

STUDIES ON THE CHEMISTRY

OF

ANNOTININE

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OF  
ANNOTININE

by

Kenneth Allan Harry Adams, B.Sc.

A Thesis

Submitted to the Faculty of Graduate Studies  
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SCOPE AND CONTENTS:

The amino acid  $C_{14}H_{19}O_4N$ , obtained from annotinine by oxidative fission of ring A, has been converted to the O,N-diacetyl-lactone anhydride  $C_{18}H_{22}O_6N$ , and the anhydride ring has been shown to be five-membered. Oxidation studies have been carried out on the N-methyl lactone  $C_{17}H_{27}O_2N$ , obtained by fission of ring A, and the oxidation products isolated and characterized. Hofmann degradation studies on the N-methyl lactone have been carried out and the products isolated from this reaction are discussed in relation to the known structure of the alkaloid.



## ACKNOWLEDGEMENTS

The author expresses his sincere appreciation to Professor D. B. MacLean for his constant interest, advice, and encouragement during the course of this research.

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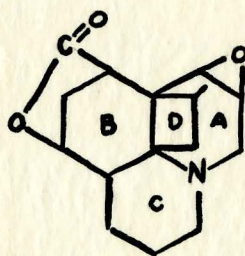


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## GENERAL INTRODUCTION

The investigations reported in this thesis were commenced at a time when the complete structure of annotinine, the major alkaloid of Lycopodium annotinum L., was yet unknown. The study was initially designed to gain more information about the relationship of the functional groups of annotinine, and to obtain chemical evidence for the detailed nature of rings C and D. During the course of this work Wiesner (2) proposed a total structure, I, for this alkaloid, and not long afterwards Przybylska and Marion (3) confirmed this structure by carrying out an X-ray diffraction study on annotinine bromohydrin.



I

At the time of this disclosure a study of the elimination of nitrogen by Hofmann degradation was being carried out. During this study reactions were encountered which were interesting in their own right and the investigation was continued on this basis. The nature of rings C and D was established mainly by inference,

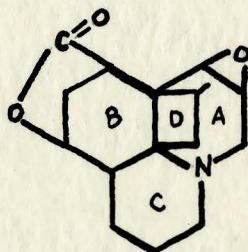


and the degradation scheme being studied at the time promised to supply more direct, chemical evidence for the proposed structure. In addition, the degradation scheme being used might have proved useful in the elucidation of the structures of the other Lycopodium alkaloids of yet unknown structure. Annotinine is the only member of this group whose structure is now firmly established.



## HISTORICAL INTRODUCTION

Annotinine,  $C_{16}H_{21}O_3N$ , I, the major alkaloid of Lycopodium annotinum L., was first isolated in 1943 by Manske and Marion (1). Annotinine, because of its availability and greater reactivity, has been more intensively studied than the other Lycopodium alkaloids. In the years following its first isolation several groups have studied the reactions of annotinine and its derivatives. A large amount of chemical evidence was obtained and on the basis of this chemical evidence Wiesner was able to deduce the structure of annotinine, which is shown below.



This structure was confirmed by Przybylska and Marion (3) who carried out an X-ray diffraction study on annotinine bromohydrin.

The chemistry of annotinine will be discussed in the following pages with special attention being given to those reactions which demonstrate the structural features of this alkaloid.

The functional groups of this alkaloid were established by Manske and Marion (4), who were able to show that annotinine contained a tertiary nitrogen, an oxide ring, a lactone ring, and a combination of four carbocyclic and heterocyclic rings.



A Kuhn-Roth oxidation (5) indicated that annotinine contained one, or less likely, two C-methyl groups.

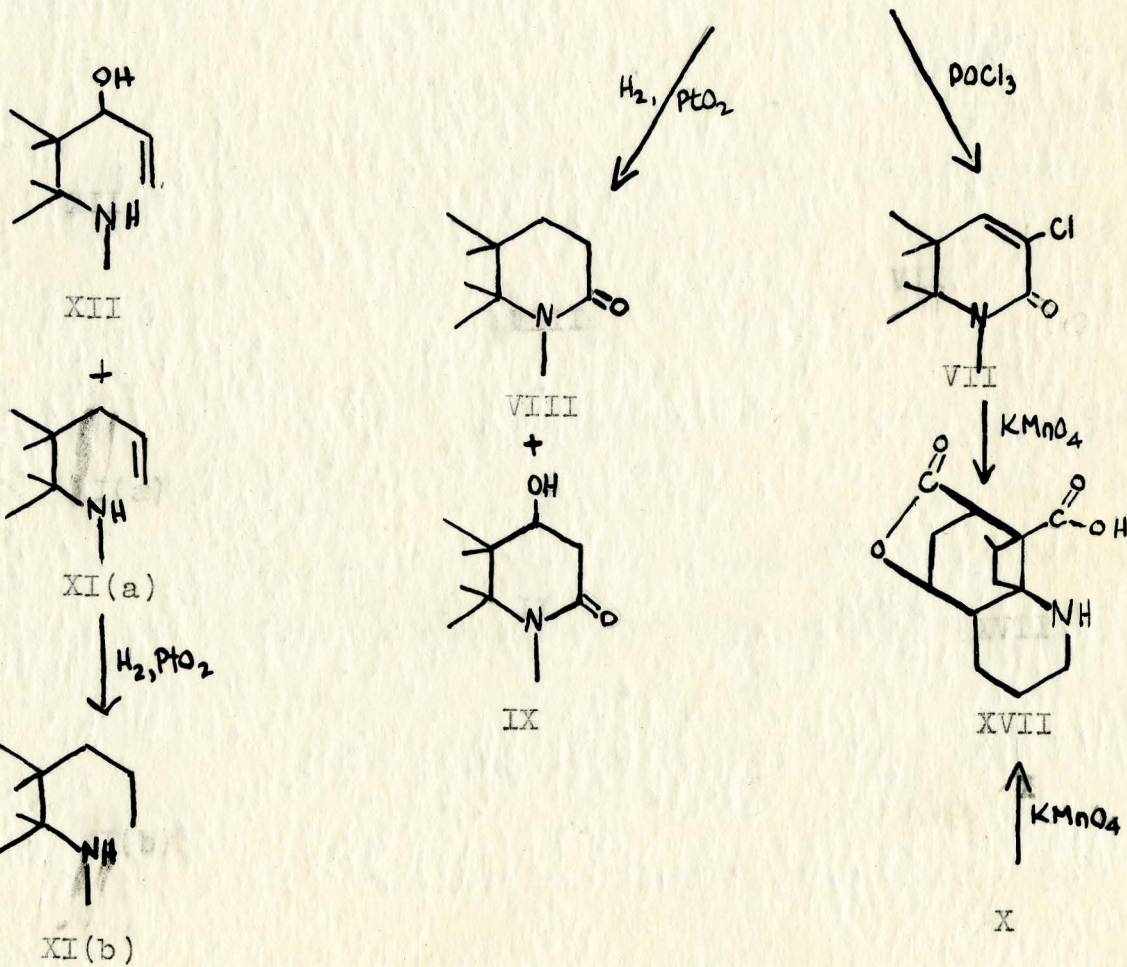
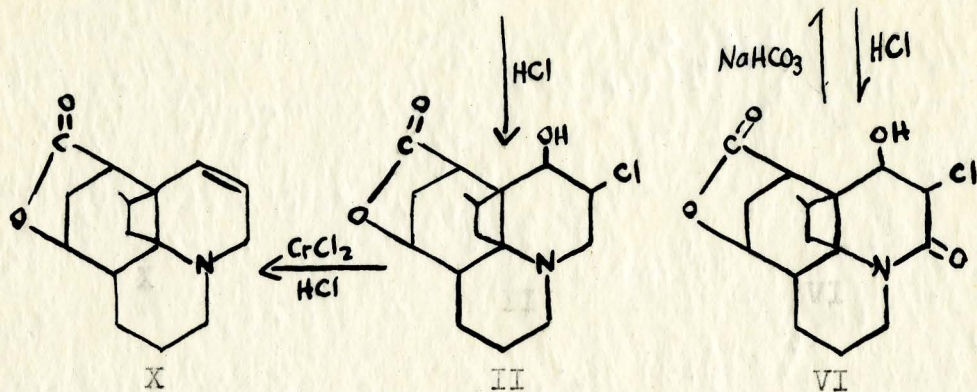
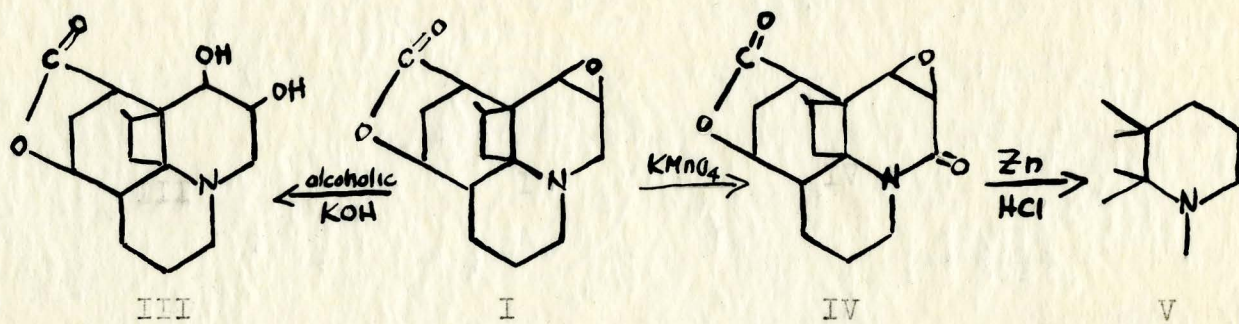
#### The Oxide Ring and Ring A

The presence of a cyclic ether structure was demonstrated by the reaction of annotinine with hydrochloric acid to form the chlorohydrin  $C_{16}H_{22}O_3NCl$ , II, and also by the action of alcoholic potassium hydroxide to give annotinine hydrate  $C_{16}H_{23}O_4N$ , III. (4) The nature of the ether ring was established by MacLean and Prime (6) and they were able to relate its position to the nitrogen atom. Annotinine was shown to react with potassium permanganate to form a neutral compound  $C_{16}H_{19}O_4N$ , IV. Compound IV was originally reported as a base, (4), and because of its conversion by Clemmensen reduction to the saturated base  $C_{16}H_{23}O_2N$ , V, was incorrectly described as a diketone. MacLean and Prime (6) pointed out that the product of oxidation of annotinine with potassium permanganate was not a base, but a neutral lactam which retained the ether ring intact. This fact indicated that annotinine contained a methylene group adjacent to the nitrogen. Annotinine lactam, IV, was converted to the corresponding chlorohydrin  $C_{16}H_{20}O_4NCl$ , VI, by treatment with concentrated hydrochloric acid (6). When VI was treated with sodium bicarbonate, hydrochloric acid was eliminated and annotinine lactam, IV, was formed. MacLean and Prime (6) pointed out that this reaction suggested that the chloro and hydroxyl groups were adjacent, and therefore that the ether ring was an epoxide ring. Annotinine lactam



chlorohydrin VI, underwent dehydration on treatment with phosphorous oxychloride or on prolonged treatment with concentrated hydrochloric acid and yielded the anhydrochloro compound  $C_{16}H_{18}O_3NCl$ , VII. Treatment of VI with hydrogen over Adams' catalyst gave a mixture of two compounds,  $C_{16}H_{21}O_3N$ , VIII, and  $C_{16}H_{21}O_4N$ , IX. In the formation of VIII, both the chloro and hydroxyl groups were eliminated and in the formation of IX, only the chloro group had been eliminated by hydrogenolysis. A similar treatment of VII yielded VIII as the sole product, formed by saturation of the double bond and hydrogenolysis of the chloro group. Annotinine chlorohydrin, II, failed to react with sodium bicarbonate, concentrated hydrochloric acid, phosphorous oxychloride and hydrogen over Adams' catalyst. The only structural difference between II and VI is the presence of the lactam carbonyl in VI. MacLean and Prime interpreted this difference in reactivity to the influence of the lactam carbonyl and suggested that the lactam carbonyl and the oxide ring were adjacent in annotinine lactam. Wiesner et al (10) pointed out that the infrared and ultraviolet spectra of VII are in agreement with the postulated conjugated lactam. This provided further evidence that the oxide ring and the lactam carbonyl groups were adjacent in annotinine lactam and that the oxide ring and the nitrogen in annotinine were separated by a single methylene group.

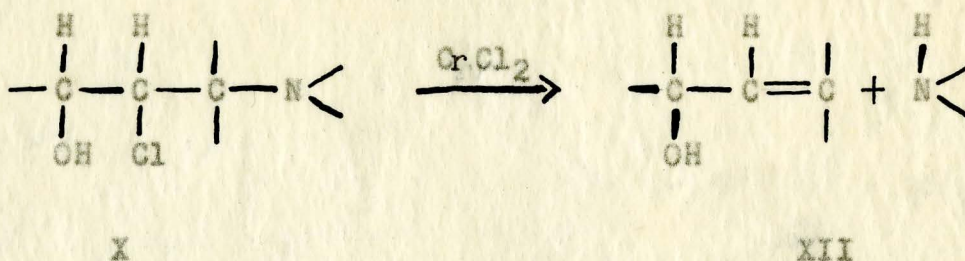




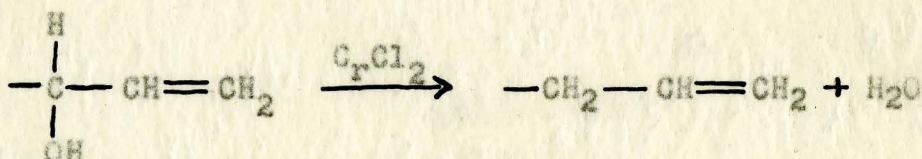


Additional evidence for the relationship of the oxide ring and the nitrogen atom was obtained from a study of the products from the reaction of annotinine chlorohydrin with chromous chloride (4). Meier, Meister and Marion (7) found that, by adjusting the reaction conditions, various proportions of three products could be isolated. The major product was an unsaturated base,  $C_{16}H_{21}O_2N$ , X, which is usually referred to as "unsaturated lactone A", but is more accurately named desoxoannotinine. Anet and Marion (8) observed that the pKa of X was 7.06, while that of its dihydro derivative was 8.48. This was in accordance with the proposed allylamine structure for X. The other products isolated from the chromous chloride reaction were two unsaturated secondary bases,  $C_{16}H_{23}O_2N$ , XI (a), referred to as "unsaturated lactone B", and  $C_{16}H_{23}O_3N$ , XII, referred to as the "hydroxylactone". Compound XII could be converted to XI(a) by treatment with chromous chloride in concentrated hydrochloric acid. Treatment of XI(a) with hydrogen over Adams' catalyst yielded the dihydro derivative  $C_{16}H_{25}O_2N$ , XI (b). Anet and Marion (8) pointed out the analogy of the chromous chloride reaction of annotinine chlorohydrin with the reaction of chromous chloride with certain steroid vic-dihalides, to yield the corresponding unsaturated compounds. Zinc in acetic acid has been shown to convert certain steroid vic-chlorohydrins to the olefins and therefore it was proposed that chromous chloride could bring about the same reaction. The formation of the "hydroxylactone", XII, is a simple extension of this reaction to  $\beta$ -chloroamines, which could react as shown below.





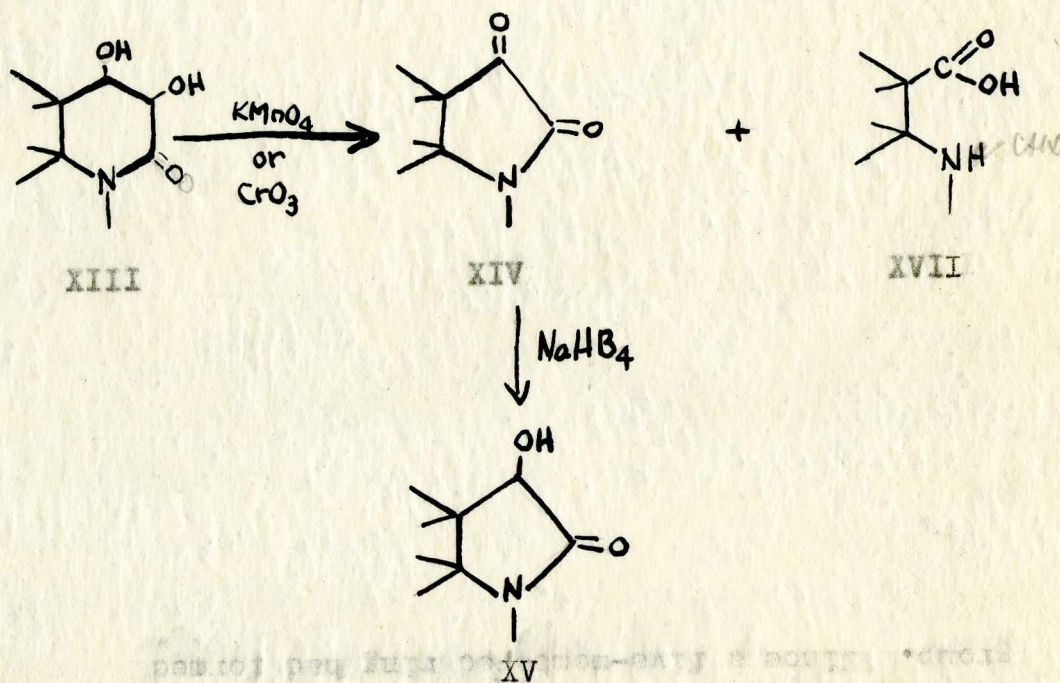
Since the "hydroxy lactone", XII, is an allyl alcohol, it would be expected to undergo reduction to "unsaturated lactone B", thus:



Evidence for the size and nature of ring A was obtained by infrared spectroscopy and by degradation experiments. The infrared spectrum of annotinine lactam, IV, suggested that the nitrogen-containing ring carrying the epoxide function was six-membered or larger (7). The six-membered character of ring A was established by Betts and MacLean (9) who carried out oxidation studies on annotinine lactamdiol, XIII, obtained by treatment of IV with dilute sulphuric acid. Compound XIII reacted with three moles of lead tetraacetate and this was taken as evidence for the arrangement of functional groups,  $\begin{array}{c} \text{O} \quad \text{OH} \quad \text{OH} \\ || \quad | \quad | \\ \text{N}-\text{C}-\text{CH}-\text{CH}- \end{array}$ . Oxidation of XII with potassium permanganate or chromic oxide yielded a mixture of two products, an acidic compound  $\text{C}_{15}\text{H}_{19}\text{O}_5\text{N}$ , XVI, and a neutral compound  $\text{C}_{15}\text{H}_{17}\text{O}_4\text{N}$ , XIV. The neutral product XIV had an infrared absorption spectrum with peaks characteristic of a  $\gamma$ -lactam, a five-membered cyclic ketone and a  $\gamma$ -lactone. Treatment of XIV with sodium borohydride in ethanol converted it to a neutral



compound  $C_{15}H_{19}O_4N$ , XV, which showed infrared absorption bands characteristic of a  $\gamma$ -lactam, a  $\gamma$ -lactone and a hydroxyl group. Since a five-membered ring had formed with the loss of one carbon atom, Maclean and Betts interpreted this as evidence for the six-membered nature of ring A. The acidic compound  $C_{15}H_{19}O_5N$ , XVI had three peaks in the carbonyl region of the infrared, attributed to amide, carboxylic acid and  $\gamma$ -lactone functions. Hydrolysis of XVI with dilute sulphuric acid yielded formic acid and the amino acid  $C_{14}H_{19}O_4N$ , XVII, in equimolar amounts. The amino acid had been previously reported by Anet and Marion (8) and by Wiesner (5). This series of reactions can be written as follows:



#### The Relationship of the Lactone and Oxide Rings

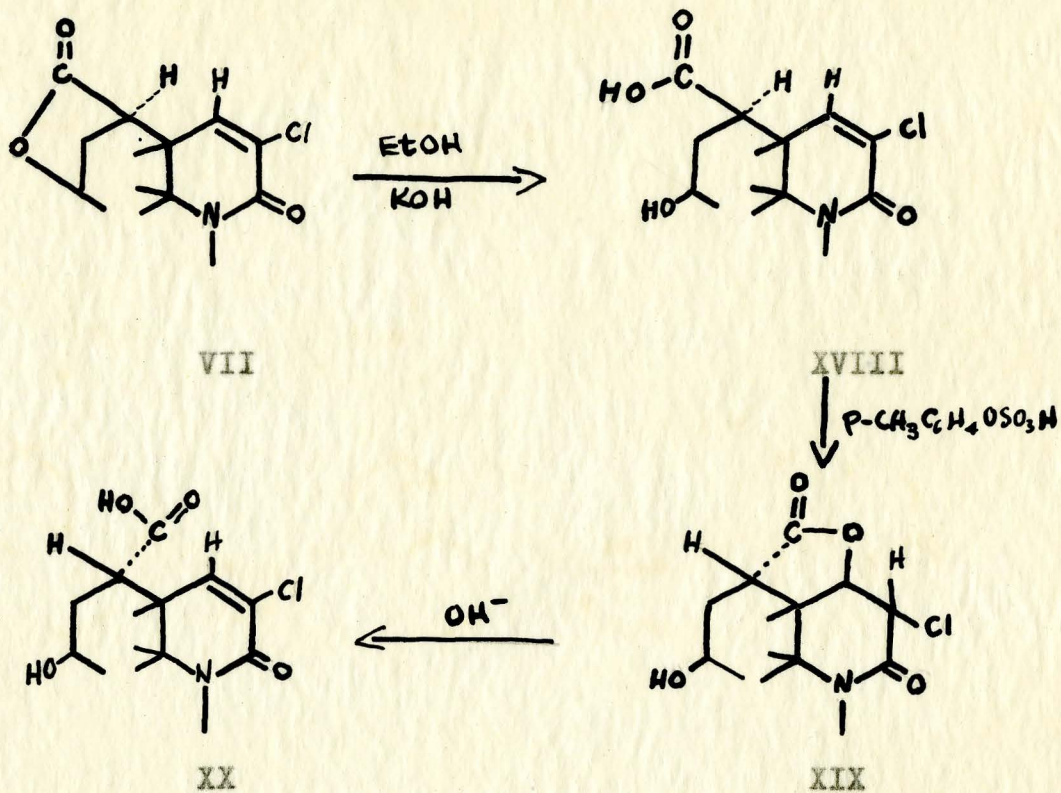
Wiesner et al. (10) subjected VII to permanganate oxidation and isolated the amino acid  $C_{14}H_{19}O_4N$ , XVII. The pKa



values of the amino acid (4.03, 9.3) and of the methyl ester (6.8) were in agreement with the values expected for a  $\beta$ -amino acid derivative. Wiesner also noted that the carbomethoxy group of the amino acid ester was resistant to saponification. This was taken as evidence for the quaternary nature of the carbon atom bearing the carboxyl group.

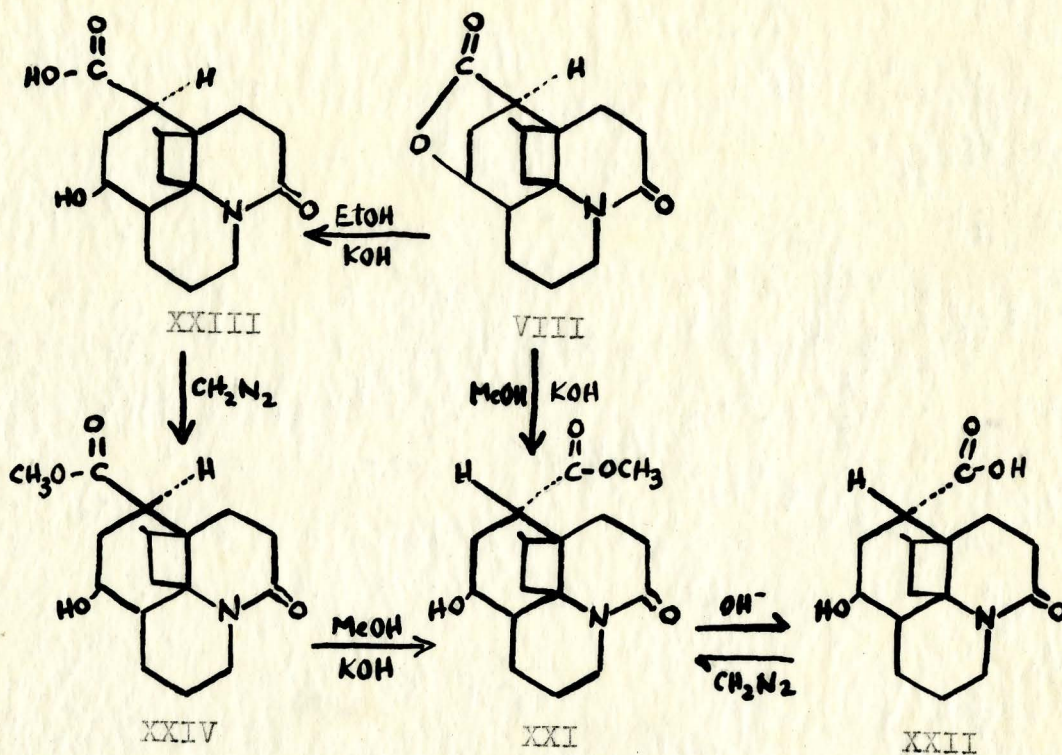
The relationship of the  $\gamma$ -lactone ring to the conjugated lactam group in the anhydro chloro compound VII was demonstrated by Wiesner et al. (10) by the following series of reactions. The lactone ring of VII was opened by mild reflux with alcoholic alkali to give the hydroxy acid  $C_{16}H_{20}O_4NCl$ , XVIII. When XVIII was gently heated under reflux with a trace of p-toluenesulphonic acid in benzene, a new hydroxy lactone  $C_{16}H_{20}O_4NCl$ , XIX was obtained. Treatment of XIX with alkali converted it to a hydroxy acid  $C_{16}H_{20}O_4NCl$ , XX, which differed from XVIII only in the configuration of the lactone carboxyl group. In order to form lactone XIX, the lactone carboxyl group must have epimerized and then added across the double bond. Evidence that a new lactone ring had been formed in XIX was obtained by converting XVIII to its O-acetyl derivative and relactonizing with p-toluenesulphonic acid. The infrared spectrum of this O-acetyl lactone showed absorption peaks at  $1780\text{ cm}^{-1}$  characteristic of a  $\gamma$ -lactone group, at  $1670\text{ cm}^{-1}$  in the lactam region and at  $1740\text{ cm}^{-1}$  attributable to an acetoxy group. This series of reactions may be represented thus:





The fact that the original configuration of the carbonyl group in annotinine is less stable than the epimeric one, and that the lactone carboxyl is attached to a secondary carbon atom was established by Wiesner and co-workers (10) by the sequence of reaction outlined below:





Mild reflux of compound VIII with potassium hydroxide in methanol gave a hydroxy methyl ester XXI. Saponification of this compound with an excess of ethanolic potassium hydroxide gave the corresponding hydroxy acid XXII, which was converted into the ester XXI by the action of ethereal diazomethane. Saponification of the lactone VIII with potassium hydroxide in ethanol gave a hydroxy acid XXIII, which was converted to its methyl ester XXIV with diazomethane. Treatment of the methyl ester XXIV with methanolic potassium hydroxide gave a quantitative yield of XXI. The ester XXI was the more stable of the two since it could not be epimerized back to ester XXIV.

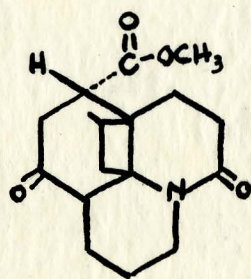
An explanation of the difference in behaviour of VIII with ethanolic and methanolic alkali was given by Wiesner (10), who



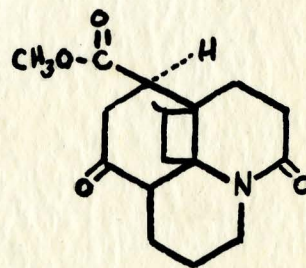
pointed out that in methanolic potassium hydroxide the concentration of methoxide ion is greater than that of hydroxide ion. Therefore methoxide rather than hydroxide ion was the attacking species in this medium and the lactone ring was opened to give the ester directly, which was then immediately isomerized to XXI. In ethanolic potassium hydroxide the concentration of hydroxide ion is greater than that of ethoxide ion, hence the lactone was attacked by hydroxide ion to form the hydroxy acid XIII. This acid was resistant to epimerization, since it was in the form of the carboxylate ion and could not enolize.

#### Ring B

Oxidation of the two hydroxy-esters, XXI and XXIV, yielded information concerning the nature of ring B. Wiesner et al. (10) oxidized XXI and XXIV with chromium trioxide in pyridine and isolated the corresponding keto-esters,  $C_{17}H_{23}O_4N$ , XXV, and  $C_{17}H_{23}O_4N$ , XXVI. Since a ketone was obtained by oxidation, the lactone hydroxyl was demonstrated to be secondary. Examination of the infrared absorption spectrum of the keto-esters indicated that the carbonyl group was in a six-membered or larger ring.



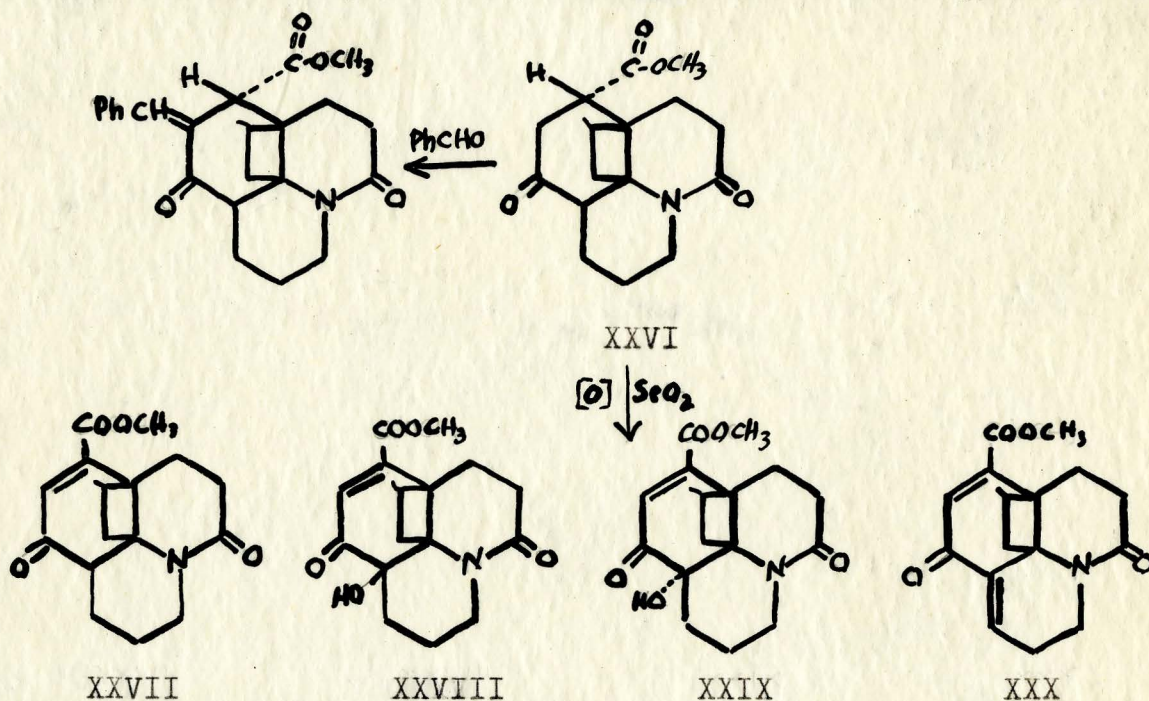
$C_{17}H_{23}O_4N$ , XXVI



$C_{17}H_{23}O_4N$ , XXV



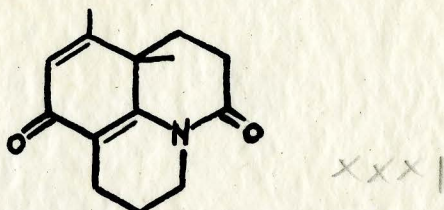
Later, Wiesner and coworkers (2) were able to establish the detailed structure of ring B. The presence of a methylene group adjacent to the carbonyl group of XXVI was demonstrated by the quantitative formation of a benzylidene derivative. The other structural features of ring B were established from a study of the selenium dioxide oxidation products of the keto-ester XXVI (2). These compounds are illustrated in the figure below:



The formation of these oxidation products can be explained in the following manner. Compound XXVII was obtained by oxidation of the  $\alpha$ -hydrogen on the methylene group of XXVI, followed by dehydration. Compounds XXVIII and XXIX arose in the same manner, and in addition the other  $\alpha$ -hydrogen was oxidized to a hydroxyl group. Compounds XXVIII and XXIX are isomeric, differing only in the configuration of the hydroxyl group. This indicated that the carbon atom to which the hydroxyl group was attached was tertiary. Compound XXX was

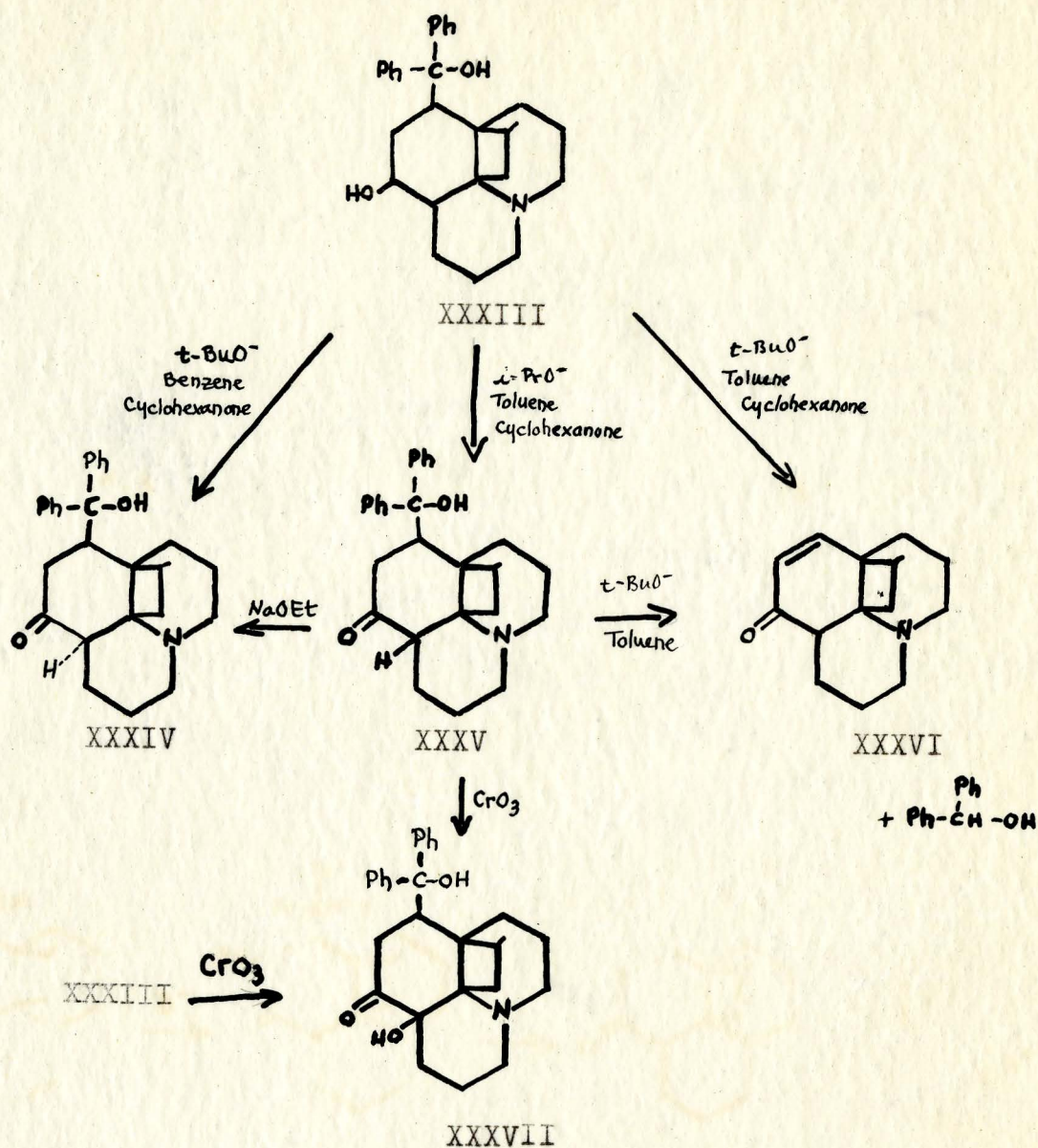


formed by dehydration of XXVIII or XXIX. Wiesner (11) pointed out that, since compound XXX was obtained instead of the more stable chromophore XXXI, it could be inferred that the nitrogen was attached to a quaternary carbon atom.



These conclusions were supported by the work of Betts and MacLean (12). Annotinine was converted to desoxodihydroannotinine, XXXII, by the method of Manske and Marion (4). Compound XXXII reacted with two moles of phenyl lithium to yield diphenyldesoxodihydroannotinine,  $C_{28}H_{35}O_2N$ , XXXIII. The reactions of XXXIII are summarized in the following figure:



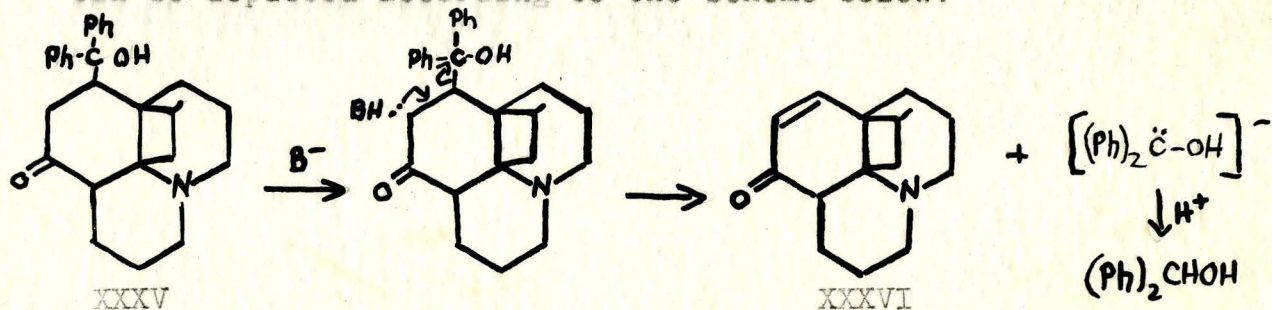


Oxidation of XXXIII by the Oppenauer procedure resulted in one of three different products, depending on the conditions used. When XXXIII was treated with potassium tertiary butoxide in refluxing benzene, in the presence of cyclohexanone, the secondary hydroxyl was oxidized to a carbonyl group and a ketone,  $\text{C}_{28}\text{H}_{33}\text{O}_2\text{N}$ , XXXIV, was obtained.



Oxidation of XXXIII with aluminum isopropoxide in refluxing toluene, in the presence of cyclohexanone, yielded a lower melting epimer of XXXIV,  $C_{28}H_{33}O_2N$ , XXXV. Compound XXXV could be converted to XXXIV by refluxing with sodium ethoxide in ethanol. Treatment of XXXIII with potassium tertiary butoxide in refluxing toluene, in the presence of cyclohexanone, yielded an unsaturated ketone  $C_{15}H_{21}ON$ , XXXVI. The infrared and ultra-violet absorption spectra of XXXVI indicated that the compound was an  $\alpha,\beta$ -unsaturated ketone with a single  $\beta$ -alkyl substituent. Compounds XXXIV and XXXV could be converted to XXXVI by treatment with potassium tertiary butoxide in refluxing toluene.

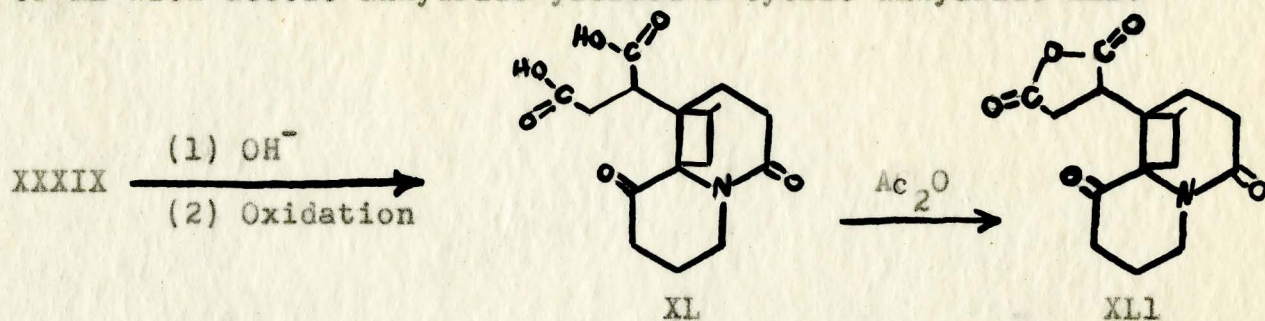
Betts and MacLean (12) showed that the diphenyl carbinol group was eliminated as benzhydrol and proposed that the mechanism was that of a reverse Michael reaction. The reaction can be depicted according to the scheme below:



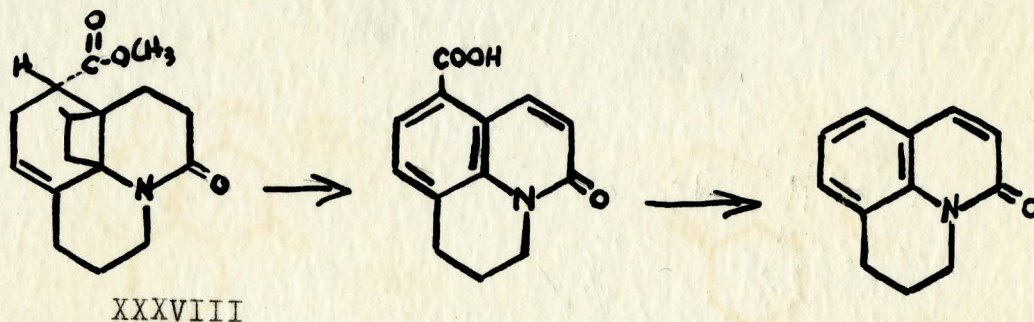
Chromic acid oxidation of the diphenyl hydroxy ketone XXXV yielded a dihydroxy ketone,  $C_{28}H_{33}O_3N$ , XXXVII. This compound was also obtainable directly from XXXIII by chromic acid oxidation. The new hydroxyl was considered to be tertiary, since it was resistant to chromic acid oxidation. From the fact that the new hydroxyl in XXXVII was likely tertiary,



duction product gave a keto-dicarboxylic acid, XL. Treatment of XL with acetic anhydride yielded a cyclic anhydride XL1.



The infrared spectrum of the keto-dicarboxylic acid XL, indicated that the carbonyl group was part of a six-membered or larger ring. The infrared absorption spectrum of the anhydride XL1, showed bands characteristic of a five-membered or strained anhydride. It was concluded therefore, that ring C was six-membered and that the two carboxyl groups were on adjacent carbon atoms. Evidence that ring C contained three methylene groups was obtained by Wiesner *et al.* (10). Oxidation of the selenium dioxide oxidation product XXVIII, by the Lemieux method with permanganate-periodate, yielded a mixture of succinic and formic acids. The succinic acid was thought to originate from ring C and therefore it must have three methylene groups. Degydrogenation experiments (21) carried out on the anhydro ester XXXVIII yielded a quinolone acid which, on decarboxylation, gave a quinolone identified by comparison with an authentic specimen.





It was concluded that annotinine contained the hexahydrojulolidine skeleton and therefore ring C was six-membered.

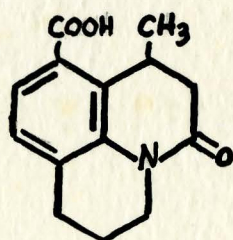
### Dehydrogenation Studies

The extensive dehydrogenation studies which have been carried out on annotinine and its derivatives have supported the evidence already established by stepwise degradation of the molecule.

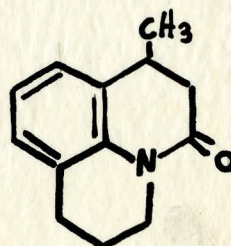
Bankiewicz, Henderson, Stonner, Valenta and Wiesner (13) dehydrogenated annotinine over selenium at 330° C and isolated 8-n-propyl quinoline. The formation of 8-n-propyl quinoline can be explained as arising from ring A and ring B, with the 8-n-propyl group coming from ring C, or from ring B and ring C with the 8-n-propyl group coming from ring A.

Anet and Marion (8) reported that a small yield of 7-methyl quinoline was isolated from the reaction mixture after dehydrogenation of annotinine over palladium at 300° C. The formation of this compound from annotinine is not easily explainable.

Anet and Marion (8) subjected the amino acid XVII to mild dehydrogenation with palladium catalyst. They isolated a lactam acid  $C_{14}H_{15}O_3N$ , XLII, which was decarboxylated to the lactam  $C_{13}H_{15}ON$ , XLIII. Wiesner (10) was able to show by synthesis that the structures of XLII and XLIII were as shown below:



XLII



XLIII

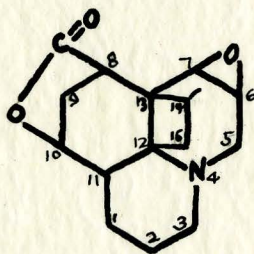


The formation of these two compounds cannot be explained simply on the basis of the known structure of annotinine. A more deep-seated rearrangement is undoubtedly involved.

Dehydrogenation (21) of the anhydroester XXXVIII yielded a quinolone acid which was identified with certainty. This was taken as evidence for the hexahydrojulolidine skeleton for the annotinine molecule.

### Ring D

The chemical degradation of ring D has not been reported but the size of this ring and its location has been inferred from the remaining structure of the molecule. It was known that there were three carbon atoms and six hydrogen atoms to be placed into the structure, and that one carbon atom and three hydrogens were a methyl group. There therefore remained a methyl substituted two-carbon chain to be assigned points of attachment. The locating of this two-carbon chain was inferred from the following evidence:



Carbon 13 appeared to be quaternary, since the ester of the Anet-Marion amino acid XVII was resistant to hydrolysis. The possible points of attachment for the other end of the two-carbon chain were carbons 1, 2, 3, 11 or 12. Since it was shown



(2, 12) that carbon 11 was tertiary, and it seemed likely that ring C contained three methylene groups (10, 13), the second point of attachment was limited to carbon 12. Wiesner (11) concluded that carbon 12 was quaternary on the basis of the product obtained on dehydration of the selenium dioxide oxidation product XXVIII. Therefore, ring D was considered to be four-membered. The C-methyl group was considered to be attached to carbon 14 from a consideration of the dehydrogenation product XLII.

The nature of rings A and B was firmly established, as was the relationship of the functional groups. The chemical evidence for the detailed nature of rings C and D was somewhat less convincing but the proposed structure was borne out by X-ray diffraction studies (3).



## RESULTS AND DISCUSSION

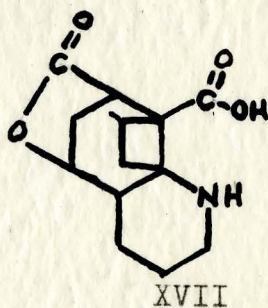
Preparation of the Five-membered Anhydride

This investigation was undertaken at a time when many features of the structure of annotinine were not established. Several structures had been proposed for the molecule but none of them accounted adequately for all the experimental facts. The dehydrogenation of the amino acid XVII to the lactam acid XLII, in relatively good yield, is a case in point. It seemed possible that the arrangement of the functional groups in the amino acid XVII might be different than previously proposed, and that a relationship might exist which would require a less complex rearrangement in going from XVII to XLII. The following study was undertaken in order to establish, by some independent method, the relationship of the lactone carboxyl and the amino acid carboxyl, in the amino acid XVII. It was reasoned that, if the cyclic anhydride between the carboxyl and the lactone carboxyl of the Anet-Marion amino acid XVII could be formed, the size of the anhydride ring would indicate the relative positions of the two groups.

The amino acid  $C_{14}H_{19}O_4N$ , XVII, was prepared by oxidation of "unsaturated lactone A" with barium permanganate. An alternative method of preparation was by permanganate oxidation of anhydroannotinine lactam chlorohydrin VI. The lactone ring of XVII was opened by treatment with aqueous barium hydroxide, and



the hydroxy dicarboxylic acid was isolated as its barium salt. When this salt was heated under reflux with acetic anhydride, a compound  $C_{18}H_{22}O_6N$ , XLIV, was isolated which, after recrystallization from acetone-ether, melted at  $240-241^{\circ}C$ . The infrared spectrum of XLIV showed bands at  $1855\text{ cm}^{-1}$  and  $1756\text{ cm}^{-1}$  attributable to a five-membered or strained cyclic anhydride, at  $1740\text{ cm}^{-1}$  attributable to an acetoxy group and at  $1665\text{ cm}^{-1}$  in the amide region. The bands attributed to the five-membered cyclic anhydride compared closely with those of succinic anhydride and with the values listed in the literature (14). The analytical data and the infrared spectrum indicated that the O, N-diacetylanhydride had formed. The formation of a five-membered anhydride indicated that the two carboxyl groups were on adjacent carbon atoms, and therefore the structure of the amino acid XVII could be written as shown below.



#### Oxidation Studies on the N-Methyl Lactone

The detailed nature of ring C and ring D was unknown at the time this work was in progress. The N-methyl lactone XLVI

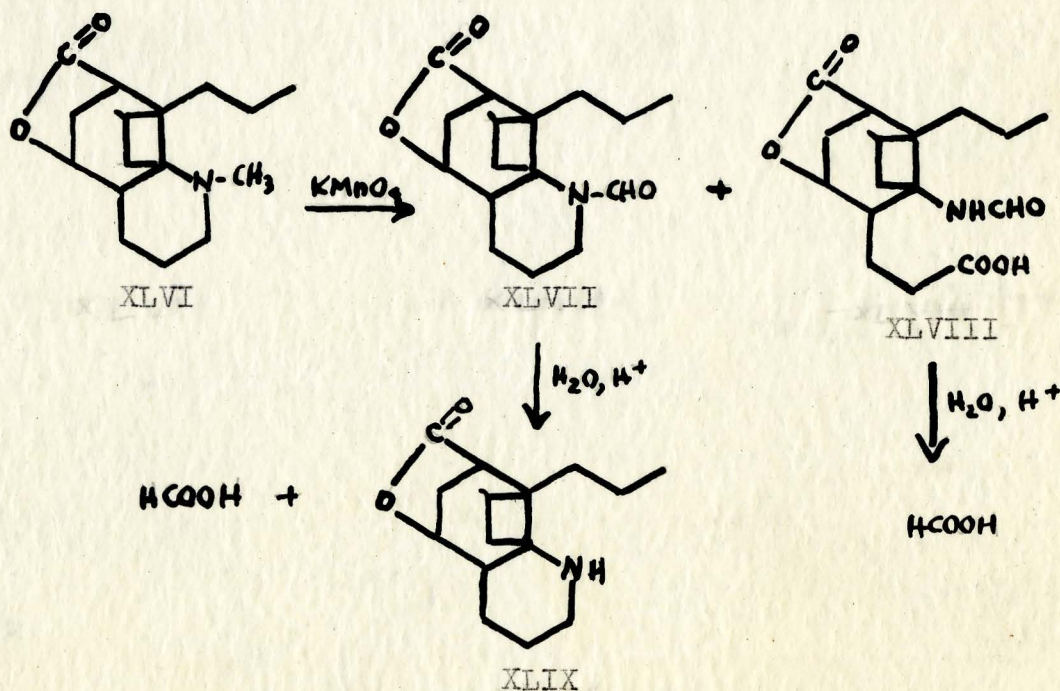


reported by Anet and Marion (8) was chosen as a suitable compound for degradation studies designed to yield information about these two rings. Compound XLVI, obtained by reduction of "Unsaturated lactone A" methosulphate, XLV, with hydrogen over Adams' catalyst, is a tricyclic compound in which, according to the authors (8), ring A has been opened, and the nitrogen atom is contained only in ring C. Oxidation studies on XLVI would yield information about the nature of ring C and Hofmann degradation would yield information about both rings C and D. Oxidation of XLVI with potassium permanganate might be expected to yield a lactam if there were a methylene group adjacent to the nitrogen atom. The position of the lactam absorption in the infrared would indicate the size of ring C. The presence of a carbonyl group in ring C would also offer opportunity for further degradation. The N-methyl lactone XLVI was treated with potassium permanganate to yield a mixture of two products, a neutral compound,  $C_{17}H_{25}O_3N$ , XLVII, and an acid  $C_{17}H_{25}O_5N$ , XLVIII. The neutral compound XLVII showed absorption bands in the infrared at  $1768\text{ cm}^{-1}$  and at  $1660\text{ cm}^{-1}$ , attributable to a  $\gamma$ -lactone group and an amide group respectively. The position of the amide absorption did not permit one to differentiate between the lactam and the N-formyl derivative of XLVI, which was a possible oxidation product. Compound XLVII was therefore treated with dilute sulphuric acid and the distillate was tested for volatile acids. The method described by Woodward and Brehm (15) for the determination of formic acid from N-formyl compounds was followed. The distillate was titrated with 0.1N sodium hydroxide and the volatile acid collected corresponded



to one mole per mole of compound XLVII. The distillate gave positive tests for formic acid. These results indicated that the N-methyl group had been oxidized to yield the N-formyl derivative. The basic compound  $C_{16}H_{25}O_2N$ , XLIX, isolated from the hydrolysate had absorption bands in the infrared at  $3320\text{ cm}^{-1}$  and at  $1760\text{ cm}^{-1}$  attributed to an N-H group and a  $\gamma$ -lactone group respectively. The melting point and the infrared spectrum of XLIX were identical with those reported by Meier, Meister and Marion (7) for "dihydro lactone B", XI(b).

The acidic product from the permanganate oxidation,  $C_{17}H_{25}O_5N$ , XLVIII, was hydrolysed in the same manner as XLVII. The volatile acid collected corresponded to one mole per mole of compound XLVIII, and the distillate gave positive tests for formic acid. Attempts to isolate the amino acid or the lactamized derivative of the amino acid proved unsuccessful. This series of reactions can be formulated in the following manner:



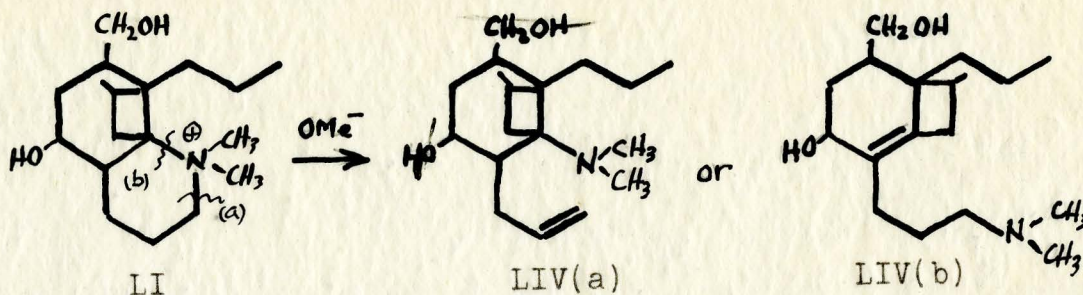


Although the oxidation did not proceed in the expected manner, some information was obtained. The isolation of the N-formyl acid XLVIII indicated that the carbon atom in ring C adjacent to the nitrogen was a methylene group. It was also established that it was ring A which had opened in the reductive decomposition of "Unsaturated lactone A" methosulphate, XLV, because the hydrolysis product XLIX proved to be identical with "dihydro lactone B", XI(b).

Hofmann Degradation Studies on the N-Methyl Dihydroxy Compound

The other approach to the problem of obtaining chemical evidence for the detailed structure of rings C and D was that of Hofmann degradation of the tricyclic compound XLVI. Compound XLVI contains a  $\gamma$ -lactone function, which would undoubtedly be opened during the course of Hofmann degradation. This would complicate the isolation of the reaction products and it was therefore considered wise to work with the reduced compound. Compound XLVI was accordingly reduced with lithium aluminum hydride to yield the dihydroxy compound,  $C_{17}H_{31}O_2N$ , L, which was converted quantitatively to the crystalline methiodide,  $C_{18}H_{34}O_2NI$ , LI. A Hofmann elimination on LI would be expected to open ring C by carbon-nitrogen bond cleavage and introduce a double bond, in either of two ways: (a) to yield a compound with an ~~endo~~exocyclic terminal double bond, or (b) to yield a compound with an endocyclic double bond. Fission (b) would appear to be less likely because of the strained nature of the transition state leading to this olefin and also because of the strained nature of the olefinic product.





Treatment of LI with potassium methoxide in absolute methanol yielded an amorphous base LIV, which could not be induced to crystallize. The methiodide of this compound was prepared with difficulty and analysed for  $C_{19}H_{36}O_2NI$ , LVI. No other crystalline derivatives of LIV could be successfully prepared. The infrared spectrum of LIV showed hydroxyl absorption but no absorption which indicated the presence of the anticipated terminal double bond, or unsaturation of any kind. Compound LIV absorbed one mole of hydrogen when treated with hydrogen at one atmosphere over Adams catalyst. This indicates the presence of a center of unsaturation. Active hydrogen determinations (Zerewitinoff) indicated the presence of a single hydroxyl group. Potentiometric titration of LIV in 50% methanol solution gave a pKa value of 9.6, which is in the range characteristic of an aliphatic trialkyl amine. Ozonolysis of LIV yielded a non-crystalline product, the infrared spectrum of which showed absorption peaks in the hydroxyl region and at  $1780\text{ cm}^{-1}$  in the carbonyl region. The latter absorption peak would be expected if compound LIV had structure LIV (b), and would be the absorption characteristic of a four-membered cyclic ketone. However, an absorption band at  $1700\text{ cm}^{-1}$  due to a non-cyclic ketone, would also be expected; such was not the case. To exclude the



possibility that the  $1780\text{ cm}^{-1}$  band was due to a  $\gamma$ -lactone function, the ozonolysis product was treated with alcoholic sodium hydroxide. The  $1780\text{ cm}^{-1}$  band was still present in the spectrum of the compound after this treatment, thus excluding the possibility of a  $\gamma$ -lactone function in the molecule. The ozonolysis results did not make it possible to say with confidence which was the correct structure for LIV.

If the structure LIV (b) were the correct one, oxidation of the secondary hydroxyl group of LIV by the Oppenauer procedure should yield a compound with a conjugated carbonyl group, which would be easily identified by infrared and ultraviolet spectroscopy. In the course of attempts to oxidize the secondary hydroxyl group of LIV with aluminum isopropoxide and cyclohexanone in refluxing toluene, an unusual result was obtained. An oily base, LV, was isolated, which showed a strong absorption band at  $1780\text{ cm}^{-1}$  in addition to the hydroxyl band at  $3400\text{ cm}^{-1}$ . The possibility of a  $\delta$ -lactone absorption was eliminated because of the strongly basic nature of the reaction mixture. The  $1780\text{ cm}^{-1}$  band might be attributed to a four-membered cyclic ketone. Compound LV was obtained in the absence of the proton acceptor cyclohexanone, that is, simply by treatment of LIV with aluminum isopropoxide in refluxing toluene. Similarly LV could be obtained directly from the methiodide LI, by treatment with potassium tertiary butoxide in tertiary butanol. Compound LV was converted to its methiodide with some difficulty and analysed for  $\text{C}_{19}\text{H}_{36}\text{O}_2\text{NI}$ . The conversion of LIV to LV appears to be an isomerization reaction which takes place in the presence of a base

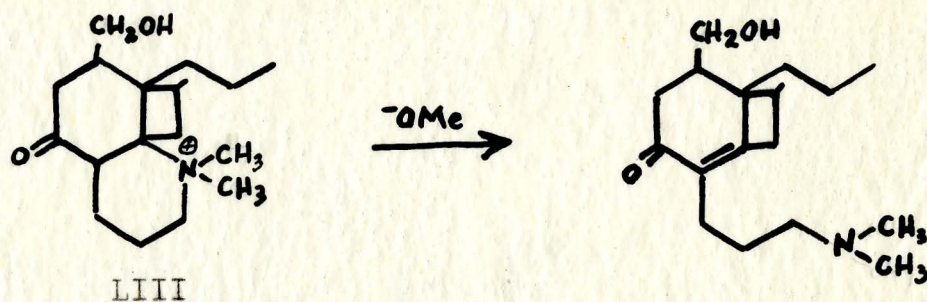


stronger than potassium methoxide. Compound LV absorbed one mole of hydrogen when treated with hydrogen at one atmosphere over Adams' catalyst. Reduction of LV with lithium aluminium hydride yielded an amorphous base which no longer showed the  $1780\text{ cm}^{-1}$  absorption band in the infrared. This is indicative of the initial presence of a carbonyl group in LV. Potentiometric titration of LV in 50% methanol gave results which indicated that LV was not a pure compound but a mixture.

The isomerization of compound LIV to compound LV is not easily explainable on the basis of the possible structures of LIV. It was thought worthwhile to carry out the Hofmann elimination and the Oppenauer oxidation in the inverse order, in order to determine whether or not the same type of isomerization would occur. The N-methyl dihydroxy compound L was oxidized by the Oppenauer procedure, with aluminum isopropoxide in refluxing benzene, in the presence of acetone. A compound LII, which showed infrared absorption bands in the hydroxyl region at  $3410\text{ cm}^{-1}$  and at  $1710\text{ cm}^{-1}$  characteristic of a six-membered ketone, was obtained. The free base could not be induced to crystallize but a crystalline methiodide,  $\text{C}_{17}\text{H}_{32}\text{O}_2\text{NI}$ , LIII, was obtained. The secondary hydroxyl of L had been oxidized to a carbonyl group and the primary hydroxyl was unaffected under the conditions of the oxidation. Treatment of the methiodide LIII, with potassium methoxide in absolute methanol, yielded an amorphous base which showed absorption bands in the infrared at  $3450\text{ cm}^{-1}$ ,  $1660\text{ cm}^{-1}$ ,  $1635\text{ cm}^{-1}$  and



weaker bands at  $3040\text{ cm}^{-1}$  and at  $1580\text{ cm}^{-1}$ , characteristic of a hydroxyl group, an  $\alpha$ - $\beta$ -unsaturated ketone and a center of unsaturation, respectively. This is qualitative evidence that the Hofmann elimination reaction introduced a double bond in conjugation with the carbonyl groups. It appeared that ring C had been opened and the double bond was in ring B, as illustrated below:



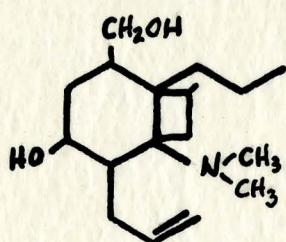
This indicates that the olefin LIV could have the double bond in the same relative position.

To ensure that carbon-nitrogen bond fission had taken place in the first Hofmann reaction, in which LIV had formed, a second Hofmann elimination was carried out on the methiodide of LIV. The formation of trimethylamine in this reaction would indicate that C-N bond fission had taken place. Treatment of LVI, the methiodide of the presumed hydroxy-olefin LIV, with potassium tertiary butoxide, yielded trimethylamine, isolated as its picrate, and a neutral oil LVII. If LIV, the product of the first Hofmann elimination gave a compound with an endocyclic double bond, a second Hofmann elimination on the methiodide of LIV should yield a neutral compound with a terminal exocyclic double bond. Ozonolysis of the neutral

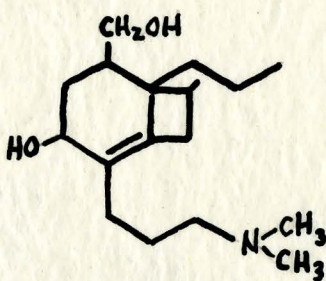


compound LVII would therefore be expected to yield formaldehyde. Compound LVII was ozonized and the reaction mixture was examined for the presence of volatile aldehydes. Acetaldehyde, identified as its dimedone derivative, was obtained as one of the products. The formation of acetaldehyde on ozonolysis necessitates the presence of the grouping  $\text{CH}_3\text{-CH}=\overset{\text{!}}{\text{C}}$  in compound LVII. This grouping could only arise by isomerization of the double bond resulting from elimination of nitrogen from the methiodide of LIV. The ultraviolet spectrum of an impure sample of LVII, the neutral product of this reaction, suggested it contained a conjugated system of double bonds. The activation provided by the increased stability of the conjugated system was apparently sufficient to cause this isomerization.

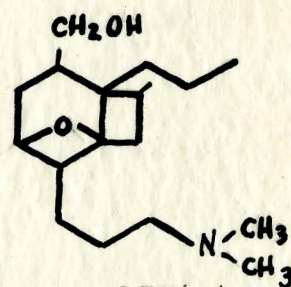
In the light of all the information available about compounds LIV and LV, several structures could be written for LIV.



LIV(a)



LIV(b)



LIV(c)

The possibility LIV(a) was excluded since no formaldehyde was isolated on ozonolysis of LIV. Structure LIV(b) is supported by the fact that the ozonolysis product showed absorption at  $1780\text{ cm}^{-1}$  in the infrared, but seems unlikely because of the strained nature of the transition state leading



to this olefin and the strained nature of the olefinic product itself. The ozonolysis of LVII, which yielded acetaldehyde as one of the products, adds support for structure LIV(b).

Structure LIV(c) was suggested by the fact that LIV analysed for a single active hydrogen, and by an analogous reaction of certain 1,3-oxides which yield an olefin and a ketone on treatment with a strong base like aluminum isopropoxide (22). The transition state for its formation would also be strained and the fact that LIV absorbed one mole of hydrogen readily at one atmosphere pressure, makes this structure seem unlikely. The ozonolysis of LIV to yield a product with a carbonyl absorption is also difficult to explain on the basis of structure LIV(c).

The structures of compounds LIV and LV have not been established in this investigation, although certain features of these reactions have been elucidated. It seems fairly certain that the first Hofmann involves a type (a) fission (cf p.27) and not a type (b) fission as one might predict. In any future study of this reaction it is suggested that more elaborate techniques be employed for the purification of the intermediates. In this investigation, chromatography on an alumina column and distillation were used but were apparently not sufficiently refined to achieve any separation. Paper chromatography could be used to determine whether or not these products were composed of more than one component and counter current extraction might be profitably employed for their purification.



## EXPERIMENTAL

### Apparatus, Methods and Materials

Infrared absorption spectra were determined using a Perkin-Elmer model 21 double-beam recording infrared spectrophotometer. Samples were mounted in nujol except where otherwise stated. Sodium chloride optics were used throughout.

Quantitative micro-hydrogenations were carried out according to the procedure described by Ogg and Cooper (17). The apparatus was supplied by A.H. Thomas Company. Active hydrogen determinations were carried out in the apparatus described by Niederl and Niederl (18). The apparatus was supplied by A.H. Thomas Company.

Basicity constants (pKa values) were determined by potentiometric titration of the bases; the solvent used in each case is stated in parenthesis. Values are those at the half-neutralization point.

The plant material was collected in the Wentworth valley of Nova Scotia and was extracted according to the procedure of Manske and Marion (1). Annotinine was obtained from the crude extract by crystallization from methanol and after recrystallization from methanol melted at 232°C.

Microanalyses were carried out in the Microanalytical Laboratories of Drs. G. Weiler and F.B. Strauss, Oxford, England. All melting points are corrected.



Unsaturated Lactone A,  $C_{16}H_{21}O_2N$ , IX

Unsaturated lactone A, IX, was prepared from annotinine chlorohydrin according to the procedure described by Manske and Marion (4). The chromous chloride was prepared from chromic chloride hexahydrate by reduction with zinc amalgam and concentrated hydrochloric acid.

Oxidation of Unsaturated Lactone A,  $C_{16}H_{21}O_2N$ , IX, with Barium Permanganate

Unsaturated lactone A was treated with barium permanganate in acetone according to the procedure described by Betts and MacLean (9). The amino acid  $C_{14}H_{19}O_4N$ , XIV, was isolated as its sulphate from the mixture of oxidation products and after recrystallization from methanol-ether, melted at  $232^{\circ}C$  with decomposition. This acid was also obtained, but in somewhat poorer yield, by oxidation of anhydroannotinine lactam chlorohydrin, VI, according to the procedure described by Wiesner et al. (5).

Hydrolysis of the Lactone Ring of the Acid  $C_{14}H_{19}O_4N$ , XIV

An aqueous solution of 0.43 g of barium hydroxide octahydrate was added to 150 mg of the amino acid sulphate. The mixture was heated under reflux for 17 hours. Excess barium ion was precipitated as barium carbonate by the addition of dry ice to the aqueous solution. The solution was filtered and evaporated to dryness under reduced pressure. The residue (310 mg), was a pale yellow water soluble solid. The addition of a drop of dilute sulphuric acid to an aqueous solution of the product yielded barium sulphate. The product was therefore the



the barium salt of the hydroxy dicarboxylic acid corresponding to XIV. The infrared absorption spectrum showed bands at  $1680\text{ cm}^{-1}$  attributed to ionized carboxyl and at  $3400\text{ cm}^{-1}$  in the hydroxyl region. The  $\gamma$ -lactone absorption band at  $1780\text{ cm}^{-1}$  was no longer present.

#### Treatment of the Barium Salt with Acetic Anhydride

A mixture of 0.4 g. of the barium salt of the hydroxy dicarboxylic acid, 0.1 g. of ammonium chloride and 5 mls. of acetic anhydride was heated under reflux for 2 hours. At the end of the reflux period, the excess acetic anhydride was removed under reduced pressure and the residue was triturated with chloroform. The chloroform solution was filtered and dried over anhydrous sodium sulphate. Evaporation of the solvent yielded a brown oil (0.2 g) which crystallized from acetone-ether. After recrystallization from acetone-ether, it melted at  $240\text{-}241^{\circ}\text{C}$ , XLIV.

Calculated for  $\text{C}_{18}\text{H}_{22}\text{O}_6\text{N}$ : C, 61.90; H, 6.61; N, 4.01 %

Found: C, 61.20, 61.31; H, 6.44, 6.28; N, 3.83, 3.95 %

The infrared spectrum showed absorption bands at  $1855\text{ cm}^{-1}$  and  $1756\text{ cm}^{-1}$ ,  $1740\text{ cm}^{-1}$  and  $1665\text{ cm}^{-1}$  characteristic of a five-membered (or strained) anhydride ring ( $1855$  and  $1756$ ), an acetoxyl group and an amide group respectively.

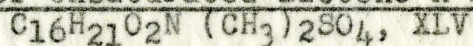
#### Unsaturated Lactone A Methosulphate, XLV

The procedure employed was essentially that of Anet and Marion (8). Freshly distilled methyl sulphate (2.3 g.) was added to a solution of 2.33 g. of unsaturated lactone A, X,



dissolved in 58 mls. of anhydrous benzene. The reaction mixture was allowed to stand at room temperature for 48 hours at which time the benzene solution was decanted from the crystalline methosulphate. The crystals were washed with several small portions of dry benzene. The crystalline methosulphate, XLIII, was not further purified but was reduced directly as described below. Further crops of the methosulphate were obtained from the benzene solution after longer periods of time.

Reduction of Unsaturated Lactone A Methosulphate



The crystalline methosulphate (1.2 g.) was dissolved in water and treated with hydrogen over Adams' catalyst at 50 p.s.i.g. for 10 hours. The resulting solution was acidified with dilute hydrochloric acid to dissolve the basic material which had precipitated, and filtered to remove the catalyst. The filtered solution was made alkaline with ammonia and exhaustively extracted with ether. The combined ether extract was dried over anhydrous sodium sulphate and concentrated on the steam cone. Colourless needles of the N-methyl compound (0.85 g.)  $C_{17}H_{27}O_2N$ , XLVI, separated after standing in the refrigerator. The compound melted at 91-92°C. The infrared spectrum showed an absorption band at  $1780\text{ cm}^{-1}$  attributable to a  $\gamma$ -lactone group.

Potassium Permanganate Oxidation of  $C_{17}H_{27}O_2N$ , XLVI

A solution of 347 mg of the N-methyl lactone XLVI, in 20 mls. of aqueous acetone, was treated with aqueous permanganate, added dropwise with frequent swirling, over a 2 hour



period, until the purple colour persisted. A little methanol was added to destroy the excess permanganate. Sulphur dioxide was bubbled through the solution to reduce the precipitated manganese dioxide. The aqueous solution was extracted four times with ether and the combined extract was washed with dilute aqueous ammonia. This aqueous ammonia solution was worked up separately as described below. The ether layer was dried over anhydrous sodium sulphate. Evaporation of the ether yielded a residue (87 mg.) which crystallized from acetone and melted at 180-181°C.

Calculated for  $C_{17}H_{25}O_3N$ : C, 70.03; H, 8.70; N, 4.81 %

Found: C, 69.26, 69.15; H, 8.62, 8.87; N, 4.04, 4.78 %

The infrared spectrum of this neutral compound, XLVII, showed bands at  $1768\text{ cm}^{-1}$  and  $1660\text{ cm}^{-1}$  attributed to a  $\gamma$ -lactone and an amide group respectively.

The combined ammonium hydroxide extract, noted above, was acidified with hydrochloric acid and extracted four times with ether. The ether extract was dried over sodium sulphate, and evaporated to dryness to yield 80 mg. of a residue, XLVIII, which was crystallized from acetone and melted at 211°C with decomposition.

Calculated for  $C_{17}H_{25}O_5N$ : C, 63.13; H, 7.79; N, 4.33 %

Found: C, 62.53, 62.67; H, 7.35, 7.45; N, 4.63, 4.74 %

The infrared spectrum of XLVIII, the acidic material, showed absorption bands at  $3350\text{ cm}^{-1}$  in the hydroxyl region,  $1760\text{ cm}^{-1}$  in the  $\gamma$ -lactone region,  $1724\text{ cm}^{-1}$  in the carboxyl region and at  $1640\text{ cm}^{-1}$  in the amide region.



Treatment of  $C_{17}H_{25}O_3N$ , XLVII with Dilute Sulphuric Acid

The procedure followed was that described by Woodward and Brehm (15) for the determination of formic acid from N-formyl derivatives. Compound XLVII, (59.5 mg.) was heated under reflux for two hours with 10 ml. of 2N sulphuric acid. The solution was then distilled, adding water throughout the distillation to maintain a constant volume. The distillate was collected in fractions and titrated with 0.1 N sodium hydroxide. The amount of volatile acid collected corresponded to 1.02 mole per mole of compound XLVII. The distillate gave a positive test for formic acid with mercuric chloride as described by Feigl (16). The distillate also reduced permanganate and bromine. The aqueous residue after distillation of the formic acid was made basic with ammonium hydroxide and extracted several times with ether. The combined extract was dried over anhydrous sodium sulphate and evaporated to dryness to yield 40 mg. of residue XLIX, which, after recrystallization from petroleum ether, melted at 133-134°C.

Calculated for  $C_{16}H_{25}O_2N$ : C, 72.96; H, 9.57%

Found: C, 72.65, 72.90; H, 9.47, 9.52%

The infrared spectrum of XLIX showed absorption bands at 3320  $cm^{-1}$  in the secondary amino region and at 1760  $cm^{-1}$  in the  $\gamma$ -lactone region. The melting point and infrared spectrum of XLIX were similar to those of dihydro lactone B, XI(b), described by Meier, Meister and Marion (7).

Treatment of Acid XLVIII with Dilute Sulphuric Acid

The acid XLVIII (72.1 mg.) was hydrolysed under the con-



ditions described above for compound XLVII. The amount of volatile acid collected corresponded to 1.05 mole per mole of XLVIII. The distillate gave positive tests for formic acid. An attempt was made to isolate a crystalline product from the hydrolysate but it proved to be unsuccessful.

Reduction of N-Methyl Lactone  $C_{17}H_{27}O_2N$ , XLVI, with Lithium Aluminum Hydride

A solution of the N-methyl lactone, XLVI, (1.60 g.) in 100 mls. of anhydrous ether was added slowly with stirring to a refluxing solution of lithium aluminum hydride (2.4 g.) in 200 mls. of anhydrous ether. The mixture was heated under reflux with stirring for 2 hours. The excess lithium aluminum hydride was decomposed by the careful addition of wet ether followed by water. The ether was decanted and the precipitated hydroxides were further washed with two 25 ml. portions of ether. The combined ether solution was dried over anhydrous sodium sulphate. Evaporation of the solvent yielded a colourless oil which crystallized on the addition of acetone. Recrystallization from acetone yielded white crystals, L, (1.37 g.) which melted at 148-149°C.

Calculated for  $C_{17}H_{31}O_2N$ ; C, 72.56; H, 11.11; N, 5.00 %

Found: C, 72.31, 72.49; H, 11.07, 10.91; N, 5.42, 5.22 %

The infrared spectrum of compound L showed bands in the hydroxyl region at  $3400\text{ cm}^{-1}$  and  $3100\text{ cm}^{-1}$ . The lactone absorption at  $1780\text{ cm}^{-1}$  was no longer present.

Preparation of the methiodide, LI, of compound L, is described on p. 46



Treatment of Methiodide  $C_{18}H_{34}O_2NI$ , LI, with Potassium Methoxide

A solution of 1.0 g. of potassium metal in 25 mls. of absolute methanol was added to a solution of 1.10 g. of methiodide, LI, in 25 mls. of absolute methanol. The mixture was heated under reflux for 3 hours. The excess methanol was removed by distillation under reduced pressure and the residue was taken up in water. The aqueous phase was extracted four times with ether and the combined extract was dried over anhydrous sodium sulphate. Evaporation of the ether yielded 0.90 g. of a nearly colourless oil, XLVIII. This oil resisted all attempts at crystallization. Attempts to prepare crystalline salts were also unsuccessful. The infrared absorption spectrum showed a hydroxyl band at  $3400\text{ cm}^{-1}$  but no double bond absorption.

Methiodide of LIV

Methyl iodide (3 mls) was added to a solution of 179 mg. of LIV in acetone. The mixture was heated under reflux for 15 minutes. Excess reagent and solvent were removed by distillation under reduced pressure. The residue was dissolved in absolute ethanol and after standing in the refrigerator for several days, a solid which melted at  $167-168^{\circ}\text{C}$  was obtained, LVI.

Calculated for  $C_{19}H_{36}O_2NI$ : C, 52.18; H, 8.24; N, 3.20 %

Found: C, 51.86, 52.48; H, 8.03, 7.78; N, 3.34, 3.08 %

Attempted Oppenauer Oxidation of LIV

A solution of 265 mg. of LIV in dry toluene was treated with 1.0 g. of aluminum isopropoxide and 1.5 mls. of cyclohexanone. The reaction mixture was heated under reflux for one



hour, cooled and poured on ice. The toluene layer was separated and the aqueous layer was further extracted three times with ether. The toluene and ether extracts were combined and washed four times with 10% hydrochloric acid. The acid washings were made alkaline with ammonium hydroxide and extracted with ether. The ether extract was dried over anhydrous sodium sulphate and taken to dryness. The residue (250 mg.) LV, was a nearly colourless oil which could not be induced to crystallize. The infrared absorption bands at  $3400\text{ cm}^{-1}$  in the hydroxyl region and at  $1780\text{ cm}^{-1}$  attributable to a four-membered cyclic ketone. A weak absorption at  $1640\text{ cm}^{-1}$  can be attributed to a double bond. Attempts to prepare a crystalline salt of the base also proved unsuccessful.

#### Potentiometric Titration of Compound LIV

Compound LIV (25 mg.) was dissolved in 10 ml. of 1:1 methanol/water and was titrated with 0.1 N hydrochloric acid. A stream of nitrogen bubbles was passed through the solution to provide agitation. pH readings were recorded after the addition of successive 0.02 ml. volumes of acid. The pKa (pH at half-neutralization) was 9.6. This value is characteristic of a tertiary base. The molecular weight of LIV as calculated from the titration data is 292. The calculated molecular weight for  $\text{C}_{18}\text{H}_{33}\text{O}_2\text{N}$  is 295.

#### Quantitative Micro-hydrogenations

The procedure used for the quantitative determination of unsaturation was that described by Ogg and Cooper (17).



The reductions were carried out in freshly distilled glacial acetic acid using a platinum oxide (Adams') catalyst. The weight of sample used was such that approximately 4 ml. of hydrogen was absorbed. The technique was checked by hydrogenation of a compound known to contain one double bond, namely maleic acid. It was found that maleic acid absorbed 1 mole of hydrogen per mole of acid in 45 minutes.

#### Microhydrogenation of Compound LIV

Compound LIV was hydrogenated in glacial acetic acid over Adams' catalyst, at one atmosphere hydrogen pressure. One mole of hydrogen per mole of compound was absorbed in 50 minutes.

#### Microhydrogenation of Compound LV

Compound LV was hydrogenated under the same conditions as described above and absorbed one mole of hydrogen per mole of compound LV in 20 minutes.

#### Active Hydrogen Determinations

Active hydrogen analyses were carried out on a micro scale using a modification of the Zerewitinoff method, as described by Niederl and Niederl (18). The Grignard reagent, methyl magnesium iodide, was prepared in isoamyl ether according to the procedure described by Smith and Shriner (19). Anisole was used as the solvent and was purified by the procedure outlined below. The commercial ether was distilled and a mid-fraction (151-153°C) was collected. This material was heated



under reflux with metallic sodium for 5 hours and then distilled through a 30 cm. Vigreux column, collecting the fraction which boiled at 152-153°C. This fraction was stored in a glass stoppered flask over phosphorus pentoxide. A sample of a 0.5 ml. of anisole dried in this manner gave a blank volume of methane of about 0.04 ml. at room temperature and 0.14 ml. after heating to 100°C and cooling to room temperature.

Known runs were carried out using p-phenylphenol as the reactive compound. A solution of p-phenylphenol (9.043 mg.) dissolved in 0.5 ml. anisole, produced 1.32 ml. of methane (corrected for blank volume) when treated with 0.5 ml. (excess) methyl magnesium iodide solution at room temperature. There was no increase in the volume of methane when the reaction was carried out at 100°C. The theoretical volume of methane from 9.043 mg. of p-phenylphenol at 24°C and 757 mm, is 1.31 ml. The average error on three runs was  $\pm 1\%$ .

#### Active Hydrogen Analysis on Compound LIV

Compound LIV was treated with methyl magnesium iodide in anisole as described above. The methane liberated at room temperature corresponded to about 1.05 active hydrogens. When the reaction was carried out at the temperature of boiling water the compound analysed for about 1.1 active hydrogens. Error  $\pm 5\%$ .

#### Treatment of LIV with Aluminum Isopropoxide in Boiling Toluene

A solution of 800 mg. of aluminum isopropoxide in anhydrous toluene was added to a solution of XLVIII (610 mg) in an-



hydrous toluene. The mixture was heated under reflux for two hours. The reaction mixture was poured on ice and the organic layer was separated. The aqueous layer was further extracted three times with ether. The combined toluene-ether extract was dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure to yield 595 mg. of a nearly colourless oil. This oil could not be induced to crystallize. The infrared absorption spectrum of the base showed bands at  $3400\text{ cm}^{-1}$  and at  $1780\text{ cm}^{-1}$ , with a weak absorption at  $1640\text{ cm}^{-1}$ . The spectrum proved to be identical with that of the product previously isolated from the attempted Oppenauer oxidation of LIV, i.e. compound LV.

#### Treatment of LI with Potassium Tertiary Butoxide in Tertiary Butanol

A solution of 143 mg. of the methiodide LI in 15 ml. of dry tertiary butanol was treated with 200 mg. of potassium tertiary butoxide at reflux temperature, for two hours. The excess alcohol was removed by distillation under reduced pressure and the residue was taken up in water. The aqueous solution was extracted several times with ether and the combined extract was dried over anhydrous sodium sulphate. Evaporation of the solvent yielded a residue (100 mg.) which had an infrared absorption spectra identical with that of the product obtained in the preceding experiment, i.e. compound LV.

#### Methiodide of Compound LV

Methyl iodide (2 ml.) was added to a solution of compound LV (200 mg.) in 3 ml. acetone. The mixture was heated under reflux for 5 minutes. On cooling a gelatinous white precipitate



separated. The precipitate was dissolved in 2 ml. absolute methanol and the solution was concentrated on the steam cone. The solution was cooled and the white precipitate (200 mg.) was removed by filtration. This poorly crystalline material melted over a range of 179-182°C.

Calculated for  $C_{19}H_{36}O_2NI$ : C, 52.17; H, 8.22; N, 3.2; I, 29.06%

Found: C, 52.19, 52.40; H, 7.85, 7.87; N, 3.19, 3.15; I, 29.1%

#### Reduction of LV with Lithium Aluminum Hydride

A solution of compound LV (233 mg.), dissolved in dry ether, was added dropwise over a 30 minute period to a vigorously stirred suspension of 120 mg. of lithium aluminum hydride in 50 ml. of anhydrous ether. The mixture was heated under reflux, with stirring, for two hours. The excess hydride was decomposed by the careful addition of water. The ether layer was decanted and the precipitated hydroxides were washed with ether. The combined ether extract was dried over anhydrous sodium sulphate. Evaporation of the solvent yielded a residue (210 mg.) which failed to crystallize. Attempts to prepare a crystalline salt also proved unsuccessful. The infrared absorption spectrum of this oil LVII showed absorption bands at  $3400\text{ cm}^{-1}$  in the hydroxyl region but the  $1780\text{ cm}^{-1}$  attributable to the four-membered cyclic ketone was no longer present.

#### Methiodide of N-Methyl Dihydroxy Compound $C_{17}H_{31}O_2N$ , L.

Methyl iodide (5 mls) was added to a solution of 1.24 g. of compound L in acetone. After standing at room temperature for 3 hours, a white crystalline precipitate separated, LI, (1.50 g.) which, after recrystallization from absolute ethanol,



melted at 181.0-181.5°C.

Calculated for  $C_{18}H_{34}O_2NI$ : C, 51.07; H, 8.28; N, 3.31%

Found: C, 51.56, 51.36; H, 8.23, 8.14; N, 3.03, 3.03%

Oppenauer Oxidation of  $C_{17}H_{31}O_2N$ , I

A solution of compound XLV, (0.60 g), 3 mls. of acetone and aluminum isopropoxide (0.90 g) in anhydrous benzene was heated under reflux for 7 hours, cooled and poured on ice. The benzene layer was separated and the aqueous layer was further extracted three times with ether. The combined benzene-ether extract was dried over anhydrous sodium sulphate. Evaporation of the solvent yielded an oil, LII, (0.54 g) which could not be induced to crystallize. The infrared spectrum had peaks at  $3400\text{ cm}^{-1}$  in the hydroxyl region and at  $1700\text{ cm}^{-1}$  in the carbonyl region.

Methiodide of Keto-Alcohol, LII

Methyl iodide (5 mls) was added to a solution of the keto-alcohol, XLIX, (0.84 g) dissolved in 5 mls. of acetone. The mixture was heated under reflux for 10 minutes, cooled and allowed to stand at room temperature for 3 hours. The excess methyl iodide and acetone were removed by distillation under reduced pressure to yield 0.71 g. of a residue, LIII, which, on recrystallization from absolute ethanol, melted at 172-173°C.

Calculated for  $C_{18}H_{32}O_2NI$ : C, 51.30; H, 7.60; N, 3.32%

Found: C, 50.93, 51.35; H, 7.22, 7.35; N, 3.32, 2.97%

Treatment of Methiodide, LIII, with Sodium Methoxide

A solution of 0.61 g. of potassium metal in 20 ml. of



absolute methanol was added to a methanolic solution of the methiodide, L. The solution was heated under reflux for two hours and the methanol removed under reduced pressure. The residue was taken up in water and extracted four times with ether. The combined ether extract was dried over anhydrous sodium sulphate. Evaporation of the solvent yielded an oily residue, LIV. The base could not be induced to crystallize. The infrared spectrum showed peaks at  $3450\text{ cm}^{-1}$  in the hydroxyl region,  $1660\text{ cm}^{-1}$  corresponding to a conjugated carbonyl group and at  $1635\text{ cm}^{-1}$  corresponding to a double bond. Weaker bands at  $3040\text{ cm}^{-1}$ , and  $1580\text{ cm}^{-1}$  could also be interpreted as double bond absorption.

#### Ozonolysis of Compound LIV in Anhydrous Methanol

Compound LIV (500 mg) was dissolved in 10 ml. anhydrous methanol. The solution was cooled in an acetone-dry ice bath and a stream of ozone enriched oxygen (5% ozone) was bubbled through it for 30 minutes. The ozonide was decomposed by hydrogenation over Adams' catalyst at 11 p.s.i.g. for 2 hours. The catalyst was removed by filtration and the methanol was removed by distillation under reduced pressure to yield 490 mg. of a colourless oil. The residue was dissolved in ether and extracted with 10% hydrochloric acid. The acid extract was basified with ammonium hydroxide and extracted several times with ether. The ether extracts were dried over anhydrous sodium sulphate and evaporated to dryness. A crystalline product could not be obtained. The infrared spectrum of the residue showed absorption peaks at  $3400\text{ cm}^{-1}$  in the hydroxyl region and at  $1780\text{ cm}^{-1}$  attributable to a cyclic four-membered ketone.



Ozonolysis of Compound LIV in Glacial Acetic Acid

Compound XLVIII (150 mg) was ozonized in glacial acetic acid at 20°C for 30 minutes as described above. Zinc dust (200 mg) was added to the solution of the ozonide and the mixture was steam distilled. The steam distillate (40 ml) was divided into two portions. To one portion was added 5 ml. of 2, 4-dinitrophenylhydrazine solution. (1g. 2, 4-DNPH., 10 ml. concentrated sulphuric acid, 25 ml. ethanol, 5 ml. water). The second portion was neutralized with sodium hydroxide and made slightly acidic with dilute acetic acid. Dimedone solution (15 g. dimedone in 25 ml. 1:3 water/ethanol) (5 ml) was added. The two solutions were allowed to stand overnight. No precipitate was obtained in either case, therefore no formaldehyde or other volatile aldehydes had been produced.

Treatment of Methiodide LVI with Potassium Tertiary Butoxide

A solution of 183 mg. of methiodide LVI in 5 ml. of dry tertiary butanol was treated with 400 mg. of potassium tertiary butoxide. The solution was heated under reflux for 4 hours under a stream of nitrogen. The nitrogen stream was bubbled into a dilute solution of hydrochloric acid to trap any volatile bases. At the end of the reflux period the alcohol was removed by distillation under reduced pressure and the residue was taken up in water. The aqueous solution was extracted several times with ether. The combined ether extract was washed with 10% hydrochloric acid and then dried over anhydrous sodium sulphate. Evaporation of the ether yielded 48 mg. of an oil, LVIII, which could not be



induced to crystallize. The infrared spectrum of this neutral compound showed absorption bands at  $3400\text{ cm}^{-1}$  in the hydroxyl region and a very weak band at  $1640$  in the double bond regions. The ultraviolet spectrum of LVIII had an absorption maximum at  $242\text{ m}$ , which is characteristic of two double bonds in conjugation. The extinction coefficient ( $3,500$ ) is much lower than that expected for such a system. This, however, could be due to the impure nature of the sample. The acid solution containing the volatile bases liberated in the reaction was evaporated to dryness under reduced pressure and the residue was treated with a concentrated sodium hydroxide solution. The gas evolved was swept over in a stream of nitrogen and was trapped in an ethereal solution of picric acid. The yellow precipitate formed was collected by suction filtration and melted at  $225\text{-}226^{\circ}\text{C}$ . The melting point showed no depression on admixture with authentic trimethylamine picrate.

#### Ozonolysis of Hofmann Product LVIII

Compound LVIII, (102 mg) was dissolved in 5 ml. glacial acetic acid. A stream of ozone enriched oxygen (5% ozone) was bubbled through the solution at room temperature for 25 minutes. The acetic acid solution was treated with zinc dust and steam distilled. The steam distillate was neutralized with sodium hydroxide and was made slightly acidic with acetic acid.



Dimedone solution (5 mls) was added. The crystalline dimedone derivative obtained was filtered with suction and after recrystallization from aqueous ethanol melted at 138-139°C. The melting point was not depressed on admixture with authentic acetaldehyde dimedone.



## SUMMARY

The amino acid  $C_{14}H_{19}O_4N$ , XVII, has been converted to the O,N-diacetyl cyclic anhydride  $C_{18}H_{22}O_6N$ , XLIV, and the anhydride ring has been shown to be five-membered, thus proving that the lactone carboxyl and the originally present amino acid carboxyl were on adjacent carbon atoms.

Oxidation studies on the N-methyl lactone  $C_{17}H_{27}O_2N$ , XLVI, have been carried out and two products were isolated. The neutral compound  $C_{17}H_{25}O_3N$ , XLVII, was shown to be the N-formyl derivative of "dihydro lactone B" XI(b). The acidic product  $C_{17}H_{25}O_5N$ , XLVIII, was shown to be the compound in which ring C had been opened, the N-methyl group oxidized to an N-formyl group and the carbon atom adjacent to the nitrogen atom oxidized to a carboxyl group. These studies established that it was ring A which was opened in the preparation of the N-methyl lactone, and that the carbon atom adjacent to the nitrogen atom in ring C was a methylene group.

A study of the Hofmann elimination reaction on the N-methyl dihydroxy compound,  $C_{17}H_{31}O_2N$ , L, was carried out. The exact nature of the olefinic product was not established but the properties of the compound were extensively studied. An isomerization reaction of the olefin LIV, to a keto-olefin LV, was encountered. This reaction is considered to be worthy of future investigation.



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