LYCOPODIUM ALKALOIDS

XVI. ANNOTINE

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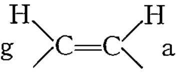
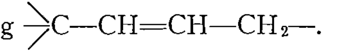
ABSTRACT

Annotine, $C_{16}H_{21}O_3N$, is shown to be pentacyclic and to contain a tertiary hydroxyl group, a lactone function, a tertiary nitrogen atom, and a dialkylated double bond. The position of the double bond and the tertiary hydroxyl group relative to the nitrogen atom has been established by Emde degradation of annotine methiodide. The presence of a lactone function is inferred from the reduction of annotine to dihydroannotinol, a hemiacetal, which reacts with 1 mole of ethyl mercaptan. The reduction of the lactone to a diol in an annotine derivative has been carried out. The chemical studies and the examination of annotine and its derivatives by modern instrumental methods allow the assignment of a plausible structure to the alkaloid.

Annotine, $C_{16}H_{21}O_3N$ (I), was first isolated from *L. annotinum* of Canadian origin by Manske and Marion and designated alkaloid L.11 (1). It was subsequently found in European plant material by Bertho and Stoll (2), who assigned the name annotine to it, and by Achmatowicz and Rodewald (3). An examination of the functional groups of annotine was carried out by Perry and MacLean (4), who concluded that the alkaloid contained a tertiary nitrogen atom, a double bond, a hydroxyl group, a ketonic carbonyl group, and a third inert oxygen function, which they ascribed to an ether linkage other than an epoxide.

In this communication we wish to report further studies on annotine by chemical and modern instrumental methods. These studies have shown that annotine is a lactone and have thereby established the nature of the third inert oxygen function. They have also allowed an assignment of the position of the double bond and the hydroxyl group in the molecule relative to the nitrogen atom and have led to the development of a plausible structure for the molecule.

Nuclear magnetic resonance (n.m.r.) spectroscopy has been used extensively in this study and has provided useful information about the functional groups and their environment in annotine and its derivatives. The n.m.r. spectrum of annotine has a quartet centered at $\tau = 4.04$ ($J = 10$ c.p.s.) with an intensity corresponding to two protons. This band indicates the presence of the grouping  $>C(H){=}C(H)<$ and is an example of an intermediate case of an AB spectrum in which the chemical shift between the two nuclei is comparable to the coupling constant between them (5). It was possible to resolve each component of the quartet into a triplet in each of which the splitting was the same. These data indicate the presence of a methylene group adjacent to the double bond, as in the grouping  $>C{-}CH{=}CH{-}CH_2{-}$.

A singlet occurring at $\tau = 8.57$ with an intensity corresponding to three protons points to the presence of a methyl group attached to a quaternary carbon. The low τ value of the C—methyl group in this system can possibly be explained by the deshielding effect of a carbonyl group beta to the methyl protons. It has been postulated that the deshielding effect in such a system is due to the anisotropy of the carbonyl group and that the deshielding effect will be greatest if the methyl group lies in the plane of the carbonyl group (5).

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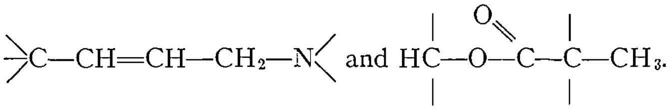
The n.m.r. spectrum also shows a band at $\tau = 5.83$, corresponding in intensity to two protons. This band in more concentrated solution appears as two overlapping peaks, which are tentatively assigned to the groupings $\rangle\mathrm{CH{-}O{-}C}\overset{\mathrm{O}}{\langle}$ and $-\!\!\!\rangle\mathrm{C{-}OH}$. It should be pointed out at this stage that the hydroxyl group in annotine is believed to be tertiary, since the substance is recovered unchanged after treatment with chromic acid or by the Oppenauer method. Other peaks in the spectrum are more difficult to assign, but one might suggest that the peak at $\tau = 6.63$, corresponding to two protons, may be attributed to a methylene group sandwiched between the double bond and the nitrogen atom, while the peak at 7.32 is due to a second methylene group adjacent to nitrogen but attached to a saturated center.

In an effort to check the assignments above, we examined the n.m.r. spectrum of dihydroannotine (II). We found that the preparatory procedure reported previously (4) gave a product of uncertain composition, but that catalytic hydrogenation over platinum in the presence of a trace of hydrochloric acid gave a pure dihydroannotine in high yield. Its n.m.r. spectrum lacks the ethylenic absorption of annotine but retains the $-\!\!\!\rangle\mathrm{C{-}CH_3}$ absorption at $\tau = 8.56$. The proton of the hydroxyl group probably gives rise to the sharp band observed in the spectrum at $\tau = 6.82$. A band of intensity equal to one proton at $\tau = 5.74$ has been assigned again to the grouping $\rangle\mathrm{CH{-}O{-}C}\overset{\mathrm{O}}{\langle}$. The half-band width of 9.9 c.p.s. for this band indicates coupling to three or four protons.

The above n.m.r. data establish that the double bond is dialkylated and joined to a methylene group on one side and probably to a quaternary center on the other side. They show that the methyl group is attached to a quaternary carbon center and that it may be beta to a carbonyl function. The presence of the peak centered at 5.83 in annotine and 5.74 in the dihydro compound implies the presence of a hydrogen attached to a carbon carrying an ester function. This observation provided the first clue that annotine might be a lactone. The following structural sequences are suggested from the n.m.r. data:



$$-\!\!\!\rangle\mathrm{C{-}CH{=}CH{-}CH_2{-}N}\langle \quad \text{and} \quad \mathrm{H\overset{|}{\underset{|}{C}}{-}O{-}\overset{O\,\backslash\!\backslash}{C}{-}\overset{|}{\underset{|}{C}}{-}CH_3}.$$

The experiments reported below support both of these structural assignments.

The sequence $-\!\!\!\rangle\mathrm{C{-}CH{=}CH{-}CH_2{-}N}\langle$ suggested by the n.m.r. spectra of annotine and dihydroannotine has been confirmed and extended by degradation of annotine methiodide. Annotine readily forms a methiodide, and attempts were made to degrade it by several methods. An attempted elimination with potassium tertiary butoxide yielded an oil which resisted all attempts at crystallization. Attempts to prepare crystalline salts were also unsuccessful. Attention was therefore turned to the Emde degradation. Although annotine methiodide did not react with sodium amalgam, it readily reacted with hydrogen over Adams' catalyst to form a crystalline saturated compound III, $C_{17}H_{27}O_3N$.

The infrared spectrum of III shows absorption at 3485 cm^{-1} in the hydroxyl region and strong absorption at 1712 cm^{-1} in the carbonyl region. The n.m.r. spectrum of III shows that no ethylenic protons are present. It does indicate, however, the presence of two C—methyl groups in the compound with absorptions at $\tau = 8.55$ and $\tau = 8.98$. The signal at $\tau = 8.55$ is a single sharp peak and corresponds to the original $-\!\!\!\rangle\mathrm{C{-}CH_3}$

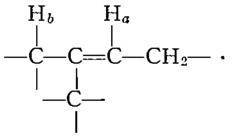
group in the alkaloid. The band at $\tau = 8.98$ is quite broad, indicating coupling with neighboring protons. This band is assigned to a methyl group at the end of an alkyl chain formed by cleavage of a C—N bond in the hydrogenolysis reaction. A single sharp peak occurring at $\tau = 7.47$ with an intensity corresponding to three protons is assigned to the N—CH$_3$ group in the compound. A broad band at $\tau = 5.73$ is also present in the n.m.r. spectrum of compound III.

Significantly, dihydroannotine methiodide did not undergo an Emde degradation when treated with hydrogen over Adams' catalyst. The driving force for the Emde degradation of annotine must be the presence of the double bond, in a position β,γ to the nitrogen. In support of the latter hypothesis it should be noted that the pK_a values of annotine and dihydroannotine were found to be 7.5 and 8.61 respectively (4). These results substantiate the n.m.r. assignment.

The presence and the nature of an alkyl side chain in the Emde degradation product III were established by a modified Kuhn–Roth oxidation (6). The volatile carboxylic acids produced were separated and identified by gas chromatography of their methyl esters. Oxidation of the Emde degradation product III yielded acetic and propionic acids, a result that established the presence of an ethyl group in the compound. When the same oxidation procedure was applied to annotine itself, there was no carboxylic acid detected by gas chromatography. Considering the evidence for a $\rightarrow$C—CH$_3$ group in the alkaloid, one would have expected acetic acid to have been produced by the oxidation—even if in low yield—because of the quaternary character of the $\rightarrow$C—CH$_3$ group.

Detailed information on the structure of the ring opened in the Emde degradation was obtained from a study of the anhydro derivative of degradation product III. Attempts to dehydrate III with thionyl chloride or phosphoric acid were unsuccessful. It was dehydrated cleanly to compound IV, $C_{17}H_{25}O_2N$, by *p*-toluenesulphonic acid or 47% hydriodic acid. The infrared spectrum of compound IV shows carbonyl absorption at 1730 cm^{-1} but it does not show any hydroxyl absorption. The ultraviolet spectrum shows no absorption characteristic of an α,β-unsaturated carbonyl system.

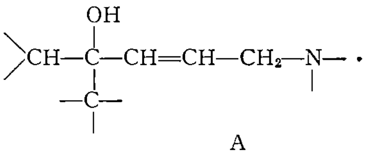
Nuclear magnetic resonance spectroscopy provided key information about the anhydro compound IV. In the n.m.r. spectrum the >N—CH$_3$ group gives rise to a single sharp peak of intensity corresponding to three protons at $\tau = 7.79$, and a $\rightarrow$C—CH$_3$ group appears as a singlet at $\tau = 8.76$. The band at $\tau = 8.96$, representing the methyl group at the end of the alkyl chain, appears as a distinct triplet, a result which establishes that the terminal methyl group is adjacent to a —CH$_2$— group. This conclusion had been reached previously from the results of the modified Kuhn–Roth oxidation of the Emde product III. The n.m.r. spectrum also shows the ubiquitous broad band at $\tau = 5.63$. Of prime importance, however, is the triplet (splitting, 6.1 c.p.s.) of relative intensity 1:2:1 at $\tau = 4.77$ corresponding to one ethylenic proton. This band is assigned to the grouping >C=CH—CH$_2$—. Each component of the triplet could be resolved into a doublet (splitting, 0.9 c.p.s.). A grouping of the following type is compatible with this observation:



The doublet can be considered to arise from spin–spin coupling between H_a and H_b with coupling constant $J_{ab} = 0.9$ c.p.s. Usually, coupling only occurs across one, two, or three bonds, although it can and does extend further in systems with double bonds.

Catalytic reduction of the anhydro compound IV led to the formation of a non-crystalline compound V, $C_{17}H_{27}O_2N$. A modified Kuhn–Roth oxidation of compound IV yielded acetic and propionic acids, whereas the saturated compound V yielded butyric acid as well as propionic and acetic acids.

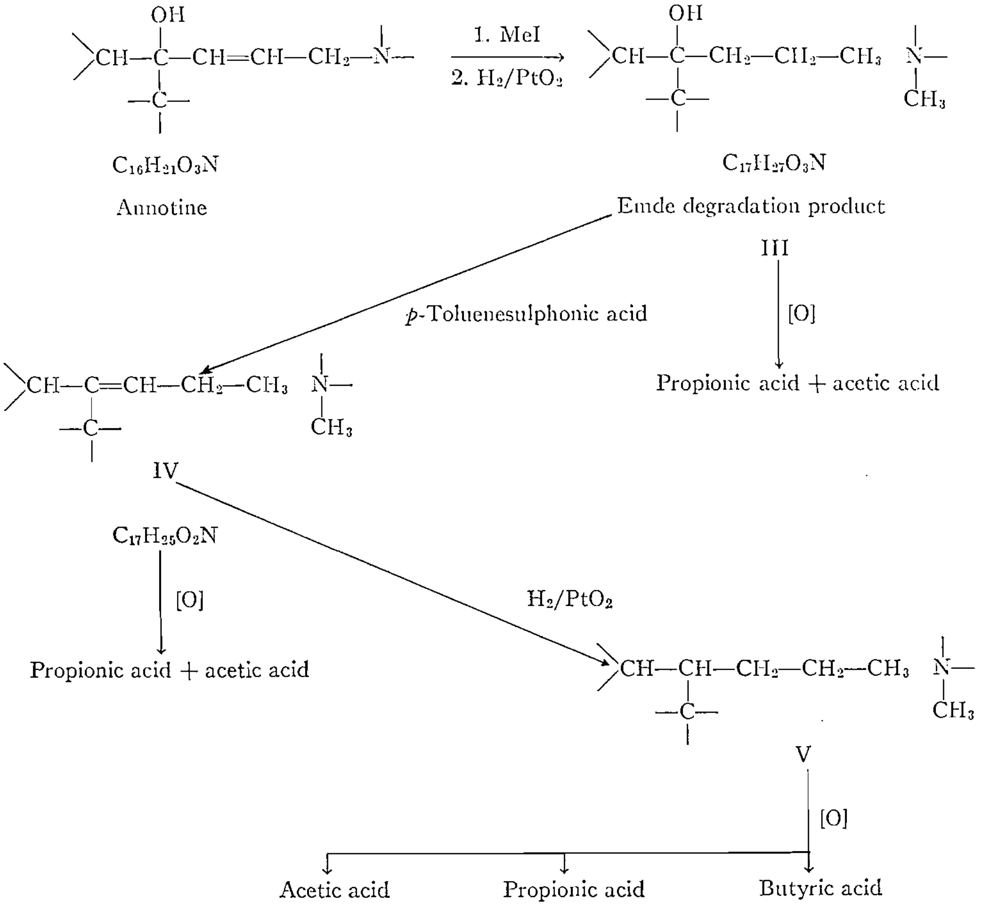
The results of the Kuhn–Roth oxidations in conjunction with information gleaned from the n.m.r. spectra of compounds derived from the Emde degradation indicate the presence of the system A in annotine:



$$\rangle CH-\underset{\underset{|}{-C-}}{\overset{OH}{C}}-CH{=}CH-CH_2-\underset{|}{N}- \cdot$$

A

The results obtained by a study of the Emde degradation of annotine are summarized in Scheme I.

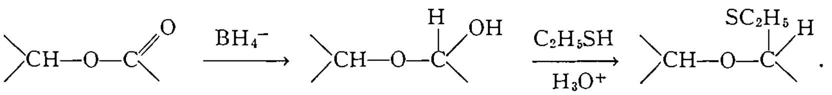


SCHEME I

It was intimated that annotine is a lactone and not a ketone, as Perry and MacLean believed (4). Their evidence for the ketonic character of the carbonyl group was based on two reactions. One of these was the treatment of annotine with sodium borohydride yielding annotinol, $C_{16}H_{23}O_3N$, which differs in composition from the starting compound by two hydrogens. Annotinol no longer had carbonyl absorption in its infrared spectrum and was converted to annotine by oxidation. The second reaction, with phenyl lithium, gave phenylannotine, $C_{22}H_{27}O_3N$. The analysis showed that a single phenyl group was incorporated and the compound showed no carbonyl absorption in its infrared spectrum. We have reexamined these two processes and have confirmed Perry and MacLean's observations. In addition, we independently established the molecular weight of phenylannotine by recording its mass spectrum, and have also examined its ultraviolet spectrum (which displayed only benzenoid absorption). There seemed little reason to doubt the presence of a ketonic carbonyl group.

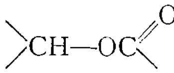
There are a number of observations, however, at variance with their assignment. The n.m.r. spectra of annotine, dihydroannotine, and the products of Emde degradation all contain a peak at $\tau = 5.7 \pm 0.1$ of an intensity of one proton. This peak is lower than might be expected of a hydrogen in a cyclic ether, $>C\mathit{H}—O—R$, but lies more in the region expected of a lactone or ester, $>C\mathit{H}—O—C\overset{\displaystyle /\!\!/ O}{<}$ (5). Secondly, we found that annotine was unreactive toward common carbonyl reagents. Thirdly, upon attempted Wolff–Kishner reduction, only a very small amount of annotine was recovered by chloroform extraction of the basic reaction mixture. It appears that annotine forms a water-soluble salt when it is heated at high temperatures with potassium hydroxide. In confirmation of this postulate, we found that annotine shows the same behavior when it is treated under conditions identical with those employed in the Wolff–Kishner reduction, but in the absence of hydrazine. Finally, a deuterium exchange study showed that there were no hydrogens in annotine that take part in enolate ion formation. At least one enolic hydrogen might be expected of a ketonic substance based on the structural framework common to the *Lycopodium* alkaloids of known structure. All the evidence noted above militates against the presence of a ketonic carbonyl group in annotine, but it does not directly support the presence of a lactone function.

There are two experiments which lend direct support to the lactone formulation for annotine. We found that dihydroannotinol, $C_{16}H_{25}O_3N$ (VI), formed by borohydride reduction of dihydroannotine, reacted with ethyl mercaptan in acid medium to form the substance $C_{18}H_{29}O_2NS$ (VII) in which a thioethyl group has apparently replaced a hydroxyl group of VI. A reaction of this nature can be readily interpreted on the basis of a lactone structure for annotine but not for a ketonic structure. Thus, the borohydride reduction of dihydroannotine would proceed to the hemiacetal, which, with ethyl mercaptan in the presence of acid, yields the product VII, as depicted by the partial formulae below:



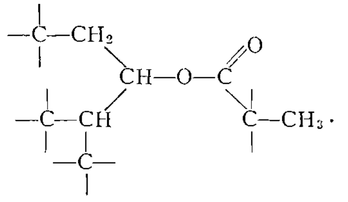
$$>CH—O—C\overset{O}{\lessdot} \xrightarrow{BH_4^-} >CH—O—\overset{H\ \ OH}{C}\!< \xrightarrow[H_3O^+]{C_2H_5SH} >CH—O—\overset{SC_2H_5\ H}{C}\!< .$$

Dihydroannotinol reverted to dihydroannotine upon Oppenauer oxidation, indicating that the reaction was a simple reduction production.

In the n.m.r. spectrum of VII there is now a singlet at $\tau = 5.17$, presumably due to the hydrogen situated on the carbon carrying the thioethyl group. It is significant that the peak present in other spectra at 5.7 ± 0.1 and assigned to the  $>CH{-}OC(=O){-}$ proton is no longer present; instead a broad peak corresponding to one proton is observed at τ = 6.19. The methyl and methylene protons of the thioethyl group appear as a triplet at τ = 8.73 and as a quartet centered at 7.35, respectively, but both underlie other absorption. The original C—methyl group has not altered appreciably in position, appearing at τ = 8.66. Apparently the two electron-attracting groups attached to the beta carbon, —SR and —OR, have a deshielding effect equivalent to a carbonyl group in the beta position. The fact that two thioethyl groups were not introduced is another indication of the stability of this cyclic system. Other examples of its unreactivity toward ring opening are the resistance of the lactone to hydrolysis and the absence of carbonyl absorption in the infrared spectra of the hemiacetal, dihydroannotinol, and the hemiketal, phenylannotine.

In the second experiment, the lactone ring was successfully reduced to a diol with lithium aluminium hydride. Perry and MacLean reported that annotine was recovered unchanged upon treatment with $LiAlH_4$ in tetrahydrofuran and we have confirmed their findings. The failure of this reaction may be due to the formation, by interaction of $LiAlH_4$ with the hydroxyl group, of an insoluble salt which separates from the medium. Accordingly, we carried out the reaction on a derivative of annotine which no longer had the hydroxyl group. Compound IV was chosen because of its relative ease of preparation. Reaction of IV with $LiAlH_4$ yielded diol VIII, $C_{17}H_{29}O_2N$. The uptake of four hydrogen atoms in the conversion IV → VIII shows that the function which underwent reduction is a lactone rather than a ketone.

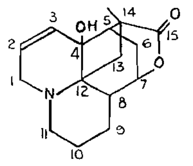
If the deductions regarding the position of the C—methyl group relative to the carbonyl carbon are correct, we may summarize these findings in expression B.



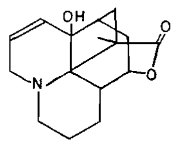
B

This formulation is compatible with the chemical and spectroscopic data presented before. Since the infrared absorption of the carbonyl group of annotine and its derivatives never appears above 1740 cm^{-1}, one must assume that the lactone is six-membered or larger.

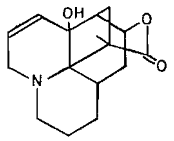
All *Lycopodium* alkaloids of established structure with a single nitrogen atom conform in structure to lycopodine (6), with the exception of annotinine (7) and lyconnotine (8). The three alkaloids are closely related, having in common a hydrojulolidine system. In lyconnotine the hydrojulolidine system is not bridged as it is in annotinine and lycopodine. Since annotine occurs in the same plant as these alkaloids and since it may be deduced from its composition that it contains a combination of four carbocyclic and (or) heterocyclic rings other than its lactone ring, it seemed likely that it might conform in skeletal structure to either lycopodine or annotinine. However, attempted incorporation of expressions A or B into the skeletal framework of either alkaloid proved impossible. Other formulations based upon the hydrojulolidine framework were therefore considered, and three of these (C, D, and E) are shown below along with the structures of annotinine, lycopodine, and lyconnotine.



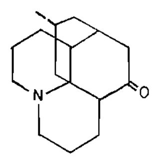
C



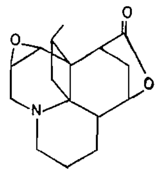
D



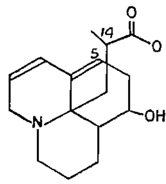
E



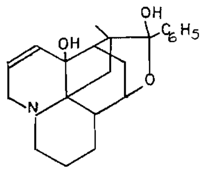
Lycopodine



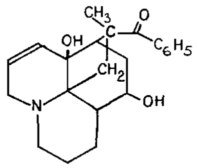
Annotinine



Lyconnotine X



IX (a)



IX (b)

The placement of the bridge in C, D, and E follows from analogy to other alkaloids of this family. This placement is supported by the n.m.r. spectrum of IV which was discussed previously. The conclusions with respect to the structure of ring D are corroborated by investigation of the pyrolysis of phenylannotine. This compound, represented by structure IX*a* and IX*b*, was heated at 300° in the presence of zinc dust. The distillate collected during the pyrolysis was examined by gas–liquid partition chromatography. It was found to consist of two substances which, from their ultraviolet spectra, appeared to be phenones. The identity of the two compounds was established by comparison of their retention times with known phenones. They proved to be propiophenone and α-methylacrylophenone. In structure IX*b* the carbon atoms participating in the formation of α-methylacrylophenone are designated.

It is appropriate to consider here whether any of the structures C, D, or E proposed for annotine is favored over the others. The conclusion, discussed previously, that dihydroannotinol and phenylannotine exist in the hemiacetal and hemiketal forms, respectively, is evidence against structure E. Examination of molecular models, based on E, of dihydroannotinol and phenylannotine reveal that considerable strain is released by opening the hemiacetal and hemiketal rings, respectively. Thus if structure E were correct, one would expect dihydroannotinol to be a hydroxyaldehyde rather than a hemiacetal and phenylannotine to exist as the hydroxyketone, provided that reduction had ceased at this stage.

Structure C is favored over D and E on the basis of the following considerations. In all *Lycopodium* alkaloids of established structure there is a methylene group present at C_{13}. There is no recorded case of substitution at this site. Recently, structure X has been proposed for lyconnotine, a new alkaloid isolated from *L. annotinum* (8). This structure bears a strikingly close relation to C. The carbon skeleton of annotine (as in C) is formally derived from X by formation of a bond between C_5 and C_{14}. Thus it may be that X and annotine are formed in the plant from a common precursor, or one may be the precursor of the other. The conversion of annotine to the carbon skeleton of X in vitro is readily visualized. Transformation of annotine to the hydroxymethyl ester (oxidation of the C_7 hydroxyl to the ketone followed by a β-elimination) leads to the carbon skeleton of X.

The configuration suggested for annotine in structure C is based upon the following considerations. Neither annotine itself nor dihydroannotine show the Bohlmann bands (9) characteristic of the *trans*-fused quinolizidine system in the 2900 cm^{-1} region of their infrared spectra. Anhydrotetrahydroannotine, obtained by dehydration of dihydroannotine over *p*-toluenesulfonic acid in xylene followed by reduction with hydrogen, does exhibit the Bohlmann bands. Reduction of the $\Delta^{3,4}$ double band would be expected to proceed from the least hindered side and to lead to the inverted configuration of C_4 and the *trans*-fused quinolizidine system. Unfortunately, neither the dehydration product nor the anhydrotetrahydroannotine were crystalline compounds and fully characterized, so that these conclusions must be accepted with some reservation. Furthermore, the hydroxyl group in dihydroannotine is intramolecularly hydrogen bonded, showing the hydroxyl absorption at 3545 cm^{-1} (which is not shifted upon dilution). This result is also compatible with the *cis*-fused quinolizidine system and is analogous to lycodoline (10), which carries a hydroxyl group in the same relative position in the hydrojulolidine system. The configuration at C_7 and C_8 follows from the stability of the lactone to hydrolysis and the stability of the hemiacetal and hemiketal forms of dihydroannotinol and phenylannotine, respectively.

EXPERIMENTAL

Apparatus and Methods

Melting points were recorded on a Kofler micro hot stage melting point apparatus and are uncorrected.

The infrared spectra were recorded on a Beckman Model IR-5 recording spectrophotometer in Nujol mull, unless otherwise stated.

Ultraviolet spectra were measured in methanol on a Perkin–Elmer Model 4000 spectracord.

The mass spectra were determined on a C.E.C.-103 mass spectrometer at an ionizing potential of 70 eV and an ionizing current of 50 μA. Samples were introduced through a heated inlet system maintained at 140 °C. The samples were run through the courtesy of Professor K. Biemann, Massachusetts Institute of Technology, Cambridge, Massachusetts.

The n.m.r. spectra were measured in deuterochloroform solutions with tetramethylsilane as an internal standard on a Varian V-4300 B high-resolution spectrometer at a frequency of 60 Mc/s.

Annotine (I)

Annotine was obtained from annotoxine which had crystallized from the mother liquors remaining after the separation of annotinine from the mixed alkaloids of *L. annotinum*. Annotoxine was readily separated into acrifoline and annotine by fractional crystallization of the hydrobromide salts.

Annotine was purified through the nitrate salt. The crude alkaloid was dissolved in the minimum volume of 2 *M* acetic acid and treated with a concentrated solution of sodium nitrate. The crystals of annotine nitrate were separated and dissolved in water, and the solution was basified with ammonia. Immediately after basification, the base precipitated in snowflake-like clusters. The base was separated by filtration and recrystallized from acetone, yielding rectangular plates which melted at 174.6–176 °C.

Calcd. for $C_{16}H_{21}O_3N$: C, 69.79; H, 7.69; N, 5.09. Found: C, 69.78; H, 7.83; N, 5.05.

The infrared spectrum in Nujol showed weak absorption at 3300 cm^{-1} in the hydroxyl region and a split peak at 1728 cm^{-1} and 1739 cm^{-1} in the carbonyl region. In chloroform solution there was absorption at 3500 cm^{-1} attributable to an intramolecularly bonded hydroxyl group, strong absorption at 1720–1730 cm^{-1} in the carbonyl region, and absorption at 1644 cm^{-1} which is assigned to unsaturation. There was also absorption at 1374 cm^{-1} attributable to a C—methyl group.

Preparation of Dihydroannotine (II)

A solution of annotine (0.050 g) in 30 ml of redistilled methanol containing 5 drops of concentrated hydrochloric acid and 0.040 g of Adams' catalyst was subjected to a hydrogen pressure of 50 p.s.i.g. for 5 h. The solution was filtered free of catalyst and the organic solvent removed under reduced pressure. Water was added to the residue, and the resulting solution was basified with ammonia and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure to yield 0.050 g of product which crystallized immediately on contact with ether. Recrystallization from acetone – petroleum ether gave colorless crystals melting at 203–204 °C.

Calcd. for $C_{16}H_{23}O_3N$: C, 69.28; H, 8.36; N, 5.05; active H, 0.36 (1). Found: C, 69.55; H, 8.71; N, 5.23; active H, 0.37.

The infrared spectrum of this material in Nujol had hydroxyl absorption at 3510 cm^{-1}, carbonyl absorption at 1728 cm^{-1}, and strong absorption at 1118 cm^{-1}. In carbon disulphide solution there was hydroxyl absorption at 3545 cm^{-1} and carbonyl absorption at 1740 cm^{-1}.

Preparation of Annotine Methiodide

A solution of annotine (0.100 g) in 5 ml of acetone and 1 ml of methyl iodide was heated under reflux and within 5 min a crystalline methiodide began to separate. The mixture was heated under reflux for 15 min longer, cooled, and allowed to stand at room temperature for 1 h. Filtration gave 0.125 g (81%) of product melting at 235.5–237 °C (with decomposition). An additional 0.021 g (13%) of product was obtained from the filtrate on further treatment with methyl iodide in acetone. The methiodide was recrystallized first from methanol–acetone and then from water to yield colorless crystals melting at 236–237 °C (with darkening).

Calcd. for $C_{16}H_{21}O_3N \cdot CH_3I \cdot H_2O$: C, 46.90; H, 5.97; N, 3.22. Found: C, 47.24; H, 6.10; N, 3.74.

Achmatowicz and Rodewald (3) report a melting point of 317° (violent explosion) and Bertho and Stoll (2) a melting point of 236–237° for annotine methiodide.

Treatment of Annotine Methiodide with Hydrogen

A solution of annotine methiodide (0.200 g) in 50 ml of distilled water containing 0.200 g of Adams' catalyst was subjected to a hydrogen pressure of 50 p.s.i.g. for 5 h. The solution was filtered free of catalyst, basified with ammonia, and extracted several times with chloroform. The combined chloroform extract was dried over sodium sulphate and evaporated under reduced pressure to yield 0.111 g (79%) of product which crystallized on standing. Recrystallization from acetone–ether yielded rectangular plates melting at 221–222 °C.

Calcd. for $C_{17}H_{27}O_3N$: C, 69.59; H, 9.28; N, 4.77. Found: C, 69.10, 69.12; H, 9.24, 9.33; N, 4.77, 4.97.

The infrared spectrum of compound III showed absorption at 3485 cm^{-1} in the hydroxyl region and strong absorption at 1712 cm^{-1} in the carbonyl region.

Modified Kuhn–Roth Oxidation of Annotine

The oxidation procedure and analytical method described by Harrison *et al.* (6) were used. Oxidation of 0.057 g (0.21 mmole) of annotine did not yield any carboxylic acid detectable by vapor phase chromatography.

Modified Kuhn–Roth Oxidation of the Emde Degradation Product III

Oxidation of 0.018 g (0.062 mmole) of the Emde degradation product III yielded 0.079 meq (64%) of volatile carboxylic acids. Vapor phase chromatography of their methyl esters revealed the presence of methyl acetate and methyl propionate in the molar ratio of 78:22 respectively.

Dehydration of III

(a) With p-Toluenesulphonic Acid

Compound III (0.146 g) was heated under reflux with *p*-toluenesulphonic acid in *o*-xylene overnight. The reaction mixture was cooled, water added, and the *o*-xylene separated and washed with dilute acid. The combined aqueous layer and acid washings were washed with ether, basified with ammonia, and extracted several times with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure to yield a crystalline material (0.121 g). Recrystallization from acetone – petroleum ether gave crystals melting at 164 °C. The compound was purified for analysis by sublimation at 80° and 0.01 mm Hg, m.p. 164 °C.

Calcd. for $C_{17}H_{25}O_2N$: C, 74.14; H, 9.15. Found: C, 73.61; H, 9.12.

The infrared spectrum of compound IV showed carbonyl absorption at 1730 cm^{-1}, but no hydroxyl absorption. The ultraviolet spectrum showed no absorption characteristic of an α,β-unsaturated carbonyl system.

(b) With Hydriodic Acid

A solution of III (0.050 g) in 5 ml of 47% hydriodic acid was heated under reflux for 6 h. The reaction mixture was cooled and poured on ice. The aqueous solution was made alkaline with ammonia and extracted several times with chloroform. The combined chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure to yield 0.040 g of a crystalline material whose infrared absorption spectrum was identical with that of the compound obtained from the treatment of compound III with *p*-toluenesulphonic acid.

Hydrogenation of IV

A solution of compound IV (0.110 g) in 40 ml of redistilled methanol containing 0.080 g of Adams' catalyst was subjected to a hydrogen pressure of 50 p.s.i.g. for 5 h. The solution was filtered free of catalyst and evaporated to dryness to yield a pale yellow oil which could not be induced to crystallize. This material was sublimed at 80 °C and 0.01 mm Hg to give a white amorphous solid.

Calcd. for $C_{17}H_{27}O_2N$: C, 73.60; H, 9.81; N, 5.05. Found: C, 73.45; H, 9.36; N, 5.22.

The infrared spectrum of compound V showed strong carbonyl absorption at 1730 cm^{-1}, but no hydroxyl absorption.

Modified Kuhn–Roth Oxidation of IV

Oxidation of 0.065 g (0.236 mmole) of IV yielded 0.40 meq (85%) of volatile carboxylic acids. Vapor phase chromatography of their methyl esters revealed the presence of methyl acetate and methyl propionate in the molar ratio of 66:34 respectively.

Modified Kuhn–Roth Oxidation of V

Oxidation of 0.060 g (0.216 mmole) of compound V yielded 0.31 meq (72%) of volatile carboxylic acids. Vapor phase chromatography of their methyl esters revealed the presence of methyl acetate, methyl propionate, and methyl butyrate in the molar ratio of 63:34:3 respectively.

Dihydroannotinol (VI)

A mixture of 0.060 g of sodium borohydride in 10 ml of ethanol and 0.200 g of dihydroannotine (II) in 10 ml of ethanol was refluxed for 4 h, cooled, and the excess borohydride destroyed by the addition of acetic acid. Most of the alcohol was removed by distillation, 50 ml of water was added, and the solution was basified with ammonia and extracted several times with chloroform. The combined chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure to yield 0.190 g of a colorless oil which crystallized on standing. Recrystallization from chloroform gave crystals melting at 186–187 °C. The infrared spectrum of this compound (dihydroannotinol) showed hydroxyl absorption at 3526 cm^{-1}, but no carbonyl absorption. The mass spectrum showed a molecular ion peak at 279.

Calcd. for $C_{16}H_{25}O_3N$: active H, 0.72 (2). Found: active H, 0.74.

Oppenauer Oxidation of Dihydroannotinol

A solution of dihydroannotinol (0.050 g) and aluminium tertiary butoxide (0.300 g) in 5 ml of toluene and 1 ml of cyclohexanone was heated under reflux for 6 h, then poured into cold dilute hydrochloric acid, and the organic layer extracted several times with dilute acid. The aqueous extract was made alkaline with 10% sodium hydroxide solution and extracted several times with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure, leaving a white solid whose infrared spectrum was the same as that of dihydroannotine.

Treatment of Dihydroannotinol with Ethyl Mercaptan

Dihydroannotinol (0.030 g) in 1 ml of concentrated hydrochloric acid (density 1.19) was treated, at 0 °C and under mechanical stirring, with ethyl mercaptan (1 ml) for 10 h. The solution was poured on a mixture of sodium hydroxide solution (40%; 2 ml) and cracked ice. The basic solution was extracted several times with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure to yield a colorless oil (0.021 g) which crystallized on standing. This substance was recrystallized from acetone to yield rectangular plates melting at 130 °C.

Calcd. for $C_{18}H_{29}O_2NS$: C, 66.82; H, 9.03; S, 9.91. Found: C, 66.93; H, 9.09; S, 10.25.

The infrared spectrum of compound VII showed hydroxyl absorption at 3410 cm^{-1}, but no carbonyl absorption. Its molecular weight was found to be 323 by mass spectrometry.

Reduction of IV with Lithium Aluminium Hydride

Compound IV (190 mg) in dry ether was added dropwise to a suspension of excess lithium aluminium hydride in dry ether. The reaction mixture was stirred at room temperature overnight and the excess hydride decomposed with moist ether. The ether solution was decanted and evaporated to yield 160 mg of viscous oil. The oil was converted to its hydrobromide salt and the latter recrystallized from methanol–acetone. It melted at 301–305°. The infrared spectrum showed hydroxyl absorption, but no carbonyl absorption. The mass spectrum of the base liberated from the hydrobromide showed a molecular ion peak at 279 corresponding to the molecular formula $C_{17}H_{29}O_2N$.

Phenylannotine

This compound was prepared by the method of Perry and MacLean (4). It was recrystallized from acetone and melted at 215–217°. The mass spectrum showed a molecular ion peak at 353. The infrared spectrum has an intense band in the hydroxyl region at 3470 cm^{-1} and a weak band at 1640 cm^{-1} attributable to non-benzenoid unsaturation. There was no absorption in the carbonyl region. In the ultraviolet spectrum there was only benzenoid absorption.

Pyrolysis of Phenylannotine

A mixture of phenylannotine (0.02 g) and zinc dust (1 g) was placed in a pyrex tube and covered with more zinc dust (1 g). The empty end of the tube was drawn to a capillary; the tube was then evacuated and the capillary sealed. The part of the tube carrying the mixture was placed in an oven and heated at about 300° for 1 h. The material which distilled in the capillary was studied by vapor phase chromatography. The following two columns were used: 5% SE-30 on Chromosorb W, 80–100 mesh, and 5% Carbowax 1500 on Chromosorb P, 60–80 mesh. The pyrolysis product consisted of two major components which had retention times identical with those of propiophenone and α-methacrylophenone, prepared by the method of Burckhalter and Fuson (11), on both columns.

The two components of the pyrolysis product mentioned above were collected together in methanol as they left the column and were thus separated from other minor constituents present in the pyrolysis mixture. The ultraviolet spectrum of the mixture of the two substances shows bands at 240 and 280 mμ.

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REFERENCES

1. R. H. F. Manske and L. Marion. Can. J. Research, B, **21**, 92 (1943).
2. A. Bertho and A. Stoll. Chem. Ber. **85**, 663 (1952)
3. O. Achmatowicz and W. Rodewald. Roczniki Chem. **32**, 485 (1958)
4. G. S. Perry and D. B. MacLean. Can. J. Chem. **34**, 1189 (1956).
5. L. M. Jackman. Applications of nuclear magnetic resonance spectroscopy in organic chemistry. Pergamon Press, New York. 1959.
6. W. A. Harrison, M. Curcumelli-Rodostamo, D. F. Carson, L. R. C. Barclay, and D. B. MacLean. Can. J. Chem. **39**, 2086 (1961).
7. K. Wiesner, Z. Valenta, W. A. Ayer, L. R. Fowler, and J. E. Francis. Tetrahedron, 4, 87 (1958).
8. F. A. L. Anet *et al.* Tetrahedron Letters, No. 14, 751 (1964).
9. F. Bohlmann. Chem. Ber. **91**, 2157 (1958).
10. W. A. Ayer and G. G. Iverach. Tetrahedron Letters, No. 3, 87 (1962).
11. J. H. Burckhalter and R. C. Fuson. J. Am. Chem. Soc. **70**, 4184 (1948).