THE ALKALOIDS OF LYCOPODIUM ANNOTINUM L.

THE ALKALOIDS OF LYCOPODIUM ANNOTINUM L. A STUDY OF DIPHENYLANNOTININE AND TWO OF THE MINOR ALKALOIDS

by

George Stanley Perry, B. Sc.

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AUTHOR: George Stanley Perry, B. Sc. (London University)

SUPERVISOR: Professor D. B. MacLean

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SCOPE AND CONTENTS:

Part (i) - The Minor Alkaloids

Annotoxine, $C_{32}H_{44}O_5N_2$, has now been isolated from a Canadian source. It has been separated into the alkaloids acrifoline, $C_{16}H_{23}O_2N$, and annotine (alkaloid L 11), $C_{16}H_{21}O_3N$. Both alkaloids have been shown to possess one hydroxyl group, one carbonyl group and one centre of unsaturation. The lack of reactivity of the third oxygen function of annotine suggests that it is present in an ether linkage. The identity of O-acetylacrifoline with alkaloid L 12 has also been proved.

Part (ii) - Diphenylannotinine

Diphenylannotinine has been shown to be $C_{28}H_{33}O_3N$. It possesses two hydroxyl groups, the third oxygen is present in an ether linkage. The exact nature of the ether linkage could not be ascertained on the basis of the experimental evidence.

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The author wishes to thank Dr. R. H. F. Manske for samples of Acrifoline and Alkaloids L 11 and L 12; also Professor K. Wiesner, of the University of New Brunswick, for a generous supply of Lycopodium annotinum mother liquors, without which the investigations reported in Part (i) of this Thesis would not have been possible.

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GENERAL INTRODUCTION

Amongst the members of the plant kingdom which have been studied with regard to their alkaloid content, the members of the Lycopodiacae have received little attention. The presence of alkaloids in the Lycopodiacae was first reported in 1881 by Boedeker (1), but it was not until 1943 that the results of the first thorough investigation were published (2). To date, about thirty different alkaloids have been isolated, although a few of these have been shown to be derivatives of each other (3). Most of the alkaloids contain sixteen carbon atoms and an examination of their molecular formulae reveals that they probably possess a tetracyclic ring structure.

Lycopodium annotinum L. has been the most thoroughly investigated species; it is found in most parts of the northern hemisphere and occurs in Canada, the northern United States and Europe. The lack of functional groups in most of the Lycopodium alkaloids renders their degradation to simpler compounds peculiarly difficult. Annotinine, the major alkaloid of L. annotinum, is well suited for study because of the presence of a lactone ring and a reactive ether linkage.

At the time the investigations reported in Part (ii) were commenced no plausible structure for annotinine had been suggested. Much work had

already been carried out in the region of the nitrogen atom and oxide ring and it was considered that an attack through the lactone ring might lead to further evidence for the structure. Annotinine reacts with phenyl lithium to form a diphenyl derivative but there are conflicting reports in the literature concerning the molecular formula of diphenylannotinine and its mode of formation. A reinvestigation of this reaction was carried out in order to clarify this situation and a number of attempts were made to degrade diphenylannotinine and its oxidation products. It was during the course of these studies that Wiesner (4) presented substantial evidence for the presently accepted structure of annotinine. With this announcement the investigations on diphenylannotinine were discontinued.

Together with annotinine there have been isolated a number of minor alkaloids; amongst these are Acrifoline, Annotine (Alkaloid L 11), and Alkaloid L 12. A number of derivatives were prepared in order to determine the exact nature of the functional groups present in these alkaloids. Ample use of the Infrared Spectrophotometer was made in interpreting structures. Efforts were also made to correlate some of the derivatives of these alkaloids with other alkaloids occurring in L. annotinum. This work is presented in Part (i) of this thesis.

HISTORICAL INTRODUCTION

Isolation of the Alkaloids

The presence of alkaloids in the Lycopodiacae was first reported by Boedeker in 1881 (1) and confirmed in 1934 by Orechov (5) and by Muszynski (6). Neither investigator carried out a detailed study and it was not until 1943 that a thorough chemical analysis of the alkaloids obtained from Lycopodium annotinum L. was carried out by Manske and Marion (2). From an extract they isolated the nine alkaloids.

Annotinine	$C_{16}H_{21}O_3N$
Obscurine	$C_{18}H_{28}ON_2$
Lycopodine	$C_{16}H_{25}ON$
Alkaloid L 8	$C_{16}H_{25}O_2N$
Alkaloid L 9a	$C_{20}H_{31}O_4N$
Alkaloid L 9b	$C_{16}H_{23}ON$
Alkaloid L 10	$C_{16}H_{27}O_2N$
Alkaloid L 11	$C_{16}H_{21}O_3N$
Alkaloid L 12	$C_{18}H_{25}O_3N$

A subsequent examination of obscurine by Moore and Marion (7) showed it to be a mixture of two alkaloids, α -obscurine, $C_{17}H_{26}ON_2$, and β -obscurine, $C_{17}H_{24}ON_2$.

During later work on L. annotinum var. acrifolium, Fern., Manske and Marion (8) isolated:-

Annotinine	$C_{16}H_{21}O_3N$
Alkaloid L 27	$C_{16}H_{23}O_2N$
Alkaloid L 28	$C_{17}H_{27}O_2N$
Alkaloid L 29	$C_{16}H_{25}O_2N$
Alkaloid L 30	$C_{16}H_{25}O_2N$
Alkaloid L 31	$C_{20}H_{29}O_4N$

while the alkaloids obscurine, L 8, L 9, L 11, and L 12 were missing. Because of the great difference in alkaloid content of the two plants, they suggested that the latter should be raised to the rank of a new species, Lycopodium acrifolium (Fern.) N. comb. Alkaloid L 27 was given the common name Acrifoline. It was later shown by Douglas, Lewis and Marion (3) that alkaloid L 8 was identical to alkaloid L 30.

In 1952, Bertho and Stoll (9) published the results of their investigations of three samples of Lycopodium annotinum of German origin. Besides annotinine and lycopodine they isolated from one extract:

Annotine	$C_{16}H_{23}O_4N$
Acrifoline	$C_{16}H_{23}O_2N$
Annotoxine	$C_{31}H_{42}O_5N_2$
A base	$C_{16}H_{25}ON$
A base	$C_{10}H_{19}ON$ or $C_{10}H_{21}ON$

From the other two samples they were able to isolate annotinine and annotine but no annotoxine.

The most recent investigation on the alkaloid content of L. annotinum is described by the Polish chemists Achmatowicz and Rodewald (10). From L. annotinum of Polish origin nine alkaloids were reported:

Annotinine	$C_{16}H_{21}O_3N$
Acrifoline	$C_{16}H_{23}O_2N$
Alkaloid L 11	$C_{16}H_{21}O_3N$
Lycopodine	$C_{16}H_{25}ON$
α-Obscurine	$C_{17}H_{26}ON_2$
β-Obscurine	$C_{17}H_{24}ON_2$
Iso-lycopodine	$C_{16}H_{25}ON$
Annotoxine	$C_{32}H_{44}O_5N_2$
Alkaloid L 8	$C_{16}H_{25}O_2N$

These workers proved that annotoxine was an equimolecular compound of acrifoline and alkaloid L 11. They were able to fractionally crystallize the methiodide and hydriodide into two components which proved identical to the methiodides and hydriodides of acrifoline and alkaloid L 11 respectively. As a further proof they were able to prepare annotoxine by mixing equimolecular amounts of acrifoline and alkaloid L 11. The formula, $C_{31}H_{42}O_5N_2$, proposed by Bertho and Stoll (9) for annotoxine was, therefore, incorrect. The Polish workers also suggested that annotine is not a new alkaloid at all but is alkaloid L 11. They compared the ultraviolet spectra, melting points and optical rotations, which were identical, c.f. annotine, m.p. 176°C, $[\alpha]_{D1}^{21}$ = -114°, alkaloid L 11, m.p. 176°C, $[\alpha]_D^{24}$ = -110.3°. In a recent communication (11) Prof. Bertho states that his group discovered in 1953 that annotoxine was a molecular compound of acrifoline and L 11 and that annotine was actually identical to Alkaloid L 11 (mixed m.p. with a sample from Dr. Manske). He therefore assumed that the sample which they originally described as a new alkaloid was simply a hydrate of L 11.

The absence of annotoxine in L. annotinum of Canadian origin, and in two of the extracts of German origin is probably due to the method of isolation. Achmatowicz and Rodewald suggest that any separation procedure based on the crystallization of the salts must lead to fractionation of acrifoline and alkaloid L 11. They have also suggested that the alkaloid fractions L 9a and L 9b of Manske and Marion (2) are probably impure acrifoline.

The Minor Alkaloids

Acrifoline, Alkaloid L 11, and Alkaloid L 12

The chemistry of acrifoline and alkaloid L 11 has not been studied in any detail but Bertho and Stoll (9) report the following results for acrifoline. The alkaloid is a tertiary base, contains no $N\text{-}CH_3$, $O\text{-}CH_3$ or OH groups but apparently contains a carbonyl group on the basis of the ultraviolet spectrum and its reaction with phenyl lithium. Lack of reactivity toward peracetic acid and selenium dioxide led them to propose a fully saturated ring system. The coupling reaction of acrifoline with diazotized aniline (Pauly reaction) suggested that perhaps a benzenoid ring might be present. Acrifoline did not undergo Hofmann degradation but there was evidence that phenylacrifoline did react. Likewise they state that acrifoline could not be hydrogenated, but give no experimental details. They also state that 2,4-dinitrophenylhydrazine gives no reaction nor does treatment of the base with benzaldehyde and sodium ethylate.

In the case of their 'annotine' the alkaloid contained no $N\text{-}CH_3$, $O\text{-}CH_3$ or OH groups but reacted with phenyl lithium to give a product, the

composition of which at the time seemed somewhat anomalous. On the basis of the revised formula, $C_{16}H_{21}O_3N$, the analysis of phenylannotine corresponds fairly well with $C_{22}H_{27}O_3N$. The alkaloid was also treated with lithium aluminium hydride to yield a base, $C_{16}H_{23}O_3N$, m.p. 162 - 163°C. They also report, with lack of experimental detail, that 'annotine' fails to react with benzaldehyde and sodium methylate, with hydrogen, or with selenium dioxide, or to undergo a Hofmann degradation of its methiodide. It also gave inconclusive results on treatment with cyanogen bromide, boiling hydrochloric acid and hydrazine.

No information on the nature of the functional groups of alkaloid L 12, $C_{18}H_{25}O_3N$, was available in the literature.

Lycopodine

Lycopodine, $C_{16}H_{25}ON$, although only a minor alkaloid in L. annotinum, is the most widely distributed of the Lycopodium alkaloids. It has been shown to be a tertiary base, and to contain no $N\text{-}CH_3$, $O\text{-}CH_3$ or OH groups. The presence of a ketone carbonyl group was demonstrated by the formation of phenyl-lycopodine (12), a hydrazone (12), and a monoxime (3). The presence of the quinoline nucleus has been suggested on the basis of selenium dehydrogenation experiments (13).

Annotinine

The Oxide Ring

Annotinine was selected for structural studies because it is the most abundant of the alkaloids of Lycopodium annotinum. It is also well suited for investigation because it possesses two reactive functional

groups through which to attack the molecule. The molecular formula is $C_{16}H_{21}O_3N$, it is a tertiary base, contains no N-CH_3, OH or O-CH_3 groups. Two of the oxygen atoms are contained in a lactone ring whilst the third was assumed to be present as an oxide ring.

The first experiments were carried out by Manske and Marion (8). They found that annotinine was soluble in hot aqueous alkali which suggested the presence of a lactone ring. The infrared spectrum confirms that it is a γ-lactone. Treatment of annotinine with alcoholic potassium hydroxide converted it into a compound $C_{16}H_{23}O_4N$, II, still containing a γ-lactone ring, called annotinine hydrate because it was formed by the addition of a mole of water. Work by Wiesner and co-workers (14) showed that treatment of annotinine with dilute sulfuric acid gave an isomeric compound, $C_{16}H_{23}O_4N$.

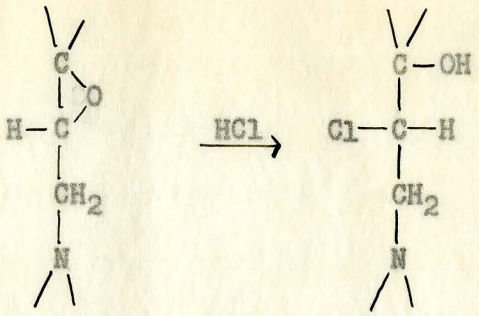
Oxidation of annotinine with aqueous potassium permanganate led to a new compound, $C_{16}H_{19}O_4N$, IV, which still retained the lactone ring. Manske and Marion (8) incorrectly described it as a diketone because Clemmensen reduction of it yielded the saturated base dihydrolactone 'A', $C_{16}H_{23}O_2N$, V. A Clemmensen reduction of annotinine led to an unexpected result in that only hydrochloric acid was added with the formation of annotinine chlorohydrin, $C_{16}H_{22}O_3NCl$, VI. Treatment of VI with alcoholic alkali gave annotinine hydrate II, and chromous chloride reduction gave the unsaturated base, unsaturated lactone A, $C_{16}H_{21}O_2N$, VII, which is easily catalytically reduced to the saturated base V.

It remained for MacLean and Prime (15) to demonstrate that the oxidation product of annotinine, $C_{16}H_{19}O_4N$, IV, was a neutral lactam, which retained the oxide ring intact. They were able to convert annotinine

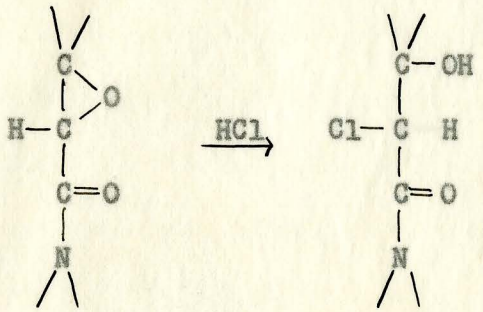
lactam into the corresponding chlorohydrin, $C_{16}H_{20}O_4NCl$, VIII, by refluxing it with hydrochloric acid. The lactam chlorohydrin, VIII, could be reconverted into IV by heating with sodium bicarbonate in acetone. Prolonged heating of IV with hydrochloric acid or under reflux conditions with phosphorous oxychloride led to the unsaturated compound, anhydro lactam chlorohydrin, $C_{16}H_{18}O_3NCl$, IX, in which a molecule of water has been eliminated.

Hydrogenation of VIII over Adams' catalyst yielded two products, $C_{16}H_{21}O_4N$, X, in which the halogen had been replaced by hydrogen, and the desoxy lactam, $C_{16}H_{21}O_3N$, XI, in which both the hydroxyl and chlorine groups had been replaced. Catalytic reduction of IX led only to XI. The corresponding annotinine chlorohydrin, VI, could not be reconverted into annotinine, dehydrated or catalytically reduced.

The difference in the reactivities of the two chlorohydrins was ascribed by MacLean and Prime to be due to the close proximity of the oxide ring to the lactam carbonyl. In the case of the lactam chlorohydrin the oxide ring was considered to be beta to the nitrogen atom. On the basis of the formation of X, and the regeneration of the lactam from the lactam chlorohydrin they proposed that the oxide ring was three-membered. The addition of HCl to annotinine and annotinine lactam would proceed by trans addition and with inversion at the carbon bearing the chloro group, according to the scheme:-

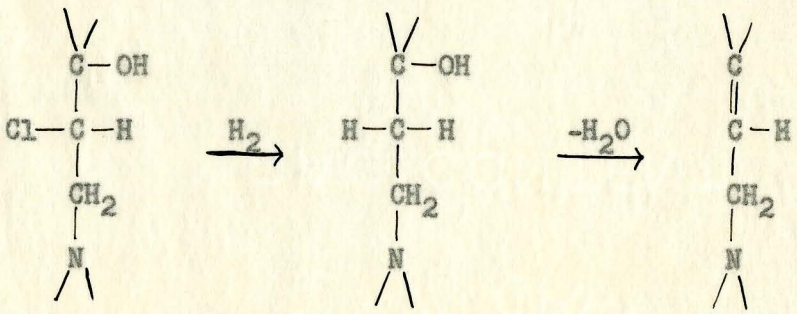


$C_{16}H_{21}O_3N$, I $C_{16}H_{22}O_3NCl$, VI



$C_{16}H_{19}O_4N$, IV $C_{16}H_{20}O_4NCl$, VIII

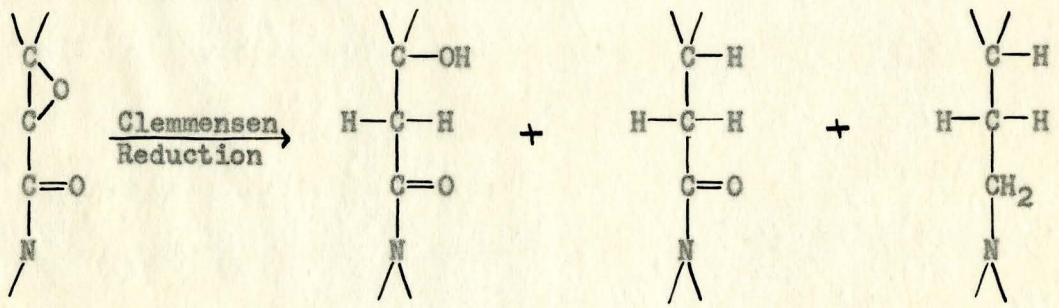
The trans dehydration of annotinine chlorohydrin should, according to theory, proceed with difficulty, but the corresponding dehydration of annotinine lactam chlorohydrin would be facilitated by epimerization of the carbon atom bearing the chloro group. Replacement of the chloro group by hydrogen should also permit trans dehydration.



$C_{16}H_{22}O_3NCl$, VI $C_{16}H_{21}O_2NCl$, VII

This was found to be the case. Manske and Marion (8) obtained the unsaturated base VII by treatment of annotinine chlorohydrin, VI, with chromous chloride. The reaction apparently proceeded through replacement of the chloro group by hydrogen, followed by trans dehydration.

When MacLean and Prime treated annotinine lactam, IV, under the conditions of the Clemmensen reduction they obtained not only the saturated base V but also a good yield of compound X, with a smaller amount of XI. Further treatment of X and XI yielded only small amounts of the saturated base V. Similar treatment of annotinine lactam chlorohydrin, VIII, and anhydro lactam chlorohydrin, IX yielded X and XI respectively, and both gave some unsaturated base VII. Both the reduction of the amide carbonyl and the replacement of the hydroxyl and chloro groups is unusual but it finds a parallel in the reduction of α-chloro acids and β-hydroxy amides. The reduction of the double bond in anhydro lactam chlorohydrin, VIII, under the conditions of the Clemmensen reduction finds an analogy in the saturation of the double bond in α,β-unsaturated acids. This reduction sequence was given as further proof for an epoxide ring and its attachment beta to the nitrogen atom. The Clemmensen reduction is represented thus:-



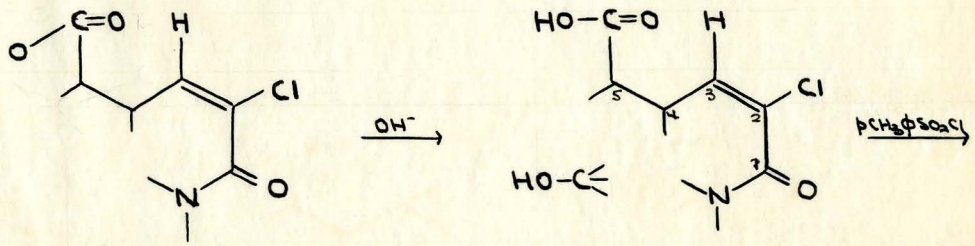
$C_{16}H_{19}O_4N$, IV $C_{16}H_{21}O_4N$, X $C_{16}H_{21}O_3N$, XI $C_{16}H_{23}O_2N$, V

Meier, Meister and Marion (16) have repeated the chromous chloride reduction of annotinine chlorohydrin, VI, and found that the reduction was more complicated than at first supposed. Besides unsaturated lactone A, VII, they obtained two unsaturated secondary bases. The formation of the secondary bases is put forward as further evidence for the β,γ position of the oxide ring. The cyclic allylamine structure for unsaturated lactone A, VII I,received further support from the comparison of the pKa values of the unsaturated base and its dihydro derivative (c.f. ref. 17). The pKa of unsaturated lactone A was 7.06, whereas its dihydro derivative was 8.48.

The Lactone Ring

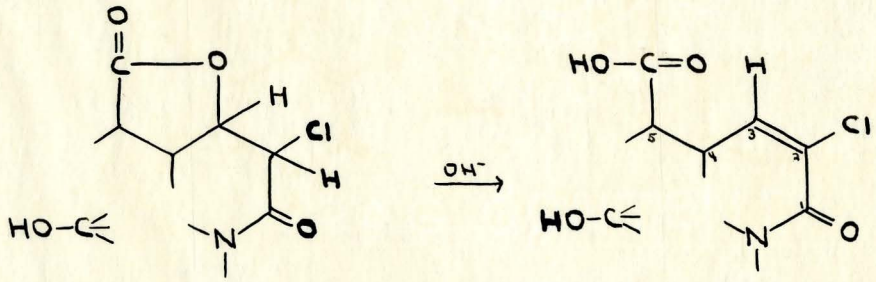
The relationship of the lactone ring to the epoxide ring has been extensively studied by Wiesner and co-workers (14, 18). As stated earlier Manske and Marion (8) had prepared annotinine hydrate, II, $C_{16}H_{23}O_4N$, by treatment of annotinine with alcoholic potassium hydroxide, followed by relactonization with dilute sulfuric acid. Wiesner et al. were able to isolate an isomeric form, annotinine hydrate, III, by treatment of annotinine with dilute sulfuric acid alone. Both hydrates possess a γ - lactone ring. The hydrate II, it is suggested by Wiesner, possesses a different lactone ring. In the formation of annotinine hydrate II the lactone and oxide rings are opened simultaneously and on subsequent treatment with dilute sulfuric acid relactonization to a new γ-lactone occurs. But in the formation of annotinine hydrate III only the oxide ring is opened so that III contains the lactone ring originally present in annotinine. Neither of the hydrates undergoes periodate oxidation although III is undoubtedly a 1,2-diol. The formation of annotinine hydrate II requires that the carbonyl group must be in close proximity to the oxide ring.

Hydrolysis of anhydrolactam chlorohydrin, IX, with alcoholic alkali does not liberate chlorine, but instead the corresponding hydroxy acid, $C_{16}H_{20}O_4NCl$, XII, is formed. Refluxing this acid in benzene with a catalytic amount of paratoluenesulfonic acid gave a new γ-lactone, $C_{16}H_{20}O_4NCl$, XIII, isomeric with XII. A possible interpretation of this reaction is that the carbonyl group interacts with the double bond. A re-opening of the lactone ring with alkali led to a hydroxy acid, XIV, isomeric with XII, and also possessing a double bond. This new hydroxy acid, XIV, is considered to be a stereoisomer of XII, probably due to epimerization about C_5. The series of reactions may be represented thus:-



$C_{16}H_{18}O_3NCl$, IX

$C_{16}H_{20}O_4NCl$, XII

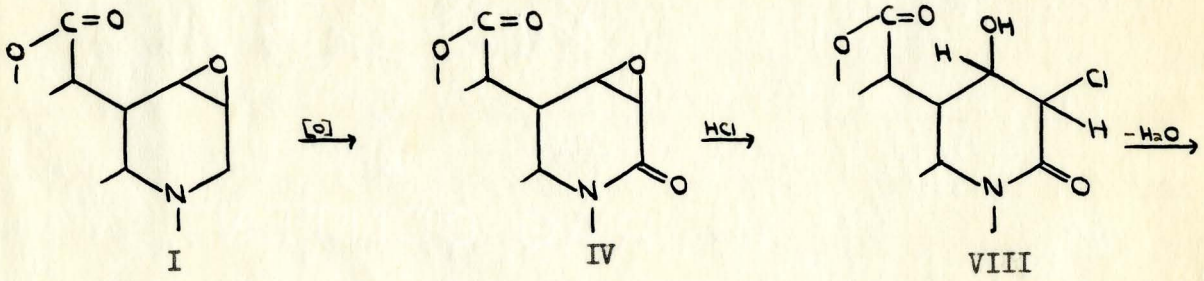


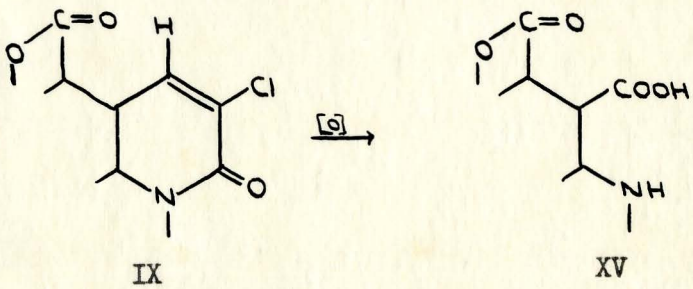
$C_{16}H_{20}O_4NCl$, XIII

$C_{16}H_{20}O_4NCl$, XIV

The formation of XIII and its hydrolysis to the isomeric hydroxy acid, XIV, can only be accounted for if the relative position of the carbonyl group and the double bond are as shown in the partial structures above.

In his paper, Wiesner (18) reported the oxidation of anhydrolactam chlorohydrin, IX, to an amino acid, $C_{14}H_{19}O_4N$, XV. The same amino acid obtained by the oxidation of unsaturated lactone A was reported by Anet and Marion (19). The loss of two carbon atoms and the formation of a carboxyl group are proof that the nitrogen atom and the oxide ring are contained in a cyclic system, and that the hydroxyl group of annotinine lactam chlorohydrin, VII, is secondary. Thus a partial structure for annotinine, and the reaction sequence to the amino acid is:-





That the lactam ring is six-membered is supported by the infrared absorption spectra of various derivatives containing the lactam group (e.g.

annotinine lactam 1645 cm.$^{-1}$).

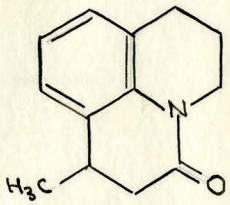
In 1955 Wiesner (4) presented proof that the hydroxyl group of the lactone ring was secondary. Annotinine desoxy lactam, $C_{16}H_{21}O_3N$, XI, which contains the original lactone ring was converted into the corresponding methyl ester, $C_{17}H_{25}O_4N$, XVI. Oxidation of XVI with chromium trioxide-pyridine complex (20) gave the keto-ester, $C_{17}H_{23}O_4N$, XVII. Therefore the hydroxyl group of XVI must be secondary.

Dehydrogenation Studies

It remained for a study of the dehydrogenation of annotinine and the amino acid, XV, to give the final clue to the structure of annotinine. When Anet and Marion (19) carried out a drastic dehydrogenation of annotinine at 300°C over palladium they obtained small amounts of 7-methyl quinoline. Dehydrogenation of the amino acid, $C_{14}H_{19}O_4N$, XV, in the presence of palladium on barium sulfate gave an acid $C_{14}H_{15}O_3N$, XVIII. This acid no longer contained a γ-lactone ring but contained in its infrared spectrum bands attributable to a carboxyl group, an amide and a carbonyl group respectively. They were able to decarboxylate this acid to a neutral compound, $C_{13}H_{15}ON$, XIX. A comparison of the ultraviolet spectra of XVIII and XIX indicated that the carboxyl group was directly attached to the chromophoric system. Lithium aluminium hydride reduction of XIX gave the weak base $C_{13}H_{17}N$, XX.

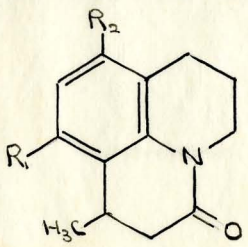
Wiesner and co-workers (21) were able to obtain 8-n-propyl quinoline, as well as another base, and a number of neutral nitrogen containing substances by the selenium dehydrogenation of annotinine. The dehydrogenation of the amino acid, XV, over palladium-charcoal catalyst (Henderson (22)) yielded not only the acid XVIII but also the decarboxylation product, XIX. Further dehydrogenation of XIX gave a neutral lactam, $C_{13}H_{13}ON$, XXI, identi-

fied with an authentic sample as 2-oxo-4-methyl-juloline (23). The ultraviolet spectrum of XVIII indicated that the carboxyl group was in a position meta to the amino group, and because the ultraviolet spectrum of XXI indicated a benzene ring then XIX is probably



$C_{13}H_{15}ON$

This was proved by the synthesis of its racemic form and by the comparison of the ultraviolet and infrared spectra. Then the acid XVIII, is

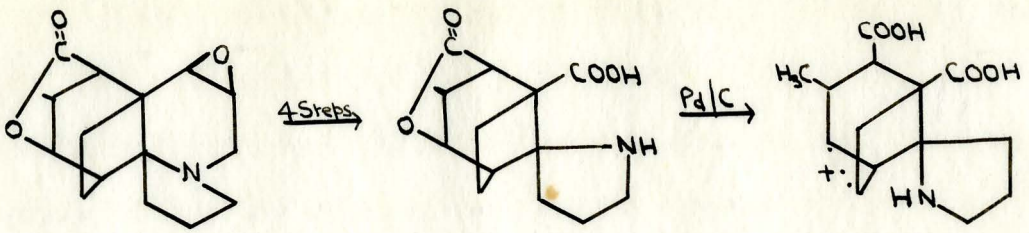


$R_1 = COOH, R_2 = H$

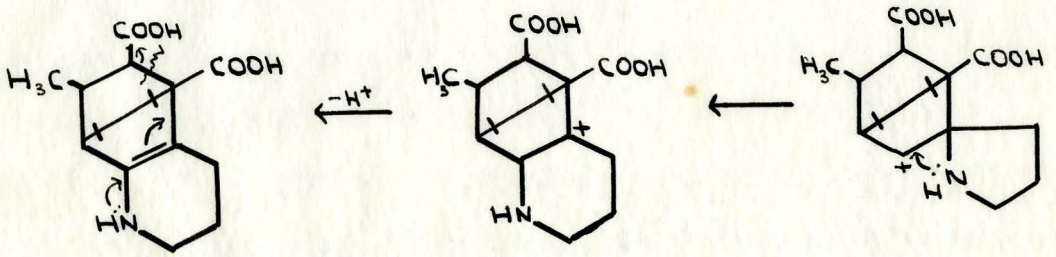
or $R_1 = H, R_2 = COOH$

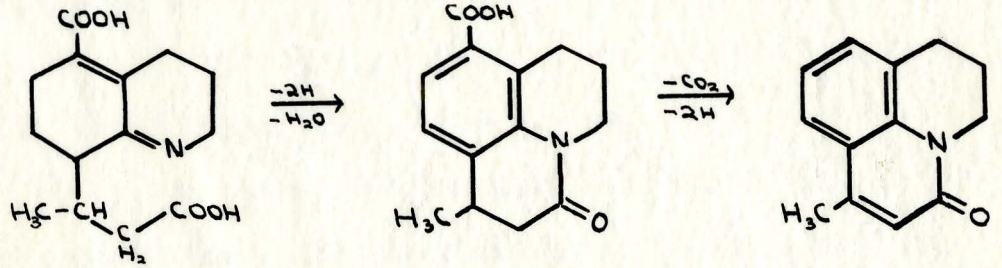
Dehydrogenation of the methyl ester of the amino acid (Bankiewicz (24)), yielded both the acid and neutral lactam already described, together with 2-oxo-4-methyl-juloline.

That a number of rearrangements occur is certain because a structure for annotinine cannot be formulated using the juloline ring system. Wiesner (4) has proposed the following sequence (see page 17) to account for the formation of 2-oxo-4-methyl-juloline.

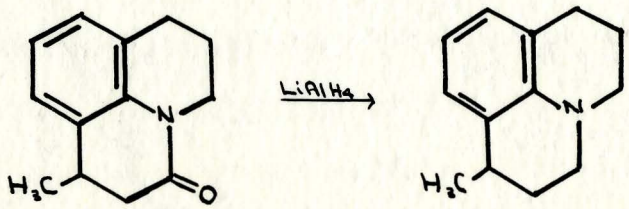


I XV





XVIII XXI



XIX XX

The structure proposed for annotinine is in accord with all the known reactions of the alkaloid. The formation of 7-methyl-quinoline, 8-n-propylquinoline and the other dehydrogenation products can all be accounted for on the basis of rearrangements of the type outlined in the sequence above.

Diphenylannotinine

In the publication of Bertho and Stoll (9) it was reported that annotinine did not react with phenyl lithium, although the presence of a carbonyl group had been established. They attributed failure to react to be due to steric effects. During the course of some work on the reaction of phenyl lithium with annotinine MacLean (25) was able to obtain a compound which analysed quite well for $C_{28}H_{33}O_3N$, although its hydrochloride salt analysed for $C_{28}H_{36}O_4NCl$. Repetition of this work by Meier and Marion (26) produced different results and they report a compound $C_{28}H_{35}O_4N$. This compound in its infrared spectrum, contained several bands in the hydroxyl region, although they do not state how many. Because the expected derivative, $C_{28}H_{33}O_3N$, had not been obtained they explain the formation of their compound as follows. The reaction mixture had been poured onto ice and it is probable that the lithium hydroxide cleaved the ether with resulting formation of a glycol. Oxidation of their diphenylannotinine, $C_{28}H_{35}O_4N$, with chromic acid in glacial acetic acid caused the loss of two hydrogens with no increase in oxygen content. The oxidation product, $C_{28}H_{33}O_4N$, IIIc, which had also been obtained by MacLean, was still basic and its infrared spectrum still contained several absorption bands in the hydroxyl region together with a strong peak at 1720 $cm.^{-1}$, indicating the presence of a

keto group. Hence the oxidation must have converted a secondary hydroxyl group into a keto group. In the similar oxidation of annotinine hydrate, II, (16), in which it had been presumed that the lactone ring was the original one present in annotinine, they state that it must have been one of the glycol hydroxyl groups which is oxidized. It was therefore assumed that in the oxidation of diphenylannotinine, $C_{28}H_{35}O_4N$, it was the same hydroxyl group which had been oxidized to a ketone. Meier and Marion state that it appeared probable, therefore, that the free hydroxyl that was originally esterified in the lactone ring was tertiary. This has now, of course, been proven incorrect (4, 27).

The above discussion describes most of the work on the alkaloids of Lycopodium annotinum which has been carried out by other workers.

DISCUSSION OF RESULTS

Part (i) The Minor Alkaloids

The only studies which have been carried out on any of the minor alkaloids are those reported by Bertho and Stoll (9). Their work indicated the absence of hydroxyl groups and unsaturation in both acrifoline and annotine. They were able, however, to establish the presence of carbonyl groups through the reaction of the alkaloids with phenyl lithium to form phenyl derivatives. According to Wittig (28), phenyl lithium is the most sensitive reagent for the carbonyl group. Most of the other tests were of a qualitative nature and did not serve to identify reactive functional groups. Unfortunately there is a lack of experimental detail concerning some of the more important reactions, e.g. catalytic hydrogenations, which in this laboratory were successfully carried out. Another anomalous reaction is that of the lithium aluminium hydride reduction of annotine where Bertho and Stoll report a compound, $C_{16}H_{23}O_3N$, m.p. 162 - 163°C. In this laboratory it was not found possible to reduce annotine with lithium aluminium hydride in either ether or tetrahydrofuran solution. The reduction was satisfactorily carried out with sodium borohydride in ethanol to yield a compound, $C_{16}H_{23}O_3N$, IIb, m.p. 131.5 - 132.5°C. It contained no carbonyl absorption bands when examined in the infrared. In a recent communication (11), Bertho has stated that their 'annotine' was most

probably a hydrate, and it seems therefore very probable that their reduction product was simply impure alkaloid L 11; their analytical results can be accommodated by $C_{16}H_{21}O_3N$ or $C_{16}H_{23}O_3N$.

The evidence presented herein proves that in both acrifoline and annotine there is present one hydroxyl group, probably not tertiary, one ketone carbonyl group and one centre of unsaturation. The lack of reactivity of the third oxygen atom in annotine leads to the conclusion that it must be present in an ether linkage. The nature of this ether linkage has not been established except that it is probably not an epoxide. It has also been proposed that in both of these alkaloids the double bond is in an allyl position to the nitrogen atom.

The investigations have further revealed that annotoxine can be isolated from L. annotinum of Canadian origin. Acrifoline although obtainable from annotoxine was readily isolated from the mother liquors* of L. annotinum by treatment of an acetone solution with hydrobromic acid. The salt which precipitated gave sufficiently pure acrifoline for experimental work. It was found possible to isolate acrifoline from seven or eight batches of L. annotinum collected in various regions of Nova Scotia and from one collected in Ontario. Thus it would seem that this alkaloid is present in all varieties of L. annotinum and there is perhaps little justification for raising the variety acrifolium to the rank of a separate species as suggested by Manske and Marion (8).

Annotoxine has been separated into acrifoline and annotine by chromatography, although the separation was incomplete. A complete separation was effected by precipitation of the insoluble acrifoline

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* Crude alkaloids remaining after the separation of annotinine

hydrobromide from an acetone solution of annotoxine. A comparison of the specific rotations of annotoxine, acrifoline, and annotine lends further proof for the complex nature of annotoxine; c.f. acrifoline $[\alpha]_{20}^{19}$ = -266.2°; annotine $[\alpha]_{D}^{20}$ = -114.5°; annotoxine, found $[\alpha]_{D}^{19}$ = -190.7°; calculated for an equimolecular compound $[\alpha]_{D}^{19}$ = -190.3°.

Annotinine, $C_{16}H_{21}O_3N$, has already been shown to contain a saturated tetracyclic ring system (4, 22, 24). Both acrifoline and annotine are C_{16} alkaloids, and with the establishment of their functional groups, can also be accommodated in a tetracyclic ring system containing one centre of unsaturation. Alkaloid L 8, $C_{16}H_{25}O_2N$, which has one hydroxyl group and one carbonyl group (25), and lycopodine, $C_{16}H_{25}ON$, which contains a ketone function (3,12) also conform to a saturated tetracyclic ring system. It seems very fortuitous that the alkaloids mentioned above can all be accommodated in a tetracyclic ring system. It might not be too presumptuous to suggest that all the C_{16} alkaloids contain a common nucleus and therefore bear a simple relationship to one another. One such simple relationship was demonstrated during the studies on acrifoline, $C_{16}H_{23}O_2N$, where alkaloid L 12, $C_{18}H_{25}O_3N$, was shown to be O-acetylacrifoline. Douglas, Lewis and Marion (3) have established the simple relationship between lycopodine, $C_{16}H_{25}ON$, and alkaloid L 14, $C_{16}H_{25}N$, (anhydrodihydrolycopodine, $>C=O \rightarrow >C<_{H}^{OH} \rightarrow >$), and between lycopodine and L 2, $C_{18}H_{29}O_2N$ (O-acetyldihydrolycopodine, $>C=O \rightarrow >C<_{H}^{OH} \rightarrow >C<_{H}^{OCOCH_3}$)

Annotoxine

Annotoxine separated as a crystalline deposit in L. annotinum mother liquors which had been stored in the refrigerator for several months. Also

more recently it has been found that by seeding the mother liquors with annotoxine, this base will crystallize. After recrystallization it melted at 196.3 - 197.3°C. In its infrared absorption spectrum it contained one band in the hydroxyl region, two bands in the carbonyl region, and bands attributable to unsaturation.

Acrifoline

Acrifoline, $C_{16}H_{23}O_2N$, in its infrared spectrum contained only a single band in the hydroxyl region with weak absorptions in the carbonyl region. However when examined in chloroform solution, the base showed very strong carbonyl absorption together with a band attributable to unsaturation. The presence of unsaturation in acrifoline was also indicated by its facile oxidation with potassium permanganate.

Acrifoline, Ia, was catalytically reduced to dihydroacrifoline, $C_{16}H_{25}O_2N$, IIa, m.p. 162.5 - 169.5°C. Although obtainable as a crystalline solid it could not be recrystallized in a form suitable for analysis but was instead converted to the hydrochloride and hydrobromide salts. It was not found possible to determine the degree of unsaturation by microhydrogenation, probably because a sufficiently high pressure could not be obtained. The infrared spectrum of the free base contained only hydroxyl and carbonyl bands. The uptake of one mole of hydrogen is indicative of only one centre of unsaturation. Lithium aluminium hydride reduction of IIa proceeded readily in ether solution to yield a base, dihydroacrifolinol, IIIa, $C_{16}H_{27}O_2N$, m.p. 195.2°C. In its infrared absorption spectrum it contained hydroxyl absorption but no bands in the carbonyl region. The addition of two more hydrogens and the lack of carbonyl absorption in the

infrared is indicative of the presence of a single carbonyl group in acrifoline.

The reduction of acrifoline, Ia, with lithium aluminium hydride in ether could not be effected, even after twelve hours at reflux temperature. However when the higher boiling solvent tetrahydrofuran was used, the reduction proceeded smoothly to give acrifolinol, $C_{16}H_{25}O_2N$, IVa, m.p. 191.1 - 192.6°C. In its infrared absorption spectrum it contained a strong hydroxyl band and a weak band attributable to unsaturation. The catalytic reduction of IVa did not give the expected compound IIIa, but instead an isomeric base, $C_{16}H_{27}O_2N$, Va, m.p. 165.5 - 167.5°C, was obtained. Its infrared spectrum was almost identical to that of IIIa indicating a definite similarity of the two. They are, in all probability, stereoisomers; the isomerism could either be about the newly formed hydroxyl group or at the site of the original unsaturation.

Treatment of acrifoline, Ia, with boiling hydrochloric acid and phosphorous oxychloride failed to effect a change in the structure. When acrifoline, Ia, was treated with boiling acetic anhydride, a mono-acetate, $C_{18}H_{25}O_3N$, VIa, m.p. 119.2 - 120.2°C, was obtained. On admixture with an authentic sample of alkaloid L 12, there was no depression of the melting point and their infrared spectra were superimposable. Alkaloid L 12 is therefore only the O-acetyl derivative of acrifoline. The formation of a monoacetyl derivative is proof for the presence of only one hydroxyl group in acrifoline. The alkaline hydrolysis of alkaloid L 12 gave acrifoline, thus demonstrating that no changes in structure had occurred during the acetylation.

The basicity constant, pKa, of acrifoline was found to be 8.34, whereas that of dihydroacrifoline IIa, was 9.13. The increase in basic strength is indicative of unsaturation in acrifoline in the allyl position to the nitrogen atom (17).

Annotine (Alkaloid L 11)

The alkaloid annotine, $C_{16}H_{21}O_3N$, has so far only been obtained in these laboratories by the breakdown of annotoxine. In its infrared absorption spectrum it contained a weak band in the hydroxyl region, a double peak in the carbonyl region and weak absorptions attributable to unsaturation. The active hydrogen determination gave a value of just under one and one-half active H. The presence of unsaturation was also indicated by the oxidation of annotine by potassium permanganate.

Contrary to the work of Bertho and Stoll (9), it was not found possible to reduce annotine with lithium aluminium hydride in either ether or tetrahydrofuran solution. Annotine was reduced with sodium borohydride in ethanol to yield the base, IIb, m.p. 113 - 129°C after recrystallization from ethanol. The compound melted sharply at 131.3°C after drying 'in vacuo' when there was a loss in weight corresponding to two moles of ethyl alcohol. The base then analysed for $C_{16}H_{23}O_3N$. In its infrared spectrum it contained a double band in the hydroxyl region and weak absorption bands attributable to unsaturation. The catalytic reduction of IIb yielded the saturated base, $C_{16}H_{25}O_3N$, IIIb, m.p. 185.8°C. The uptake of one mole of hydrogen is supporting evidence for the presence of a single centre of unsaturation. Catalytic hydrogenation of annotine over Adams' catalyst yielded dihydroannotine, $C_{16}H_{23}O_3N$, IVb, m.p. 202.0 - 202.5°C. In its

infrared spectrum it contained a single band in the hydroxyl region, a single band in the carbonyl region, but no absorption bands due to unsaturation.

The action of hydrochloric acid on annotine led to an unexpected result. The compound which was isolated as the hydrochloride salt analysed for $C_{16}H_{21}O_3NHCl$, Vb, m.p. greater than 300°C. It was not the hydrochloride of annotine as was shown by a comparison of their infrared spectra where shifts of 10 $cm.^{-1}$ were found in the carbonyl region as well as distinct differences in the fingerprint region. The crude base gave a negative test for halogen (Beilstein test). Therefore, either a rearrangement or isomerization has taken place and the present state of knowledge does not allow an interpretation of this reaction.

An attempt was made to prepare the oxime of annotine in order to determine the number of carbonyl groups present but annotine failed to form an oxime under the conditions employed.

Treatment of annotine, Ib, with acetic anhydride gave, but in poor yield, a mono-acetyl derivative, $C_{18}H_{23}O_4N$, VIb, m.p. 182.7°C. The formation of a mono-acetate is strong evidence for the presence of a single hydroxyl group, probably not tertiary, in annotine. In its infrared spectrum it contained absorption bands attributable to an acetoxy group, a carbonyl group and unsaturation. The alkaline hydrolysis of VIb regenerated annotine; therefore no side reactions had occurred during the acetylation. Annotine would thus appear to be unaffected by alkali.

Treatment of annotine, Ib, with phenyl lithium gave phenylannotine, $C_{22}H_{27}O_3N$, VIIb, m.p. 214.0°C, (Bertho and Stoll (9) report 215 - 216°C). The infrared spectrum contained no bands attributable to a carbonyl group.

The formation of a mono-phenyl carbinol is further proof for the presence of a single carbonyl group in annotine. It was noted that phenylannotine was highly solvated when recrystallized from acetone or methanol.

A determination of the basicity constant (pKa) of annotine revealed that it was a weaker base than dihydroannotine. The values were 7.5 and 8.61, respectively. This increase in basic strength is again strong evidence for the presence of the allylamine structure in annotine.

The lack of reactivity of the third oxygen function means that it is probably present as an ether linkage. The failure to add the elements of hydrochloric acid, to react with alkali, to reduce with either lithium aluminium hydride or sodium borohydride, are indications that it is not an epoxide. If the third oxygen atom is in an ether linkage then annotine conforms to a tetracyclic ring system containing one centre of unsaturation.

Part (ii) - Diphenylannotinine

The reaction between annotinine, $C_{16}H_{21}O_3N$, and phenyl lithium had not been previously studied in any detail. The early work of MacLean (25) indicated that the reaction product, diphenylannotinine, had the molecular formula, $C_{28}H_{33}O_3N$, and was formed by normal reaction of phenyl lithium at the lactone carbonyl. The recent work of Meier and Marion (26) contradicted this result and they reported a compound, $C_{28}H_{35}O_4N$.

The first object of this investigation was to establish, unequivocally, the exact nature of the reaction and then to attempt a degradation of diphenylannotinine in an effort to gain more information about the structure of annotinine. The latter objective was not pursued after the proposal of a structure for annotinine was made by Wiesner (4).

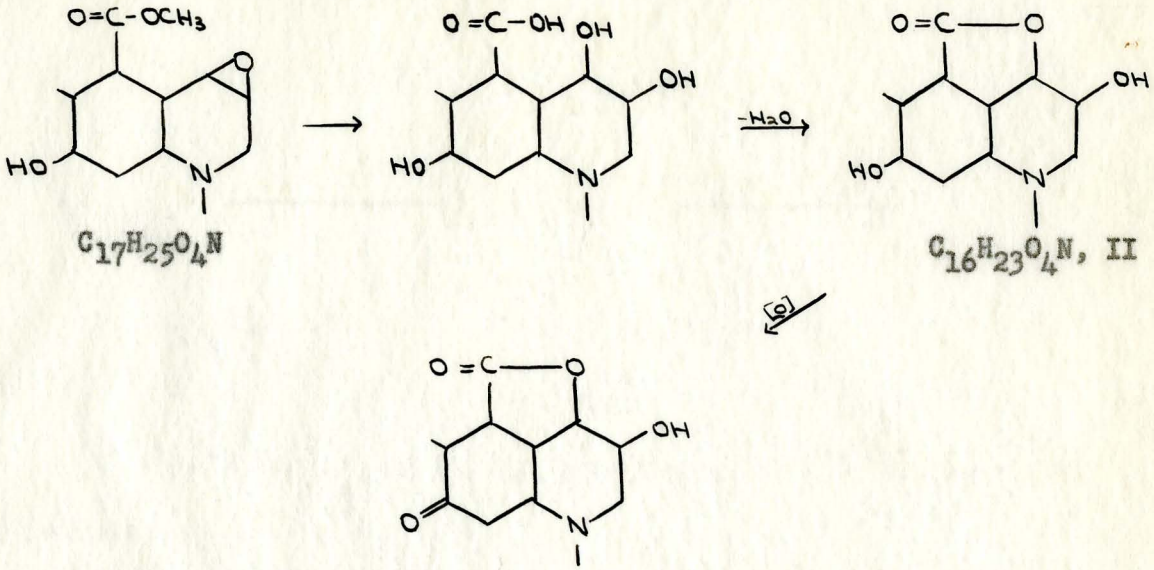
Diphenylannotinine was prepared according to the method of Meier and Marion (26). For the first time diphenylannotinine was obtained in a crystalline form. It was crystallized from benzene, although it was obviously highly solvated because it lost about 20 per cent of its weight after drying 'in vacuo'. The undried compound exhibited a double melting point at 128 and 236°C, neither of which was particularly sharp. After drying it exhibited only the single melting point at about 236°C. Diphenylannotinin e analysed closely for $C_{28}H_{33}O_3N$, although it was 0·9 per cent high on carbon. A number of analyses were carried out by two different analytical laboratories and a good agreement was obtained.

However, the analytical data can be reconciled with a solvated compound, $C_{28}H_{33}O_3N \cdot 1/3C_6H_6$, but cannot be accommodated by an O_4 formulation. In its infrared absorption spectrum it contained two bands in the hydroxyl region but no bands in the carbonyl region.

Diphenylannotinine formed a crystalline hydrochloride which gave analytical results in excellent agreement with $C_{28}H_{35}O_4N \cdot HCl$. This salt is probably a monohydrate, $C_{28}H_{33}O_3N \cdot HCl \cdot H_2O$.

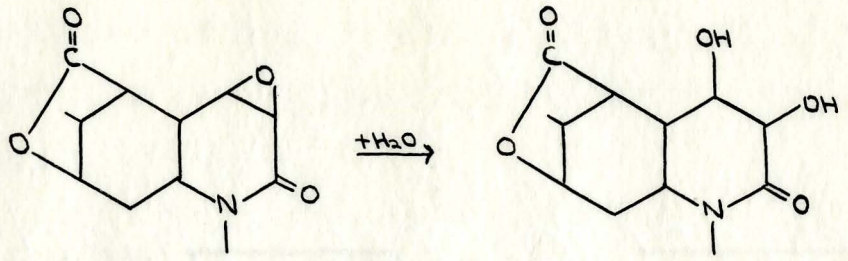
The Chromic Acid Oxidation Product

The chromic acid oxidation of diphenylannotinine apparently offered further evidence against an O_3 formulation because a ketonic compound (infrared spectrum), $C_{28}H_{33}O_4N$, IIIc, m.p. 242.1°C, was formed. This at first seemed to indicate that diphenylannotinine must certainly be an O_4 compound. However, in two other annotinine derivatives the opening of the ether linkage by chromic acid has been reported. The first case is that reported by Stonner (27) who found that the methyl ester derived from annotinine, $C_{17}H_{25}O_4N$, was converted on treatment with chromic acid anhydride in glacial acetic acid into annotinine hydrate II, $C_{16}H_{23}O_4N$. This reaction probably proceeds by a sequence of the following type:



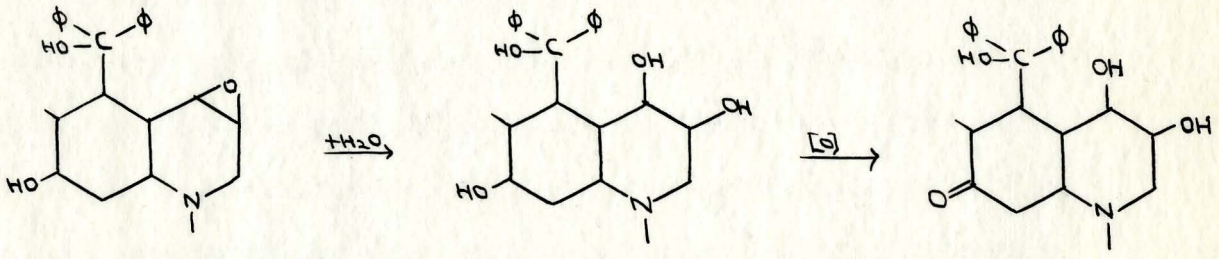
The further oxidation of annotinine hydrate II to the ketone, $C_{16}H_{21}O_4N$, had already been reported by Meier, Meister and Marion (16). The hydroxyl group oxidized is probably the one originally esterified in the lactone ring. The second case was reported by MacLean (25). He found that when annotinine lactam, $C_{16}H_{19}O_4N$, IV, was treated with chromic acid anhydride in glacial acetic acid, it was converted into a compound, $C_{16}H_{21}O_5N$. This new compound contained in its infrared spectrum the usual bands at 1615 cm.$^{-1}$ and 1762 cm.$^{-1}$ in the amide and lactone carbonyl regions. There were two new bands in the hydroxyl region at 3250 cm.$^{-1}$ and 3518 cm.$^{-1}$ indicating the presence of two hydroxyl groups.

This reaction proceeds by hydration of the oxide ring, thus:-



$C_{16}H_{19}O_4N$, IV $C_{16}H_{21}O_5N$

By anology with the above, the chromic acid oxidation may be formulated:-



$C_{28}H_{33}O_3N$, IIc $C_{28}H_{33}O_4N$, IIIc

The presence of three hydroxyl groups in IIIc is supported by the infrared analysis, although an active hydrogen determination indicated only two hydroxyl groups. Treatment of IIIc with hydrochloric acid, or alcoholic alkali, yielded only unchanged starting material. An attempt was made to dehydrate it with acetic anhydride but the product obtained was an acetate (identified by the presence of characteristic

absorption bands in the infrared). That dehydration had not occurred during the acetylation was confirmed by the hydrolysis of the acetate when IIIc was obtained. Lithium aluminium hydride reduction of IIIc gave the weak base, $C_{28}H_{35}O_4N$, IVc, m.p. 260.9°C, which was probably solvated.

The Oppenauer Oxidation Product

During attempts to obtain larger amounts of IIIc, the Sarett oxidation procedure (20), which utilizes the pyridine-chromic acid anhydride complex, was tried. Yields were very poor, generally in the order of 5 to 10 per cent. The Oppenauer method of Woodward et al.(29) was then attempted. Using benzophenone and potassium tert.-butoxide in benzene gave yields in the order of 30 per cent of a new ketonic substance, $C_{28}H_{31}O_3N$, Vc, m.p. 244.2 - 245.2°C. Yields of 50 to 60 per cent were obtained when cyclohexanone in toluene was substituted for benzophenone and benzene. In its infrared spectrum it contained a single band in the hydroxyl region together with strong absorption in the carbonyl region. The presence of a single hydroxyl group was supported by the active hydrogen determination. The ketonic base, Vc, was presumably formed by oxidation of the secondary hydroxyl group which was originally esterified in the lactone ring, the ether linkage remaining unaffected. The formation of this O_3 oxidation product is added support for the O_3 formulation for diphenylannotinine. Treatment of Vc with concentrated hydrochloric acid or with hydrogen over Adams' catalyst gave only unchanged starting material. The failure to take up hydrogen is proof for the absence of olefinic unsaturation

in the Oppenauer oxidation product. It had been considered that dehydration might have occurred. Lithium aluminium hydride reduction was difficult to effect, requiring forty-eight hours for complete reduction. The resulting product possessed an infrared spectrum identical to that of diphenylannotinine and gave a hydrochloride identical to that of diphenylannotinine hydrochloride, $C_{28}H_{35}O_4N \cdot HCl$, although there were indications of the presence of another product. Reduction with sodium borohydride proceeded more readily. The product obtained had an infrared spectrum superimposable on that of diphenylannotinine. The hydrochloride salt, however, depressed the melting point of diphenylannotinine hydrochloride. Its analysis can be best accommodated with $C_{28}H_{35}O_4N \cdot HCl$ and it is probably an isomer of diphenylannotinine hydrochloride. The reduction back to an isomer of diphenylannotinine is even further evidence for an O_3 formulation for the product of the reaction between annotinine and phenyl lithium.

Added evidence for the unusual action of chromic acid in glacial acetic acid in opening the oxide ring was obtained when it was found that chromic acid treatment of Vc resulted in the formation of IIIc, in good yield.

In another attempt to prove that diphenylannotinine was not an O_4 compound, annotinine hydrate II, $C_{16}H_{23}O_4N$, was treated with phenyl lithium. In no experiment was a compound corresponding to $C_{28}H_{35}O_4N$ isolated; although in one experiment there was obtained a derivative which gave analytical results conforming to a C_{22} compound.

Interpretation of Basicity Data

The specific rotations and basicity constants of diphenyl-

annotinine and its derivatives were determined (see below). It has been possible to partially correlate the basicity data with the proposed reaction sequences, but the interpretation of the optical data must be deferred until specific rotation data is available for other annotinine derivatives.

Compound	m.p. °C	$[\alpha]_D$	pKa
Annotinine			
$C_{16}H_{21}O_3N$, I	232°	-276.6°(10)	5.8 (27)
$C_{28}H_{33}O_3N$, IIc	236°	+7.8°	5.9
$C_{28}H_{33}O_4N$, IIIc	242°	+55.8°	4.2
$C_{28}H_{35}O_4N$, IVc	260.9°	+13.5°	5.4
$C_{28}H_{31}O_3N$, Vc	245.2°	+90.8°	3.3

The similarity of the basicity constants of annotinine and diphenylannotinine, IIc, seemed to indicate that no drastic changes had occurred in the region of the nitrogen atom. If the oxide ring had undergone fission, a rise in pKa would be expected following the removal or modification of the electron attracting oxide ring. A rise from 5.8 to 8.4 in pKa was observed when annotinine was completely reduced with lithium aluminium hydride (27). The introduction of an electron attracting carbonyl group into diphenylannotinine, as in Vc, where the carbonyl group was assumed to be beta to the nitrogen atom, would be expected to result in a slight decrease in basicity. Such a decrease (2.6) was found. In IIIc it is assumed that the oxide ring is no longer present and an increase in basicity should result, although this would be offset by the beta carbonyl group. A slight decrease (1.7) was actually found.

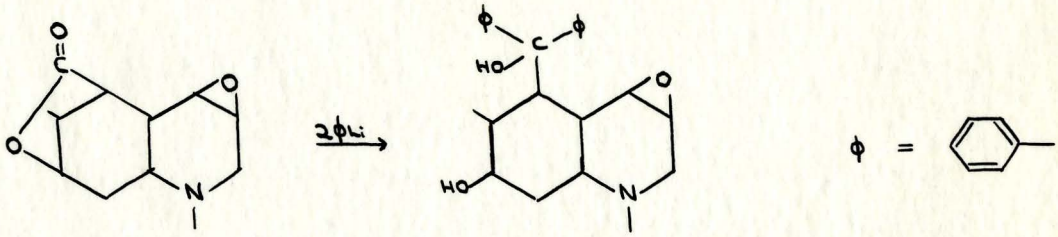
The reduction product, IVc, which no longer possesses the carbonyl group should be a stronger base than diphenylannotinine. An increase in basicity was not found, in fact, a slight decrease (0.5) was actually observed. This decrease cannot be explained.

It should be borne in mind that steric factors might play an important role in determining the basic strangths of these compounds and not too much reliance should be placed on an interpretation of them.

Modes of Formation of an O_3 Compound

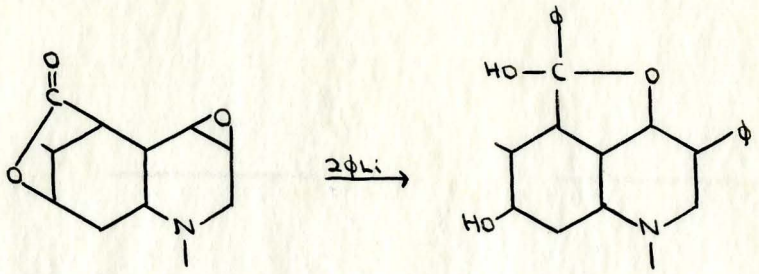
Because the experimental evidence seemed to indicate that an epoxide ring was no longer present in diphenylannotinine, it was necessary to examine possible O_3 formulations. The infrared data indicated two hydroxyl groups; the third oxygen atom was presumed to be bound in an ether linkage. The following sequences were considered:-

(a) Normal attack of phenyl lithium on the lactone carbonyl group, with retention of the epoxide ring



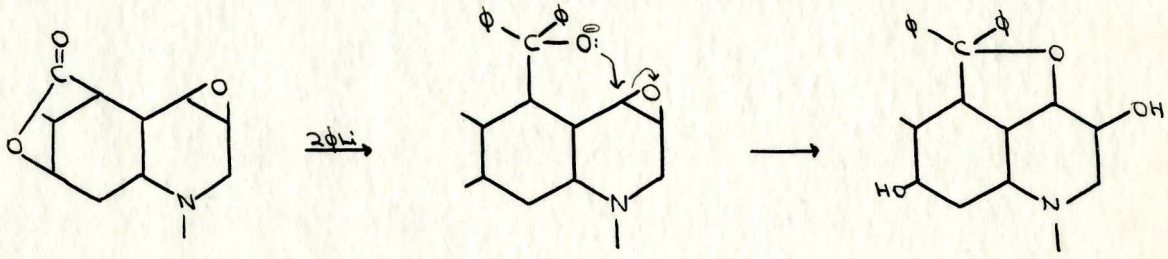
$C_{16}H_{21}O_3N$, I $\qquad$ $C_{28}H_{33}O_3N$

(b) Attack of phenyl lithium on the oxide ring and the lactone carbonyl group to give a hemiketal



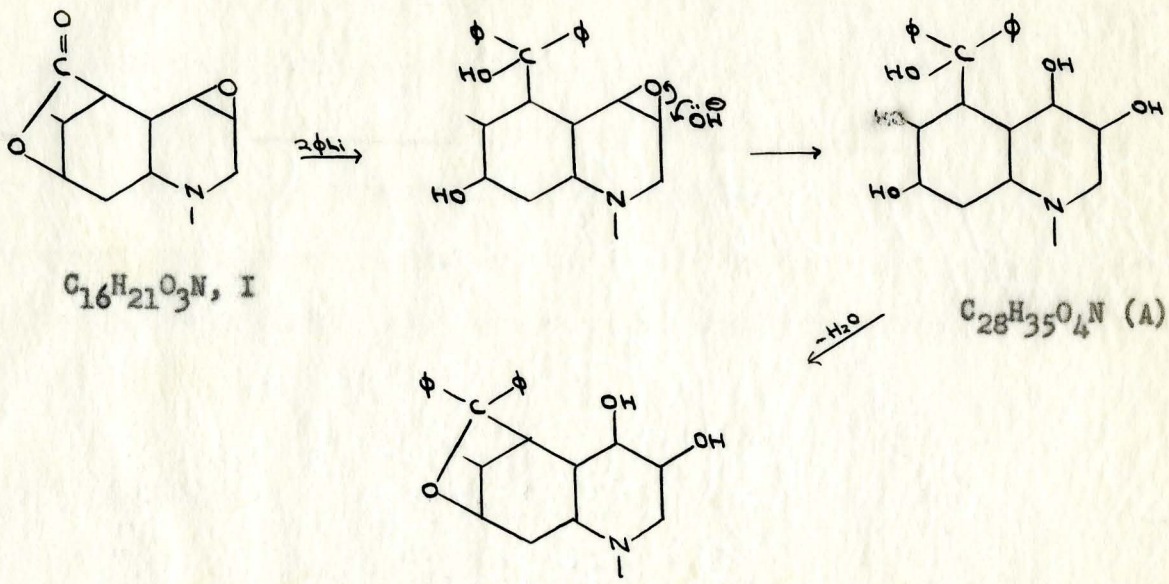
$C_{16}H_{21}O_3N$ $C_{28}H_{33}O_3N$

(c) Attack of phenyl lithium on the lactone carbonyl group, followed by internal attack by the intermediate anion on the oxide ring



$C_{16}H_{21}O_3N$ $C_{28}H_{33}O_3N$

(d) Attack of phenyl lithium on the lactone ring, followed by attack of hydroxyl ion on the epoxide ring as suggested by Meier and Marion (26) and then dehydration



$C_{16}H_{21}O_3N$, I

$C_{28}H_{35}O_4N$ (A)

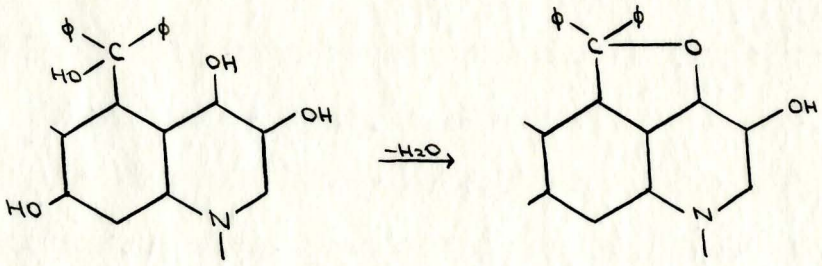
$C_{28}H_{33}O_3N$

On the basis of the presently accepted structure of annotinine (4, 22, 24), the intermediate A would be the diphenylannotinine of Meier and Marion. Each formulation will now be discussed in the light of the experimental evidence at hand.

Sequence (b) is untenable since diphenylannotinine failed to give any ketonic tests with hydrazine or phenylhydrazine which would be expected of a compound of this type. This sequence may also be dismissed because drastic oxidation of diphenylannotinine yielded benzophenone and benzoic acid. Benzophenone can only arise if the two phenyl residues are attached to the same carbon atom as in (a), (c) and (d). The benzoic acid is not considered to be of primary origin. Slack and Waters (30)

have reported benzoic acid, as well as the major product, benzophenone, as products of the mild chromic acid oxidation of diphenylmethane.

A sequence such as (d) is also unlikely for the following reasons. This formulation involves three steps, the latter two requiring hydration and dehydration. For two such reactions to be carried out simultaneously is somewhat unusual, in fact probably impossible. If a dehydration step were involved it would be more likely to proceed in the opposite direction as was found during the relactonization of annotinine hydrate II (8, 18, 27).



The resulting product would then be that proposed by sequence (c).

The suggestion of Meier and Marion (26) that there is attack by hydroxyl ion on the epoxide ring during the isolation of diphenylannotinine may be discounted for the following reasons. If the oxide ring were opened by hydroxyl ion then when the reaction mixture is decomposed by hydrochloric acid, a chlorohydrin should be formed, since chloride ion would now be the predominant ionic species. However it was found that the same product was isolated when the reaction mixture was decomposed by either hydrochloric acid or water.

Diphenylannotinine does not react with concentrated hydrochloric acid, lithium aluminium hydride, or with more phenyl lithium; neither does the Oppenauer oxidation product, Vc, react with hydrochloric acid. Treatment of diphenylannotinine with phosphorous oxychloride in pyridine gave a product containing chlorine, but because the desired dehydration had not occurred (there were no bands due to olefinic unsaturation when the compound was examined in the infrared), it was not examined further. Thus it would appear that diphenylannotinine possesses neither an epoxide ring nor does the tertiary hydroxyl group appear to react and thus sequence (a) would appear to be untenable. However, steric factors might play an important role and thus account for the non-reactivity of the ether linkage.

A sequence such as (c) would account for the non-reactivity of the ether linkage towards lithium aluminium hydride and phenyl lithium. But if diphenylannotinine is a substituted tetrahydrofuran then it might be expected to react with hydrochloric acid to form a chlorohydrin. A structure such as this would also account for the difficulty in dehydrating diphenylannotinine and the Oppenauer oxidation product.

Conclusions

Diphenylannotinine is an O_3 compound in which the two phenyl groups are attached to the same carbon atom. The unreactive nature of the ether linkage leads to the conclusion that it might be present as a five membered ring or that the original epoxide ring is still present but due to steric hindrance is unable to react further.

EXPERIMENTAL

Apparatus, Methods, and Materials

Infrared spectra were determined using a Perkin-Elmer model 21B double beam Infrared recording spectrophotometer. Samples were mounted in nujol* except where otherwise stated. Basicity constants (pKa values) were determined by potentiometric titration of the bases or their salts; the solvent used in each case is stated in parenthesis. Values are those at the half-neutralization point. Molecular rotations were measured in a 10 cm. micro-tube using a Hilger polarimeter.

Samples for analysis were dried at 78°C in high vacuum. 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.

Fisher chromatographic grade alumina, and Woelm Grade 1 alumina were used for chromatography.

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* Otherwise known as heavy mineral oil

Part (i) - Minor Alkaloids

Isolation of Annotoxine

From the mother liquors from the separation of annotinine there was deposited, after several months standing in the refrigerator, a crystalline precipitate. The crude alkaloid obtained by filtration melted at 190 - 191°C. Following recrystallization from acetone or methanol it melted at 196.3 - 197.3°C. A mixed melting point with a sample of annotoxine kindly supplied by Dr. Bertho showed no depression and their infrared spectra were identical.

Anal. Calc. for $C_{32}H_{44}O_5N_2$: C, 71.61; H, 8.27; N, 5.22.

Found: C, 72.13, 72.12; H, 8.53, 8.28; N, 4.86, 4.80.

$[\alpha]_D^{19} = -187.1°$ (c, 1% chloroform)

Bertho and Stoll (9) $[\alpha]_D^{20} = -179°$ (c, 1% chloroform)

Achmatowicz and Rodewald (10) $[\alpha]_D^{19} = -185.6°$ (c, 1% chloroform)

The infrared spectrum contained absorption bands in the hydroxyl region at 3400 cm.$^{-1}$, in the carbonyl region at 1730 cm.$^{-1}$, and 1695 cm.$^{-1}$, and a weak band at 1665 cm.$^{-1}$ attributable to unsaturation.

Separation of Annotoxine

(a) By Chromatography. Annotoxine, 300 mg., was dissolved in chloroform and absorbed on a column of alumina (Woelm). The column was eluted with benzene and 10 ml. fractions collected. Annotine (alkaloid L 11) passed through the column with benzene and from the combined fractions

130 mg. of pure annotine was obtained. The column was then eluted with 2 vol. per cent methanol in benzene to yield a first sample, m.p. 164.5 - 170.5°C, of impure annotoxine (70 mg.). This was followed by 100 mg. of acrifoline. Even after several attempts it was not possible to effect a complete separation by this method.

(b) Through the Hydrobromide Salts. To a solution of 3.0 gm. of annotoxine in 100 ml. of hot acetone was added a solution of hydrobromic acid (48 per cent) until just acid. There was immediate precipitation of a crystalline salt. After filtering and drying, it weighed 1.66 gm. Concentration of the mother liquors yielded a further 0.3 gm. The combined hydrobromide salts were dissolved in 100 ml. of water, basified with ammonia and extracted several times with chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate, and concentrated 'in vacuo' to yield 1.48 gm. (100 per cent) of acrifoline, identified by mixed melting point and infrared spectrum with an authentic sample.

Evaporation of the mother liquors from the separation of acrifoline hydrobromide yielded a crystalline residue which was then dissolved in 100 ml. water. The solution was basified with ammonia and extracted with chloroform several times. The combined extracts were dried over anhydrous sodium sulfate and evaporated to dryness to give 1.60 gm. (100 per cent) of alkaloid L 11, identified by mixed melting point and infrared spectrum with an authentic sample.

Acrifoline

Acrifoline was available from annotoxine in the manner described

above. The main source, however, was the mother liquors of annotinine. These were concentrated 'in vacuo', dissolved in acetone and treated with hydrobromic acid when the insoluble acrifoline hydrobromide precipitated.

Acrifoline was obtained as a white solid which rapidly assumed a pink colour on exposure to air. It melted over a wide range, 99 - 104°C, even after several recrystallizations from ether.

Anal. Calc. for $C_{16}H_{23}O_2N$: C, 73.56; H, 8.81; N, 5.36.

Found: C, 73.20, 73.20; H, 9.06, 8.92; N, 4.75, 5.00.

pKa = 8.34 (50% methanol-water)

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

Bertho and Stoll (9) $[\alpha]_D^{20}$ = -250° (c, 1% ethyl acetate)

Achmatowicz and Rodewald (10) $[\alpha]_D^{21}$ = -264.8° (c, 1% acetone)

The infrared absorption spectrum contained a strong band in the hydroxyl region at 3310 $cm.^{-1}$, but only very weak bands in the carbonyl region. In chloroform solution there were very strong bands at 1700 $cm.^{-1}$ and 1675 $cm.^{-1}$. These two bands are assigned to carbonyl (ketone) and unsaturation respectively.

Attempts to determine the degree of unsaturation by microhydrogenation over palladium catalyst in glacial acetic acid, and over Adams' catalyst in methanol were unsuccessful.

Acrifoline was readily oxidized by potassium permanganate. It was recovered unchanged after refluxing for an hour with either concentrated hydrochloric acid or phosphorous oxychloride.

Annotine (Alkaloid L 11)

This alkaloid has, so far, only been obtained by the breakdown

of annotoxine. After recrystallization from methanol it melted at 173.6 - 175.6°C. It was identified by mixed melting point and infrared spectrum with an authentic sample. Like acrifoline, it was oxidized by potassium permanganate.

Anal. Calc. for $C_{16}H_{21}O_3N$: Active H,(1) 0.37, (2) 0.73.

Found: Active H,0.48, 0.51.

pKa = 7.5 (50% methanol-water)

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

Bertho and Stoll (9) $[\alpha]_D^{21}$ = -114° (c, 1% chloroform)

Achmatowicz and Rodewald (10) $[\alpha]_D^{24}$ = -110.3° (c, 1% acetone)

The infrared absorption spectrum contained a weak band in the hydroxyl region at 3300 cm.$^{-1}$ and a strong double peak at 1725 cm.$^{-1}$ and 1712 cm.$^{-1}$ in the carbonyl region. In carbon disulfide solution there was absorption at 3540 cm.$^{-1}$ and 3020 cm.$^{-1}$ attributable to a single hydroxyl group and one centre of unsaturation respectively. There was also strong absorption at 1741 cm.$^{-1}$ due to a ketone carbonyl group, and bands at 1695 cm.$^{-1}$ and 1656 cm.$^{-1}$ attributable to unsaturation. In chloroform solution there was a broad band in the carbonyl region at 1720 - 1730 cm.$^{-1}$, and a band at 1644 cm.$^{-1}$ which is assigned to unsaturation.

Attempts to determine the degree of unsaturation by microhydrogenation were unsuccessful.

Reduction of Acrifoline, Ia* with Hydrogen

Acrifoline, 300 mg., was dissolved in 50 ml. of redistilled methanol, treated with 0.10 gm. Adams' catalyst and subjected to a hydro-

* Acrifoline derivatives will be designated 'a'.

gen pressure of 50 p.s.i.g. for three hours. After filtering free of catalyst, followed by concentration 'in vacuo' there remained a yellow oil. This was dissolved in ether and filtered to free it from a little insoluble material. The concentrated ether solution deposited almost colourless crystals of IIa after several hours standing in the refrigerator. Several recrystallizations from ether gave a crystalline solid melting at 162.5 - 169.5°C. The free base (IIa), in pure form, was difficult to obtain in good yield. Consequently the combined mother liquors were evaporated to dryness, dissolved in acetone and converted into the hydrochloride and hydrobromide salts for analysis. Both salts had melting points greater than 300°C.

Anal. Calc. for $C_{16}H_{25}O_2N \cdot HCl$: C, 64.09; H, 8.74; N, 4.67.

Found: C, 63.51, 63.48; H, 8.70, 8.71; N, 4.80, 4.85.

Calc. for $C_{16}H_{25}O_2N \cdot HBr$: C, 55.81; H, 7.61.

Found: C, 55.50, 55.43; H, 7.46, 7.42.

The infrared spectrum of the hydrochloride contained a strong band at 3240 $cm.^{-1}$ in the hydroxyl region, and at 1704 $cm.^{-1}$ in the carbonyl region. The hydrobromide contained bands at 3300 $cm.^{-1}$ and 1702 $cm.^{-1}$.

pKa = 9.13 (hydrochloride salt and water)

The infrared spectrum of the free base contained strong absorptions at 3500 $cm.^{-1}$ in the hydroxyl region, and 1679 $cm.^{-1}$ in the carbonyl region.

Reduction of $C_{16}H_{25}O_2N$, IIa, with Lithium Aluminium Hydride

To a solution of 100 ml. of anhydrous ether containing 10 ml. of 2.76 M $LiAlH_4$ in ether there was slowly added a solution of 500 mg. of IIa in 50 ml. of dry ether. The mixture was stirred under reflux for eight

hours. Excess $LiAlH_4$ was destroyed by careful addition of aqueous ether, the ether layer was decanted, dried over anhydrous sodium sulfate and concentrated 'in vacuo'. The yellow oily residue, 400 mg. (80 per cent theory) of IIIa, after recrystallization from methanol-acetone melted at 195.2°C.

Anal. Calc. for $C_{16}H_{27}O_2N$: C, 72.40; H, 10.26; N, 5.28.

Found: C, 71.80, 72.01; H, 10.12, 10.46; N, 4.79, 4.80.

Its infrared absorption spectrum contained a strong band at 3300 $cm.^{-1}$ in the hydroxyl region, but no bands attributable to carbonyl or unsaturation.

Reduction of Acrifoline, Ia, with Lithium Aluminium Hydride

Acrifoline, 1.50 gm., was dissolved in 100 ml. of dry tetrahydrofuran (dried over sodium); to this solution was added 5 ml. of a 2.76 M solution of $LiAlH_4$ in ether, and the reaction mixture refluxed, with stirring, for seven hours. Excess $LiAlH_4$ was destroyed by the careful addition of aqueous tetrahydrofuran and the reaction mixture filtered. On contact with air it became very dark brown in colour. After concentrating 'in vacuo', the dark brown residue was dissolved in 50 ml. dilute acetic acid, basified with ammonia and extracted several times with chloroform. The combined chloroform extracts were dried over sodium sulfate. Concentration 'in vacuo' gave 1.2 gm. of a light green foam, which after recrystallization from methanol yielded colourless crystals of IVa, m.p. 191.1 - 192.6°C.

Anal. Calc. for $C_{16}H_{25}O_2N$: C, 73.10; H, 9.57; N, 5.32.

Found: C, 72.40, 72.62; H, 9.57, 9.75; N, 4.84, 4.93.

In its infrared spectrum it contained a strong band at 3266 cm.$^{-1}$ in the hydroxyl region, and a weak peak at 1425 cm.$^{-1}$, attributable to unsaturation.

Treatment of $C_{16}H_{25}O_2N$, IVa, with Hydrogen

A solution of 300 mg. of IVa in 50 ml. of redistilled methanol containing 0.10 gm. of Adams' catalyst was treated with hydrogen at a pressure of 50 p.s.i.g. for three hours. The solution was then filtered free of catalyst and concentrated 'in vacuo' to yield 300 mg. of Va which was recrystallized from acetone-methanol. The compound melted at 165.5 - 167.5°C. A mixed melting point with IIIa was depressed forty degrees.

Anal. Calc. for $C_{16}H_{27}O_2N$: C, 72.40; H, 10.26; N, 5.28.

Found: C, 72.17, 72.25; H, 10.03; N, 4.70, 4.80.

The infrared spectrum contained a strong absorption band at 3290 cm.$^{-1}$ in the hydroxyl region. The spectrum was almost identical to that of IIIa.

Treatment of Acrifoline, Ia, with Acetic Anhydride

A mixture of 500 mg. of acrifoline, Ia, was refluxed with 20 ml. of acetic anhydride for two hours. After allowing to cool, it was shaken with ice water to hydrolyse excess acetic anhydride, basified with ammonia and extracted several times with chloroform. The combined extracts were dried over sodium sulfate and concentrated 'in vacuo'. The residue was chromatographed on alumina (Fisher) eluting with chloroform. A colourless band passed through the column and concentration yielded 250 mg. (43 per cent theory) of a white solid. It was dissolved in 20 ml. hot

petroleum ether (60-80) and filtered to free it from a little insoluble material. The filtrate was concentrated to about 3 ml. and set aside to crystallize. Small clusters of pale yellow crystals formed, m.p. 114 - 117°C. After a further recrystallization, the melting point was raised to 118 - 120°C. In admixture with an authentic sample of alkaloid L 12 $C_{18}H_{25}O_3N$, there was no depression of the melting point. Their infrared absorption spectra were superimposable. There were no bands due to hydroxyl, but instead two new bands at 1740 cm.$^{-1}$ and 1230 cm.$^{-1}$ were present which are attributable to the acetoxy group.

Alkaline Hydrolysis of Alkaloid L 12, $C_{18}H_{25}O_3N$

Alkaloid L 12, 150 mg., was refluxed for two hours with 15 ml. of alcoholic sodium hydroxide. Most of the alcohol was then removed by distillation, the residue dissolved in 25 ml. water and then extracted several times with chloroform. Evaporation of the dried extracts yielded 132 mg. (98 per cent theory) of a pale yellowish oil which crystallized immediately on contact with ether. After recrystallization from ether it melted at 98 - 99°C and gave no depression of the melting point in admixture with a sample of acrifoline.

Reduction of Annotine, Ib*, with Sodium Borohydride

To a solution of 0.10 gm. of sodium borohydride in 20 ml. of ethanol was added a solution of 500 mg. Ib in 15 ml. ethanol, and the reaction mixture refluxed for one hour. After allowing to cool, a few drops of glacial acetic acid were added to destroy excess borohydride. Most of the alcohol was distilled off, 50 ml. of water added, and the solution then

* Annotine derivatives will be designated 'b'.

basified with ammonia. Chloroform extraction yielded 450 mg. of a viscous oil which crystallized after 18 hours standing. Several crystallizations from ethanol yielded a crystalline compound which softened at 113 - 114.5°C but was not completely molten until 129°C. It was dried at 78°C and 0.01 mm. Hg. pressure. The compound, IIb, lost 27 per cent of its weight and then melted sharply at 131.5 - 132.5°C.

Anal. Calc. for $C_{16}H_{23}O_3N$: C, 69.28; H, 8.36; N, 5.06.

Found: C, 69.34, 69.25; H, 8.17, 8.20; N, 5.45.

The compound, $C_{16}H_{23}O_3N \cdot 2C_2H_5OH$ would show a 25 per cent loss in weight.

In its infrared absorption spectrum the base contained a broad band in the hydroxyl region at 3550 $cm.^{-1}$, and weak bands at 1650 $cm.^{-1}$ and 1403 $cm.^{-1}$ attributable to unsaturation. In carbon disulfide solution there were two bands in the hydroxyl region at 3510 $cm.^{-1}$ (strong) and 3380 $cm.^{-1}$ (broad). There was also a band at 3022 $cm.^{-1}$ attributable to unsaturation.

Treatment of $C_{16}H_{23}O_3N$, IIb, with Hydrogen

A solution of 300 mg. of IIb in 50 ml. of redistilled methanol containing 20 mg. of Adams' catalyst was treated with hydrogen at 50 p.s.i.g. for three hours. The solution was filtered free of catalyst and on evaporation to dryness there remained 300 mg. of a brown viscous oil which crystallized immediately on contact with ether. After several recrystallizations from methanol the compound, IIIb, melted at 185.8°C.

Anal. Calc. for $C_{16}H_{25}O_3N$: C, 68.78; H, 9.02; N, 5.01.

Found: C, 68.35, 68.35; H, 9.07, 8.95; N, 4.82, 5.08.

The infrared absorption spectrum in carbon disulfide solution contained bands in the hydroxyl region at 3500 cm.$^{-1}$ (strong), and a broad band at 3290 cm.$^{-1}$, but no bands in the 3000 cm.$^{-1}$ region (c.f. IIb).

Treatment of Annotine, Ib*, with Hydrogen

A solution of 100 mg. Ib in 50 ml. of redistilled methanol containing 50 mg. of Adams' catalyst was subjected to a hydrogen pressure of 50 p.s.i.g. for three hours. The solution was filtered free of catalyst and concentrated 'in vacuo'. The residue, 100 mg., crystallized immediately on contact with ether. Several recrystallizations from methanol yielded small white crystals of IVb, m.p. 201.5 - 202.5°C.

Anal. Calc. for $C_{16}H_{23}O_3N$: C, 69.28; H, 8.36; N, 5.06.

Found: C, 69.48, 69.33; H, 8.12, 8.20; N, 4.95.

pKa = 8.61 (methanol)

The infrared absorption spectrum contained a strong band at 3200 cm.$^{-1}$ in the hydroxyl region, and at 1728 cm.$^{-1}$ in the carbonyl region.

Treatment of Annotine, Ib, with Hydrochloric Acid

Annotine, 250 mg., was refluxed for two hours with 20 ml. of 6 M hydrochloric acid. The acid solution was allowed to cool to room temperature, basified with ammonia, and then extracted several times with chloroform. The combined chloroform extracts were dried over sodium sulfate, and concentrated 'in vacuo' to yield 200 mg. of a viscous brown residue. It could not be induced to crystallize. The residue also gave

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* Annotine derivatives will be designated 'b'.

a negative test for halogen (Beilstein test). Its infrared absorption spectrum contained bands at 3400 cm.$^{-1}$, 1735 cm.$^{-1}$, and 1662 cm.$^{-1}$, tentatively assigned to hydroxyl, carbonyl, and unsaturation respectively. The residue yielded a crystalline hydrochloride, Vb, which melted at a temperature greater than 300°C.

Anal. Calc. for $C_{16}H_{21}O_3N \cdot HCl$: C, 61.64; H, 7.07; N, 4.50.

Found: C, 61.62, 61.36; H, 6.91, 6.98; N, 4.24.

The infrared absorption spectrum of the hydrochloride contained a strong band at 3220 cm.$^{-1}$ in the hydroxyl region, a strong band at 1740 cm.$^{-1}$ with a shoulder at 1695 cm.$^{-1}$ in the carbonyl region, and weak absorption at 1420 cm.$^{-1}$ which is probably unsaturation.

Preparation of Annotine Hydrochloride

Annotine hydrochloride was obtained from the aqueous methanolic solution following the determination of the pKa value of the alkaloid. The solution was evaporated to dryness and the crystalline residue dissolved in 0.5 ml. of methanol. Following the addition of 3 ml. acetone, the hydrochloride crystallized as very small needles, m.p. greater than 300°C. Its infrared spectrum contained a strong band at 3220 cm.$^{-1}$ in the hydroxyl region, strong carbonyl absorption at 1731 cm.$^{-1}$ with a shoulder at 1703 cm.$^{-1}$. There was no band in the 1400 cm.$^{-1}$ region. A comparison of the spectrum with that of Vb revealed that the major absorption bands were almost identical, but differences of 9 cm.$^{-1}$ were found in the carbonyl region. There was, however, unmistakable non-identity in the finger-print region.

Attempt to prepare an Oxime of Annotine

The method used was that of Douglas, Lewis, and Marion (3).

Annotine, 500 mg., was dissolved in 20 ml. of ethyl alcohol, and 2.5 ml. of 1 M hydroxylamine and 3.0 ml. of 1 M sodium acetate solutions added. The resulting solution was then heated on the steambath for three hours. After allowing to cool to room temperature, one drop of 10 N sodium hydroxide was added, but even after standing for twenty-four hours in the refrigerator, no crystallization had taken place. The solution was evaporated to about one-half of its original volume and again set aside in the refrigerator. A crystalline deposit formed which showed no depression of the melting point on admixture with the starting material.

Treatment of Annotine with Acetic Anhydride

To 15 ml. of acetic anhydride was added 450 mg. of annotine, Ib, and the solution heated under reflux. After about fifteen minutes the reaction mixture had become very dark and the reaction was stopped after a further fifteen minutes. Excess acetic anhydride was decomposed by shaking with ice water, the solution basified with 20 per cent sodium hydroxide solution and then extracted with chloroform until the extracts were colourless. The combined extracts were dried over anhydrous sodium sulfate and concentrated 'in vacuo'. It was then absorbed on a column of alumina (Fisher) and eluted with chloroform. A colourless band rapidly passed through the column; evaporation of the solution gave about 120 mg. of a crystalline compound, VIb, which after recrystallization from methanol melted at 180.7 - 182.2°C.

Anal. Calc. for $C_{18}H_{23}O_4N$: $\%CH_3CO$, 13.56.

Found: $\%CH_3CO$, 12.00, 12.65.

In its infrared absorption spectrum it contained strong bands at

1732 cm.$^{-1}$ and 1250 cm.$^{-1}$ attributable to the acetoxy group; and a band at 1715 cm.$^{-1}$ due to the carbonyl group.

Alkaline Hydrolysis of Acetylannotine, VIb

A solution of 50 mg. of VIb in 10 ml. of alcoholic sodium hydroxide was refluxed for one hour on the steam bath. The solution was evaporated almost to dryness, diluted with water (25 ml.) and extracted several times with chloroform. The dried chloroform extracts were concentrated 'in vacuo' to yield 25 mg. of a viscous residue, which could not be induced to crystallize. Its infrared spectrum was identical to that of annotine. The residue was dissolved in 2 ml. of acetone and acidified with one-half drop of concentrated hydrochloric acid, and seeded with a crystal of annotine hydrochloride (see page 51). The flask immediately became a mass of crystals. The infrared absorption spectrum was superimposable on that of annotine hydrochloride, and there was no depression of the mixed melting point.

Treatment of Annotine, Ib, with Phenyl Lithium

Phenyl lithium was prepared from 0.35 gm. lithium and 3.7 gm. of bromobenzene in 30 ml. of anhydrous ether. To the phenyl lithium was slowly added a solution of 900 mg. of annotine in 100 ml. ether-benzene. During the addition a white complex separated out. The reaction mixture was refluxed for one hour following the addition of the annotine. The cooled reaction mixture was decomposed by pouring it onto ice containing glacial acetic acid. The organic layer was separated, and the acid aqueous solution washed once with 50 ml. ether. The aqueous solution was then basified with 20 per cent sodium hydroxide solution whereupon a white precipitate formed; this was extracted with chloroform. The combined

chloroform extracts were dried over sodium sulfate; concentration 'in vacuo' yielded 1.30 gm. (93 per cent theory) of a white crystalline product. The compound was refluxed with 250 ml. of acetone until dissolved, then concentrated to about 50 ml., and set aside to crystallize. The crystalline compound obtained softened at 130°C but it was not completely molten until 213 - 214°C. It was recrystallized again from acetone but without change in the melting point. Upon drying the compound, it lost 24 per cent of its weight. $C_{22}H_{27}O_3N \cdot 2CH_3COCH_3$ would require a 24.7 per cent loss in weight. The infrared spectrum of the undried compound contained very strong absorption in the carbonyl region at 1710 cm.$^{-1}$. A sample was recrystallized from methanol to yield a product, m.p. 214°C. In the infrared, it contained no bands in the carbonyl region. It, too, was solvated because on drying 'in vacuo' it lost 27.6 per cent of its weight corresponding to $C_{22}H_{27}O_3N \cdot 4CH_3OH$.

In its infrared spectrum the dry material contained no bands in the carbonyl region. There was a strong band in the hydroxyl region at 3470 cm.$^{-1}$, and a weak band at 1640 cm.$^{-1}$ attributable to non-benzenoid unsaturation. There were strong bands in the finger-print region attributable to a benzene ring.

Anal. Calc. for $C_{22}H_{27}O_3N$: C, 74.75; H, 7.70.

Found: C, 74.30, 74.32; H, 7.69, 7.70.

Part (ii) - Diphenylannotinine

Isolation of Annotinine

Several batches of Lycopodium annotinum collected in Nova Scotia and one collected in Algonquin Park, Ontario, were extracted by the method of Manske and Marion (2). It was found that the yield of annotinine from collections of plant material made during the latter part of the summer of 1955 was in the order 0.12 - 0.17 per cent. The material collected in the spring gave a yield of 0.10 per cent together with a high yield of sugars, not yet identified.

Treatment of Annotinine with Phenyl Lithium

To a solution of phenyl lithium, prepared from 1.2 gm. lithium and 14.5 gm. bromobenzene in 100 ml. of anhydrous ether, there was added a solution of 3.0 gm. annotinine in 70 ml. of ether-benzene. There was immediate precipitation of a white complex which soon dissolved. After the addition of the annotinine (fifteen minutes), the reaction mixture was refluxed for one and one-half hours, allowed to cool to room temperature, poured onto ice-water and the organic layer separated. The aqueous layer was extracted several times with fresh portions of ether. The combined extracts were dried over sodium sulfate and then concentrated 'in vacuo'. The residue was dissolved in benzene, absorbed on a column of alumina (Fisher), and eluted with benzene until no more diphenyl, a by-product, was obtained. Diphenylannotinine, IIc*, was then eluted from

* Diphenylannotinine derivatives will be designated 'c'.

the column with 2 vol. per cent methanol in chloroform. Removal of the solvent yielded a pale yellow residue (5.0 gm.) which was dissolved in 70 ml. of hot benzene and set aside for twelve hours, after which time a cluster of small pale yellow plate-like crystals had formed. They possessed a double melting point at 128 and 236°C, neither of which was particularly sharp. For analysis a sample was dried at 118°C at 0.5 mm. Hg pressure for twelve hours, when it had lost 21 per cent of its weight indicating that the compound, IIc, was highly solvated. It then exhibited a single melting point at about 236°C.

Anal. Calc. for $C_{28}H_{35}O_4N$: C, 74.83; H, 7.85; N, 3.12.

Calc. for $C_{28}H_{33}O_3N$: C, 77.93; H, 7.71; N, 3.25.

Calc. for $C_{28}H_{33}O_3N \cdot 1/3C_6H_6$: C, 78.74; H, 7.71; N, 3.06.

Calc. for $C_{28}H_{33}O_3N \cdot 1/2C_6H_6$: C, 79.11; H, 7.71; N, 2.98.

Found: C, 78.90; H, 8.07; N, 3.30.[1]

Found: C, 78.85, 78.83, 78.83, 78.87; H, 7.65, 7.69, 7.32, 7.49.[2]

pKa = 5.9 (50% methanol-water)

$[\alpha]_D^{19} = +7.8°$ (c, 5% chloroform)

The infrared spectrum contained a strong band in the hydroxyl region at 3400 $cm.^{-1}$, but only weak aromatic absorption at 1600 $cm.^{-1}$. In carbon disulfide solution there were two absorption bands in the hydroxyl region at 3603 $cm.^{-1}$ and 3460 $cm.^{-1}$. The three typical bands for a mono-substituted benzene compound were found at 3080 $cm.^{-1}$, 3052 $cm.^{-1}$, and 3021 $cm.^{-1}$.

The hydrochloride salt was prepared by dissolving 500 mg. of diphenylannotinine, IIc, in 10 ml. of acetone and adding a few drops of

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1. Drs. F. Weiler and F. B. Strauss, Oxford, England.

2. Mr. A. E. Ledingham, Guelph, Ontario.

concentrated hydrochloric acid until the solution was just acid. There was immediate precipitation of the salt which was recrystallized several times from methanol-acetone for analysis, m.p. 292.2 - 293.2°C.

Anal. Calc. for $C_{28}H_{33}O_3N \cdot HCl \cdot H_2O$: C, 69.19; H, 7.47; N, 2.88

Found: C, 69.15, 68.95; H, 7.45, 7.40; N, 2.80, 2.70.

Treatment of diphenylannotinine, IIc, with concentrated hydrochloric acid, phenyl lithium, and lithium aluminium hydride led to no new products.

Treatment of Diphenylannotinine, IIc, with Phosphorous Oxychloride

In 25 ml. of dry pyridine was dissolved 500 mg. of IIc, to the resulting solution was added 5 ml. phosphorous oxychloride which was allowed to stand for thirty-six hours. To the solution was added a little water to hydrolyse excess phosphorous oxychloride. It was then concentrated 'in vacuo', sodium hydroxide solution added till the solution was alkaline, and finally extracted with benzene. The combined benzene extracts were dried over anhydrous sodium sulfate, and concentrated to dryness 'in vacuo'. The dark brown residue was warmed with 25 per cent sulfuric acid, in which it was insoluble. It was extracted from the acid solution with chloroform; evaporation of the solvent yielded an oily residue which was crystallized from methanol. After several recrystallizations it melted at 276°C, with darkening at 220°C. The compound gave a positive test for halogen (Beilstein test). In its infrared spectrum it contained hydroxyl absorption at 3385 $cm.^{-1}$ and an unassigned band at 2350 $cm.^{-1}$. Since the desired unsaturated compound had not been obtained the compound was not submitted for elementary analysis.

Chromic Acid Oxidation of Diphenylannotinine, IIc

To a solution of 700 mg. of IIc in 3 ml. of glacial acetic acid was rapidly added a mixture of 400 mg. chromic acid anhydride in 5 ml. glacial acetic acid containing just sufficient water to dissolve the CrO_3. There was immediate separation of a dark brown sludge which soon dissolved. The reaction mixture was immediately poured onto excess ammonium hydroxide in 100 gm. ice and exhaustively extracted with chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate and then finally filtered through alumina. Concentration of the filtrate yielded 380 mg. (54 per cent theory) of a viscous yellow oil which crystallized immediately on contact with methanol. After several recrystallizations the compound, IIIc, was obtained as small white needles, m.p. 242.1°C. (Meier and Marion (26) reported 250°C.)

Anal. Calc. for $C_{28}H_{33}O_4N$: C, 75.15; H, 7.43; N, 3.13; Active H (2) 0.44.

Found: C, 75.60, 75.36; H, 7.05, 6.81; N, 3.49; Active H, 0.42, 0.48.

pKa = 4.16 (acetone)

$[\alpha]_D^{20} = +55.8°$ (c, 5% chloroform)

The infrared absorption spectrum contained a band in the carbonyl region at 1722 $cm.^{-1}$ and in the hydroxyl region at 3300 $cm.^{-1}$. In carbon disulfide solution it contained three bands in the hydroxyl region at 3595 $cm.^{-1}$, 3462 $cm.^{-1}$, and 3320 $cm.^{-1}$.

Treatment with concentrated hydrochloric acid or alcoholic alkali yielded only unchanged starting material.

Reduction of $C_{28}H_{33}O_4N$, IIIc, with Lithium Aluminium Hydride

To a solution of 250 mg. of IIIc in 30 ml. of dry ether was added

a solution of 0.10 gm. of $LiAlH_4$ in 50 ml. dry ether and the mixture refluxed for one and one-half hours. The excess $LiAlH_4$ was destroyed by careful addition of aqueous ether, the reaction mixture was poured onto 100 gm. ice containing 5 ml. of concentrated hydrochloric acid. The solution was basified with ammonia and extracted several times with ether. The combined ether extracts were dried over sodium sulfate and concentrated 'in vacuo' to yield 200 mg. of a white crystalline compound, IVc. After recrystallization from methanol it melted at 260.9°C. Upon drying the compound, it lost 11 per cent of its weight, $C_{28}H_{35}O_4N \cdot 2CH_3OH$, would require a 12.4 per cent loss in weight.

Anal. Calc. for $C_{28}H_{35}O_4N \cdot 1/2CH_3OH$: C, 73.68; H, 7.81; N, 3.02.

Found: C, 73.96, 73.87; H, 7.77, 7.73; N, 3.23, 3.04.

pKa = 5.4 (50% methanol-water)

$[\alpha]_D^{19} = +13.5°$ (c, 5% chloroform)

The infrared spectrum contained weak absorption in the hydroxyl region at 3350 $cm.^{-1}$.

Treatment with concentrated hydrochloric acid yielded only unchanged starting material.

Oxidation of Diphenylannotinine with Chromic Acid Anhydride in Pyridine

Several samples of diphenylannotinine were treated with the chromic acid-pyridine reagent of Sarett (20). The same oxidation product, IIIc, was obtained but the yields were low (5 - 10 per cent) over the range of the experimental conditions employed.

Oppenauer Oxidation of Diphenylannotinine, IIc

Diphenylannotinine, 1.0 gm., was added to a suspension of 0.6 gm.

of potassium tert.-butoxide in a mixture of 3.0 gm. dry cyclohexanone and 35 ml. of anhydrous toluene. The mixture, which became bright yellow, was refluxed for one-half hour. After allowing to cool to room temperature, the mixture was poured on ice, the organic layer separated, and the aqueous layer extracted with fresh toluene. The combined toluene extracts were washed three times with 25 ml. portions of 10 per cent hydrochloric acid. The combined acid extracts were washed once with ether, then basified with ammonia and exhaustively extracted with chloroform. The dried chloroform extract was concentrated on the steam bath prior to absorption on a column of alumina (Fisher). Elution with 2 vol. per cent methanol in chloroform yielded, after evaporation of the solvent, 610 mg. (61 per cent theory) of a crystalline product, Vc. Following recrystallization from methanol, it melted at 244.2 - 245.2°C. A mixed melting point with IIIc was completely liquid at 213°C.

Anal. Calc. for $C_{28}H_{31}O_3N$: C, 78.29; H, 7.28; N, 3.26; Active H (1) 0.27

Found: C, 77.84, 77.81; H, 7.42, 7.45; N, 3.04, 3.02; Active H, 0.23.

pKa = 3.35 (acetone)

$[\alpha]_D^{19}$ = + 90.8° (c, 5% chloroform)

The infrared absorption spectrum contained strong carbonyl absorption at 1706 $cm.^{-1}$ and weak hydroxyl absorption at 3300 $cm.^{-1}$. In carbon disulfide there was a single hydroxyl band at 3595 $cm.^{-1}$.

Treatment of Vc with concentrated hydrochloric acid and hydrogen yielded only unchanged starting material.

Reduction of $C_{28}H_{31}O_3N$, Vc, with Lithium Aluminium Hydride

The Oppenauer oxidation product, 200 mg., in 50 ml. of anhydrous ether was treated with 2.0 ml. of a 2.5 M solution of $LiAlH_4$ in ether and refluxed for forty-eight hours; shorter periods of reflux gave incomplete reduction. The excess $LiAlH_4$ was destroyed with moist ether, the ether layer separated, dried, and concentrated 'in vacuo'. The residue, 150 mg., was dissolved in chloroform and absorbed on a column of alumina (Fisher), eluting with chloroform. Several fractions were collected and the major fraction, 80 mg., separated and examined. It was amorphous and had an infrared spectrum identical to that of diphenylannotinine. Treatment of an acetone solution of this fraction with concentrated hydrochloric acid gave an immediate precipitate of a crystalline hydrochloride. Two types of crystals appeared to be present, one dense and granular, and the other feather-like. The former, which comprised the major fraction, did not depress the melting point of diphenylannotinine hydrochloride. There was not enough of the second fraction to determine its properties.

Reduction of $C_{28}H_{31}O_3N$, Vc, with Sodium Borohydride

To a solution of 0.10 gm. of $NaBH_4$ in 25 ml. of ethanol was added 500 mg. of Vc. After allowing to stand overnight, a few drops of glacial acetic acid were added to destroy excess hydride. Most of the alcohol was distilled off and the residue dissolved in water, basified with ammonia, then chloroform extracted. The dried extracts yielded 450 mg. of a glassy product whose infrared spectrum was superimposable on that of diphenylannotinine. The hydrochloride was prepared in the usual manner

(acetone and HCl) and recrystallized from methanol-acetone. It melted at 289°C, but depressed the melting point of diphenylannotinine hydrochloride twenty-five degrees.

Anal. Calc. for $C_{28}H_{33}O_3N \cdot HCl \cdot H_2O$: C, 69.19; H, 7.47.

Found: C, 68.20, 68.55; H, 7.27, 7.50.

Chromic Acid Oxidation of $C_{28}H_{31}O_3N$, Vc

To a solution of 800 mg. of Vc in 5 ml. of glacial acetic acid was added a solution of 700 mg. chromic acid anhydride in 8 ml. of glacial acetic acid containing just sufficient water to dissolve the CrO_3. After five minutes, the reaction product was poured onto excess ammonia in ice, and exhaustively extracted with chloroform. The dried extracts were combined and filtered through alumina. Concentration of the filtrate yielded 600 mg. of a brown viscous oil which crystallized immediately on contact with methanol. After recrystallization from methanol it melted at 241.1 - 242.1°C; a mixed melting point with the starting material was liquid at 219°C, whilst a mixed melting point with a sample of IIIc was undepressed. Their infrared spectra were identical.

Oxidation of Diphenylannotinine, IIc, with Potassium Dichromate in Sulfuric Acid

Diphenylannotinine, 1.5 gm., was treated with 5.0 gm. of potassium dichromate in 20 ml. of 50 per cent sulfuric acid. The mixture was directly distilled until oily droplets were no longer present in the distillate. Water was added to keep the level in the reaction flask approximately constant. The distillate was ether extracted, the extracts dried over sodium sulfate, and concentrated to yield 30 mg. of residue. The

residue was partitioned between ether and sodium bicarbonate solution. The ether soluble portion yielded about 10 mg. of a yellowish oil which had an infrared spectrum identical to that of benzophenone. When this oil was treated with a hot solution of 2,4-dinitrophenylhydrazine in alcohol a yellow precipitate formed, m.p. 230.7 - 235.7°C. The mixed melting point with an authentic sample of benzophenone-2,4-dinitrophenylhydrazone was 238 - 240°C. The recorded melting point of the hydrazone is 238°C. The infrared absorption spectra of the two hydrazones were identical.

The bicarbonate phase was acidified and ether extracted. Evaporation of the ether yielded a yellowish solid which, after recrystallization from water, melted at 120.2 - 121.2°C and did not depress the melting point of benzoic acid.

The acidic solution from the reaction flask was basified with ammonia and extracted several times with chloroform. The combined chloroform extracts were dried over sodium sulfate, and concentrated 'in vacuo' to yield 20 mg. of an oily residue which crystallized on contact with methanol. After recrystallization from methanol it proved to be identical (infrared spectrum and mixed melting point) with IIIc.

Treatment of Annotinine Hydrate, II, with Phenyl Lithium

Phenyl lithium prepared in the manner described above from 0.13 gm. of lithium was treated with 0.36 gm. of annotinine hydrate prepared in the manner described by Manske and Marion (8). The hydrate was introduced into the system by continuous extraction from a soxhlet with refluxing ether. About twelve hours was required for the addition because of

the low solubility of annotinine hydrate in ether. The mixture was decomposed on ice-hydrochloric acid, the aqueous layer separated, basified with ammonia, and extracted with chloroform. Evaporation of the chloroform yielded a viscous residue which deposited a few crystals on treatment with methanol. Some 30 mg. of material was separated which melted at 190 - 192.1°C, showed hydroxyl absorption in the infrared at 3530 $cm.^{-1}$, and weak unassignable bands at 3200 $cm.^{-1}$, 1720 $cm.^{-1}$, and 1750 $cm.^{-1}$. The compound was probably contaminated with starting material.

The mother liquors from the separation of this compound deposited a second crop of crystals on treatment with ether. About 100 mg. of this compound which melted at 214 - 216°C was obtained. On admixture with the starting material and diphenylannotinine, samples were liquid at 198.4 and 181.7°C respectively. The mixed melting point with the previously isolated compound was 207.5°C; and the infrared spectra were almost identical indicating a similarity of the two.

The compound formed a hydrochloride which after recrystallization from methanol-acetone melted at 238°C.

Anal. Found: C, 67.58, 67.47; H, 7.62, 7.65; N, 3.78, 3.69.

Inspection of these figures does not allow calculation of a reasonable formula for the compound.

Treatment of $C_{28}H_{33}O_4N$, IIIc, with Acetic Anhydride

A mixture of 500 mg. of IIIc in 30 ml. acetic anhydride was refluxed for four hours. It was allowed to cool to room temperature, the excess acetic anhydride was hydrolysed with water, basified with ammonia, and exhaustively extracted with chloroform. The combined chloroform

extracts were dried over sodium sulfate and concentrated 'in vacuo'. The concentrated solution was absorbed on a column of alumina (Fisher) and eluted with chloroform. A pale yellow band passed rapidly through the column and was collected; evaporation of the solvent yielded 350 mg. of a pale yellow solid, VIc, which crystallized on contact with ether. Its infrared spectrum contained no bands in the hydroxyl region; there was a broad peak at 1732 $cm.^{-1}$, with a shoulder at 1770 $cm.^{-1}$. A strong band at 1240 $cm.^{-1}$ was attributed to an acetoxy group. In chloroform solution there was a broad absorption band at 3500 $cm.^{-1}$, a double peak at 1735 and 1720 $cm.^{-1}$, and weak bands at 1680 $cm.^{-1}$, 1655 $cm.^{-1}$, and 1600 $cm.^{-1}$. The compound was not purified further but was used directly in the following experiment.

Alkaline Hydrolysis of VIc

The compound, 300 mg., was refluxed for two hours with 10 ml. of alcoholic sodium hydroxide. The alcohol was distilled off, water added, and the yellow suspension extracted with chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate and concentrated 'in vacuo' to yield 250 mg. of a yellow oil which crystallized immediately on contact with methanol, melting point, 240 - 242.1°C; it did not depress the melting point of IIIc.

SUMMARY

Part (i) - The Minor Alkaloids

Annotoxine, $C_{32}H_{44}O_5N_2$, has for the first time been isolated from Lycopodium annotinum of Canadian origin. It was separated into acrifoline, $C_{16}H_{23}O_2N$, and annotine, $C_{16}H_{21}O_3N$, (alkaloid L 11) by chromatography and also through the hydrobromide salts.

Acrifoline was shown to possess one hydroxyl group, one ketone carbonyl group, and one centre of unsaturation. A determination of the basicity constants of acrifoline and dihydroacrifoline suggested that acrifoline is probably an allylamine. Acrifoline was acetylated with acetic anhydride to form the monoacetate, $C_{18}H_{25}O_3N$, which was identical to alkaloid L 12 previously isolated by Manske and Marion from Lycopodium annotinum L. (2). Acrifoline could not be dehydrated with concentrated hydrochloric acid or phosphorous oxychloride.

Annotine (alkaloid L 11) was also shown to contain a single hydroxyl group, one ketone carbonyl group, and one centre of unsaturation. A comparison of the basicity constant of annotine and dihydroannotine revealed that annotine may also be an allylamine. The non-reactivity of the third oxygen function suggests that it is present as an ether linkage which is probably not an epoxide ring.

Part (ii) - Diphenylannotinine

Annotinine, $C_{16}H_{21}O_3N$, was treated with phenyl lithium to yield diphenylannotinine which was crystallized from benzene. The compound was solvated, even after drying 'in vacuo'. It analysed very closely for $C_{28}H_{33}O_3N$, IIc, and the analytical data cannot be reconciled with the O_4 compound $C_{28}H_{35}O_4N$ reported by Meier and Marion (26). The Oppenauer oxidation of diphenylannotine yielded the weak ketonic base, $C_{28}H_{31}O_3N$, Vc, which contained one hydroxyl group. Its reduction back to $C_{28}H_{33}O_3N$ confirms the O_3 formulation for diphenylannotinine. Chromic acid oxidation of Vc yielded the weak ketonic base $C_{28}H_{33}O_4N$, IIIc, which could be obtained directly by the oxidation of diphenylannotinine. Hydride reduction of IIIc yielded $C_{28}H_{35}O_4N$, IVc. Neither IIc, IIIc, IVc, or Vc could be dehydrated under the conditions employed.

The infrared spectrum of diphenylannotinine indicated the presence of two hydroxyl groups and that of the Oppenauer oxidation product indicated one hydroxyl group which was supported by the active hydrogen determination. The third oxygen is probably present in an ether linkage, which may be the original epoxide ring of annotinine.

BIBLIOGRAPHY

1. Boedeker, K.; Ann. 208, 363 (1881). Quoted in reference (9).

2. Manske, R. H. F., Marion, L.; Can. J. Research B21, 92 (1943)

3. Douglas, B., Lewis, D. G., Marion, L.; Can. J. Chem., 31, 272 (1953)

4. Wiesner, K.; Summer Seminar on Natural Product Chemistry, University of New Brunswick, 1955

5. Orechov, A.; Arch. Pharm. 272, 673 (1934), Chem. Abst. 28, 5596 (1934).

6. Muszynski, J.; Arch. Pharm. 273, 452 (1935), Chem. Abst. 30, 1178 (1936)

7. Moore, B. P., Marion, L.; Can. J. Chem., 31, 952 (1953)

8. Manske, R. H. F., Marion, L.; J. Am. Chem. Soc., 69, 2126 (1947)

9. Bertho, A., Stoll, A.; Ber., 85, 663 (1952)

10. Achmatowicz, O., Rodewald, W.; Roczniki Chem., 29, 509 (1955)

11. Bertho, A.; private communication, January, 1956

12. MacLean, D. B., Manske, R. H. F., Marion, L.; Can. J. Research B28, 460(1950)

13. Marion, L., Manske, R. H. F.; Can. J. Research B20, 153 (1942)

14. Henderson, D. R., Stonner, F. W., Valenta, Z., Wiesner, K.; Chem. and Ind. 852 (1954)

15. MacLean, D. B., Prime, H. C.; Can. J. Chem., 31, 543 (1953)

16. Meier, H. L., Meister, P. D., Marion, L.; Can. J. Chem., 32, 268 (1954)

17. Vexlearschi, G., Rumpf, P.; Compt. rend., 236, 939 (1953)

18. Henderson, D. R., Stonner, F. W., Valenta, Z., Wiesner, K.; Chem. and Ind. 544 (1954)

19. Anet, F. A. L., Marion, L.; Can. J. Chem. 33, 849 (1955), Chem. and Ind., 1232 (1954)

20. Poos, G. I., Arth, G. E., Beyler, R. E., Sarett, L. H.; J. Am. Chem. Soc., 75, 427 (1953)

21. Bankiewicz, C., Henderson, D. R., Stonner, F. W., Valenta, Z., Wiesner, K.; Chem. and Ind., 1068 (1954)

22. Henderson, D. R.; Ph. D. Thesis, University of New Brunswick, 1955

23. Reissert, A.; Ber., 24, 845 (1891)

24. Bankiewicz, C.; unpublished work quoted in (22)

25. MacLean, D. B.; unpublished results

26. Meier, H. L., Marion, L.; Can. J. Chem., 32, 280 (1954)

27. Stonner, F. W.; Ph. D. Thesis, University of New Brunswick, 1955

28. Wittig, G.; Angew. Chem., 53, 24 (1941)

29. Woodward, R. B., Wendler, N. L., Brutschy, F. J.; J. Am. Chem. Soc., 67, 1425 (1945)

30. Slack, R., Waters, W. A.; J. Chem. Soc. 1666, (1948)