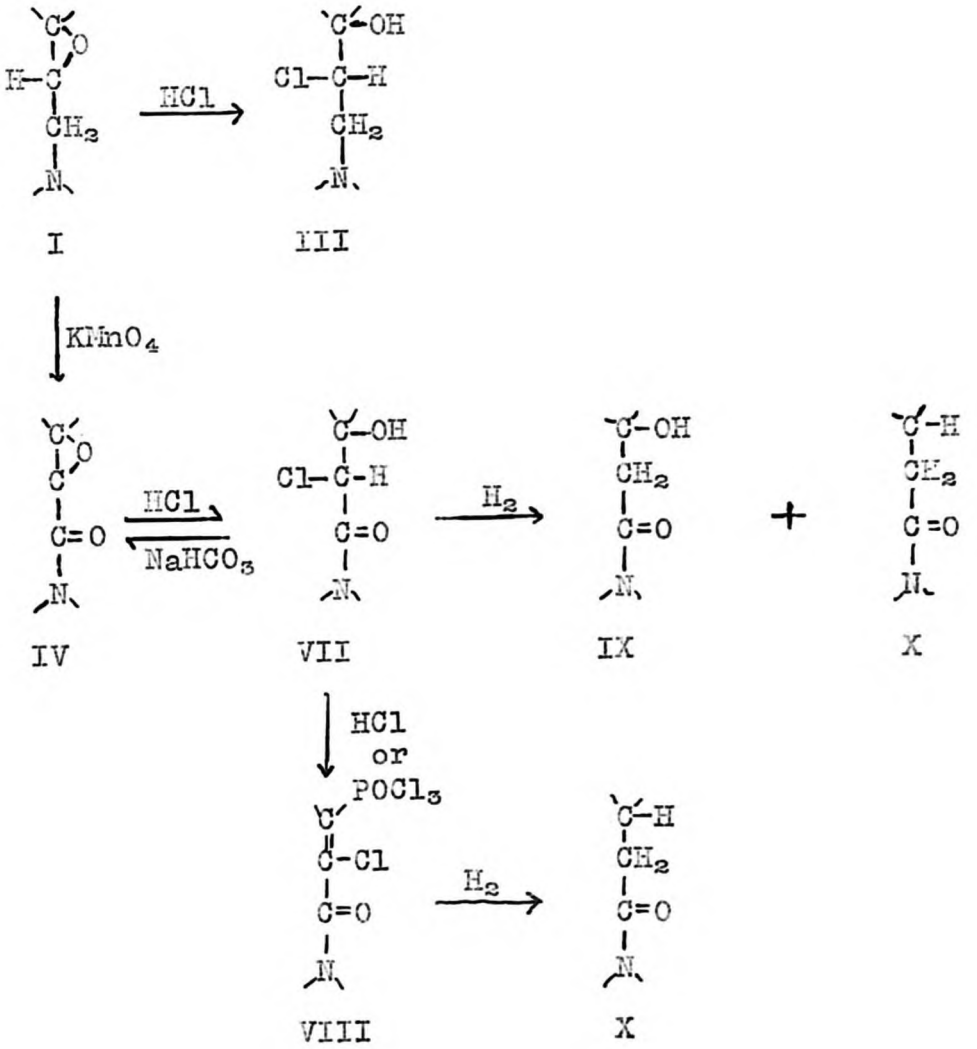
The Relationship of the Ether Ring to the Nitrogen Atom

After the derivation of the functional groups in annotinine, work was directed to the determination of the relationship of these functional groups to one another. MacLean and Prime (15) established the relationship of the oxide ring to the nitrogen atom. They re-examined the permanganate oxid-

ation product of annotinine, IV, and found that it was not a base but an amide, and observed also that the oxide ring was still present in this compound. The oxidation product, IV, now called annotinine lactam, reacted with concentrated hydrochloric acid to give a chlorohydrin, $C_{16}H_{20}O_4NCl$, VII, formed by fission of the oxide ring with the formation of hydroxyl and chloro groups. The lactam chlorohydrin VII, was dehydrated either by prolonged heating under reflux with hydrochloric acid or by treatment with phosphorus oxychloride. The product in both cases was the unsaturated compound $C_{16}H_{18}O_3NCl$, VIII. Treatment of the lactam chlorohydrin with hydrogen over Adams catalyst gave the compounds $C_{16}H_{21}O_4N$, IX, formed by hydrogenolysis of the chloro group, and $C_{16}H_{21}O_3N$, X, formed by hydrogenolysis of both the hydroxyl and the chloro groups. Similar treatment of the anhydro compound VIII with hydrogen yielded compound X. Annotinine lactam was reformed when the lactam chlorohydrin was heated under reflux with sodium bicarbonate in acetone.

MacLean and Prime (15) attempted to carry out the same reactions with annotinine chlorohydrin III, that they had with annotinine lactam chlorohydrin. They found that the oxide ring was not reformed when annotinine chlorohydrin was heated with sodium bicarbonate. Annotinine chlorohydrin was not dehydrated with hydrochloric acid or with phosphorus oxychloride and failed to react with hydrogen over Adams catalyst. They attributed the increased lability of the chloro and hydroxyl groups of the lactam chlorohydrin to the amide group which was present in that compound, but which was absent in annotinine chlorohydrin. They

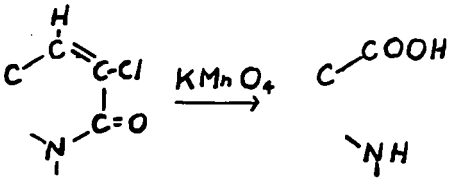
concluded that the ether ring was situated adjacent to the carbonyl carbon of the amide group and that the chloro and hydroxyl groups of the chlorohydrins were adjacent to one another. The ether ring of annotinine would then be an epoxide ring. The reactions outlined above are represented below.



MacLean and Prime (15) accounted for the facile dehydration of the lactam chlorohydrin by suggesting that the carbon carrying the chloro group was isomerized by the enolization of

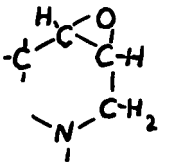
the adjacent amide linkage in the ionizing medium. Isomerization of this carbon atom was not possible in annotinine chlorohydrin and it did not dehydrate when it was treated under conditions similar to those which dehydrated the lactam chlorohydrin.

MacLean and Prime (15) and Meier, Meister and Marion (16) concluded that the hydroxyl group of the lactam chlorohydrin was tertiary because the compound was not oxidized by potassium permanganate or by chromic acid. However, Henderson, Stonner, Valenta and Wiesner (17) oxidized the anhydro compound VIII with permanganate and isolated an amino acid, $C_{14}H_{19}O_4N$, XI, which still contained the γ-lactone ring. Their oxidation was formulated as follows:



VIII XI

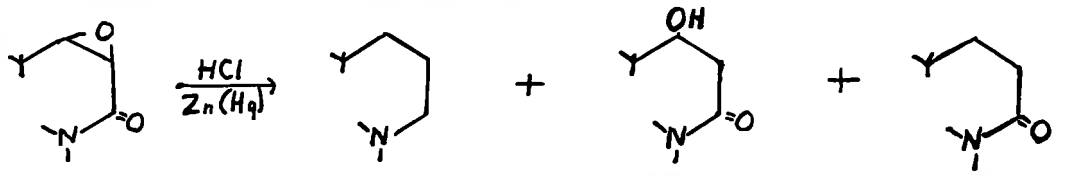
Therefore the carbon carrying the hydroxyl group in the chlorohydrin must be secondary. The methyl ester of the amino acid XI did not saponify and therefore it was proposed (16) that the carbomethoxy group was tertiary. The following partial structure for annotinine was proposed:



That the above partial structure was part of a six- or larger membered ring was indicated by the position of the absorption

band of the amide group in the infrared spectrum of annotinine lactam (16, 17).

The above partial structure for annotinine was used to explain two reactions reported by Manske and Marion (11), namely the Clemmensen reduction of annotinine lactam and the chromous chloride reduction of annotinine chlorohydrin. MacLean and Prime (15) on repeating the Clemmensen reduction of annotinine lactam isolated besides the saturated base $C_{16}H_{23}O_2N$, V, the neutral compound $C_{16}H_{21}O_4N$, IX, in good yield, and a small amount of the compound $C_{16}H_{21}O_3N$, X.



Annotinine Lactam — V — IX — X

Clemmensen reduction of compounds IX and X separately yielded only small amounts of the compound V, so they were not considered to be intermediates in the reduction of annotinine lactam. Similar treatment of the lactam chlorohydrin gave compounds IX and X and some of compound V. MacLean and Prime concluded that the Clemmensen reduction of annotinine lactam involved a direct reduction of an amide to an amine which was unusual for this type of reaction. The removal of the chloro and the hydroxyl groups was not typical of this reaction, but had been reported for α-chloro acids and α-chloro ketones and for β-hydroxy acids (15). Meier, Meister and Marion (16) also studied the Clemmensen reduction of annotinine lactam. They isolated the

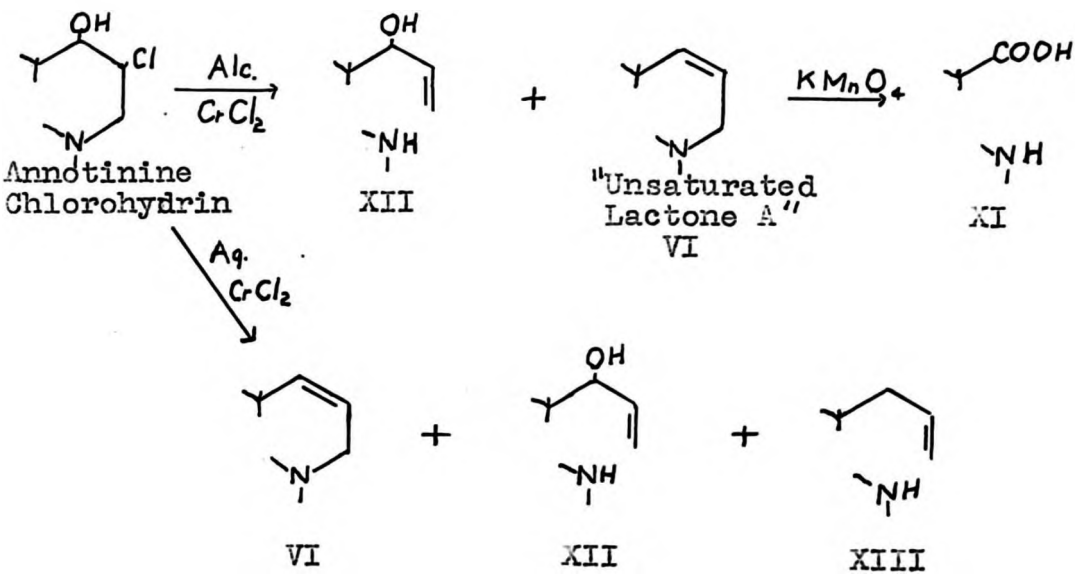
base V and some lactam chlorohydrin but none of compounds IX or X. They concluded that the amide group was reduced because it was strongly activated due to the close proximity of other (unnamed) functional groups.

Meier, Meister and Marion (16) investigated the chromous chloride reduction of annotinine chlorohydrin and isolated as well as the unsaturated base $C_{16}H_{21}O_2N$, VI, two other compounds. The composition of the product depended upon the solvent used and the concentration of hydrochloric acid. Treatment of annotinine chlorohydrin with an alcoholic solution of chromous chloride yielded a product which was two-thirds compound VI and one-third a hydroxy compound $C_{16}H_{23}O_3N$, XII. Compound XII, called "hydroxylactone", was at first thought to be the simple dehalogenated product of annotinine chlorohydrin. Later Anet and Marion (19) found that it contained unsaturation and had a terminal methylene group. It yielded a dihydro derivative on hydrogenation and formaldehyde on ozonolysis. Treatment of "hydroxylactone" with acetic anhydride produced a neutral O,N-diacetyl derivative. Anet and Marion (19) proposed that the unsaturated base VI, usually called "unsaturated lactone A" was a cyclic allylamine. They observed that its pKa was 7.06 while the pKa of its dihydro derivative was 8.48. The proposed cyclic allylamine structure was supported by chemical evidence. The amino acid, $C_{14}H_{19}O_4N$, XI, was isolated as a product of the permanganate oxidation of "unsaturated lactone A".

When Meier, Meister and Marion (16) treated annotinine

chlorohydrin with an aqueous solution of chromous chloride and hydrochloric acid the product consisted of "hydroxylactone" (34.6 per cent), "unsaturated lactone A" (33.2 per cent) and a compound designated as "unsaturated lactone B", XIII, (3.8 per cent). Anet and Marion (19), who assigned the empirical formula $C_{16}H_{23}O_2N$ to "unsaturated lactone B", found that it had a terminal methylene group. It formed a neutral N-acetyl derivative which yielded formaldehyde on ozonolysis. The dihydro derivative of "unsaturated lactone B" had N-H absorption at 3325 cm^{-1}. in its infrared spectrum. When the "hydroxylactone" was treated with chromous chloride "unsaturated lactone B" was obtained.

The chromous chloride reactions were represented as follows:



Anet and Marion (19) proposed a satisfactory explanation for the chromous chloride reactions. They noted that chromous chloride would replace halogen by hydrogen and reduce vic-dihalides to

the corresponding unsaturated compounds. In these reactions chromous chloride behaved like zinc in neutral or acidic solution, which was known to convert vic-chlorohydrins to olefins. It would be expected therefore that chromous chloride would convert β-chloroalcohols to olefins, which would account for the formation of "unsaturated lactone A". They proposed that β-chloroamines would be expected to behave similarly, thus:

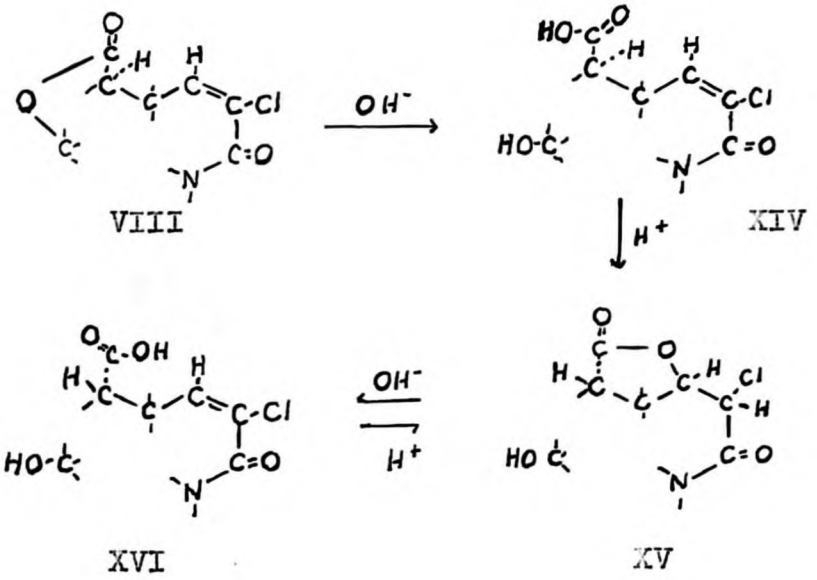
$$-\overset{|}{\underset{Cl}{C}}-\overset{|}{C}-N< \quad \xrightarrow{CrCl_2} \quad >C=C< \quad H-N<$$

The latter reaction would then account for the formation of the "hydroxylactone" and "unsaturated lactone B".

The Relationship of the Lactone Ring to the Ether Ring

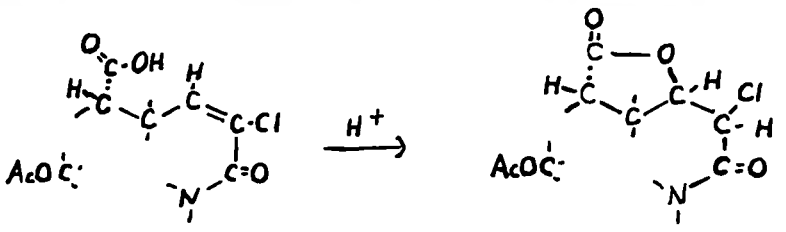
Valenta, Stonner, Bankiewicz and Wiesner (17, 18) extended the partial structure of anhotinine to include the lactone ring. They heated the anhydro compound, $C_{16}H_{18}O_3NCl$, VIII, under mild reflux with alcoholic alkali and obtained a hydroxy acid $C_{16}H_{20}O_4NCl$, XIV, which still had the conjugated lactam group, but no lactone ring. When the acid XIV was heated under reflux with a trace of p-toluenesulphonic acid in benzene a hydroxy lactone $C_{16}H_{20}O_4NCl$, XV, was formed. The infrared spectrum of XV lacked conjugated lactam absorption, but contained γ-lactone absorption. It was proposed that a new lactone ring was present in XV, formed by addition of the carboxyl group of XIV across the conjugated double bond. Treatment of the hydroxy lactone XV with alkali caused a base-catalysed β-elimination, which resulted in the formation of a hydroxy acid $C_{16}H_{20}O_4NCl$, XVI. The infrared spectrum of the acid XVI was similar to but not identical with that of the first

hydroxy acid XIV. They (18) suggested that the carboxyl group in the acid XVI had a configuration opposite to that of the acid XIV. Relactonization of the acid XVI gave compound XV. The above reactions were formulated in the following manner:



VIII XIV XVI XV

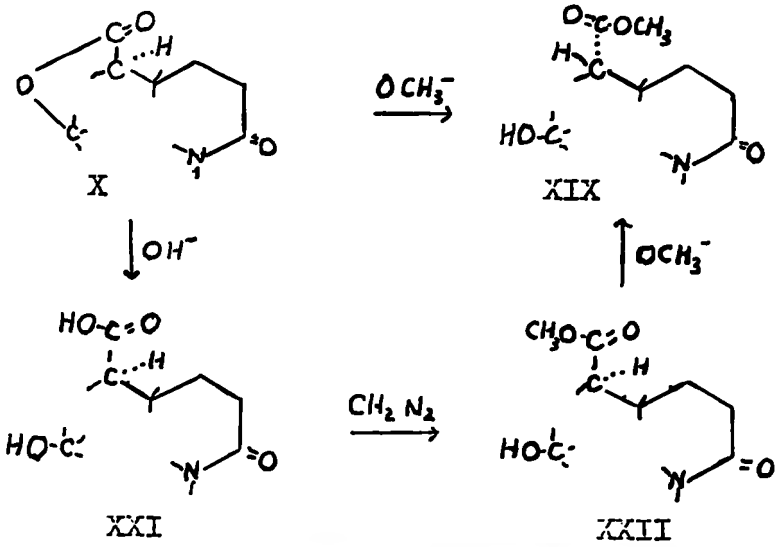
The postulate that a new γ-lactone ring had formed in compound XV was confirmed in the following manner (18). The hydroxyl group of compound XVI was acetylated and the acetyl derivative XVII, was lactonized. The resulting compound $C_{18}H_{22}O_5NCl$, XVIII, had γ-lactone absorption, but no conjugated lactam absorption in its infrared spectrum.



XVII XVIII

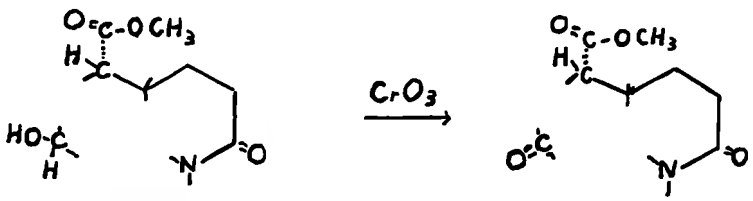
Valenta, Stonner, Rankiewicz and Wiesner (18) had suggested that the carboxyl group of the acid XVI had a configur-

ation opposite to that of the acid XIV. In the following sequence of reactions they confirmed that the carboxyl group derived from the lactone ring was readily epimerised. Compound X was heated under mild reflux with methanolic potassium hydroxide and a hydroxy methyl ester, $C_{17}H_{25}O_4N$, XIX, was isolated. The ester XIX was saponified with ethanolic potassium hydroxide to give the hydroxy acid $C_{16}H_{23}O_4N$, XX. The acid XX was converted back to the ester XIX by treatment with diazomethane. However when compound X was refluxed with ethanolic potassium hydroxide a hydroxy acid XXI was isolated, which when treated with diazomethane gave a methyl ester XXII isomeric with the ester XIX. The ester XXII was converted into the ester XIX by treatment with methanolic potassium hydroxide. They offered the following explanation for the above sequence of reactions. In methanolic potassium hydroxide the concentration of methoxide ions was considerably higher than the concentration of hydroxide ions. The methoxide ions caused the lactone ring to open giving the ester XXII which immediately isomerized under the reaction conditions to the ester XIX. In ethanolic potassium hydroxide the lactone ring was attacked by hydroxide ions rather than by ethoxide ions and the corresponding acid XXI was formed. This acid was present in the reaction mixture as the carboxylate ion which would be stable to epimerization. Treatment of the acid XXI with diazomethane gave the unstable ester XXII. This work not only established the secondary nature of the lactone carboxyl, but also the fact that the original configuration of the carboxyl group in annotinine was less stable than the epimeric one.



X XIX XXI XXII

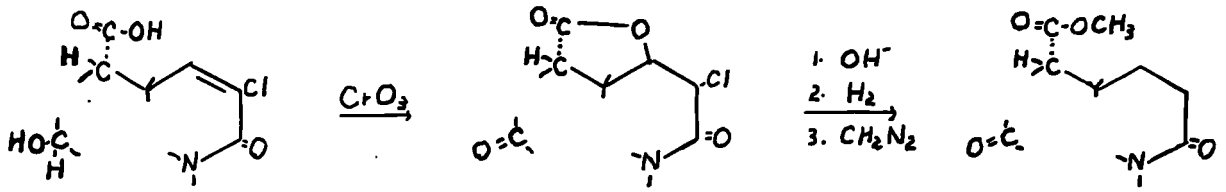
Information about the hydroxyl group derived from the lactone ring was obtained by Valenta, Stonner, Dankiewicz and Wiesner (18). They oxidized the two hydroxy esters XIX and XXII with chromium trioxide in pyridine and obtained the corresponding keto esters XXIII and XXIV. This indicated that the hydroxyl group contained in the lactone ring was secondary in nature. The position of the ketonic carbonyl absorption bands in the infrared spectrum of the keto-esters indicated that the keto group was in a six-membered ring or larger.



XIX XXIII

The keto ester XXIII was also obtained by another method (18). The unsaturated acid XVI was oxidized with chromium trioxide in pyridine and a product was obtained from which a keto lactone XXV was isolated. The keto lactone XXV was dissolved in alcoholic alkali which caused β-elimination of the lactone. The solution

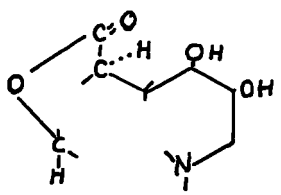
was hydrogenated over palladium-charcoal and the acidic product was treated with diazomethane. The keto ester XXIII was obtained.



XVI XXV XXIII

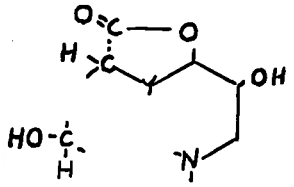
Martin-Smith, Greenhalgh and Marion (20) also observed the epimerization of the carboxyl group originating from the lactone ring of annotinine. They treated annotinine for a short time with boiling aqueous barium hydroxide and obtained a hydroxy amino acid, $C_{16}H_{23}O_4N$, XXVI. The acid was converted to its methyl ester XXVII with diazomethane. The ester XXVII was readily converted to an isomeric ester XXVIII by treatment with potassium methoxide in methanol. The ester XXVIII was also obtained when annotinine itself was treated with potassium methoxide in methanol.

The ease of epimerization of the carboxyl group of the lactone ring and its relactonization to form a new γ-lactone ring cast doubt on the originally proposed structure of annotinine hydrate, shown below. Annotinine hydrate was prepared by Manske and Marion (11) by treating annotinine with alcoholic alkali.

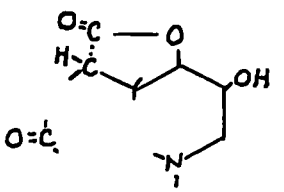


Therefore the structure of annotinine hydrate was reinvestigated. Meier, Meister and Marion (16) oxidized the hydrate with chromic

acid and isolated a compound, $C_{16}H_{21}O_4N$, XXIX, which still contained the γ-lactone ring and one hydroxyl, the other hydroxyl had been oxidized to a keto group. Valenta, Stonner, Bankiewicz and Wiesner (18) found that the compound XXIX lacked the properties of an alpha-ketol. They proposed the following partial structures for annotinine hydrate, II, and its oxidation product XXIX:

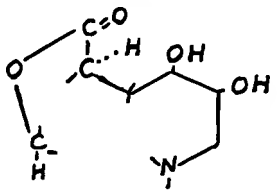


II



XXIX

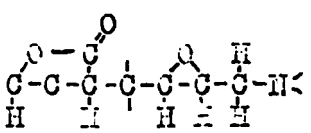
They (17) treated annotinine with dilute sulphuric acid and isolated a compound XXX which was an isomer of annotinine hydrate. They postulated that XXX had the following partial structure:



XXX

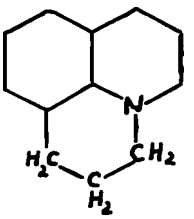
Support for this partial structure was found in the fact that compound XXX on permanganate oxidation was converted into the amino acid XI, $C_{14}H_{19}O_4N$ (20).

On the basis of the chemical evidence which has been presented, the following partial structure for annotinine was written (21)



Dehydrogenation Experiments

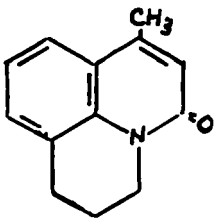
In order to obtain information about the size and arrangement of the rings in annotinine, dehydrogenation experiments were carried out on the alkaloid and two of its derivatives. Bankiewicz, Henderson, Stonner, Valenta and Wiesner (14) dehydrogenated annotinine over selenium at 330°C and isolated 8-n-propylquinoline from the reaction product. Anet and Marion (19) reported that they isolated 7-methylquinoline when they dehydrogenated annotinine over palladium at 300°C. It was concluded that a hydrogenated quinoline system was probably present in annotinine (21). The n-propyl group in the 8 position of the quinoline nucleus indicated that the following arrangement of atoms might be present in annotinine with one nitrogen ring having three adjacent methylene groups:



It is difficult to explain the formation of 7-methylquinoline on the basis of the known structure of annotinine.

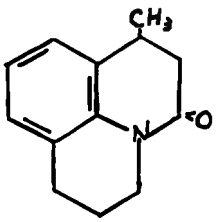
The amino acid XI, $C_{14}H_{19}O_4N$, was dehydrogenated over palladium on barium sulphate at 200-250°C by Anet and Marion (19). An optically active acid $C_{14}H_{15}O_3N$, XXXI, and a small amount of its decarboxylated derivative $C_{13}H_{15}ON$, XXXII, were isolated. The acid XXXI no longer contained γ-lactone absorption in its infrared spectrum but did have absorption for carboxyl and amide groups and for a benzene ring. The neutral compound XXXII was

obtained from the acid XXXI by heating the acid over copper powder. A comparison of the ultraviolet spectrum of the acid XXXI with that of compound XXXII indicated that the carboxyl group in the acid XXXI was directly attached to the benzene ring. Valenta, Wiesner, Bankiewicz, Henderson and Little (22) have carried out an extensive investigation of the products of the dehydrogenation of the amino acid XI. They isolated, on repeating the dehydrogenation, besides the acid XXXI and the neutral lactam XXXII, a second neutral compound $C_{13}H_{13}ON$, XXXIII. The compound XXXIII, which was also obtained by dehydrogenation of the neutral lactam XXXII over palladium charcoal at 270°C, was shown by synthesis to have the following structure:



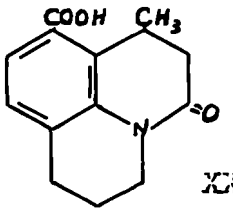
XXXIII

The neutral lactam XXXII was then found to be one of the optical isomers of



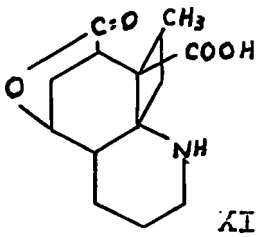
XXXII

They (22) established that the structure of the acid XXXI was the following by synthesis of its racemate.



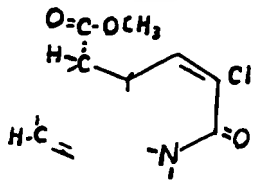
XXXI

The acid XXXI was produced from the amino acid XI with retention of all the carbon atoms and without loss of its optical activity. Therefore one would expect that no deep-seated rearrangements of XI had occurred in the dehydrogenation reaction. However, on the basis of the now known structure of XI (shown below) a simple explanation of the conversion of the amino acid, XI, into the compound XXXI is not possible. An ingenious explanation has been proposed by Wiesner (23).



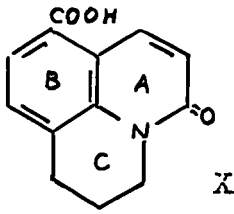
XI

In another dehydrogenation reaction Wiesner, Valenta, Ayer and Kankiewicz (24) used the anhydro ester $C_{17}H_{20}NO_3Cl$, XXXIV, prepared by heating the methyl ester of the acid XVI under reflux in xylene with phosphorus pentoxide (21).



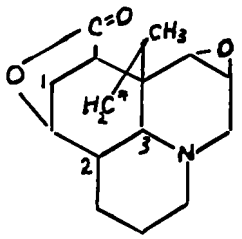
XXXIV

The anhydro ester XXXIV was dehydrogenated over palladium charcoal at 290°C and a quinolone acid $C_{13}H_{11}O_3N$, XXXV, was obtained. The structure of XXXV was determined by decarboxylation to a quinolone of known structure and by comparison of the ultraviolet and infrared spectra of its dihydro derivative with those of compound XXXI.

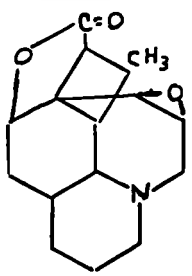


XXXV

The quinolone acid XXXV was formed from the anhydro ester XXXIV with the loss of three carbon atoms from the carbon skeleton. Kiesner, Valenta, Ayer and Bankiewicz (24) postulated that these three carbon atoms were part of a ring which had bridged ring B (see above) in the anhydro ester XXXIV. In the dehydrogenation reaction this ring was eliminated with the formation of the aromatic ring. In July, 1956, they (24) proposed that annotinine had one of the following structures, where C* was joined to one of C(1), C(2) or C(3). The connection between C* and C(2) was preferred.

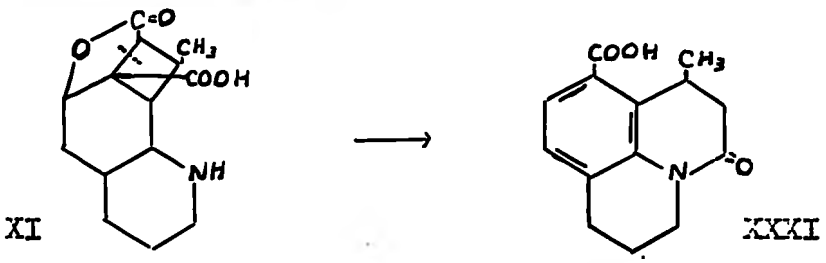


In April, 1957, Martin-Smith, Greenhalgh and Marion (20) proposed the following structure for annotinine:



They stated that the products of the dehydrogenation of annotinine and its derivatives can be rationalised just as readily on the basis of this structure as on the structures

proposed by Wiesner, Valenta, Ayer and Bankiewicz (24). The formation of the lactam acid XXXI from the amino acid XI would involve rupture of the lactone ring, opening of the four-membered ring in the position indicated, aromatization of the six-membered carbon ring and lactamization of the liberated carboxyl group with the secondary amino group.

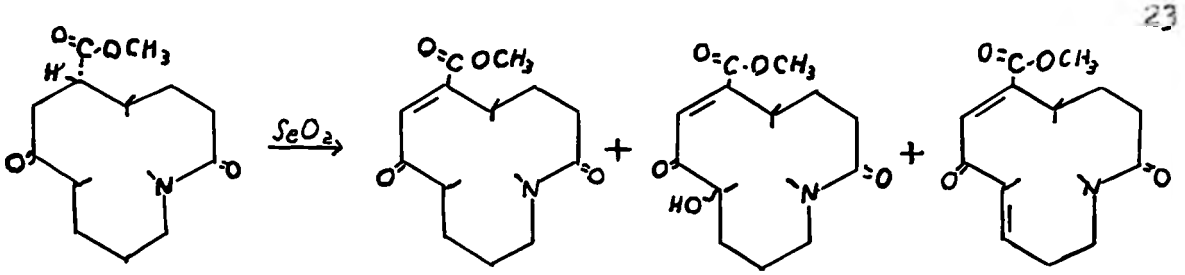


XI XXXI

A more complicated mechanism was needed to explain the formation of the lactam acid XXXI from the amino acid XI on the basis of the structures proposed by Wiesner, Valenta, Ayer and Bankiewicz (24). From the chemical evidence presented and the dehydrogenation studies it was impossible to assign a complete structure to annotinine.

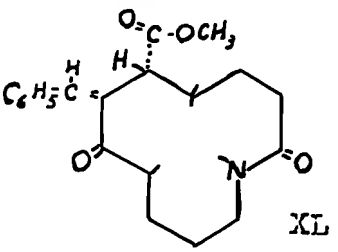
The Structure of Annotinine

In May, 1957, Wiesner, Ayer, Fowler and Valenta (2) presented chemical evidence which eliminated all but one of the proposed structures for annotinine. They treated the keto ester XXIII with selenium dioxide in dioxane and isolated four products which were given the following partial structures:



XXIII XXXVI XXXVII, XXXVIII XXXIX

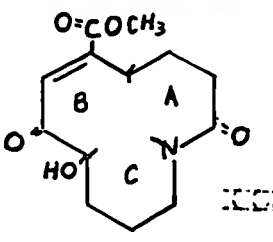
Compounds XXXVII and XXXVIII were considered to be epimeric at the carbon bearing the hydroxyl group. This eliminated C(2) (see above) as a terminal position of the fourth ring in annotinine. The presence of the system $\overset{O}{\overset{\|}{C}}\text{-}\underset{H}{\underset{|}{C}}\text{=}C\text{-}CO_2CH_3$ invalidated the structure proposed by Martin-Smith, Greenhalgh and Marion (20). Treatment of the compound XXIII with benzaldehyde gave a benzylidene derivative XL.



XL

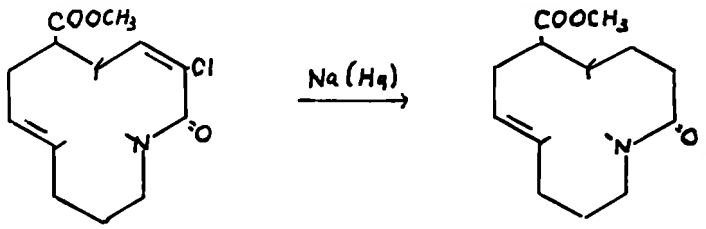
This indicated that a methylene group was situated alpha to the carbonyl group in XXIII. Thus C(1) was eliminated as a terminal position of the fourth ring in annotinine.

Diesner, Ayer, Fowler and Valenta (2) oxidised the compound XXXVIII by the Lemieux method with periodate-permanganate and isolated formic and succinic acids. Since the succinic acid could not arise from rings A or B, it must have come from ring C. This supported the assumption already made that this ring contained three adjacent methylene groups.



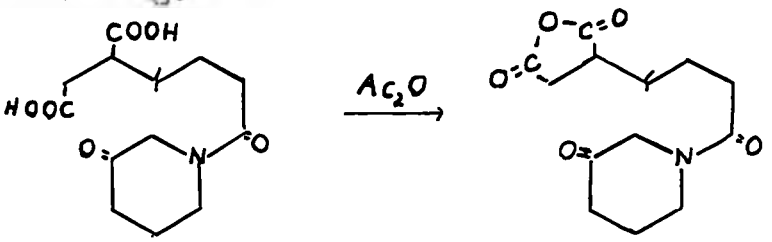
XXXVIII

Wiesner (23) reduced the anhydro ester XXXIV with sodium amalgam and obtained the compound XLI.



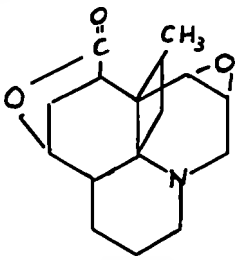
XXXIV XLI

Oxidation of the compound XLI yielded compound XLII which, on treatment with acetic anhydride, gave compound XLIII. The infra-red spectrum of XLIII indicated that the keto group was part of a six-membered ring.



XLII XLIII

According to Wiesner, Ayer, Fowler and Valenta (2) the above chemical evidence indicated that annotinine must have the following structure. The other structures which had been proposed had been eliminated.



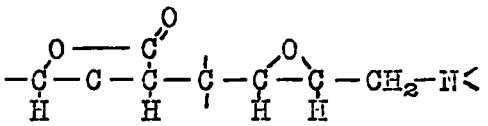
Annotinine

Later in the same year Przybylska and Marion (3) carried out an X-ray investigation on annotinine bromohydrin and verified that the structure above for annotinine proposed by Wiesner, Ayer, Fowler and Valenta was correct.

DISCUSSION OF RESULTS

Introduction

The relationship of the functional groups of annotinine had been established when the work presented in this thesis was commenced in 1955. MacLean and Prime (15) had determined the position of the ether ring relative to that of the nitrogen atom and had postulated that the ether ring was three-membered. The relationship of the γ-lactone function to the other functions in the molecule had been determined by Valenta, Stonner, Bankiewicz and Wiesner (17, 18). These relationships can be expressed in the following manner:



Dehydrogenation studies on annotinine indicated that a hydrogenated quinoline nucleus might be present in annotinine (14, 19).

The infrared spectrum of annotinine lactam, the permanganate oxidation product of annotinine, indicated that the heterocyclic ring carrying the ether function (ring A) was six-membered or larger (16, 17). The first part of this investigation was initiated in order to establish with certainty the size of ring A in annotinine.

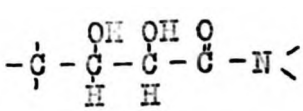
The second part of the work presented in this thesis was started with the intention of finding out whether the carboxyl and hydroxyl groups contained in the lactone function were on the

same or on different rings. Infrared spectral data indicated that the ring carrying the hydroxyl group of the lactone function was six-membered or larger (18), but there was no chemical evidence to indicate whether the carboxyl group was on the same ring.

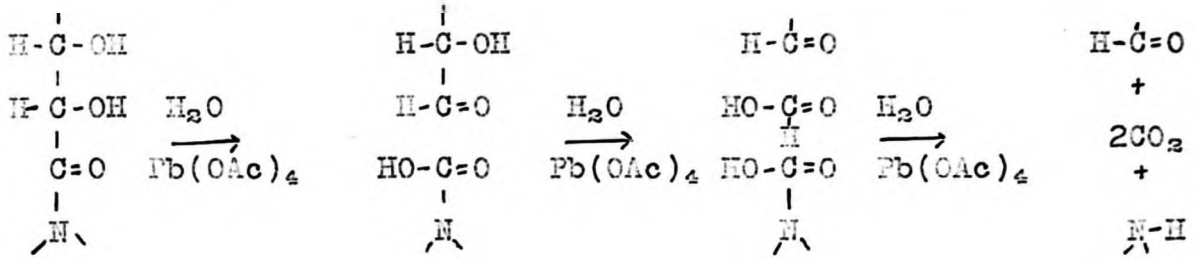
The final part of this investigation was concerned with attempts to relate the structure of annotinine to that of other Lycopodium alkaloids, in particular to the structure of lycopodine, the most widely distributed of these alkaloids. Most of the alkaloids isolated from the various species of the genus Lycopodium have 16 carbon atoms and a single nitrogen atom. Therefore one might expect that these alkaloids would have the same or nearly the same carbon skeletal arrangement, and differ principally in the number, type and position of their functional groups.

The Structure of Ring A

Studies on the structure of ring A began with annotinine lactam which was prepared by permanganate oxidation of annotinine using the method of MacLean and Prime (15). When annotinine lactam was heated under reflux with dilute sulphuric acid, the oxide ring was opened and the dihydroxy compound, annotinine lactamdiol, $C_{16}H_{21}O_5N$, XLIV, was isolated. Annotinine lactamdiol reacted slowly with three moles of lead tetraacetate. This can be easily explained if the following arrangement of atoms were present in annotinine lactamdiol:



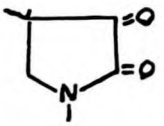
Two moles of lead tetraacetate would be used for cleavage and the third would oxidize the liberated formic acid (25, 26).



The reaction of lead tetreacetate with annotinine lactamdiol confirmed that the hydroxyl groups were adjacent and vicinal to the amide group. This was the first time that the bond between the carbons bearing the hydroxyl groups derived from the oxide ring was cleaved by glycol-cleavage reagents.

The oxidation of annotinine lactamdiol with potassium permanganate and with chromium trioxide was carried out in order to elucidate the size of ring A. Treatment of annotinine lactamdiol with chromium trioxide in acetic acid yielded a neutral compound $C_{15}H_{17}O_4N$, XLV, and an acidic compound $C_{15}H_{19}O_5N$, XLVI. The two compounds, XLV and XLVI, and the amino acid $C_{14}H_{19}O_4N$, XI, were isolated when potassium permanganate was the oxidizing agent.

The infrared spectrum of the neutral compound XLV contained bands at 1708, 1760 and 1780 cm.$^{-1}$. The band at 1780 cm.$^{-1}$ was due to the γ-lactone function. The bands at 1708 and 1760 cm.$^{-1}$ are best explained if it is assumed that the nitrogen is part of a five-membered ring. The band at 1708 cm.$^{-1}$ would then be due to a γ-lactam function and the one at 1760 cm.$^{-1}$, to a five-membered cyclic ketone. The following arrangement of atoms would be present in the neutral compound, XLV:

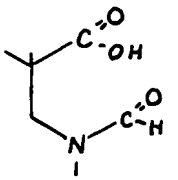


XLV

Sodium borohydride reduces ketones but not lactam carbonyls or lactones. Therefore one might expect the product of reduction of compound XLV with sodium borohydride would still contain the γ-lactone and γ-lactam functions and have a hydroxyl group in place of the ketone. The infrared spectrum of the compound XLVII, $C_{15}H_{19}O_4N$, the borohydride reduction product of compound XLV, contained hydroxyl absorption at 3335 $cm.^{-1}$, γ-lactone absorption at 1781 $cm.^{-1}$, γ-lactam absorption at 1682 $cm.^{-1}$ and no ketone absorption. Thus the above partial structure for the compound XLV must be correct. Compound XLV, with a γ-lactam ring, was formed from annotinine lactamdiol with the loss of one carbon atom. Therefore the heterocyclic ring in annotinine lactamdiol having the lactam function and the two hydroxyl groups was six-membered.

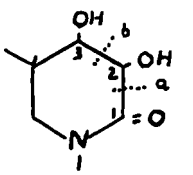
Examination of the infrared spectrum of the acidic compound $C_{15}H_{19}O_5N$, XLVI, derived from the oxidation of annotinine lactamdiol, revealed the presence of bands at 1610, 1705 and 1775 $cm.^{-1}$. The bands at 1705 and 1775 $cm.^{-1}$ can be assigned to a carboxyl group and to the γ-lactone group respectively, which leaves the band at 1610 $cm.^{-1}$ to be assigned to an amide group. Hydrolysis of the acid XLVI with dilute sulphuric acid gave formic acid in equimolar amount and the amino acid $C_{14}H_{19}O_4N$, XI, identified as its methyl ester. Therefore

the acid XLVI must be an N-formyl derivative of the amino acid XI and have the partial structure:



XLVI

The formation of the compounds XLV, XLVI and XI on oxidation of annotinine lactamdiol can be explained in the following way:



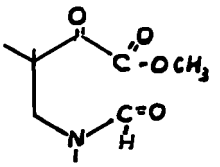
Annotinine Lactamdiol

Initial fission of the above system at (a) would yield a carbamic acid which would lose carbon dioxide giving a secondary amine. Oxidation of the intermediate secondary amine at carbon 3 would yield an α-keto acid which by cyclization would give compound XLV with the γ-lactam function. Fission of annotinine lactamdiol at (b) and oxidation at centers 2 and 3 would give an N-oxalyl carboxylic acid which in the medium would decarboxylate to give the acid XLVI. Either XLV or XLVI or their progenitors would yield the amino acid XI by hydrolysis or oxidation or a combination of the two.

The acid XLVI was also isolated on permanganate oxidation of "unsaturated lactone A", which was prepared by the chromous chloride reduction of annotinine chlorohydrin (11). The

oxidation of "unsaturated lactone A" was carried out in order to find an explanation for the anomalous results of the action of cyanogen bromide on "unsaturated lactone A". Anet and Marion (19) proposed that "unsaturated lactone A" was a cyclic allylamine. Therefore the reaction of "unsaturated lactone A" with cyanogen bromide would be expected to proceed with ease to give an N-cyano allylbromide. However, when "unsaturated lactone A" was treated with cyanogen bromide, about 80 percent of it was recovered unchanged. The remaining 20 percent was converted into an unidentified amorphous material, the infrared spectrum of which contained very little N-cyano absorption. This inertness of "unsaturated lactone A" to action of cyanogen bromide suggested that it might have a vinylamine rather than an allylamine structure. In order to differentiate between these two possible structures, "unsaturated lactone A" was oxidized with barium permanganate. Three oxidation products were isolated, two acids and the amino acid $C_{14}H_{19}O_4N$, XI. One acid was the N-formyl compound $C_{15}H_{19}O_5N$, XLVI, isolated previously from the oxidation of annotinine lactamdiol. The second acid was converted into its methyl ester, $C_{17}H_{21}O_6N$, XLVIII, and isolated as such. The infrared spectrum of the ester XLVIII had the usual γ-lactone peak at 1780 $cm.^{-1}$ as well as single peaks at 1743 and 1710 $cm.^{-1}$ which were in the region of absorption of carbomethoxy and ketone groups respectively, and a double peak at 1633 and 1645 $cm.^{-1}$ in the amide absorption region. Hydrolysis of the ester

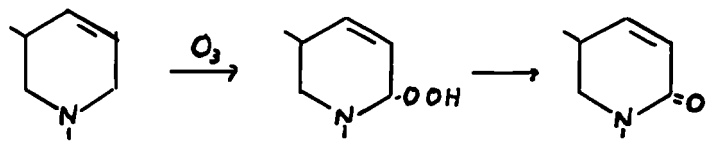
XLVIII with dilute sulphuric acid yielded an equimolar amount of formic acid and a trace of the neutral compound XLV. The above evidence indicated that the ester XLVIII had the following partial structure:



XLVIII

The products of the permanganate oxidation of "unsaturated lactone A" can be explained just as readily on the basis of a vinylamine structure as on an allylamine structure. It was thought that ozonolysis might differentiate between the two possible positions of the double bond. If the vinylamine group were present, a neutral N-formyl compound would be isolated and if "unsaturated lactone A" were an allylamine the product would be a basic dialdehyde. However, neither of these compounds was isolated when "unsaturated lactone A" was ozonized. Instead, the product was a neutral compound $C_{16}H_{19}O_3N$, XLIX, the infrared spectrum of which showed absorption for a conjugated lactam. It proved to be identical with the compound $C_{16}H_{19}O_3N$, prepared by MacLean and Prime (15) by dehydration of the hydroxy lactam IX and isolated also by Anet and Marion (19) when they treated annotinine lactam chlorohydrin with chromous chloride. The formation of the compound XLIX on ozonolysis of "unsaturated lactone A" can be rationalized more readily on the basis of an allylamine structure than the alternative vinylamine formulation. Ozone might be expected to react with "unsaturated lactone A" at the methylene

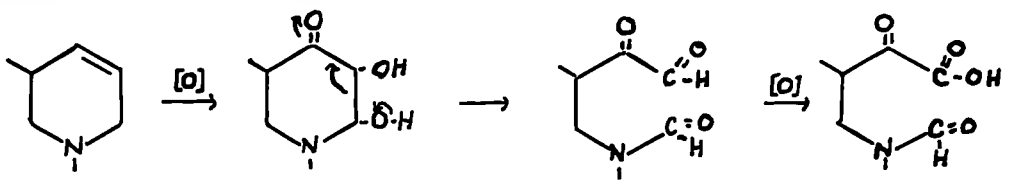
carbon to form a hydroperoxide from which compound XLIX would be produced by decomposition.



"Unsaturated Lactone A"

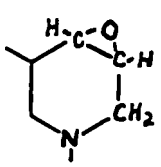
XLIX

The products of the permanganate oxidation of "unsaturated lactone A" can be explained on the basis of the allylamine structure in the following way. Oxidation of "unsaturated lactone A" at the methylene group between the double bond and the nitrogen atom followed by hydroxylation of the double bond would yield annotinine lactamdiol. Further oxidation of annotinine lactamdiol to give the N-formyl acid XLVI was described previously. The amino acid XI was formed by complete oxidation and hydrolysis. The formation of the acid, which on esterification gave compound XLVIII, can be accounted for by the following sequence:



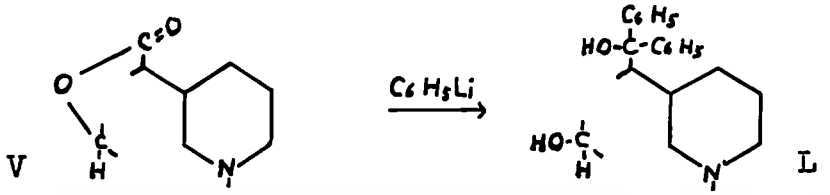
"Unsaturated Lactone A"

From the results of the reactions of annotinine lactamdiol and of "unsaturated lactone A" the structure of ring A of annotinine can be written with certainty as:



The Structure of Ring B

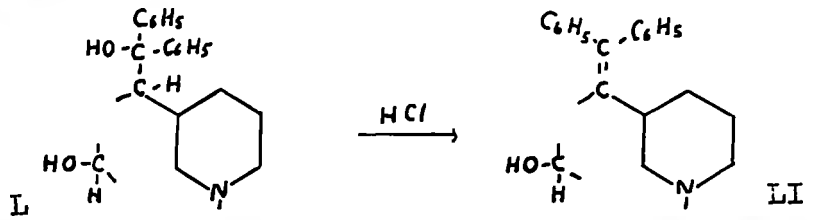
Valenta, Stonner, Bankiewicz and Wiesner (18) established that the ring carrying the hydroxyl function of the γ-lactone ring in annotinine was six-membered or larger. There was no chemical evidence to indicate whether the carboxyl group and the hydroxyl group derived from the γ-lactone function were on the same or different rings. It was thought that for a study of this problem a derivative of annotinine in which ring A contained no functional groups should be used, since several examples of the interaction of the carboxyl group, derived from the lactone ring, with functional groups in ring A, had been observed (18, 20). Accordingly "unsaturated lactone A" was converted by reduction into its dihydro derivative, $C_{16}H_{23}O_2N$, V, subsequently called desoxodihydroannotinine. This compound reacted readily with phenyllithium in ether to give diphenyldesoxodihydroannotinine, $C_{28}H_{35}O_2N$, L.



Diphenyldesoxodihydroannotinine had phenyl absorption at 2580 Å, $\log\varepsilon = 2.8$, in its ultraviolet spectrum and hydroxyl absorption at 3600 and 3150 $cm.^{-1}$ and phenyl absorption at 1590 $cm.^{-1}$ in its infrared spectrum. A study of the reactions of this compound gave the desired information.

Diphenyldesoxodihydroannotinine readily lost a mole of water when it was refluxed with methanolic hydrochloric acid.

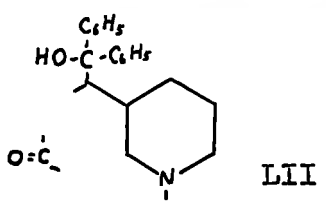
The product $C_{28}H_{33}ON$, LI, did not take up hydrogen over Adams catalyst and was thought at first to be a cyclic ether formed by loss of water between the two hydroxyl groups, rather than an unsaturated compound. However, the infrared spectrum of the compound LI contained a band at 3630 $cm.^{-1}$ in the hydroxyl absorption region and a double band at 1600 and 1580 $cm.^{-1}$ which indicated the presence of a conjugated phenyl group (27). The ultraviolet spectrum of compound LI showed strong absorption in the region of 2200-2800 Å ($\log \varepsilon = 3.6-4.0$) and was similar to the ultraviolet spectrum of 1,1-diphenylethylene. The dehydration of diphenyldesoxodihydroannotinine, L, is illustrated in the following way:



L LI

This supported the evidence of Valenta, Stonner, Bankiewicz and Wiesner (18) that the carboxyl group derived from the lactone ring was secondary in nature.

Oxidation of diphenyldesoxodihydroannotinine by the Oppenauer procedure, using aluminum isoproxide and cyclohexanone in toluene gave the expected hydroxy ketone $C_{28}H_{33}O_2N$, LII.

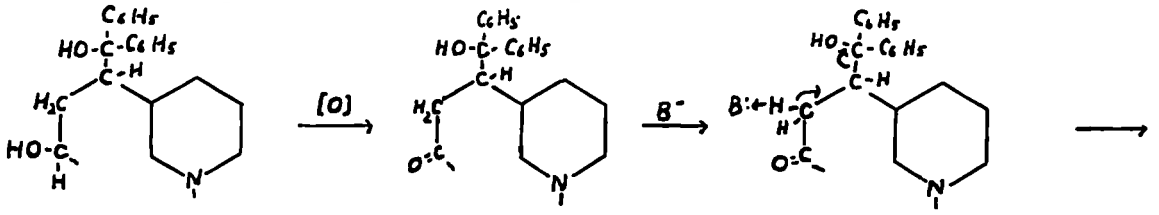


LII

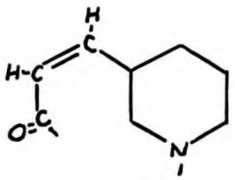
However, oxidation by the modified Oppenauer procedure, using potassium tertiary butoxide and cyclohexanone in refluxing toluene,

gave an unexpected result. Elimination of the diphenyl carbinol group had occurred to yield a basic compound $C_{15}H_{21}ON$, LIII. The infrared spectrum of compound LIII had bands at 1680 and 1625 $cm.^{-1}$ which are in the regions of absorption of a conjugated ketone and a double bond respectively. The ultraviolet absorption spectrum of compound LIII had, in addition to a weak carbonyl band at 3180 A, a strong band at 2280 A (log ϵ = 3.85) which is characteristic of an α,β-unsaturated ketone with a single β-alkyl substituent (28). Therefore the grouping $O=\underset{R}{\overset{}{C}}-\overset{H}{\overset{|}{C}}=\overset{H}{\overset{|}{C}}-R$ must be present in the compound LIII.

The unusual nature of the reaction leading to the conjugated ketone LIII made it worthy of more detailed study. Accordingly the hydroxy ketone LII, which was considered to be an intermediate in the reaction, was treated with potassium tertiary butoxide in refluxing toluene under a nitrogen atmosphere. The conjugated ketone LIII and benzohydrol in a quantitative yield were isolated. When the above reaction was carried out in air a mixture of benzohydrol and benzophenone resulted. The latter was isolated as its 2,4-dinitrophenylhydrazone. The elimination of the diphenyl carbinol group from diphenyldesoxodihydroanhotinine and from the hydroxy ketone LII very likely proceeded similarly to a reverse Michael reaction and can be depicted according to the following sequence:



L LII



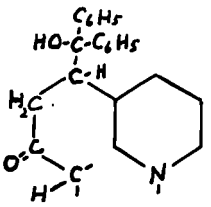
LIII

$$+ \quad [(C_6H_5)_2-\ddot{C}OH]^- \xrightarrow{H^+} (C_6H_5)_2-CHOH$$

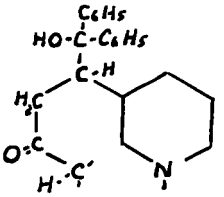
The mode of formation of the conjugated ketone LIII and the ultraviolet spectrum of the same compound provided two facts about the structure of annotinine, first that the hydroxyl and carboxyl groups derived from the γ-lactone function were on the same ring and second that the carbon between the carbon bearing the potential hydroxyl group and the carbon bearing the potential carboxyl group had two hydrogens.

Treatment of diphenyldesoxodihydroannotinine with potassium tertiary butoxide and cyclohexanone in refluxing benzene for fifteen minutes produced a hydroxy ketone $C_{28}H_{33}O_2N$, LIV, which was isomeric with the hydroxy ketone LII. Compound LII melted at 201°C, its specific rotation in chloroform (21°C) was +100.7 and its infrared spectrum had bands at 3500, 1700 and 1595 $cm.^{-1}$ due to hydroxyl, ketone and phenyl absorptions respectively. Compound LIV melted at 223°C, its specific rotation in chloroform (21°C) was -60.2 and its infrared spectrum had bands at 3420, 1710 and 1595 $cm.^{-1}$ due to hydroxyl, ketone and phenyl absorptions respectively. The melting point of a mixture of the two compounds LII and LIV was lowered. The hydroxy ketone LII, when it was heated under reflux with sodium ethoxide in ethanol overnight, was partially converted into the hydroxy ketone LIV. Therefore the two hydroxy ketones LII and LIV must be simple epimers of each other. The more rapid epimerization induced by potassium t-butoxide must be due to its greater basic strength and greater

ionic character. The most likely site in the molecule for epimerization to occur in the hydroxy ketone LII was at a position alpha to the ketone group. It has already been shown that one carbon alpha to the ketone group was secondary, therefore the other alpha carbon must be tertiary.

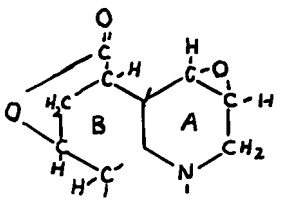


LII



LIV

On the basis of the chemical evidence presented in this discussion the following partial structure can be written for annotinine:

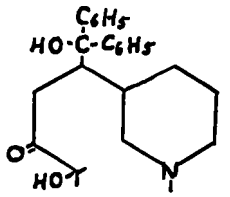


The same conclusions regarding the structure of ring B were drawn by Wiesner, Ayer, Fowler and Valenta (2) at the time that the work just described was being completed. Indeed it was at the same time that they proposed what proved to be the correct structure for annotinine.

The reaction of diphenyldesoxodihydroannotinine with chromium trioxide was also investigated in the course of this study and an anomalous result was observed. Diphenyldesoxodihydroannotinine reacted with chromium trioxide to give a compound

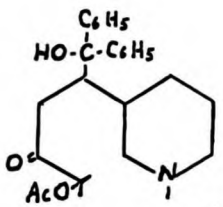
$C_{28}H_{33}O_3N$, LV, which had one oxygen more and two hydrogens less than the starting material. The compound LV was examined in order to gain some insight into its composition. It was found to have two active hydrogens, therefore two hydroxyl groups must be present in LV. The infrared spectrum of the compound LV showed a broad band in the hydroxyl region and a band at 1720 $cm.^{-1}$ in the ketone region as well as phenyl absorption. The keto group in compound LV was likely derived from the oxidation of the secondary hydroxyl group of diphenyldesoxodihydroannotinine. One hydroxyl group of LV would be the tertiary hydroxyl group already present in the starting material. The new hydroxyl group must be tertiary, otherwise it would have been oxidized in the reaction medium. One activated position at which an hydroxyl group might have been introduced was on a tertiary carbon alpha to the nitrogen to yield a carbinolamine. However, compound LV did not behave like a carbinolamine. It did not form anhydronium salts and it formed a methiodide. Both compound LV and its hydrochloride gave the same trihydroxy compound $C_{28}H_{35}O_3N$, LVI, when treated with lithium aluminum hydride. The only other activated position at which an hydroxyl group might have been introduced was the tertiary carbon alpha to the carbonyl group to yield an α-hydroxyketone. Evidence for an α-hydroxyketone grouping in compound LV was found in a comparison of its infrared spectrum with that of compound LII. The carbonyl absorption of compound LV was at 1720 $cm.^{-1}$ while the carbonyl absorption of compound LII was at 1700 $cm.^{-1}$. The difference of 20 $cm.^{-1}$ implied

a close structural relationship between the two groups in which the hydroxyl function was alpha and axial to the carbonyl function. The following partial structure for the compound LV can be written:

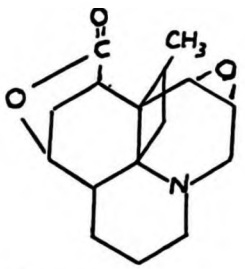


LV

Evidence that the tertiary carbon alpha to the carbonyl group was involved in the formation of compound LV was shown by the different behaviour of the compounds LII and LIV, which are epimeric at that carbon, to chromium trioxide oxidation. Compound LII, on treatment with chromium trioxide, gave the compound LV, while compound LIV was recovered unchanged on similar treatment. Lead tetraacetate converted compound LII into a new compound, LVII, which had hydroxyl, ketone and acetate absorption in its infrared spectrum. Compound LVII gave compound LV on hydrolysis with alkali, so it must have been an O-acetyl derivative of compound LV.



LVII



Annotinine

At this time Przybylska and Marion (3) published the results of the X-ray analysis of annotinine bromohydrin, which confirmed the structure of annotinine previously proposed by Wiesner, Ayer, Fowler and Valenta (2) on the basis of chemical

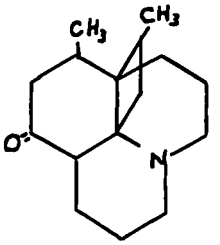
evidence. The conclusions which have been reached in this investigation are in complete agreement with the above structure. Structural studies on the alkaloid were discontinued and an effort was made to relate the structure of annotinine to that of other Lycopodium alkaloids.

The Relationship of the Structure of Annotinine to the Structures of other Lycopodium Alkaloids

Lycopodine, $C_{16}H_{25}ON$, the most widely distributed Lycopodium alkaloid, occurred in nearly all of the species of the genus Lycopodium investigated so far, and was found with annotinine in L. annotinum L.(1). One might expect that annotinine and lycopodine would have the same carbon skeletal arrangement, since alkaloids occurring in the same plant are frequently related in some simple way.

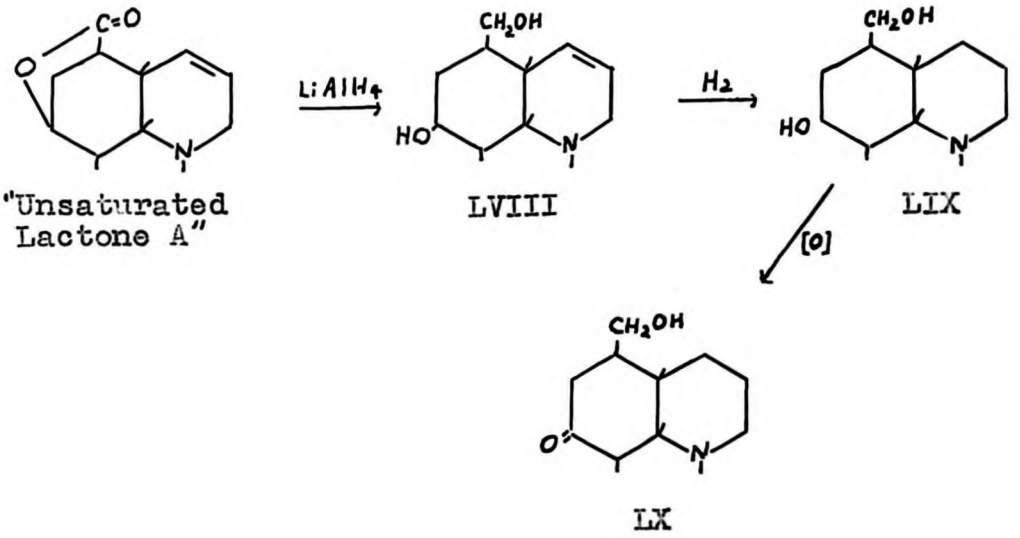
Lycopodine contains a tertiary nitrogen, a ketone group and a combination of four carbocyclic and heterocyclic rings (29). If annotinine and lycopodine were to have the same carbon skeletal arrangement, the simplest relationship between the two alkaloids would be for the carbonyl group of lycopodine to be in the position of a potential hydroxyl group in annotinine. According to Barclay (30), it is unlikely that the carbonyl group and the nitrogen atom are in the same six-membered ring in lycopodine. Therefore the carbon carrying the potential hydroxyl group of the lactone ring of annotinine might be the carbon of the carbonyl group in lycopodine. The carboxyl group

of the lactone ring might be in the form of a methyl group in lycopodine. Using these premises, the following is a possible structure for lycopodine:



Attempts were made to convert anzotinine into a compound of this structure.

A suitable compound with which to begin this study was "unsaturated lactone A". It was reduced by the method of Prime (31) with lithium aluminum hydride to give the unsaturated dihydroxy compound $C_{16}H_{25}O_2N$, LVIII. Hydrogenation of compound LVIII over Adams catalyst gave the dihydroxy compound $C_{16}H_{27}O_2N$, LIX. Compound LIX was oxidized by the Oppenauer procedure, using aluminum isopropoxide and acetone in benzene, and yielded the hydroxy ketone $C_{16}H_{25}O_2N$, LX. The above reactions can be represented by the following partial formulae:



The only difference between the structure of the compound LX and the structure postulated for lycopodine was the presence of the primary hydroxyl group in compound LX. Various attempts to replace this hydroxyl group with a hydrogen atom proved fruitless. A tosylate was prepared by treating compound LX with p-toluenesulphonyl chloride in pyridine. It was expected that reduction of the tosylate with lithium aluminum hydride would replace the O-tosyl group with hydrogen. However, hydride reduction of the tosylate of LX produced a compound which apparently contained a primary hydroxyl group. The compound isolated after the reduction product was oxidized by the Oppenauer procedure contained both hydroxyl and carbonyl absorption in its infrared spectrum. The reduction of tosylates with lithium aluminum hydrice usually results in hydrocarbons, but the production of alcohols is not unknown (32).

Attempts were made to replace the primary hydroxyl of compound LX with halogen, which in turn could be replaced with hydrogen by hydrogenation over palladium. The replacement of the O-tosyl group with halide ion has been observed (33). However when the tosylate of compound LX was treated with sodium iodide in refluxing acetone it was recovered unchanged. In two other experiments compound LX was treated with phosphorus tribromide in pyridine and in chloroform. Less than 50 per cent recovery of basic material was realized in both cases. The infrared spectrum of the recovered material indicated that it was mostly compound LX, contaminated with a small amount of its phosphite ester.

Compound LX was recovered unchanged after it had been heated under reflux overnight with a 4:1 solution of 48 per cent hydrobromic acid and 85 per cent phosphoric acid. Treatment of the hydroxy ketone LX with thionyl chloride did not give the expected chloro ketone. There was isolated instead a mixture of the starting material, a small amount of material which was probably a conjugated ketone because there were bands at 1680 and 1570 $cm.^{-1}$ in its infrared spectrum, and a substance containing sulphur. The conjugated ketone was produced in only small yield and it could not be induced to crystallize. The sulphur-containing material produced sulphur dioxide when it was heated under reflux with dilute sulphuric acid and was probably a sulphite ester of compound LX.

Since the hydroxyl group of compound LX would not undergo replacement, it was decided to see if it could be removed by dehydration. However, compound LX resisted all dehydration attempts. It was recovered unchanged after being heated under reflux with p-toluenesulphonic acid in xylene. Very little basic material was recovered when compound LX was treated with phosphorus oxychloride.

An explanation can be given for the resistance of the hydroxyl group of compound LX to displacement or elimination. The hydroxyl group of LX is primary and therefore the preferred mode of reaction would be by backside attack of the reacting species (S_N2 mechanism) rather than through carbonium ion formation followed by reaction with the other species (S_N1 mechanism). According to the molecular model of annotinine depicted by Przybylska and Marion (3) the cyclobutane ring and the γ-lactone

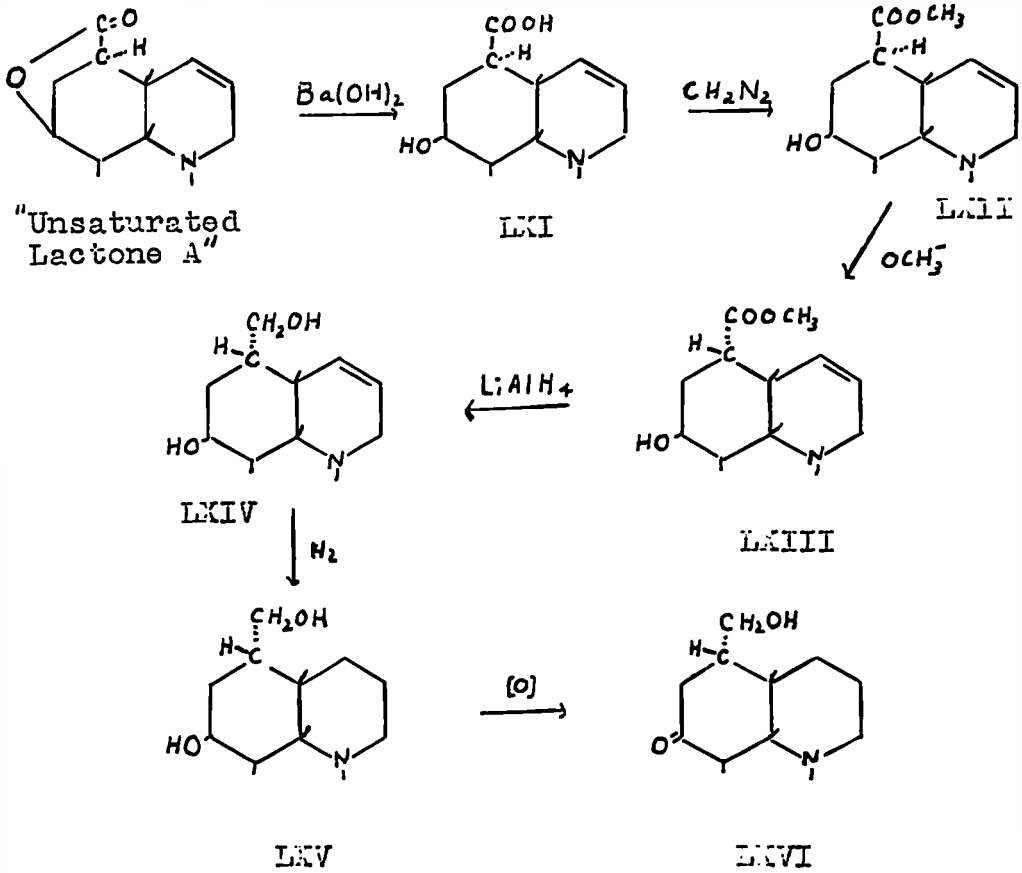
ring are beside each other, with the C-methyl group on the cyclobutane ring pointing toward the lactone ring. Therefore one would expect that the hydroxymethyl group ($-CH_2OH$), derived from the lactone carboxyl group, would be sterically hindered and backside attack at its carbon or the carbon alpha to it would be difficult.

Since the attempts described above to replace the hydroxyl group of compound LX with hydrogen were unsuccessful, it was of interest to prepare the compound which had the hydroxymethyl group in the opposite configuration. One might expect that an hydroxymethyl group which was in the configuration epimeric to the one in compound LX would be less hindered because it would be on the side of the molecule opposite to the cyclobutane ring. Valenta, Stonner, Bankiewicz and Wiesner (18) had already observed that the carboxyl group derived from the lactone ring epimerized easily and that the new configuration of the carboxyl group was more stable than the natural one.

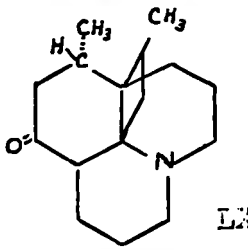
The compound in which the hydroxymethyl group was in the opposite configuration to that of compound LX was prepared in the following way. "Unsaturated lactone A" was heated under reflux with aqueous barium hydroxide which opened the lactone ring. The resulting amino acid LXI was esterified with diazomethane, giving the methyl ester LXII. The carbomethoxy group of the compound LXII was epimerized by refluxing it with potassium methoxide in methanol. The resulting ester, LXIII, was reduced with lithium aluminum hydride and the unsaturated

dihydroxy compound $C_{16}H_{25}O_2N$, LXIV, was isolated. Evidence that compound LXIV was the epimer of compound LVIII was obtained by the comparison of the infrared spectra of the hydrochlorides of the two compounds. They were distinctly non-identical. The addition of the hydrochloride of compound LVIII to that of compound LXIV lowered its melting point.

Compound LXIV was hydrogenated over Adams catalyst yielding the dihydroxy compound $C_{16}H_{27}O_2N$, LXV. The hydroxy ketone $C_{16}H_{25}O_2N$, LXVI, the epimer of compound LX, was obtained when the dihydroxy compound LXV was oxidized by the Oppenauer procedure using aluminum isopropoxide and cyclohexanone in toluene. The above reactions can be illustrated by following partial formulae:



The primary hydroxyl group of compound LXVI was readily replaced by a bromine atom when LXVI was heated under reflux with a 4:1 solution of 48 per cent hydrobromic acid and 85 per cent phosphoric acid. Treatment of the product, $C_{16}H_{24}ONBr$, LXVII, with hydrogen over palladium on calcium carbonate, yielded the compound $C_{16}H_{25}ON$, LXVIII, in which the bromine atom had been replaced with a hydrogen atom.



LXVIII

The structure of compound LXVIII was epimeric with the structure originally postulated for lycopodine. A comparison of the two compounds was made, however, in case lycopodine had the epimeric structure. Although compound LXVIII could not be induced to crystallize, its perchlorate crystallized easily and melted at 221-225°C. This was 60° lower than the melting point of lycopodine perchlorate. The infrared spectra of the perchlorate of compound LXVIII and lycopodine perchlorate were distinctly non-identical.

There was a chance that lycopodine and compound LXVIII differed only in the configuration of the tertiary carbon alpha to the carbonyl group. If this were the case, then the same anhydro compound would be isolated when the dihydro derivatives of lycopodine and compound LXVIII were dehydrated. This is based on the assumption that dehydration would occur towards the tertiary carbon, which has been observed for other annotinine derivatives (21, 23).

Douglas, Lewis and Marion (9) had already prepared anhydrodihydrolycopodine, $C_{16}H_{25}ON$, by the action of phosphorus pentachloride in boiling xylene on dihydrolycopodine, the hydroxy compound produced on hydride reduction of lycopodine. In this investigation it was found that anhydrodihydrolycopodine can also be prepared by treatment of dihydrolycopodine with either thionyl chloride or concentrated hydrochloric acid. By the same sequence of reactions compound LXVIII was converted first into its dihydro derivative $C_{16}H_{27}ON$, LXIX, then into the anhydro compound $C_{16}H_{25}N$, LXX. The infrared spectra of compound LXX and its perchlorate were not similar to the corresponding spectra of anhydrodihydrolycopodine and its perchlorate. The perchlorates of compound LXX and anhydrodihydrolycopodine melted 60° apart.

The only alternative remaining was the comparison of the ring system of lycopodine with that of annotinine. Presumably, desoxodihydrolycopodine could be obtained by hydrogenation of anhydrodihydrolycopodine. The preparation of desoxodihydrolycopodine was of interest for another reason. It would have the same empirical formula, $C_{16}H_{27}N$, which had been reported for the alkaloid L4, isolated by Manske and Marion (6, 34) from L. fabelliforme Fernald. Dihydrolycopodine as well as its O-acetate and lycopodine had been isolated from the same plant. However, anhydrodihydrolycopodine resisted hydrogenation over Adams catalyst and Raney nickel at 100°C and higher temperatures and 1000 p.s.i.g. hydrogen. Therefore the formation of anhydrodihydrolycopodine from dihydrolycopodine may involve a more

complex change than simple dehydration.

Since no relationship between annotinine and lycopodine was established, an effort was made to correlate the structure of annotinine with that of other Lycopodium alkaloids. In the course of the work just described several compounds derived from annotinine were isolated (see Table 1) which were isomeric with some of the Lycopodium alkaloids. The alkaloids which have been isolated from twelve species of the genus Lycopodium are listed in Table 2. Some of the alkaloids are simple derivatives of others (9, 10), but none have been related to annotinine. In order to determine if a relationship existed between the structure of annotinine and that of another alkaloid, the melting point and infrared spectrum, if available, of the alkaloid or its perchlorate were compared with that of the isomeric annotinine derivative.

The alkaloids L8 and acrifoline occur in the plant, L. annotinum with annotinine (1, 11, 12, 13). It was found that the alkaloid L8, $C_{16}H_{25}O_2N$, which contained a hydroxyl group and a carbonyl group, was not identical with either compound LX or its epimer, compound LXVI. An isomer of acrifoline was not prepared but several compounds were prepared which were isomeric with some derivatives of acrifoline prepared by Perry and MacLean (10). Hydrogenation of acrifoline gave dihydroacrifoline which contained a carbonyl group and a hydroxyl group. It was not identical with either of the isomeric compounds, LX or its epimer, compound LXVI. When dihydroacrifoline was reduced with lithium

aluminum hydride, the dihydroxy compound, α-dihydroacrifolinol, $C_{16}H_{27}O_2N$, resulted. A comparison of this compound with the compound LIX or its epimer, compound LXV, showed that there was no identity. Acrifolinol, $C_{16}H_{25}O_2N$, which was prepared by lithium aluminum hydride reduction of acrifoline, contained two hydroxyl groups and a double bond. It was not the same as the isomeric compound, LVIII or its epimer, compound LXIV. Treatment of acrifolinol with hydrogen over Adams catalyst gave β-dihydroacrifolinol, $C_{16}H_{27}O_2N$, which was not identical with either the dihydroxy compound LIX or its epimer, compound LXV.

A comparison was made of alkaloids, not found in the same plant as annotinine, with isomeric annotinine derivatives. The melting points of the alkaloids L34 and clavatine were found to be higher than those of the isomeric compounds LVIII, LX and LXIV. Also the perchlorates of the alkaloids L23, L25 and pseudoselagine melted higher than the perchlorates of the isomeric compounds LVIII, LX, LXIV and LXVI. The perchlorates of the alkaloids L13, L24 and the alkaloid, $C_{16}H_{25}ON$, isolated from L. annotinum L. by Bertho and Stoll (12), had higher melting points than the perchlorate of the isomeric compound LXVIII. Both the perchlorates of the alkaloid L16 and of the isomeric compound LXVIII melt in the vicinity of 220°C, however their infrared spectra were different and mixture of the two depressed the melting point 35°C. The perchlorate of the alkaloid L22 had a higher melting point, and the perchlorate of the alkaloid L10 had a lower melting point than the perchlorate of the isomeric compound LXIX. A definite correlation between the structure of annotinine and the structure of another Lycopodium alkaloid has yet to be made.

TABLE I

Some Compounds derived from Annotinine Isomeric with other *Lycopodium* Alkaloids

Number of Compound	Formula of Compound	M.P. Base	M.P. Perchlorate	No. of Functional Groups >C=O	>C=C<	-ĊOH
LVIII	$C_{16}H_{25}O_2N$	165°C	204°C	-	1	2
LIX	$C_{16}H_{27}O_2N$	181	175	-	-	2
LX	$C_{16}H_{25}O_2N$	126	227	1	-	1
LXIV	$C_{16}H_{25}O_2N$	108	229	-	1	2
LXV	$C_{16}H_{27}O_2N$	-	227	-	-	2
LXVI	$C_{16}H_{25}O_2N$	-	212	1	-	1
LXVIII	$C_{16}H_{25}ON$	-	225	1	-	-
LXIX	$C_{16}H_{27}ON$	-	235	-	-	1
LXX	$C_{16}H_{25}N$	-	168	-	1	-

TABLE II

The Lycopodium Alkaloids

ıber of aloid	Name of Alkaloid	Formula of Alkaloid	M.P. Base	M.P. $HClO_4$	Relationship to other Alkaloids	Source of Alkaloids
-	Lycopodine	$C_{16}H_{25}ON$	116°C	283°C		a,b,c,d,e, f,g,h,i,l,m
L1	Complanatine	$C_{16}H_{27}ON$	169	194	Dihydrolyco-podine (30)	b
L2	-	$C_{18}H_{29}O_2N$	97	231	Acetate of L1 (9)	b
L3	-	$C_{18}H_{31}O_2N$	-	246		b
L4	-	$C_{16}H_{27}N$	-	225		b
L5	-	$C_{18}H_{28}O_2N$	-	282		b
L6	α-Obscurine	$C_{17}H_{26}ON_2$	283	-		b,c,e
	β-Obscurine	$C_{17}H_{24}ON_2$	322	-		b,c,e
L7	Annotinine	$C_{16}H_{21}O_3N$	232	267		c,i
L8	-	$C_{16}H_{25}O_2N$	180	318		c,m
L9	-	$C_{16}H_{23}ON$	-	276		c
	-	$C_{20}H_{31}O_4N$	98	274		c
.10	-	$C_{16}H_{27}ON$	-	223		c
.11	Annotine	$C_{16}H_{21}O_3N$	174	239		c
.12	-	$C_{18}H_{25}O_3N$	119	244	Acetate of Acrifoline(10)	c
.13	-	$C_{16}H_{25}ON$	130	274		d,e,f,g,h
.14	-	$C_{16}H_{25}N$	-	238	Anhydrodihydro-lycopodine(9)	d
.15	-	$C_{20}H_{31}O_4N$	-	231		d
.16	-	$C_{16}H_{25}ON$	-	221		e
.17	-	$C_{18}H_{27}O_3N$	-	296		e
.18	-	$C_{11}H_{19}ON$	-	-		f
.19	-	-	231	-		f

TABLE II (continued)

The Lycopodium Alkaloids

umber of kaloid	Name of Alkaloid	Formula of Alkaloid	M.P. Base	M.P. $HClO_4$	Relationship to other Alkaloids	Source of Alkaloids
L20	-	$C_{17}H_{27}O_2N$	259°C	271°C		g
L21	-	$C_{13}H_{21}ON$	-	301		g
L22	-	$C_{16}H_{27}ON$	198	254		g
L23	-	$C_{16}H_{25}O_2N$	161	300		g
L24	-	$C_{16}H_{25}ON$	-	278		g
L25	-	$C_{16}H_{25}O_2N$	-	297		g
L26	-	$C_{15}H_{25}ON$	171	-		h
L27	Acrifoline	$C_{16}H_{23}O_2N$	104	266		c,i,m
L28	-	$C_{17}H_{28}O_2N$	-	211		i
L29	-	$C_{16}H_{23}O_2N$	-	273		i
L30	-	$C_{16}H_{25}O_2N$	178	311	Identical with L8 (9)	i
L31	-	$C_{20}H_{29}O_4N$	-	217		i
L32	Cernuine	$C_{16}H_{26}ON_2$	106	110		k
L33	-	-	218	-		k
L34	-	$C_{16}H_{25}O_2N$	236	-		l
L35	-	$C_{14}H_{21}ON$	133	-		l
-	Saururine	$C_{10}H_{19}N$	-	-		j
-	Sauroxine	$C_{17}H_{26}ON_2$	198	-		j
-	Clavatine	$C_{16}H_{25}O_2N$	213	-		f
-	Clavatoxine	$C_{17}H_{27}O_2N$	185	-		f
-	Annotoxine	$C_{32}H_{44}O_3N_2$	197	227	Molecular compound of L11 and Acrifoline (13)	c

TABLE II (Continued)

The *Lycopodium* Alkaloids

mber of kaloid	Name of Alkaloid	Formula of Alkaloid	M.P. Base	M.P. $HClO_4$	Relationship to other Alkaloids	Source of Alkaloids
-	-	$C_{16}H_{25}ON$	-	234°C		c
-	-	$C_{16}H_{19(21)}ON$	-	-		c
-	Pseudoselagine	$C_{16}H_{25}O_2N$	163	295	May be identical with L23(40)	m
-	Lycotine	$C_{17}H_{24}N_2$	118			c

Sources of the Alkaloids:
a - *L. complanatum* L. (4)
b - *L. flabelliforme* Fernald. (6,34)
c - *L. annotinum* L. (1,12,13,41)
d - *L. tristachyum* Pursh. (34)
e - *L. obscurum* L. (35)
f - *L. clavatum* L. (5,36)
g - *L. lucidulum* Michx. (37)
h - *L. sabinaefolium* Willd. (38)
i - *L. annotinum* var. *acrifolium* Fern. (11)
j - *L. saururus* Lam. (8)
k - *L. cernuum* L. (7)
l - *L. densum* Labile (39)
m - *L. selago* L. (40)

EXPERIMENTAL

Methods and Materials

The source of annotinine was L. annotinum collected in the Wentworth Valley, Nova Scotia. The crude alkaloids which were obtained from the plant by the method of Manske and Marion (6), were dissolved in methanol. Annotinine separated in crystalline form and was isolated by filtration. It melted at 230°C and was used in most reactions without further purification.

The infrared spectra were determined using a Perkin-Elmer Model 21 recording spectrophotometer. The samples were mounted in nujol unless otherwise stated. The ultraviolet spectra were determined in ethanol with a Beckmann Model DU spectrophotometer. The optical rotations were measured in 20 cm. tubes using a Hilger polarimeter.

The microanalyses were carried out in the Microanalytical Laboratories of Drs. G. Weiler and F.B. Strauss of Oxford, England, and by Mr. A.E. Ledingham of the Dominion Rubber Company, Guelph, Ontario.

All melting points are corrected.

Experimental Procedures

Preparation of Annotinine Lactamdiol

Annotinine lactam (2.0 g.), prepared by permanganate oxidation of annotinine using the procedure of MacLean and Prime (15), was heated under reflux overnight with 40 ml. of 10 per cent sulphuric acid containing a few drops of dioxane to

facilitate solution. The dioxane was boiled off, the solution was cooled, made alkaline with ammonium hydroxide, and extracted four times with chloroform. The combined chloroform extract was dried over sodium sulphate, and taken to dryness. The residue (1.64 g.) was recrystallized from acetone and melted at 238-239°C.

Calc. for $C_{16}H_{21}O_5N$: C, 62.54; H, 6.84; N, 4.56%

Found: C, 62.78; H, 6.82; N, 4.76%

The infrared spectrum of annotinine lactamdiol has two bands in the hydroxyl region at 3530 $cm.^{-1}$ (sharp) and 3300 $cm.^{-1}$ (broad), one in the lactone region at 1770 $cm.^{-1}$ and one in the lactam region at 1620 $cm.^{-1}$

Treatment of Annotinine Lactamdiol with Lead Tetraacetate

Annotinine lactamdiol (0.1537 g.) was dissolved in 50 ml. of glacial acetic acid and the solution was mixed with 50 ml. of 0.15 N lead tetraacetate in glacial acetic acid. Aliquots (10 ml.) were removed at regular intervals and added to 20 ml. of a buffer solution which contained 5 g. of potassium iodide and 62.5 g. of sodium acetate per 250 ml. of solution according to the procedure of Hockett and McClenahan (42). The excess iodine was titrated with 0.02 N sodium thiosulphate. A blank was prepared simultaneously omitting only the sample, and run concurrently. The results are given in Table III.

TABLE III

The Reaction of Annotinine Lactamdiol with Lead Tetraacetate

Time in Hours	Mole Ratio of $Pb(OAc)_4$ Consumed
24	0.35
48	0.79
72	1.17
91	1.48
117	1.94
143	2.33
167	2.65
191	2.84
215	3.06
238	3.10

Chromium Trioxide Oxidation of Annotinine Lactamdiol

A solution of 0.30 g. of annotinine lactamdiol in 5 ml. of glacial acetic acid was warmed to 60°C and a solution of chromium trioxide in acetic acid and water was added until the yellow color of unreacted chromium trioxide persisted. Excess oxidant was destroyed with methanol and the reaction mixture was taken to dryness under reduced pressure. Dilute ammonium hydroxide and chloroform were added to the residue, the mixture was shaken, the chloroform layer was separated and the aqueous layer was extracted three more times with chloroform. The combined chloroform extract was washed with dilute hydrochloric acid, dried over anhydrous sodium sulphate, and evaporated to dryness. The residue (0.11 g.), after several crystallizations from ether, melted at 197-199°C.

Calc. for $C_{15}H_{17}O_4N$: C, 65.49; H, 6.23%

Found: C, 65.79; H, 6.24%

The infrared spectrum of compound XLV in chloroform solution had three bands in the carbonyl region at 1708, 1760 and 1780 $cm.^{-1}$ attributed to γ-lactam, 5-membered cyclic ketone and γ-lactone functions respectively.

The alkaline aqueous solution from the chloroform extraction above was acidified with sulphuric acid and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate, and taken to dryness. The residue (0.03 g.), after recrystallization from ether, melted at 205°C.

Calc. for $C_{15}H_{19}O_5N$: C, 61.43; H, 6.48; N, 4.77%

Found: C, 61.59; H, 6.48; N, 4.46%

The infrared spectrum of compound XLVI had bands at 1610, 1705 and 1775 $cm.^{-1}$ attributed to amide, carboxyl and γ-lactone functions respectively.

The methyl ester of compound XLVI was prepared by treatment of a methanolic solution of the acid with a solution of diazomethane in ether. The ester, remaining after evaporation of the solvents, was recrystallized from ether and melted at 122-123°C.

Calc. for $C_{16}H_{21}O_5N$: C, 62.54; H, 6.84; N, 4.56; OMe, 10.06%

Found: C, 62.45; H, 6.76; N, 4.41; OMe, 10.15%

The infrared spectrum of the ester had a double peak at 1653 and 1665 $cm.^{-1}$ in the amide region, a peak at 1730 $cm.^{-1}$ assigned to the carbomethoxy group and a peak at 1768 $cm.^{-1}$ attributed to the γ-lactone function.

Potassium Permanganate Oxidation of Annotinine Lactamdiol

A solution of 0.50 g. of annotinine lactamdiol in 50 ml. of glacial acetic acid was treated with an aqueous solution of

potassium permanganate until the pink color was persistent. The excess permanganate was destroyed with methanol and the mixture was taken to dryness under reduced pressure. The residue was suspended in water and the solution was treated with sulphur dioxide to reduce the manganese dioxide. The acidic solution was then exhaustively extracted with chloroform. The chloroform extract was washed several times with dilute ammonium hydroxide, dried over anhydrous sodium sulphate and taken to dryness. The residue (0.12 g.) was identical with the γ-lactam XLV. The alkaline aqueous solution was made acid with hydrochloric acid and extracted with chloroform. The dried chloroform extract, on evaporation, yielded 0.10 g. of the acid XLVI. The acid solution from the first chloroform extraction was continuously extracted with ether to yield a small sample of the sulphate of the amino acid XI identified by its melting point and its infrared spectrum.

Reduction of the Compound $C_{15}H_{17}O_4N$, XLV, with Sodium Borohydride

A solution of compound XLV (0.10 g.) in 10 ml. of ethanol was added slowly with stirring to a solution of sodium borohydride (0.20 g.) in 10 ml. of ethanol at room temperature. The reaction mixture was stirred for two hours before the excess borohydride was decomposed first with formaldehyde, then with acetic acid until faintly acid. The solution was evaporated to dryness under reduced pressure. Dilute ammonium hydroxide was added to the residue and the alkaline solution was extracted with chloroform. The dried chloroform extract was evaporated to dryness to yield 0.09 g. of residue which, after three recrystallizations from acetone, melted at 254-255°C.

Calc. for $C_{15}H_{19}O_4N$: C, 64.95; H, 6.90%

Found: C, 64.90; H, 6.96%

The infrared spectrum of compound XLVII had absorption bands at 3335 $cm.^{-1}$ in the hydroxyl region, at 1682 $cm.^{-1}$ in the γ-lactam region and at 1781 $cm.^{-1}$ in the γ-lactone region.

Hydrolysis of the Acid XLVI

The procedure followed was that of Woodward and Brehm (43) for the determination of formic acid from N-formyl derivatives. The acid (0.216 g.) was heated under reflux for two hours with 20 ml. of 1 N sulphuric acid. The solution was then distilled, water being added to keep the volume approximately constant. The distillate was collected in fractions and titrated with 0.1 N sodium hydroxide. The amount of volatile acid collected corresponded to 0.95 mole per mole of the original acid XLVI. A sample of the aqueous distillate gave a positive test for formic acid with mercuric chloride as described by Feigl (44). The distillate also reduced permanganate and bromine.

The aqueous residue after removal of the formic acid was treated alternately with barium hydroxide and sulphuric acid to remove inorganic ions. Evaporation of the solution yielded an amino acid residue which was converted to its methyl ester by diazomethane. The amino acid methyl ester was identical with the methyl ester of the amino acid XI prepared from "unsaturated lactone A". A mixture of the two compounds showed no depression in melting point and their infrared spectra were identical.

Treatment of "Unsaturated Lactone A" with Cyanogen Bromide

"Unsaturated lactone A", prepared by chromous chloride reduction of annotinine chlorohydrin in concentrated hydrochloric acid using the procedure of Manske and Marion (11), was treated with excess cyanogen bromide in anhydrous benzene at room temperature, at 60°C and at the reflux temperature of benzene. In each case approximately 80 per cent of the starting material was recovered. The remaining 20 per cent was recovered as a neutral amorphous precipitate which showed absorption in the infrared at 1770 $cm.^{-1}$ in the lactone region and at 1650 $cm.^{-1}$ (unassigned). There was only slight absorption in the cyanamide region at 2200 $cm.^{-1}$. Attempts to purify this compound proved unsuccessful.

Oxidation of "Unsaturated Lactone A" with Barium Permanganate

"Unsaturated lactone A" (1.86 g.) was dissolved in acetone and the solution was treated with aqueous barium permanganate until the pink color persisted. The excess permanganate was destroyed with methanol. The manganese dioxide was collected by filtration, suspended in water and reduced with sulphur dioxide. The organic solvents in the filtrate from the separation of the manganese dioxide were removed by distillation under reduced pressure. The two aqueous solutions were combined, a little sulphuric acid was added and the resulting acid solution was exhaustively extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate, and evaporated to dryness. Upon addition of acetone to the residue, a crystalline precipitate separated (0.40 g.) which after recrystallization from acetone melted at 205°C. The melting point was not depressed in admixture

with the acid $C_{15}H_{19}O_5N$, XLVI, prepared by oxidation of annotinine lactamdiol. Their infrared spectra were identical.

The acetone mother liquors from the separation of the acid XLVI were freed of acetone, taken up in methanol, and treated with a solution of diazomethane in ether. After the solution had been concentrated, 0.08 g. of crystalline ester separated, which, after recrystallization from methanol, melted at 193-195°C with some decomposition.

Calc. for $C_{17}H_{21}O_6N$: C, 60.89; H, 6.27; N, 4.18; OMe, 9.25%.
Found: C, 60.90; H, 6.77; N, 4.11; OMe, 9.35%.

The infrared spectrum of the ester XLVIII had a double peak at 1633 and 1645 $cm.^{-1}$ in the amide region and single peaks at 1710, 1743 and 1780 $cm.^{-1}$ corresponding to carbonyl, carbomethoxy and γ-lactone groups respectively.

Continuous ether extraction of the aqueous solution from the chloroform extraction above yielded the sulphate of the amino acid $C_{14}H_{19}O_4N$, XI.

Hydrolysis of the Ester $C_{17}H_{21}O_6N$, XLVIII

The ester XLVIII (0.215 g.) was hydrolyzed with 20 ml. of 1 M sulphuric acid by the procedure described previously for the acid XLVI. The amount of volatile acid collected in the distillate corresponded to one mole per mole of ester used. The distillate, containing the sodium salt of the volatile acid, was concentrated under reduced pressure, made acid with sulphuric acid and redistilled. The distillate gave a positive test for formic acid with mercuric chloride as described by Feigl (44), and also reduced permanganate and bromine.

The aqueous solution containing the hydrolyzed compound was extracted with chloroform. The dried chloroform extract yielded, on evaporation of the chloroform a small residue (0.02 g.), which had an infrared spectrum identical with that of compound XLV. Recrystallization of the residue from ether yielded a few crystals which did not depress the melting point of compound XLV.

Ozonolysis of "Unsaturated lactone A"

A solution of 0.36 g. of "unsaturated lactone A" in 35 ml. of anhydrous ethyl acetate was treated with excess ozone at -30°C (the ozone concentration in the oxygen stream was approximately 3.5 per cent and the time of treatment was 45 minutes). Adams catalyst (0.025 g.) was added to the cold solution, which was immediately placed under 10 p.s.i.g. hydrogen for 2 hours. The catalyst was removed by filtration and the filtrate taken to dryness. The residue was dissolved in chloroform and washed with aqueous ammonia and dilute hydrochloric acid. Evaporation of the dried chloroform solution yielded a residue (0.23 g.) which was dissolved in benzene and absorbed on a column of alumina (Fisher, 80-200 mesh). Elution with one volume per cent methanol in benzene yielded 0.18 g. of a compound which melted at 174°C after recrystallization from ether. Its infrared spectrum had bands at 1600 and 1660 $cm.^{-1}$ attributed to a conjugated lactam function and at 1785 $cm.^{-1}$ attributed to the γ-lactone group. This compound, XLIX, proved to be identical with the compound $C_{16}H_{19}O_3N$ formed by dehydration of the hydroxy lactam IX by MacLean and Prime (15). The infrared spectra of the two compounds were identical and a mixture of the two showed no depression in

melting point.

Preparation of Diphenyldesoxodihydroannotinine

Desoxodihydroannotinine was prepared, by the hydrogenation of "unsaturated lactone A" over Adams catalyst, by the procedure of Manske and Marion (11). A solution of phenyllithium in ether was prepared by adding 40 g. of freshly distilled bromobenzene to 4.0 g. of lithium suspended in 150 ml. of anhydrous ether. After the addition was complete the mixture was stirred and heated under reflux for one hour. To this solution was added slowly 6 g. of desoxodihydroannotinine dissolved in 100 ml. of anhydrous ether. The reaction mixture was heated under reflux for two hours, cooled and decomposed in ice-hydrochloric acid. Some undissolved hydrochloride separated at this point. The ether layer was separated and washed several times with dilute hydrochloric acid. The acid washings, the original aqueous solution and the undissolved hydrochloride were combined and the mixture was made alkaline with ammonium hydroxide and extracted with ether. The ether extract was dried over anhydrous sodium sulphate and evaporated to dryness to yield a residue, which, on addition of acetone, deposited 3.5 g. of crystalline product. The acetone mother liquors were treated with concentrated hydrochloric acid to yield a crystalline hydrochloride. The total recovery as base and as hydrochloride represented a yield of 75-80 per cent. The base after recrystallization from acetone melted at 218-220°C.

Calc. for $C_{28}H_{35}O_2N$: C, 80.57; H, 8.39%

Found: C, 80.39; H, 8.75%

The ultraviolet spectrum of diphenyldesoxodihydroannotinine had λmax at 2580 Å, log ϵ = 2.8. The infrared spectrum showed hydroxyl absorption at 3600 (sharp) and 3150 cm.$^{-1}$ (broad) and weak phenyl absorption at 1590 cm.$^{-1}$. $[\alpha]_D^{21}$ -106° (c, 1% in chloroform).

Dehydration of Diphenyldesoxodihydroannotinine

A solution of 0.15 g. of diphenyldesoxodihydroannotinine in 2.5 ml. of concentrated hydrochloric acid and 7.5 ml. of methanol was heated under reflux for two hours and then evaporated to dryness under reduced pressure. The residue crystallized on addition of acetone to give a crystalline hydrochloride (0.14 g.) which, after recrystallization from methanol-acetone, melted at 295°C with decomposition. The hydrochloride was converted to the free base by suspension in aqueous ammonia and extraction with ether. The dried ether extract on evaporation yielded a residue which, after recrystallization from methanol, melted at 189-190°C.

Calc. for $C_{28}H_{33}ON$: C, 84.21; H, 8.27; N, 3.50%

Found: C, 83.84; H, 8.61; N, 3.70%

The infrared spectrum of the anhydro compound LI in chloroform solution had hydroxyl absorption at 3630 cm.$^{-1}$, phenyl absorption at 1600 cm.$^{-1}$ and at 1580 cm.$^{-1}$. Its ultraviolet spectrum had strong but broad absorption in the region 2200-2800 Å, log ϵ = 4.0-3.6. No definite band was detectable.

When the anhydro compound LI was shaken with hydrogen at 50 p.s.i.g. in methanol over Adams catalyst, it failed to take up hydrogen and was recovered unchanged.

Oxidation of Diphenyldesoxodihydroannotinine with Aluminum Isopropoxide and Cyclohexanone

Diphenyldesoxodihydroannotinine (0.50 g.) was dissolved in 30 ml. of dry toluene and 1.20 g. of aluminum isopropoxide and 1.50 ml. of cyclohexanone were added. The solution was refluxed for 1 hour and 15 minutes, cooled and poured on ice. The mixture was shaken, the toluene layer was separated and the aqueous layer was extracted three times with ether. The toluene layer and the ether extract were combined and washed four times with 10 per cent hydrochloric acid. The acid washings were extracted once with ether, made alkaline with ammonium hydroxide and extracted with ether. The ether extract was dried over anhydrous sodium sulphate and evaporated to dryness. The residue crystallized on addition of ether. The product (0.35 g.), after two recrystallizations from ether, melted at 201°C.

Calc. for $C_{28}H_{33}O_2N$: C, 80.93; H, 8.00; N, 3.37%

Found: C, 80.98; H, 7.73; N, 3.25%

The infrared spectrum of compound LII had phenyl absorption at 1595 $cm.^{-1}$, carbonyl absorption at 1700 $cm.^{-1}$ and a band in the hydroxyl region at 3500 $cm.^{-1}$. $[\alpha]_D^{27} = +100.7$ (c, 1% in chloroform).

Oxidation of Diphenyldesoxodihydroannotinine with Potassium Tertiary Butoxide and Cyclohexanone

A solution of 0.20 g. of diphenyldesoxodihydroannotinine, 0.28 g. of freshly prepared potassium tertiary butoxide, 1.0 ml. of cyclohexanone and 20.0 ml. of dry toluene was heated under reflux for 1 hour, cooled and poured into ice water. The mixture was shaken, the toluene layer was separated and the aqueous layer was extracted three times with ether. The toluene layer and the

ether extract were combined and extracted several times with 10 per cent hydrochloric acid. The acid extract was washed with ether, made alkaline with ammonium hydroxide and extracted with ether. The ether extract was dried over anhydrous sodium sulphate and evaporated to dryness. The residue (0.10 g.) was dissolved in acetone and the solution treated with concentrated hydrochloric acid. A crystalline hydrochloride separated, which after recrystallization from acetone, melted at 261-262°C.

Calc. for $C_{15}H_{21}ON.HCl$: C, 67.26; H, 8.28; N, 5.57%

Found: C, 67.61; H, 8.49; N, 5.80%

The hydrobromide was prepared by a similar procedure and recrystallized from acetone-methanol. It melted at 267-268°C.

Calc. for $C_{15}H_{21}ON.HBr$: C, 57.68; H, 7.10%

Found: C, 57.54; H, 6.90%

The ultraviolet spectrum of the hydrochloride of compound LIII showed weak absorption at 3180 A, $\log\varepsilon$= 1.6 (carbonyl) and had maximum absorption at 2260 A, $\log\varepsilon$= 3.85 (conjugated carbonyl). Its infrared spectrum had peaks at 1680 $cm.^{-1}$ (conjugated carbonyl) and 1625 $cm.^{-1}$ (unsaturation).

In another experiment carried out in refluxing benzene for 15 minutes, only a trace of the conjugated ketone was obtained. The basic fraction consisted instead of a compound, isolated in a yield of 25 per cent, which crystallized from ether and melted at 223°C.

Calc. for $C_{28}H_{33}O_2N$: C, 80.93; H, 8.00; N, 3.37%

Found: C, 80.83; H, 8.02; N, 3.12%

The infrared spectrum of compound LIV had absorption at 3420 $cm.^{-1}$ in the hydroxyl region, 1710 $cm.^{-1}$ in the carbonyl region

and 1595 $cm.^{-1}$ in the phenyl region. Its ultraviolet spectrum was similar to that of the starting material, λmax at 2580 Å, $\log \varepsilon = 2.75$. $[\alpha]_D^{21} = -60.2$ (c, 1% in chloroform). A mixture of compounds LIV and LII melted before 190°C. Their infrared spectra were not similar.

The mother liquors remaining after the separation of compound LIV yielded, on concentration, a substance which melted over a wide range and was probably a mixture of compounds LIV and LII.

Treatment of Compound LII with Potassium Tertiary Butoxide

The compound $C_{28}H_{33}O_2N$, LII, (0.33 g.) was dissolved in 30 ml. of dry toluene, 0.45 g. of freshly prepared potassium tertiary butoxide was added and the solution was heated under reflux for 30 minutes under an atmosphere of oxygen-free nitrogen. The reaction mixture was worked up for recovery of the conjugated ketone LIII as described in the previous experiment. The non-basic material remaining in the toluene-ether solution was recovered by evaporation of the solvents under reduced pressure. The residue (0.15 g.) had an infrared spectrum identical with authentic benzohydrol. It was recrystallized from petroleum ether and melted at 70°C either alone or in admixture with an authentic sample of benzohydrol.

The basic material yielded a hydrochloride identical with the hydrochloride of the conjugated ketone LIII.

When the reaction was carried out in air rather than in nitrogen, the non-basic fraction was a mixture of benzohydrol and benzophenone. The latter was identified by conversion to

its 2,4-dinitrophenylhydrazone and comparison with an authentic specimen.

Treatment of Compound LII with Sodium Ethoxide

The compound $C_{28}H_{33}O_2N$, LII, (0.13 g.) was heated under reflux overnight in a dilute solution of sodium ethoxide in ethanol. The solution was evaporated to dryness under reduced pressure, water was added to the residue and the mixture was extracted with ether. The ether extract was dried over anhydrous sodium sulphate and evaporated nearly to dryness. The remaining solution was seeded with a crystal of compound LIV and after two hours, 0.02 g. of crystalline needles was separated from the solution by filtration. They melted at 220°C and did not depress the melting point of compound LIV. Also their infrared spectrum was identical with that of compound LIV. The residual material obtained from the mother liquors melted over a wide range and was likely a mixture of compounds LII and LIV.

Oxidation of Diphenyldesoxodihydroannotinine with Chromium Trioxide

A solution of 1.00 g. of diphenyldesoxodihydroannotinine in 20 ml. of glacial acetic acid was warmed to 50-60°C and treated with a concentrated aqueous solution of chromium trioxide until the yellow color of excess chromium trioxide was persistent. The excess oxidizing agent was destroyed with methanol and the solution was taken to dryness under reduced pressure. Water was added to the residue, the mixture was made basic with ammonium hydroxide and exhaustively extracted with ether. The ether

extract was dried over anhydrous sodium sulphate and evaporated to dryness. The residue was dissolved in methanol and acidified with concentrated hydrochloric acid, whereupon the hydrochloride of the product (0.92 g.) separated in crystalline form. Recrystallization from methanol yielded a compound which melted at 276°C.

The infrared spectrum of the hydrochloride of compound IV had absorption at 1595 cm.$^{-1}$ in the phenyl region, 1720 cm.$^{-1}$ in the ketone region and broad absorption at 3350-3420 cm.$^{-1}$ in the hydroxyl region. Its ultraviolet spectrum had λ max at 2600 Å, log ε = 2.69.

The base was regenerated from the hydrochloride by treatment with aqueous ammonia and extraction with ether. It was recrystallized from methanol to yield a compound which melted at 208°C.

Calc. for $C_{28}H_{33}O_3N$: C, 77.94; H, 7.65; N, 3.25; act H (2), 0.46%
Found: C, 78.09; H, 7.77; N, 3.05; act. H, 0.47%

The infrared spectrum of compound IV showed phenyl absorption at 1590 cm.$^{-1}$, carbonyl absorption at 1720 cm.$^{-1}$ (shoulder at 1728 cm.$^{-1}$) and hydroxyl absorption at 3450 and 3500-3550 cm.$^{-1}$. Its ultraviolet spectrum had λ max at 2600 Å, log ε = 2.90. $[\alpha]_D^{27}$ = +43.0 (c, 1% in chloroform).

Compound IV (0.20 g.) was treated with 3 ml. of methyl iodide and the solution allowed to stand at room temperature for 2 days. The crystalline solid which separated (0.05 g.) was recrystallized from acetone and melted at 245°C.

Calc. for $C_{28}H_{33}O_3N.CH_3I$: C, 60.71; H, 6.32; N, 2.44%
Found: C, 60.67; H, 6.38; N, 2.91%

The infrared spectrum of the methiodide of compound LV had phenyl absorption at 1600 $cm.^{-1}$, carbonyl absorption at 1720 $cm.^{-1}$ and a double peak in the hydroxyl region at 3250 $cm.^{-1}$ and 3430 $cm.^{-1}$.

Oxidation of Compound LII with Chromium Trioxide

A solution of 0.11 g. of compound LII in 5 ml. of glacial acetic acid was treated with a concentrated aqueous solution of chromium trioxide at 50-60°C until there was excess oxidant. After 10 minutes the excess chromium trioxide was destroyed with methanol and the reaction mixture worked up for recovery of basic material as described for the chromium trioxide oxidation of diphenyldesoxodihydroannotinine. The product (0.09 g.) was dissolved in methanol and its hydrochloride prepared. The hydrochloride, after recrystallization from methanol, melted at 273°C and did not depress the melting point of the hydrochloride of compound LV. Its infrared spectrum was similar to that of the hydrochloride of compound LV.

Attempted Oxidation of Compound LIV with Chromium Trioxide

A solution of compound LIV (0.04 g.) in glacial acetic acid was treated with a concentrated aqueous solution of chromium trioxide at 50-60°C. The reaction mixture was worked up for basic material as described for the chromium trioxide oxidation of diphenyldesoxodihydroannotinine. The product after recrystallization from ether melted at 223°C and proved to be identical with the starting material, compound LIV.

Reduction of Compound LV with Lithium Aluminum Hydride

A solution of 0.40 g. of compound LV in 40 ml. of anhydrous ether was added slowly to a solution of 1.0 g. of lithium aluminum hydride in 30 ml. of anhydrous ether. The reduction mixture was refluxed for 2.5 hours. The excess hydride was destroyed with wet ether followed by the addition of a few ml. of water. The ether solution was separated and evaporated to dryness to yield 0.34 g. of residue. The product was dissolved in acetone and acidified with concentrated hydrochloric acid. The hydrochloride, obtained upon evaporation of the acetone, after two recrystallizations from methanol, melted at 272°C. The base was regenerated from the hydrochloride by treatment with dilute ammonium hydroxide and extraction with ether. It was recrystallized twice from ether and melted at 209°C.

Calc. for $C_{28}H_{35}O_3N$: C, 77.56; H, 8.13%

Found: C, 77.59; H, 8.18%

The infrared spectrum of compound LVI showed absorption at 1595 $cm.^{-1}$ in the phenyl region and at 3430 $cm.^{-1}$ (broad) and 3620 $cm.^{-1}$ (slight) in the hydroxyl region.

Treatment of the hydrochloride of compound LV with lithium aluminum hydride by the same procedure also yielded the compound LVI.

Reaction of Compound LII with Lead Tetraacetate

A solution of 0.10 g. of compound LII in 50 ml. of glacial acetic acid was treated with 50 ml. of a solution of lead tetraacetate in acetic acid (saturated at room temperature). The

reaction mixture was allowed to stand overnight at room temperature. The excess lead tetraacetate was destroyed with glycol and the solution was taken to dryness under reduced pressure. The residue was treated with dilute hydrochloric acid and the solution was washed with ether. The acid solution was then made alkaline with ammonium hydroxide and extracted with ether. The residue, remaining on evaporation of the dried ether extract, amounted to 0.08 g.. It was recrystallized from petroleum ether (B.P. 40-60°C) and melted at 165-168°C. The infrared spectrum of the product, compound LVII, showed hydroxyl absorption at 3510 $cm.^{-1}$, carbonyl absorption at 1712 and 1728 $cm.^{-1}$ (double peak), acetate absorption at 1747 and 1250 $cm.^{-1}$, and phenyl absorption at 1600 $cm.^{-1}$.

The acetate LVII (0.07 g.) was added to 10 ml. of 10 per cent sodium hydroxide and treated with sufficient methanol to bring about solution. The solution was refluxed for 1.5 hours and the methanol was removed under reduced pressure. The remaining solution was extracted with ether. The dried ether extract was evaporated to dryness and the hydrochloride of the product prepared in methanol. The hydrochloride (0.02 g.) did not depress the melting point of the hydrochloride of compound LV and the infrared spectra of the two hydrochlorides were identical.

Reduction of "Unsaturated Lactone A" with Lithium Aluminum Hydride

"Unsaturated lactone A" (2.0 g.) was treated with 1 g. of lithium aluminum hydride in ether by the method of Prime (31). The product (1.7 g.), which melted at 165°C, did not depress the melting point of the compound, $C_{16}H_{25}O_2N$, isolated by Prime.

The infrared spectrum of compound LVIII contained hydroxyl absorption at 3430 $cm.^{-1}$ (sharp) and 3240 $cm.^{-1}$ (broad) and double bond absorption at 1650 $cm.^{-1}$. The perchlorate of compound LVIII was prepared by the addition of perchloric acid to a solution of the compound in ether. After recrystallization from acetone-ether, the perchlorate melted at 204°C.

Hydrogenation of Compound LVIII

A mixture of compound LVIII (1.7 g.), 100 ml. of methanol and 0.2 g. of Adams catalyst was shaken with 50 p.s.i.g. hydrogen overnight. The catalyst was removed by filtration and the filtrate was evaporated to dryness. The residue was crystallized from acetone yielding 1.1 g. of product which melted at 180-181°C. Its infrared spectrum had bands at 3450 $cm.^{-1}$ (sharp) and 3140 $cm.^{-1}$ (broad) in the hydroxyl region. The hydrochloride was prepared by treating a sample of the base, dissolved in acetone, with concentrated hydrochloric acid. After recrystallization from methanol-acetone the hydrochloride of the product melted at 274°C with decomposition.

Calc. for $C_{16}H_{27}O_2N.HCl$: C, 63.66; H, 9.35; N, 4.64%

Found: C, 63,53; H, 9.19; N, 4.54%

The infrared spectrum of the hydrochloride of compound LIX had a double peak at 3390 and 3300 $cm.^{-1}$ in the hydroxyl region. The perchlorate of compound LIX was prepared in ether and after recrystallization from acetone-ether, it melted at 175°C.

Oppenauer Oxidation of Compound LIX

A solution consisting of 0.30 g. of compound LIX, 25 ml. of dry benzene, 3 ml. of dry acetone and 1.0 g. of aluminum isopropoxide was heated under reflux for six hours. The reaction mixture was cooled and poured into ice-water. A little dilute sodium hydroxide was added, the mixture was shaken and the benzene layer was separated. The aqueous layer was exhaustively extracted with chloroform. The chloroform extract and the benzene layer were combined and extracted four times with 10 per cent hydrochloric acid. The acid extract was washed twice with ether, made alkaline with ammonium hydroxide, and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated to dryness. The residue, after recrystallization from acetone, melted at 126°C.

Calc. for $C_{16}H_{25}O_2N$: C, 73.00; H, 9.57%

Found: C, 73.02; H, 9.52%

There was absorption at 3150 $cm.^{-1}$ in the hydroxyl region and at 1703 $cm.^{-1}$ in the carbonyl region in the infrared spectrum of compound LX.

The perchlorate of compound LX was prepared by treating a sample of the base in ether with perchloric acid. After recrystallization from acetone-ether, it melted at 227°.

Unsuccessful Attempts to Replace the Hydroxyl Group of Compound LX with a Hydrogen Atom

(a) Preparation and Reactions of the Tosylate of Compound LX

A solution of 0.10 g. of compound LX, 1.0 ml. of dry pyridine and 0.08 g. of p-toluenesulphonyl chloride was allowed

to stand in the refrigerator for four days. The pyridine was removed under reduced pressure and dilute hydrochloric acid was added to the residue. The resulting acid solution was washed with ether and then made alkaline with ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated to dryness. The residue (0.17 g.) could not be induced to crystallize from any of the common solvents. Its infrared spectrum (film) showed no hydroxyl absorption, but had absorption at 1710 $cm.^{-1}$ in the carbonyl region, 1603 $cm.^{-1}$ in the phenyl region and two bands at 1370 and 1185 $cm.^{-1}$ which are characteristic of sulphonates.

Treatment of the tosylate of compound LX with lithium aluminum hydride in anhydrous ether yielded an amorphous reduction product which could not be induced to crystallize. Its infrared spectrum (film) contained a broad band at 3370 $cm.^{-1}$ in the hydroxyl region and no carbonyl or phenyl absorption. However, Oppenauer oxidation of the material in toluene, using aluminum isopropoxide and cyclohexanone, gave a substance showing both hydroxyl absorption at 3350 $cm.^{-1}$ and carbonyl absorption at 1700 $cm.^{-1}$ in its infrared spectrum (film). Thus the hydride must have cleaved the O-S bond rather than the O-C bond. The infrared spectrum of this hydroxy ketone was similar to, but not identical with, that of compound LX.

When the tosylate of compound LX was heated under reflux in acetone with sodium iodide, no displacement of the tosyl group with iodide ion occurred.

(b) Unsuccessful Attempts to Replace the Hydroxyl Group of Compound LX with a Halogen Atom

A solution consisting of compound LX (0.10 g.), phosphorus tribromide (0.10 g.) and pyridine (2 ml.) was allowed to stand at room temperature overnight. The pyridine was removed under reduced pressure and the residue was taken up in water. The solution was made alkaline with ammonium hydroxide and extracted with chloroform. The residue (0.03 g.) remaining after evaporation of the dried chloroform extract was starting material contaminated with a small amount of a substance which showed phosphate absorption in its infrared spectrum (film). The same result was obtained when chloroform was used as the solvent.

When the compound LX was heated under reflux overnight with a 4:1 solution of 48 per cent hydrobromic acid and 85 per cent phosphoric acid, the starting material was recovered.

Treatment of the hydrochloride of compound LX with thionyl chloride at room temperature yielded a mixture of starting material, a small amount of amorphous material with absorption at 1680 and 1570 $cm.^{-1}$ (conjugated ketone) in its infrared spectrum, and a substance containing sulphur. This last product produced sulphur dioxide on hydrolysis with dilute sulphuric acid.

(c) Unsuccessful Attempts to Dehydrate Compound LX

The compound LX was recovered unchanged when it was heated under reflux overnight in benzene with p-toluenesulphonic acid.

Very little basic material was recovered when compound LX was treated with phosphorus oxychloride in pyridine.

Preparation of Compound LXIV

(a) Treatment of "Unsaturated Lactone A" with Barium Hydroxide

"Unsaturated lactone A" (3.0 g.) was heated under reflux for two hours with 18.0 g. of barium hydroxide in 90 ml. of water. Solid carbon dioxide was added until the precipitation of the barium ion was complete. After removal of the barium carbonate by filtration, the filtrate was taken to dryness under reduced pressure.

(b) Esterification of the Amino Acid

The residue isolated above was dissolved in methanol and the solution was treated with excess diazomethane in ether. After two hours a small amount of undissolved material was removed by filtration and the filtrate was taken to dryness.

(c) Epimerization of the Methyl Ester

The product from the above reaction was dissolved in 40 ml. of absolute methanol and the solution was added to a solution of 3.5 g. of potassium in 50 ml. of absolute methanol. The reaction mixture was then heated under reflux for four hours. The methanol was removed under reduced pressure, the residue was treated with water and the resulting solution was extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated to dryness.

(d) Reduction of the Epimerized Ester with Lithium Aluminum Hydride

The ester isolated above was dissolved in 50 ml. of

anhydrous ether and added to a solution of 1.5 g. of lithium aluminum hydride in 100 ml. of anhydrous ether. The reaction mixture was refluxed for two hours and then the excess lithium aluminum hydride was destroyed with water. The ether layer was separated and the aqueous layer was washed several times with ether. The ether solutions were combined, dried over anhydrous sodium sulphate and the ether was removed by evaporation. A small sample of the product was recrystallized from petroleum ether and melted at 100-106°C. The remainder of the product was dissolved in acetone and the hydrochloride was prepared by addition of concentrated hydrochloric acid to the solution. After recrystallization from methanol-acetone the hydrochloride melted at 263°C. The total yield was 70 per cent.

Calc. for $C_{16}H_{25}O_2N.HCl$: C, 64.07; H, 8.47; N, 4.67%

Found: C, 64.40; H, 8.83; N, 4.44%

The infrared spectrum of the hydrochloride of compound LXIV had strong absorption at 3320 $cm.^{-1}$ in the hydroxyl region and weak absorption at 1650 $cm.^{-1}$ in the unsaturation region. Its infrared spectrum was not identical with the infrared spectrum of the hydrochloride of compound LVIII. The melting point of the hydrochloride of compound LXIV was lowered when it was mixed with the hydrochloride of compound LVIII. This is evidence that epimerization of the carbomethoxy group took place when the methyl ester, isolated above, was treated with potassium methoxide.

The perchlorate of compound LXIV was prepared by treating a sample of the base in ether with perchloric acid. After several recrystallizations from acetone-ether, the perchlorate melted at 227-229°C.

Calc. for $C_{16}H_{25}O_2N.HClO_4$: C, 52.81; H, 7.20; N, 3.85%
Found: C, 52.98; H, 7.20; N, 4.00%

Hydrogenation of Compound LXIV

Compound LXIV (1.0 g.) was dissolved in 30 ml. of methanol, Adams catalyst was added and the mixture was shaken overnight with hydrogen at 50 p.s.i.g.. The catalyst was removed by filtration, and the filtrate was taken to dryness. The residue was dissolved in ether and the solution was acidified with perchloric acid. The perchlorate, which separated after recrystallization from acetone-ether, melted at 227°C. A 90 per cent yield was obtained.
Calc. for $C_{16}H_{27}O_2N.HClO_4$: C, 52.50; H, 7.71; N, 3.83%
Found: C, 52.41; H, 7.72; N, 4.14%

The hydrochloride of compound LXV was prepared by the treatment of a sample of the base in acetone with concentrated hydrochloric acid. After recrystallization from methanol-acetone, it melted at 292°C with decomposition. The infrared spectrum of the hydrochloride of compound LXV had strong absorption at 3300 $cm.^{-1}$ in the hydroxyl region.

Oppenauer Oxidation of Compound LXV

Compound LXV (1.00 g.) was dissolved in 100 ml. of dry toluene and 3.0 g. of aluminum isopropoxide and 5 ml. of cyclohexanone were added. The solution was heated under reflux for one hour, cooled and poured into ice. A few milliliters of dilute sodium hydroxide were added, the mixture was shaken and the toluene layer was separated. The aqueous layer was then exhaustively

extracted with chloroform. The chloroform extract and the toluene layer were combined and washed four times with 10 per cent hydrochloric acid. The acid solution was washed with ether, made alkaline with ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and the chloroform was removed by evaporation. The residue was dissolved in acetone and its hydrobromide prepared. This was recrystallized from methanol-acetone yielding 0.43 g. of salt melting at 259°C.

Calc. for $C_{16}H_{25}O_2N.HBr$: C, 55.80; H, 7.61; N, 4.07%

Found: C, 55.86; H, 7.70; N, 4.37%

In the infrared spectrum of the hydrobromide of compound LXVI there was absorption at 3375 $cm.^{-1}$ in the hydroxyl region and at 1700 $cm.^{-1}$ in the carbonyl region.

The perchlorate of compound LXVI was prepared in ether and after recrystallization from acetone-ether, it melted at 212°C.

Calc. for $C_{16}H_{25}O_2N.HClO_4$: C, 52.81; H, 7.20; N, 3.85%

Found: C, 53.18; H, 7.17; N, 3.50%

Preparation of Compound LXVII

The hydrobromide of compound LXVI (0.40 g.) was heated under reflux in a solution of 3.0 ml. of 48 per cent hydrobromic acid and 2.0 ml. of 85 per cent phosphoric acid for nine hours. The reaction mixture was cooled and poured on ice. The aqueous solution was made alkaline with ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated to dryness. The product (0.36 g.), after recrystallization from methanol, melted at 123°C.

Calc. for $C_{16}H_{24}ONBr$: C, 58.88; H, 7.41; N, 4.29%
Found: C, 58.43; H, 7.17; N, 4.40%

The infrared spectrum of compound LXVII had a band at 1700 $cm.^{-1}$ in the carbonyl region. There was no absorption in the hydroxyl region.

Preparation of Compound LXVIII

The crude bromide, LXVII, (0.36 g.) was dissolved in 50 ml. of methanol, 0.35 g. of potassium hydroxide and 0.20 g. of 5 per cent palladium on calcium carbonate were added, and the mixture was shaken with hydrogen at 50 p.s.i.g. for 13 hours. The catalyst was removed by filtration and the filtrate was taken to dryness under reduced pressure. Water was added to the residue and the alkaline solution was extracted with chloroform. The chloroform extract was washed with water, dried over sodium sulphate and evaporated to dryness. The perchlorate of the product was prepared by treating a solution of the residue (0.29 g.) in ether with perchloric acid. It was recrystallized from acetone-ether and melted at 221-225°C.

Calc. for $C_{16}H_{25}ON.HClO_4$: C, 55.26; H, 7.54; N, 4.03%
Found: C, 55.46; H, 7.45; N, 4.22%

The infrared spectrum of the perchlorate of compound LXVIII contained a band at 1710 $cm.^{-1}$ in the carbonyl region. This spectrum was not similar to that of lycopodine perchlorate.

Preparation of Anhydrodihydrolycopodine

(a) Dehydration of Dihydrolycopodine with Thionyl Chloride

The hydrochloride of dihydrolycopodine (0.20 g.) was added in small portions to 2.0 ml. of thionyl chloride. After two hours

the excess thionyl chloride was removed under reduced pressure, and the residue was dissolved in water. The aqueous solution was washed with ether, made alkaline with ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and the chloroform was removed by evaporation. The perchlorate of the product was prepared in ether. It melted at 237°C and was identical with the perchlorate of anhydrodihydrolycopodine prepared by the method of Douglas, Lewis and Marion (9) by treatment of dihydrolycopodine with phosphorus pentachloride in boiling xylene.

(b) Dehydration of Dihydrolycopodine with Hydrochloric Acid

Dihydrolycopodine (0.30 g.) was heated under reflux for three hours with 6.0 ml. of concentrated hydrochloric acid. The cooled solution was made alkaline with dilute ammonium hydroxide and extracted with chloroform. The dried chloroform extract yielded 0.28 g. of anhydrodihydrolycopodine on evaporation.

Reduction of Compound LXVIII with Lithium Aluminum Hydride

Compound LXVIII (0.15 g.) was dissolved in 20 ml. of anhydrous ether and the solution was added to 40 ml. of anhydrous ether containing 0.2 g. of lithium aluminum hydride. The reaction mixture was refluxed for two hours and then the excess hydride was destroyed with water. The ether layer was separated and the aqueous layer was washed with ether. The ether solutions were combined, dried over anhydrous sodium sulphate and concentrated to dryness. The residue (0.16 g.) was dissolved in ether and the solution was treated with perchloric acid. The perchlorate which separated was recrystallized from acetone-ether. It melted at

233-235°C.

Calc. for $C_{16}H_{27}ON.HClO_4$: C, 54.90; H, 8.07%

Found: C, 54.81; H, 8.12%

The infrared spectrum of the perchlorate of compound LXIX contained absorption at 3470 $cm.^{-1}$ in the hydroxyl region.

Dehydration of Compound LXIX

The hydrochloride of compound LXIX (0.07 g.) was treated with thionyl chloride at room temperature using the method described for the preparation of anhydrodihydrolycopodine. The perchlorate of the product (0.05 g.) was prepared and after recrystallization from acetone-ether, melted at 168-171°C. Unfortunately good analytical results for this compound were not obtained. However, the infrared spectrum of the perchlorate showed no hydroxyl absorption. It was not identical with the infrared spectrum of the perchlorate of anhydrodihydrolycopodine.

Unsuccessful Attempts to Hydrogenate Anhydrodihydrolycopodine

Anhydrodihydrolycopodine was recovered unchanged from the hydrogenation reactions attempted under the following conditions:

1, In methanol with Adams catalyst at 1050 p.s.i.g. hydrogen, 110°C for 7 hours.

2, In acetic acid with Adams catalyst at 1050 p.s.i.g. hydrogen, 100°C for 11 hours.

3, In ethanol with Raney nickel at 1250 p.s.i.g. hydrogen, 145°C for 10 hours.

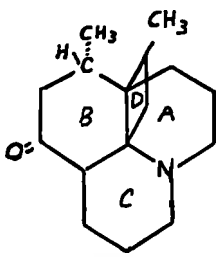
SUMMARY

Annotinine lactamdiol, $C_{16}H_{21}O_5N$, was prepared by treating annotinine lactam, the permanganate oxidation product of annotinine, with dilute sulphuric acid. The reaction of annotinine lactamdiol with three moles of lead tetraacetate confirmed the presence of an epoxide ring in annotinine. Evidence that ring A in annotinine was six-membered was obtained by the isolation of a compound containing a five-membered lactam ring, $C_{15}H_{17}O_4N$, from the products of the oxidation of annotinine lactamdiol with potassium permanganate or chromium trioxide. The ozonolysis of "unsaturated lactone A" confirmed that this compound had a cyclic allylamine structure.

The detailed structure of ring B in annotinine was elucidated by a study of the reactions of diphenyldesoxodihydroannotinine, prepared by the reaction of the dihydro derivative of "unsaturated lactone A" with phenyllithium. Oxidation of diphenyldesoxodihydroannotinine by the Oppenauer procedure using aluminum isopropoxide gave the expected hydroxy ketone $C_{28}H_{33}O_2N$. When potassium tertiary butoxide in refluxing toluene was used, a conjugated ketone $C_{15}H_{21}ON$ and benzohydrol were formed. This reaction and the ultraviolet spectrum of the conjugated ketone indicated that in annotinine the carbon atoms bearing the potential hydroxyl and carboxyl groups of the γ-lactone ring were part of the same carbocyclic ring and had a methylene group between them. A second hydroxy ketone, epimeric with the first hydroxy ketone at a tertiary carbon alpha to the carbonyl, was obtained by the

Oppenauer oxidation of diphenyldesoxodihydroannotinine using potassium tertiary butoxide in benzene. This indicated that in annotinine the second carbon alpha to the carbon carrying the hydroxyl group of the -lactone function was tertiary. Oxidation of diphenyldesoxodihydroannotinine with chromium trioxide gave a dihydroxy ketone, $C_{28}H_{33}O_3N$. The structure of this compound was elucidated.

Unsuccessful attempts were made to relate the structure of annotinine to the structure of lycopodine. Annotinine was converted into a compound of the following structure which was isomeric but not identical with lycopodine. An attempt to prepare the compound with the methyl group on ring B in the epimeric configuration was unsuccessful.



Several derivatives of annotinine were compared with isomeric Lycopodium alkaloids in an attempt to relate the structure of annotinine to that of other alkaloids. No definite correlations were established.

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