STRUCTURAL AND SYNTHETIC STUDIES ON LYCOPODIUM ALKALOIDS

STRUCTURAL AND SYNTHETIC STUDIES ON LYCOPODIUM ALKALOIDS

By

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SCOPE AND CONTENTS:

An investigation of flabelliformine, an alkaloid obtained from *Lycopodium flabelliforme*, led to the establishment of its structure.

A study of the mass spectra of annotine (a minor alkaloid present in *Lycopodium annotinum*) and several of its derivatives in combination with degradative work gave support to a total structure proposed previously for the alkaloid.

A synthetic approach to the *Lycopodium* alkaloids was considered. 1-Carbomethoxy-3-methyl-7-methoxybicyclo [3.3.1] non-3-en-9-one, a compound which appeared suitable as an intermediate in the synthesis, was prepared in low yield. The difficulty encountered in the purification of this compound, coupled with the low yield in which it was obtained, indicated the impracticality of this approach to a total synthesis of a *Lycopodium* alkaloid.

ACKNOWLEDGEMENTS

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

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PART I

STRUCTURAL STUDIES

HISTORICAL INTRODUCTION

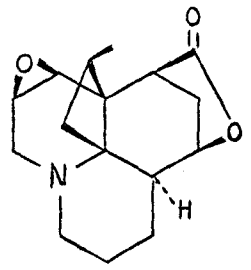
The Lycopodiaceae received very little attention as a source of alkaloids for over sixty years after the first isolation of a Lycopodium alkaloid (lycopodine) in 1881 (1).

In the nineteen-forties Manske and Marion undertook a thorough investigation of the alkaloidal content of many Lycopodium species. They reported the isolation of over thirty alkaloids (2-11). In more recent years many new Lycopodium alkaloids have been isolated (12-25), though the efforts of most investigators have been mainly directed to the elucidation of the structures of known alkaloids.

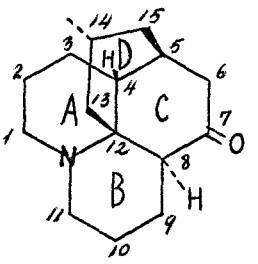
The structure of annotinine, shown in Figure 1, was the first to be elucidated. Wiesner and co-workers reported the structure of this alkaloid in 1958 (28).

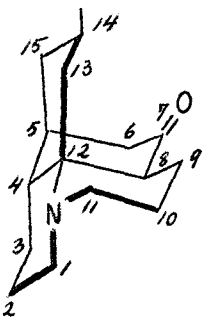
Two years later Harrison and MacLean proposed a structure for lycopodine (30) (Fig. 1), the most abundant alkaloid of this group. The disclosure of the structure of lycopodine marked the beginning of a relatively fertile period in Lycopodium alkaloid research. The structures of many alkaloids were determined in rapid succession. Today we know the structures of twenty-eight alkaloids; they are given in Figure 1 together with relevant references.

A glance at Figure 1 reveals the presence of four structural types. One of them is present only in annotinine. Another - the most common - is exemplified by lycopodine. A third structural type is found in the binitrogeneous bases α- and β-obscurine, lycodine flabellidine, de-N-methyl-α-obscurine

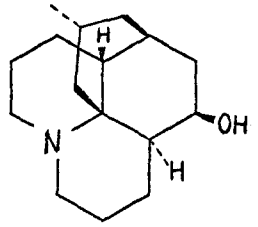


Annotinine
(26,27,28,29)

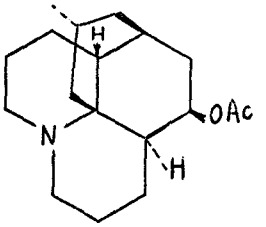




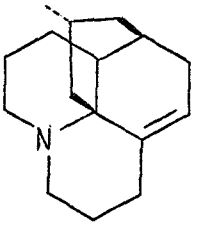
Lycopodine
(30,31,32,29)



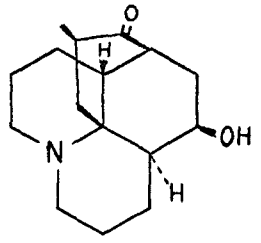
Dihydrolycopodine
(30,31,32)



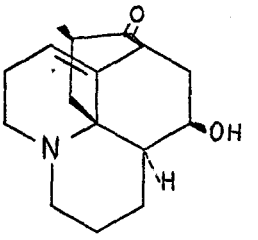
Alkaloid L.2
(30,31,32)



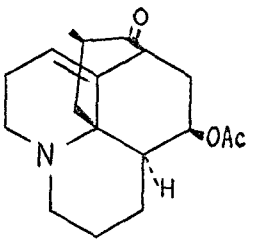
Alkaloid L.14
(30,31,32)



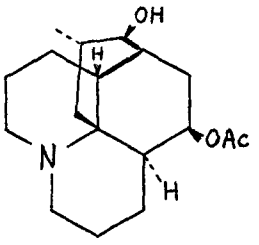
Annofoline
(33,32,34)



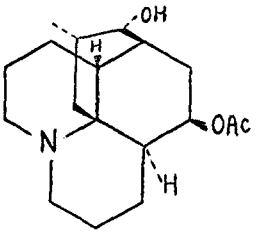
Acrifoline
(35,32,36)



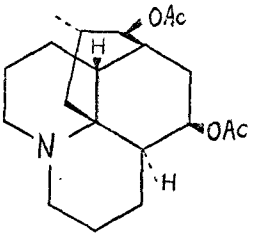
Acetylacrifoline
(35)



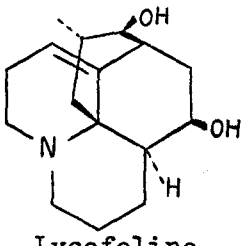
Fawcettiine
(37,34)



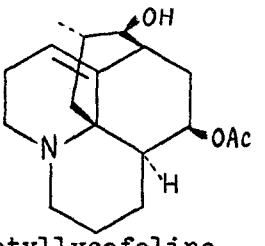
Lofoline
(33,37,34)



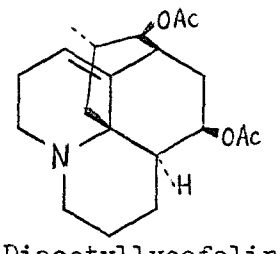
Acetylfawcettiine
(37)



Lycofoline
(20,38,39)

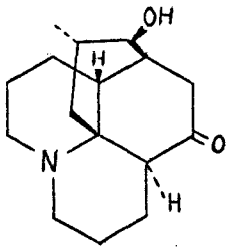


Acetyllycofoline
(20,39)

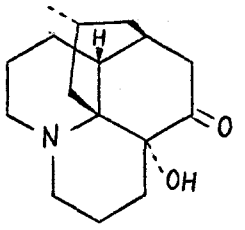


Diacetyllycofoline
(20,39)

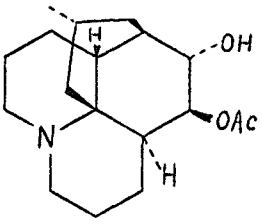
Figure 1. Lycopodium Alkaloids of Known Structure



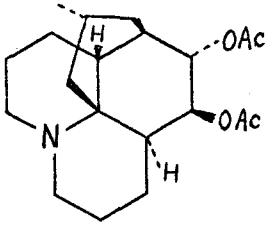
Clavolonine
(37,34)



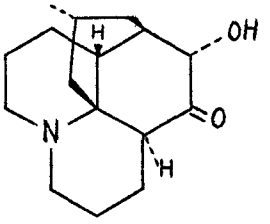
Flabelliformine
(22)



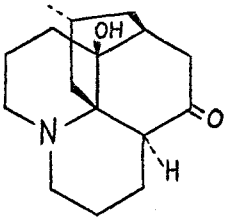
Lycoclavine
(23)



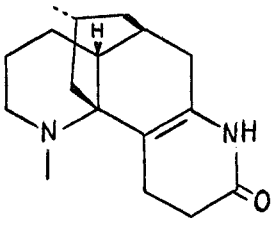
Acetyllycoclavine
(23)



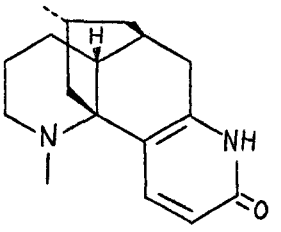
Alkaloid L.20
(40)



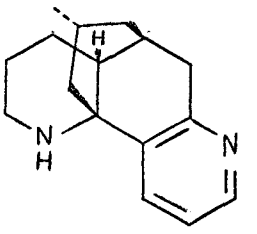
Lycodoline
(36)



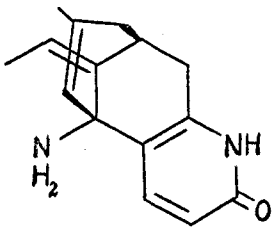
α-Obscurine
(41,42)



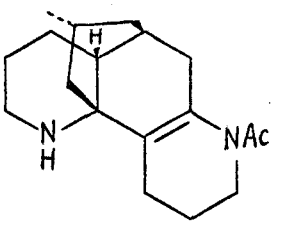
β-Obscurine
(41,42)



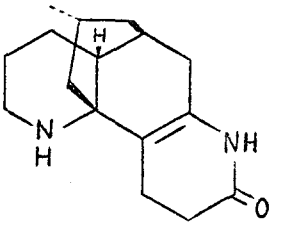
Lycodine
(41,43,44)



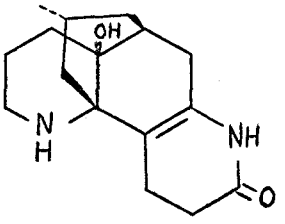
Selagine
(18)



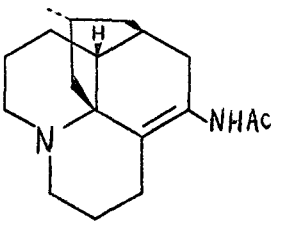
Flabellidine
(25)



De-N-methyl-α-obscurine
(25)



Hydroxy-de-N-methyl-α-obscurine
(25)



Flabelline
(24)

Figure 1. (Continued)

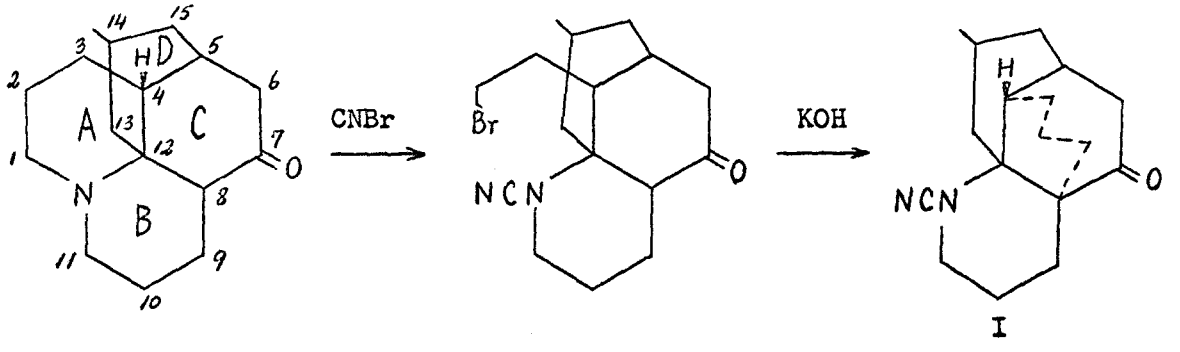
and hydroxy-de-N-methyl-α-obscurine. Lastly, the fourth structural type is found in selagine, which is closely related to the obscurines.

All Lycopodium alkaloids have some similarity in structure. This fact led Conroy to suggest (45) a single scheme accounting for their biogenesis. Conroy's scheme is formulated in Figure 2 where the formation of three structural types is shown.

The Structure of Lycopodine

A part of this thesis deals with the structural investigation of flabelliformine, an alkaloid obtained from L. Flabelliforme. Since this alkaloid is closely related in structure to lycopodine (Fig. 1), we shall briefly focus our attention on the structure of the latter. The degradative work leading to the constitution of lycopodine has been reviewed elsewhere (46), and will not be dealt with here. This section is concerned only with the stereochemistry of the alkaloid. To begin with, we should point out that bonds C_{12}-C_{13} and C_5-C_{15} are necessarily located on the same side of the hexahydrojulolidine ring system. For the purpose of studying the relative stereochemistry of lycopodine we shall give arbitrarily the asymmetric centers C_5 and C_{12} the configuration shown in Figure 1.

The work of Harrison et al (31) established the relative configuration at C_4. Lycopodine was transformed to compound I by the reaction sequence shown:



I

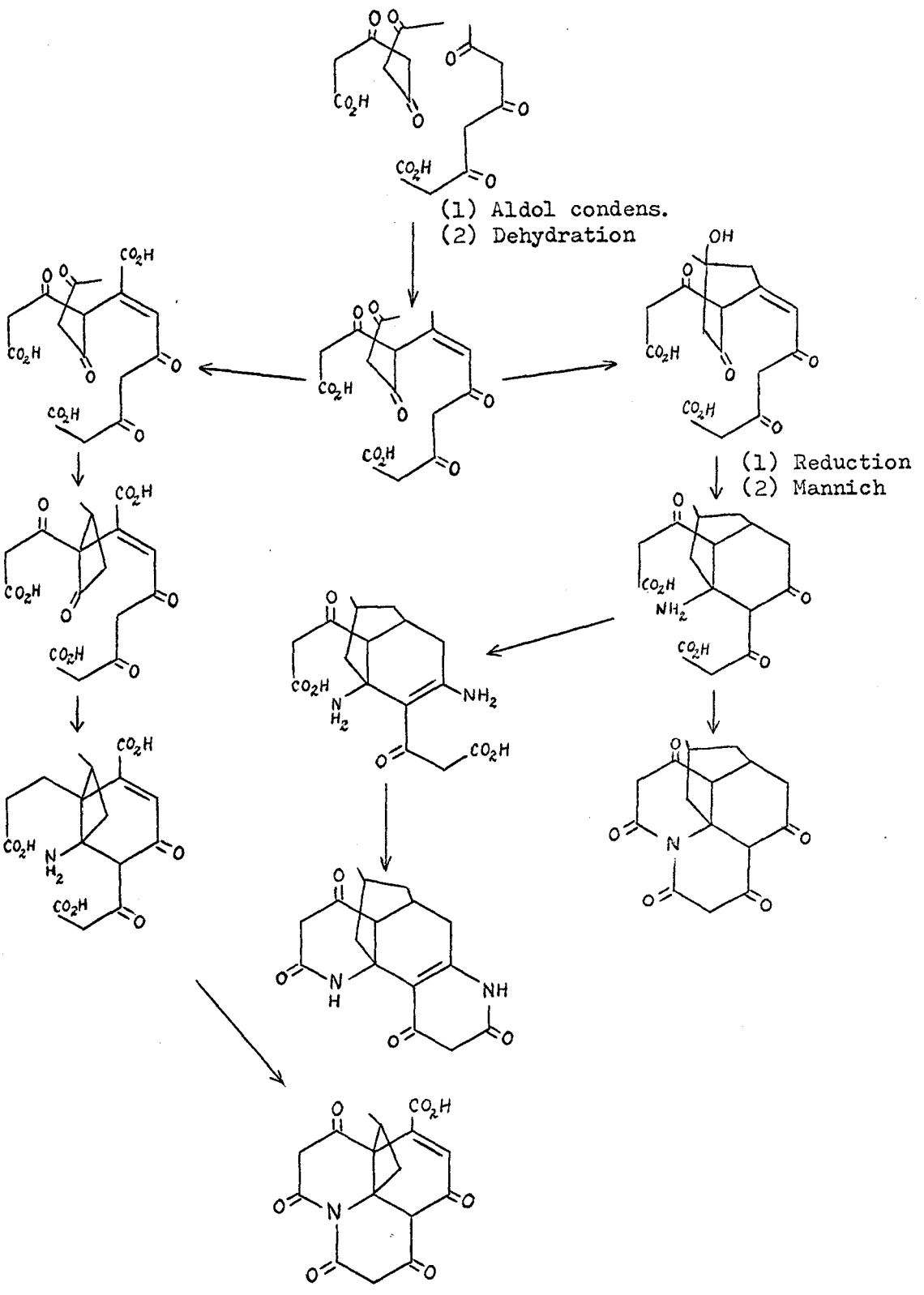
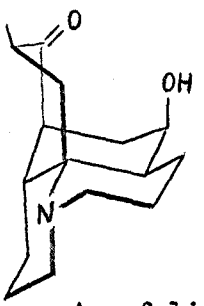


Figure 2. The Postulated Biogenesis of Lycopodium Alkaloids

In this compound the bridge joining carbon atoms 4 and 8 is located behind ring C: the alternate position is stericaly impossible. The C_4 hydrogen must therefore project in front of the ring, both in compound I and in lycopodine.

The stereochemistry at C_8 was easily established. Lycopodine does not epimerise when treated with alkali, and therefore has the most stable configuration at C_8, i.e. that in which rings B and C are joined in the trans manner. The conclusion is thus reached that the hydrogen at C_8 projects to the rear, as shown in Figure 1.

The only feature of the relative stereochemistry of lycopodine left to be considered is the configuration at C_{14}. Evidence pertaining to the configuration at this asymmetric center was presented by Anet (32). He initially established the relative configuration at C_{14} of annofoline, another Lycopodium alkaloid (Fig. 1), which he then converted to a lycopodine derivative. The infrared spectrum of annofoline was found to have a carbonyl band of abnormally low intensity, thus suggesting the presence of a hemiketal grouping. Formation of a hemiketal is only possible when ring D exists in a boat conformation. Anet proved next that annofoline is more stable than its C_{14} epimer and thus concluded that the methyl group is equatorial and not in a highly hindered axial position.



Annofoline

The transformation of annofoline to anhydrodihydrolycopodine is formulated in Figure 3. The first and fourth steps require some attention. Reduction of annofoline with sodium borohydride affords a mixture of two products, which are designated α- and β- dihydroannofoline. Reduction with sodium borohydride under neutral conditions, or with lithium aluminum hydride in ether, gives only the α-isomer while sodium borohydride reduction in the presence of sodium hydroxide affords as much as 50% of the β-isomer. This observation indicates that the β-isomer is not a reduction product of annofoline but of a ketone having the opposite configuration at C_{14}. The fourth step is a Wolff-Kishner reduction. Anet, in his argument assumes that in this step the C_{14} asymmetric center retains the configuration of β-dihydroannofoline. However, this may not be so: although molecular models indicate that the olefinic ketone shown in Figure 3 has the more stable configuration, it is possible that by the action of sodium hydroxide it is converted to a small extent to the epimeric ketone which may then undergo rapid reduction producing anhydrodihydrolycopodine. If this were the case, the latter compound and lycopodine would have a configuration at C_{14} opposite to that shown in Figure 3. This possibility, however, is weakened by the fact that the Wolff-Kishner reduction was reported to yield only one product.

The absolute configuration of lycopodine was established by Wiesner and co-workers (29) through a study of its rotation dispersion curve. The ORD curve of the alkaloid has a maximum at 307 mμ, a result which, by the application of the octant rule, leads to the absolute configuration shown in Figure 1. This configuration is in agreement with the absolute configuration of annotinine, which was determined by the same authors.

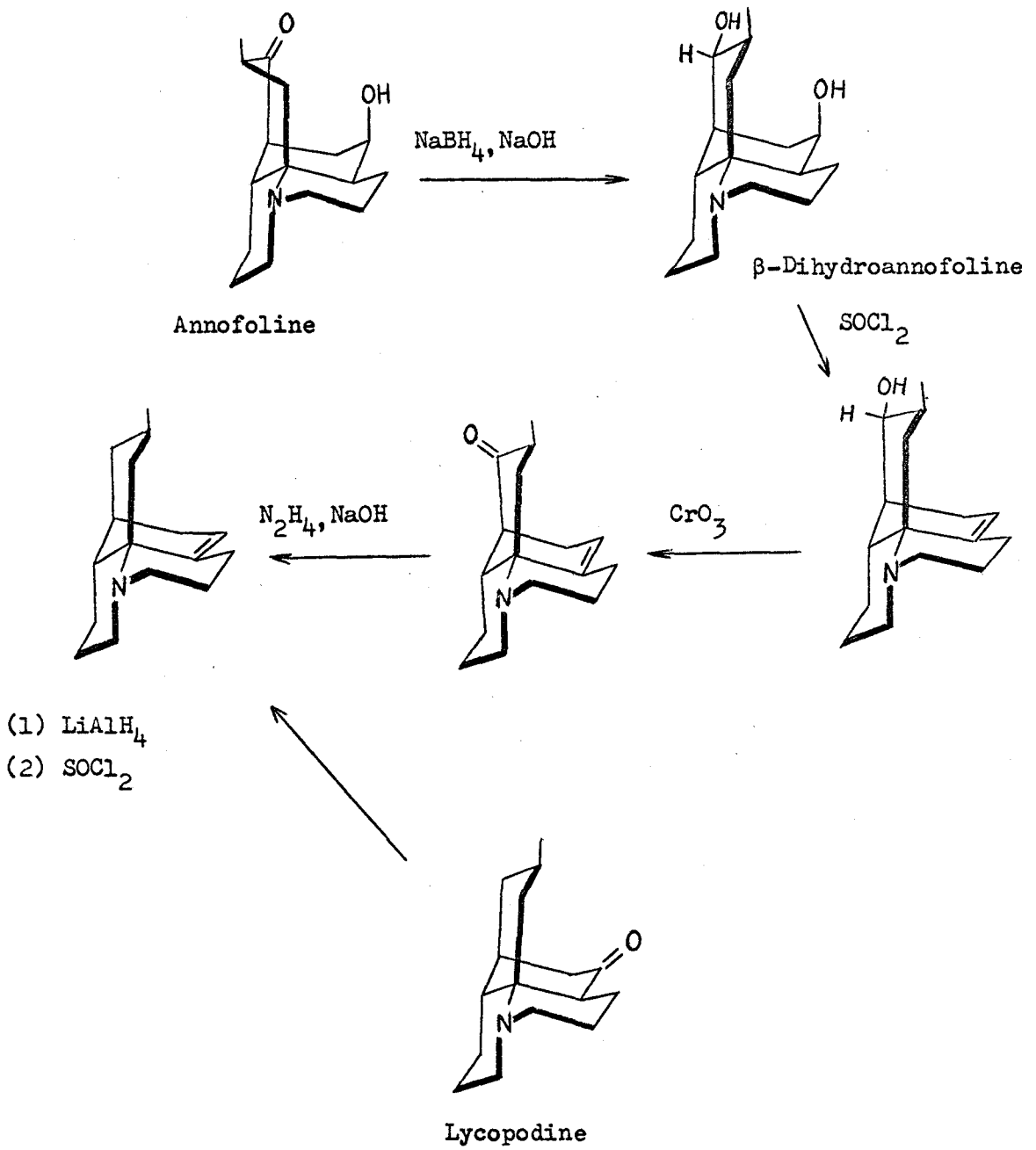


Figure 3. Transformations Establishing the Configuration of Lycopodine at C_{14}

Structural Studies on Annotine

As a study of the mass spectra of annotine and its derivatives is presented in this thesis together with some degradative work, we shall give here an account of the work done previously on this alkaloid with the purpose of establishing its structure.

Annotine II, $C_{16}H_{21}O_3N$ was first isolated from L. annotinum by Manske and Marion (4).

Perry and MacLean investigated (47) the functional groups of the alkaloid and established that it contains a carbonyl group, a double bond and a hydroxyl group. The third oxygen atom they ascribed to an ether linkage other than an epoxide.

Later, the investigations performed on annotine by Szarek (48) allowed an assignment of the position of the double bond and the hydroxyl group relative to the nitrogen atom, and established that the third oxygen is present in a lactone function.

Considerable information was gleaned from the NMR spectrum of annotine. A quartet present at $\tau = 4.04$ (J = 10 c/s), of intensity corresponding to two protons, indicates the presence of the grouping $\overset{H}{>}C = C\overset{H}{<}$; it 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. It was possible to resolve the components of the quartet into triplets having the same coupling constant. Thus, it was concluded that the grouping $>C\text{-}CH = CH\text{-}CH_2-$ is present in the molecule. A singlet present at $\tau = 8.57$ with an intensity corresponding to three protons indicates the presence of a methyl group attached to a quaternary carbon. The low τ-value of this peak may be

due to the deshielding effect of a carbonyl group attached to the same carbon atom as the methyl group. The NMR spectrum of annotine shows a band corresponding to two protons at $\tau = 5.83$; it consists of two overlapping peaks, which have been assigned to the groupings $>CH\text{-}O\text{-}C(=O)<$ (v.i.) and $->C\text{-}OH$.

Dihydroannotine III (the product formed on catalytic hydrogenation of annotine) lacks absorption corresponding to ethylenic protons. It shows a single sharp peak at $\tau = 8.56$ due to the $->C\text{-}CH_3$ group, a band at $\tau = 6.82$ attributed to the hydroxyl proton and a band at $\tau = 5.74$ which has been assigned to the grouping $>\underline{C}H\text{-}O\text{-}C(=O)-$. The last band has a half-band width of 9.9 c/s indicating coupling to three or four protons.

Annotine methiodide, when treated with hydrogen over Adams' catalyst, undergoes the Emde degradation to yield compound IV, $C_{17}H_{27}O_3N$. The occurrence of the degradation together with physical and chemical investigation of compound IV and its derivatives established the correlation between the nitrogen atom, the double bond and the hydroxyl group. It was found that dihydroannotine methiodide, unlike the methiodide of annotine, fails to undergo the Emde degredation. This fact indicates that the double bond provides the driving force for the reaction and is, therefore, β,γ to the nitrogen atom. The NMR spectrum of compound IV shows the absence of ethylenic protons. Two bands centered at $\tau = 8.55$ and $\tau = 8.98$ indicate the presence of two methyl groups. The first is a single sharp peak, and thus corresponds to the methyl group present in the alkaloid. The other band is broad, indicating coupling with neighboring protons; it is assigned to a methyl group at the end of an alkyl chain formed in the Emde degredation. The absence of this methyl group

in annotine indicates that it arose from a methylene group adjacent to the nitrogen atom. Our knowledge of the structure of the alkyl chain in compound IV was substantiated by a modified Kuhn-Roth oxidation. The identification of acetic acid and propionic acid, as reaction products, demonstrated the presence of an ethyl group.

Before we present the evidence which establishes the position of the hydroxyl group relative to the nitrogen atom and the double bond, it should be pointed out that annotine is believed to be a tertiary alcohol: it is recovered unchanged after treatment with both chromic acid and aluminum tertiary butoxide in cyclohexanone (Oppenauer). Dehydration of the Emde degradation product (IV) yields compound V, $C_{17}H_{25}O_2N$. Modified Kuhn-Roth oxidation of this compound resulted in the formation of acetic acid and propionic acid, while compound VI, obtained by catalytic hydrogenation of compound V, yielded butyric acid as well as the above mentioned acids. It is clear that the grouping $\overset{\alpha}{CH_3}\overset{\beta}{CH_2}\text{-}\overset{\gamma}{CH} = \overset{\delta}{C}\!\!<$ is present in compound V, and that the hydroxyl group in annotine is joined to the <u>delta</u> carbon atom. The NMR spectrum of the anhydro compound V shows a triplet at $\tau = 4.77$ corresponding to one ethylenic proton. Each component of the triplet could be resolved into a doublet. The grouping $-CH_2-CH = \underset{\underset{-C-}{|}}{C}\text{-}CH<$ is compatible with these results.

The experimental data cited this far indicate the presence of the following structural sequence in annotine:

$$>N\text{-}CH_2\text{-}CH = CH\text{-}\underset{\underset{-C-}{|}}{\overset{OH}{\overset{|}{C}}}\text{-}CH-$$

The reactions described above are summarized in Figure 4.

The reactions reported by Perry and MacLean (47) in support of the presence of a ketonic carbonyl in annotine can be explained equally well by a lactone formulation. One of these reactions is the sodium borohydride reduction of the alkaloid to form annotinol VII, $C_{16}H_{23}O_3N$. If a lactone group were present in annotine, compound VII would be a hemiacetal. Similarly the other reaction, that of annotine with phenyl lithium producing phenylannotine VIII, $C_{22}H_{28}O_3N$, can be accommodated by both formulations.

The first indication that a lactone function is present in annotine, rather than a ketonic carbonyl and a cyclic ether, came from the observation that the NMR spectra of annotine and many of its derivatives contain a peak corresponding to one proton at $\tau = 5.7 \pm 0.1$. The τ-value of this peak is lower than would be expected of a hydrogen in a cyclic ether $>\underline{C}H\text{-}O\text{-}R$, but lies in the region expected of a lactone or ester $>\underline{C}H\text{-}O\text{-}\overset{\overset{O}{\|}}{C}\text{-}$. The ORD curve of annotine is also in agreement with a lactone structure; it shows a plain curve whereas a ketone should give a Cotton effect curve.

The presence of a lactone function was substantiated by chemical evidence. Firstly, it was shown that annotine forms a water soluble salt when heated at high temperatures for prolonged periods in strongly alkaline solution. Secondly, dihydroannotinol IX, $C_{16}H_{25}O_3N$, prepared by borohydride reduction of dihydroannotine III, reacts with ethyl mercaptan in acid medium to form a substance X, $C_{18}H_{29}O_2NS$, in which a thio ethyl group has apparently replaced a hydroxyl group of the starting material (IX). A reaction of this nature can be readily interpreted by a lactone structure for annotine but not by a ketonic structure. Thus, borohydride reduction of dihydroannotine III affords the hemiacetal IX which, with ethyl mercaptan in the presence of acid,

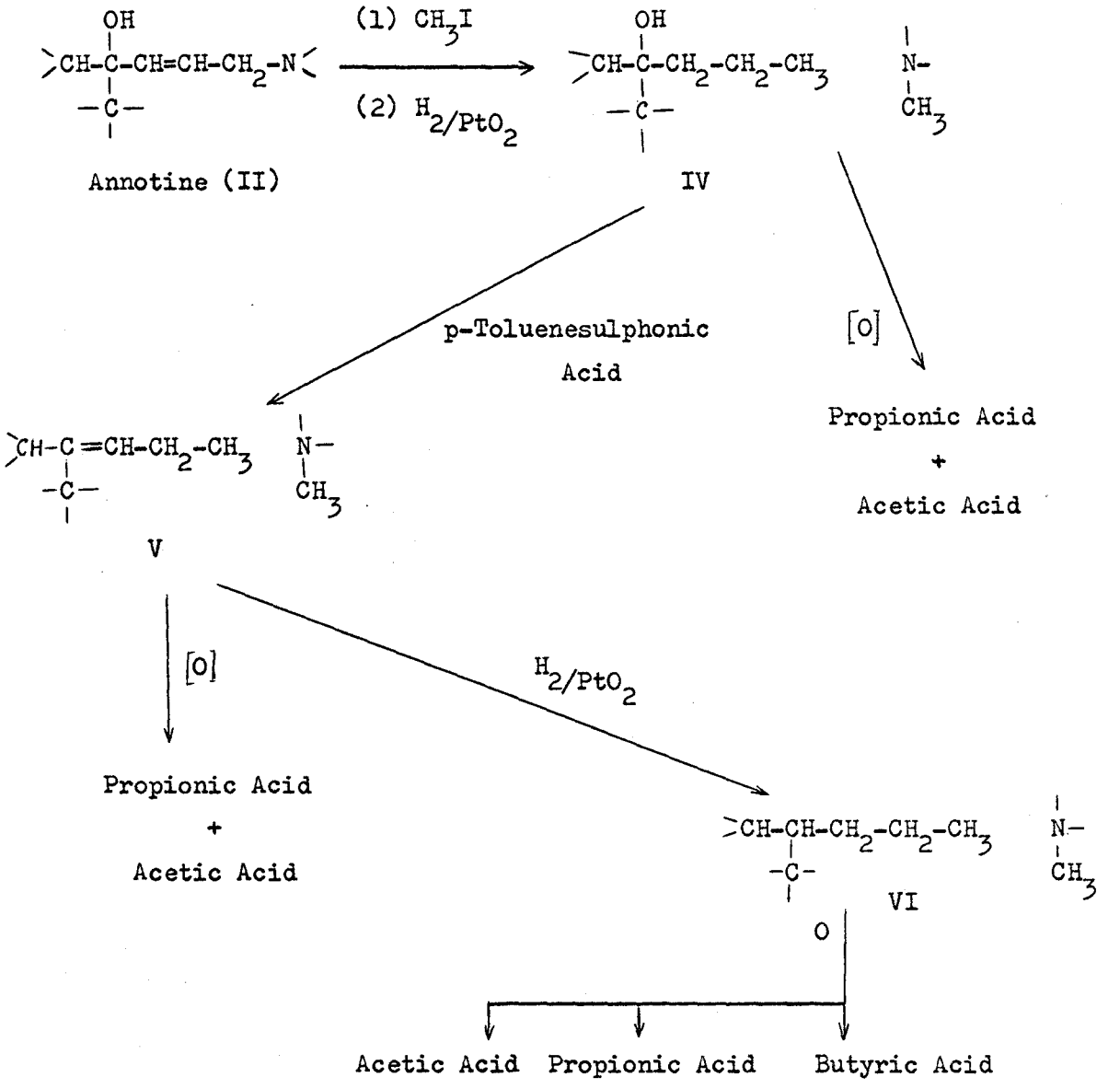
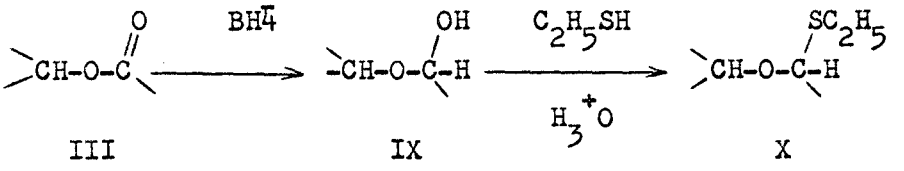


Figure 4. The Degradation of Annotine

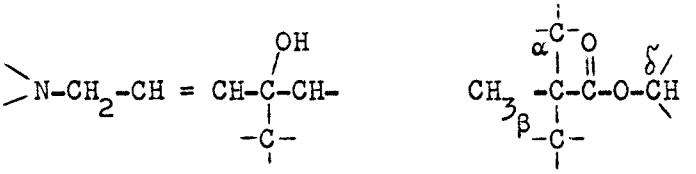
yields the product X, as depicted below:



III IX X

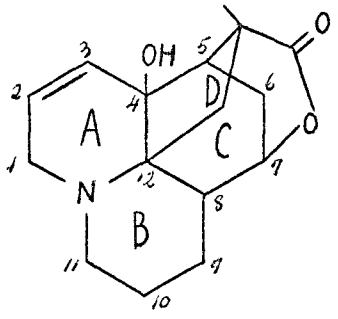
Thirdly, the anhydro compound V was reduced to a diol with lithium aluminum hydride. Perry and MacLean reported that annotine is recovered unchanged when treated with lithium aluminum hydride in tetrahydrofuran. The failure of this reaction may be due to the formation of an insoluble salt by interaction of the reducing agent with the hydroxyl group. Compound V, which lacks a hydroxyl group, undergoes reaction to form compound XI, $C_{17}H_{29}O_2N$. The uptake of four hydrogen atoms in the conversion V→XI shows that the function being reduced is a lactone rather than a ketone. A split carbonyl band in the infrared spectrum of annotine, consisting of peaks situated at 1728 and 1739 cm^{-1}, indicates that the lactone ring is six-membered.

To summarize, the work discussed in this section points to the presence of the following fragments in annotine:

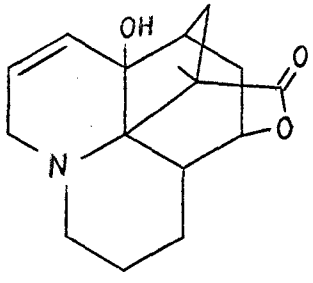


In considering various possible structures for the alkaloid, we should place more weight on those which contain the hexahydrojulolidine ring system, present in all Lycopodium alkaloids with a single nitrogen atom. There are many "hexahydrojulolidine structures" which fit the molecular formula of annotine and encompass the two fragments mentioned earlier. However, there is a further gradation of preference. In all Lycopodium alkaloids of known

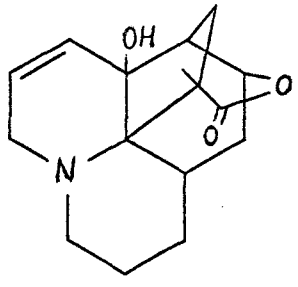
structure carbon to carbon bonds emanate from atoms C_5 and/or C_4 and C_{12} of the hexahydrojulolidine system; furthermore, functional groups have never been found on ring B. If we restrict the list of "hexahydrojulolidine structures" to those conforming with the above characteristics the following three possibilities remain:



IIA



IIB



IIC

The Mass Spectra of the Lycopodium Alkaloids

A study (49) of the mass spectra of the Lycopodium alkaloids revealed that a close relationship exists between the modes of fragmentation upon electron impact of all alkaloids examined. This can best be shown by a consideration of the mass spectrum of dihydrolycopodine followed by a brief discussion of the spectra of other Lycopodium alkaloids.

The main peaks present in the spectrum of dihydrolycopodine, shown in Figure 5(a), are at m/e 249(M); 206(M-43); 192(M-57); 174(M-75) and 145 (M-103). The peak of greatest intensity at 192 mass units is attributed to the ion formed (as depicted in Figure 6) by expulsion of the bridge joining atoms C_5 and C_{12} in the alkaloid plus a hydrogen atom. Loss of water from the m/e 192 fragment affords the ion of m/e 174. Subsequent elimination of ethylene proceeding as shown by the arrows, gives the ion of m/e 146.

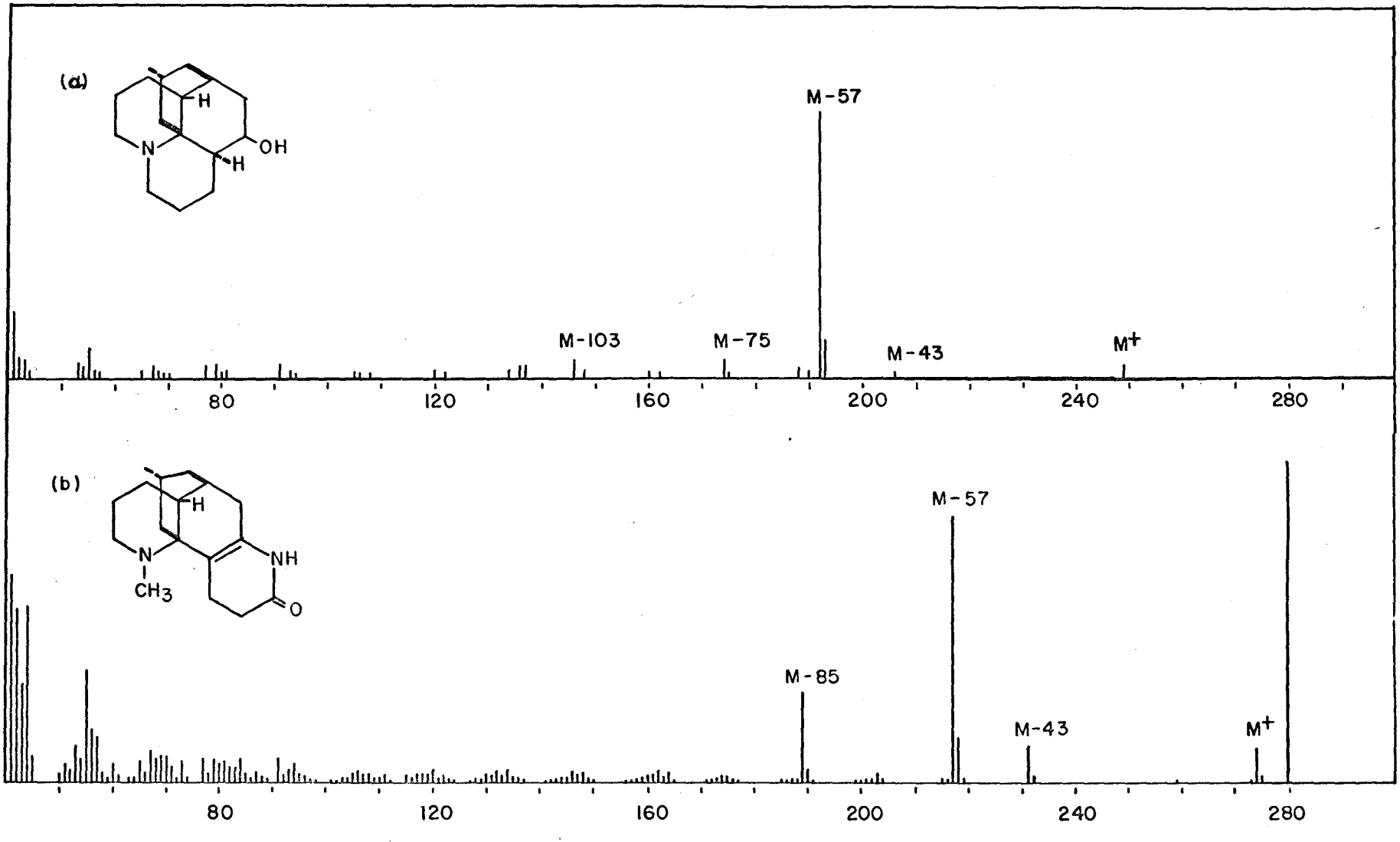


Figure 5. The Mass Spectra of (a) Dihydrolycopodine (b) α-Obscurine

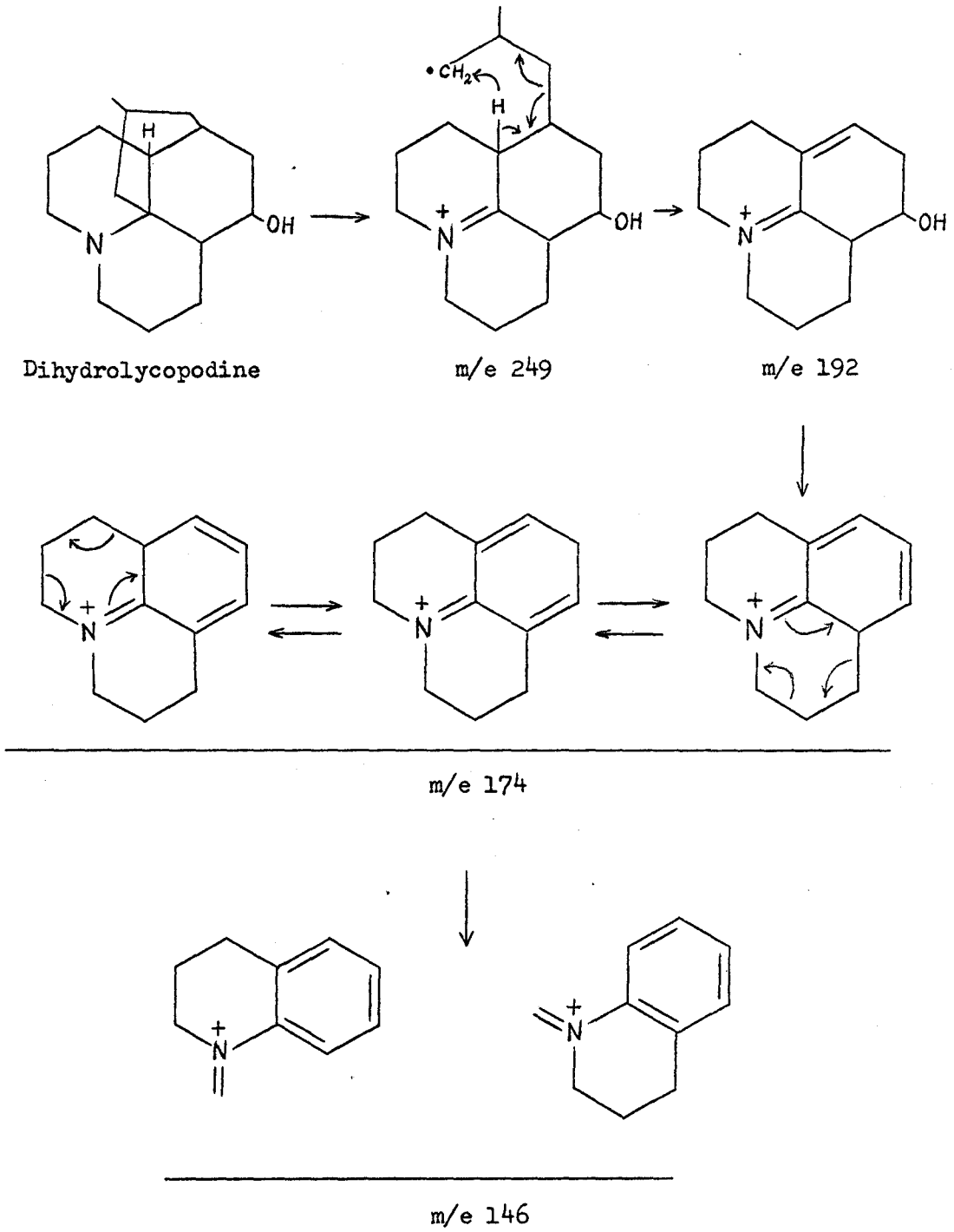


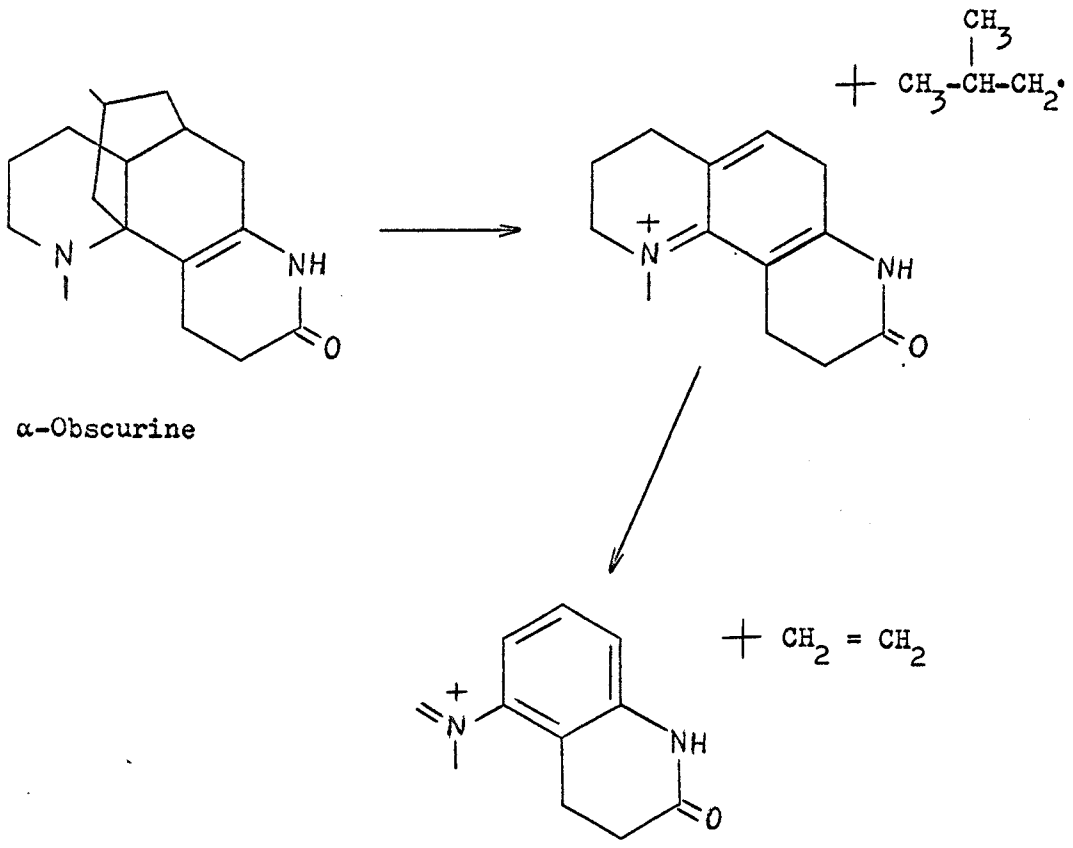
Figure 6. The Fragmentation of Dihydrolycopodine

The mass spectra of anhydrodihydrolycopodine and acetyl-dihydrolycopodine indicate the occurrence of an analogous fragmentation process. The molecular ions of both alkaloids loose the bridge grouping together with a hydrogen atom. In the case of anhydrodihydrolycopodine this process results in the formation of the ion of m/e 174 (M-57). The production of this ion by fragmentation of acetyldihydrolycopodine requires an additional step: loss of acetic acid. The m/e 174 ion is then degraded to the ion of m/e 146.

The dominating fragmentation process of lycopodine involves formation of a M-57 ion, as in the case of the alkaloids already considered; but further fragmentation follows a different route because the formation of a double bond in the 7, 8-position is not possible.

A study of the mass spectra of annofoline, lofoline and clavolonine shows that substituents on the bridge do not alter appreciably the fragmentation pattern exhibited by dihydrolycopodine. However, as might be expected, alkaloids, such as lycodoline and acrifoline, lacking the C_4 hydrogen, decompose by a scheme differing considerably from that shown in Figure 6.

The mass spectra of the binitrogeneous bases indicate a degradation mechanism similar to that of dihydrolycopodine. The spectrum of α-obscurine is given in Figure 5(b); the degradation of the alkaloid is depicted in the next page. Annotinine also appears to decompose by a process commencing by expulsion of the bridge. Thus, the conclusion drawn from the work described in this section is that the loss of the bridge dominates the fragmentation process of all Lycopodium alkaloids examined.



α-Obscurine

DISCUSSION OF RESULTS

THE STRUCTURE OF FLABELLIFORMINE

Manske and Marion investigated the alkaloidal content of L. flabelliforme and reported (2) the isolation of eight alkaloids from this species. Their work, together with subsequent research performed in these laboratories (22, 24, 25), established that the plant contains lycopodine, dihydrolycopodine, acetyldihydrolycopodine, alkaloid L.3, alkaloid L.4, α- and β-obscurine, flabellidine, nicotine, lycodine, annotinine, clavolonine, de-N-methyl-α-obscurine, de-N-methyl-hydroxy-α-obscurine and flabelliformine.

The essentials of the work leading to the structure of flabelliformine have already been reported (22). In this section we shall give a detailed account of this work, after considering briefly the isolation of the alkaloid.

The isolation of flabelliformine is based on the fact that it is less soluble in cold ether than the other alkaloids obtained from the crude alkaloid extract after the separation of lycopodine by column chromatography. The material remaining undissolved after treatment of the alkaloid mixture with ether crystallized from acetone-methanol but could not be purified by recrystallization from various solvents, by sublimation, column chromatography or through the preparation of a perchloric acid salt. However, when the mixture was dissolved in acetone and treated with hydrobromic acid, a hydrobromide separated from which a pure base - flabelliformine XII - was obtained. Dihydrolycopodine was isolated from the mother liquors.

The difficulty encountered in effecting a separation of the two alkaloids suggests that they are strongly linked in a molecular complex. Actually, Ayer and Law showed (23) that flabelliformine and dihydrolycopodine combine in equimolar quantities to form a molecular complex, which was found to be identical with a substance isolated by the same workers from L. Clavatum var. megastachyon.

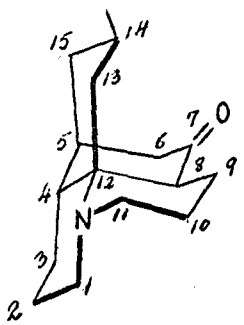
Analysis of flabelliformine was found to fit the formula $C_{16}H_{25}O_2N$. The infrared spectrum of the alkaloid, measured in chloroform solution, shows ketonic carbonyl absorption at 1705 cm^{-1} and a hydroxyl band at 3560 cm^{-1}. The presence of a carbonyl group was confirmed by reduction of the alkaloid with sodium borohydride to a diol XIII, $C_{16}H_{27}O_2N$. The nature of the hydroxyl group was studied next. Flabelliformine was found to resist oxidation: it was recovered unchanged after treatment with chromium trioxide and underwent dehydration (to produce compound XIV, see below), instead of oxidation, when heated with aluminum tertiary butoxide in the presence of cyclohexanone. Thus, we conclude that the hydroxyl group is attached to a tertiary carbon atom. This conclusion is supported by a study of the NMR spectrum of flabelliformine. The spectrum shows an intense peak at $\tau = 9.15$ (due to methyl group protons) and other bands at lower field; but there is no absorption below a τ-value of 6.5. If the grouping $>$CH-OH were present, absorption below this value would be expected.

Treatment of the alkaloid with hydrogen iodide replaced the hydroxyl group with hydrogen. The reaction product was found to be identical with lycopodine. Evidently, then, flabelliformine is formally derived from lycopodine by replacement of a methine hydrogen by a hydroxyl group. There

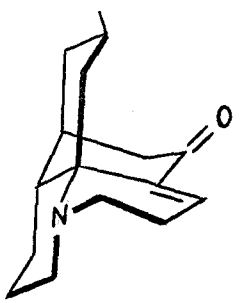
are four tertiary carbon atoms in lycopodine and hence four possible constitutional formulas can be written at this stage for flabelliformine.

The position of the hydroxyl group in the molecule was established as follows: dehydration of the alkaloid, effected by both phosphoric acid and p-toluenesulphonic acid, afforded a non-crystalline product XIV which was converted to a crystalline methiodide XV, $C_{17}H_{26}ONI$. Compound XIV has the properties of an α,β-unsaturated ketone. The ultraviolet spectrum of this substance shows maximum absorption at 245 mμ and the infrared spectrum, measured in carbon tetrachloride solution, has bands of equal intensity at 1685 and 1617 cm^{-1}. The NMR spectrum of compound XIV, has a triplet at a τ-value of 3.01, which is attributed to a proton on the β-carbon of an α,β-unsaturated carbonyl system (50, 35, 46).

Since compound XIV is converted to lycopodine on mild hydrogenation over a platinum catalyst, its formation very likely occurs without alteration of the carbon skeleton present in the alkaloid. The double bond in this compound must occupy the 8,9 position, as shown:



Lycopodine

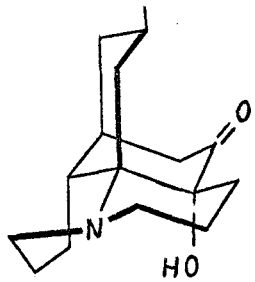


XIV

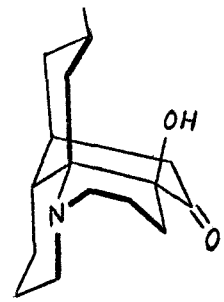
A double bond in the alternative conjugated position would result in a very large distortion of bond angles. The hydroxyl group in flabelliformine is therefore attached to C_8. The reactions described above are summarized in Figure 7.

The conversion of flabelliformine XII to lycopodine proves that both alkaloids have the same configuration at C_4, C_5, C_{12} and C_{14}. Deduction of the configuration at C_8 was based on spectral data. Cookson and Dandegaonker have shown (51) that the absorption of ultraviolet light by α-hydroxy ketones depends on the geometry of the systems: substitution of a hydroxyl group for an equatorial hydrogen atom shifts the maximum 12 mμ to shorter wave-length, whereas an axial hydroxyl group causes a shift of 15-23 mμ to longer wave-length. Thus, a comparison of the ultraviolet spectra of flabelliformine and lycopodine would reveal whether the hydroxyl group in the former is in an axial or an equatorial position. The ultraviolet spectra of the two alkaloids have maxima at 303 and 284 mμ respectively. The conclusion that the hydroxyl group occupies an axial position is supported by a comparison of the carbonyl frequencies in the infrared spectra. The infrared spectrum of lycopodine shows carbonyl absorption (chloroform solution) at 1693 cm^{-1} whereas that of flabelliformine has a band at 1705 cm^{-1}. Introduction of an equatorial hydroxyl group would be expected to lead to a lowering of the carbonyl frequency as a result of hydrogen bonding (52).

The data presented up to this point can be explained by two structures:



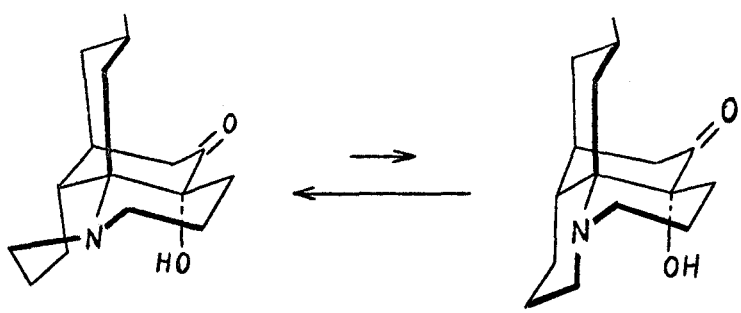
XII



XIIA

A choice between them was made through further examination of the infrared spectrum of flabelliformine. The peak at 3560 cm^{-1} lies in the region expected of an hydroxyl group forming an intramolecular hydrogen bond. The intramolecular nature of the bonding was further supported by the observation that the peak is not displaced on dilution. Since the hydroxyl group is in an axial position, bonding with the neighbouring carbonyl group can be excluded; and must, therefore, involve the nitrogen lone pair. Structure XII, alone, fulfils the spatial requirements for such an interaction.

In the equilibrium shown below, predominance of the conformation



XIIa XIIb

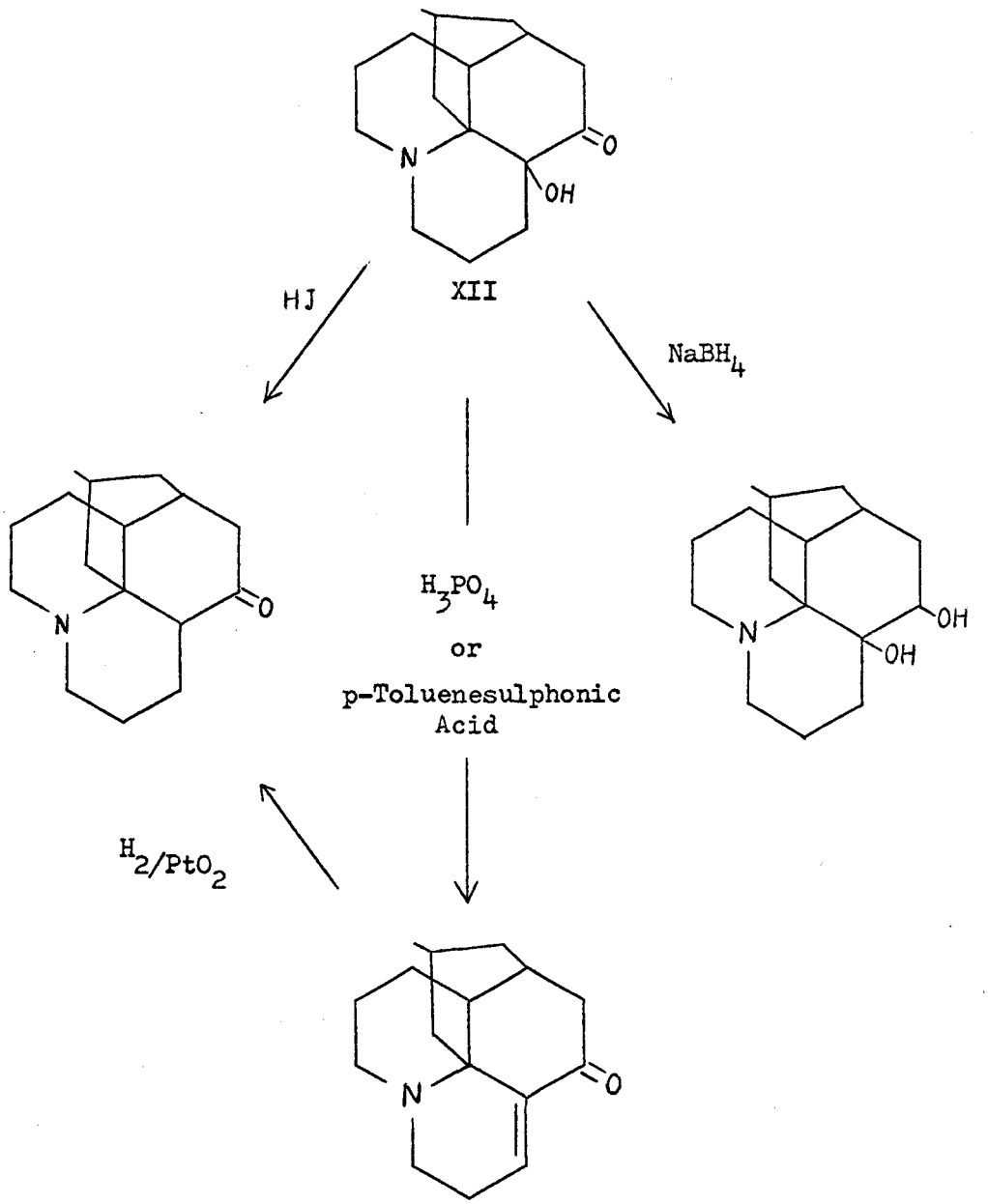
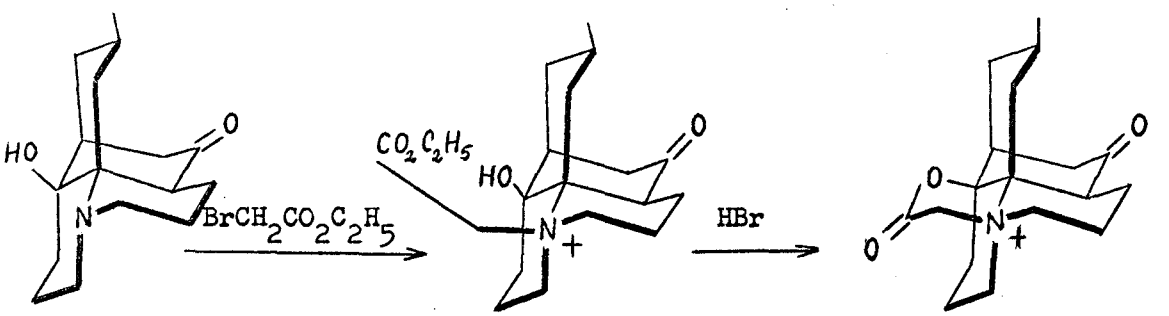


Figure 7. Reactions of Flabelliformine XII

XIIa, in which ring B is in a boat form, can be inferred from a consideration of steric hindrance effects: in the other conformation there is strong interaction between the hydroxyl group and the hydrogen atoms at 1 and 3. A band of low intensity at 3640 cm^{-1}, in the spectrum of flabelliformine, suggests that some hydroxyl groups do not participate in hydrogen bonding. This fact is in accord with the presence of form XII_b.

The proximity of the hydroxyl group to the nitrogen lone pair in lycodoline was demonstrated by Ayer and Iverach (36) by the following transformation:



Lycodoline

An attempt to confirm the existence of an analogous relationship in flabelliforme by a related method was unsuccessful. Flabelliformine, when reacted with methyl iodoacetate, was converted to the quaternary salt XVI. The material obtained by treatment of the latter with hydrobromic acid could not be induced to crystallize and was thus not characterised.

STUDIES ON THE STRUCTURE OF ANNOTINE

THE MASS SPECTRA OF ANNOTINE AND SOME OF ITS DERIVATIVES

The scarcity of annotine makes its study by degradation extremely difficult. For this reason we thought that the alkaloid could be profitably investigated by the mass spectrometric method which is particularly well suited to structural studies on substances available in small amount.

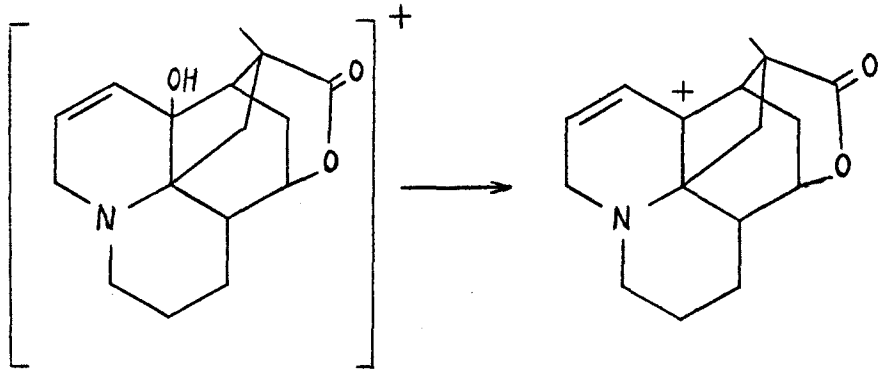
We anticipated that expulsion of the bridge (forming ring D) would dominate the fragmentation of annotine, as in fact it dominates the fragmentation of other Lycopodium alkaloids. Thus, it was hoped that the mass spectrometric study of annotine and its derivatives would shed light on the structure of the bridge present in these compounds. Besides annotine and dihydroannotine two groups of derivatives were studied each of which arise from these two compounds by change only in the lactone function. Phenylannotine is related to annotine; dihydroannotinol and dihydroannotine thioacetal to dihydroannotine.

As we shall see further on, the mass spectra can be interpreted well by assuming that annotine is represented by any one of the three structures IIA, IIB and IIC proposed for the alkaloid (p. 16), and thus the probability that one of them is correct is strengthened. Henceforth in all formulations we shall depict annotine by structure IIA. The mechanisms for fragmentation which will be proposed must be considered tentative. A more detailed study with specifically labeled compounds would be required to test the mechanistic proposals. The mass spectra are plotted in terms of

relative abundance, with the most intense peak being taken as 100%.

The Mass Spectrum of Annotine

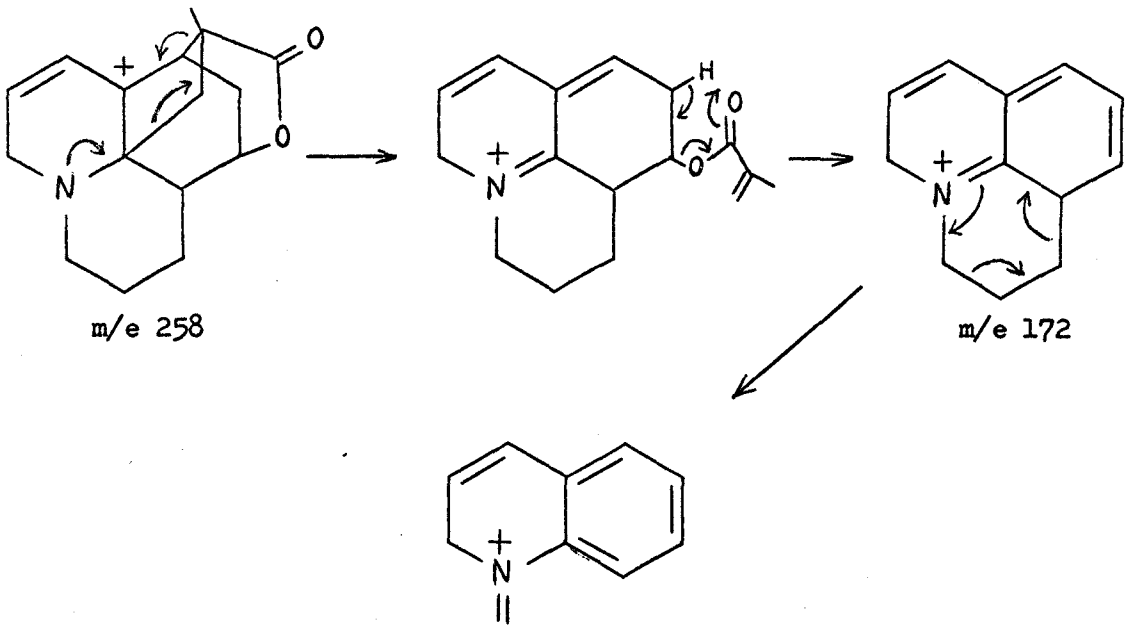
The mass spectrum of annotine, shown in Figure 8(a), may be explained by two fragmentation processes. In one process the first step involves loss of the hydroxyl group giving rise to an ion of mass 258.



m/e 275 m/e 258

A metastable peak* at m/e 242.0 is in accord with this change: the value of m/e calculated for the transformation 275 → 258 is m* = 242.0. The peaks observed at m/e 172 and 144 may be considered to arise from the ion of mass 258:

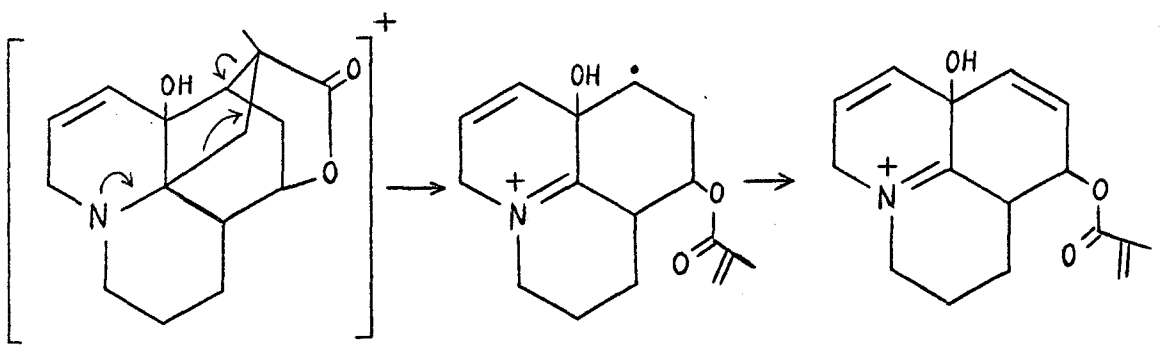
(*) Metastable peaks are observed in a spectrum when ions of mass m_1 decompose in the accelerating region near the exit slit to ions of mass m_2. The approximate value of m/e, m*, at which such ions are recorded in the spectrum may be calculated by applying the relation $m^* = m_2^2/m_1$ (53).



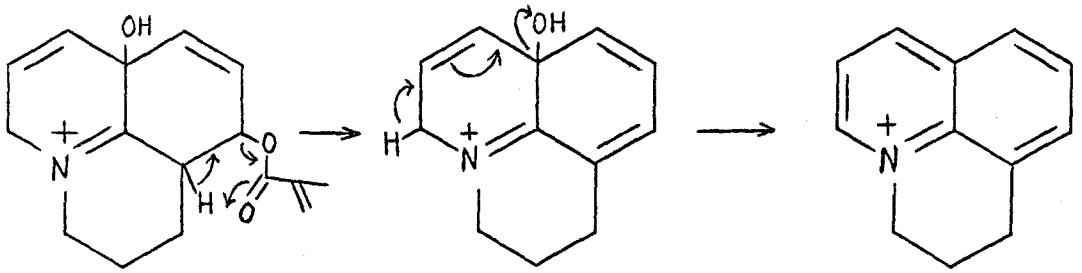
m/e 258 m/e 172 m/e 144

A metastable peak associated with the change 258→172 is observed at m/e 115 (m* calc. = 114.6). The last step is analogous to the process 174→146 encountered in the fragmentation of dihydrolycopodine and other Lycopodium alkaloids (p.16).

The peaks at m/e 274, 188 and 170 may be accounted for by the second fragmentation process:



m/e 275 m/e 274



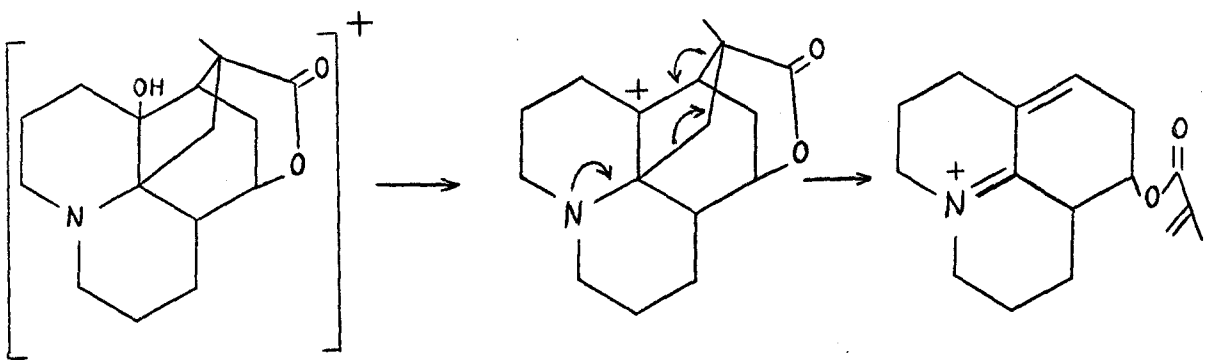
m/e 274 m/e 188 m/e 170

A metastable peak corresponding to the change 274→188 appears at m/e 129.5 (m* calc. = 129.0).

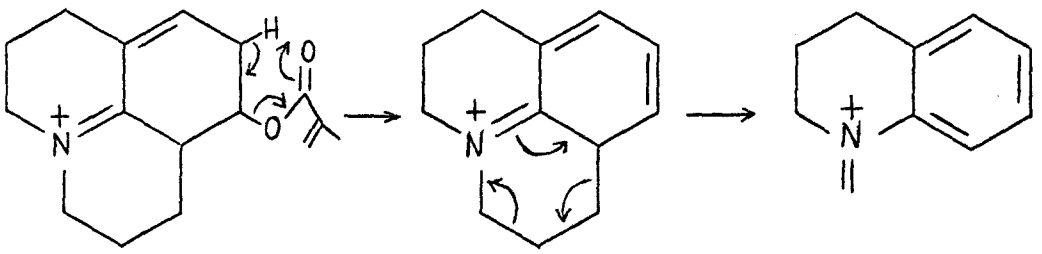
A peak present at m/e 231 is very likely due to the loss of carbon dioxide from the molecular ion. The presence of this peak, therefore, corroborates the presence of a lactone function in annotine.

The Mass Spectrum of Dihydroannotine (Fig. 8(b))

The resemblance of the mass spectrum of dihydroannotine (III) to that of annotine demonstrates that both compounds undergo an analogous fragmentation upon electron impact. One of the two fragmentation processes discussed in the previous section explains the presence of peaks at m/e 260, 174 and 146:



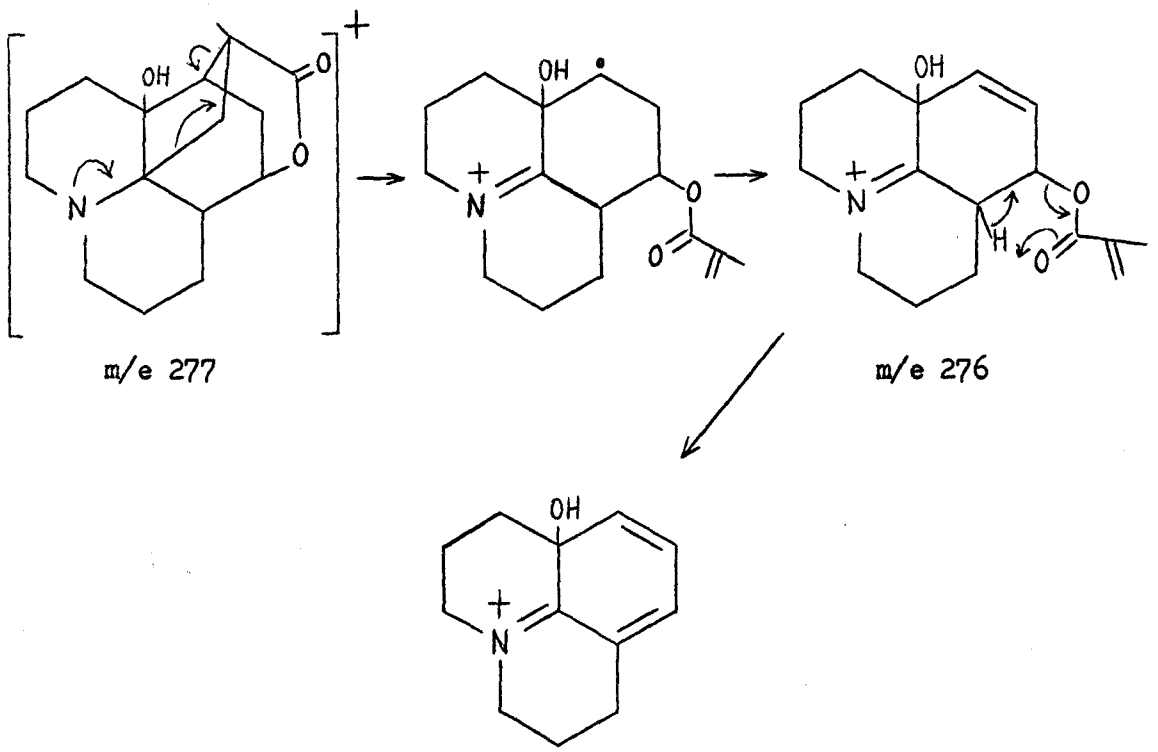
m/e 277 m/e 260



m/e 260 m/e 174 m/e 146

Metastable peaks are observed at m/e 244.5, 116.8 and 123.0 corresponding, respectively, to the transformations 277→260 (m* calc. = 244.0), 260→174 (m* calc. = 116.4) and 174→146 (m* calc. = 122.5).

The other fragmentation process when applied to dihydroannotine gives rise to ions of mass 276 and 190:



m/e 277 m/e 276 m/e 190

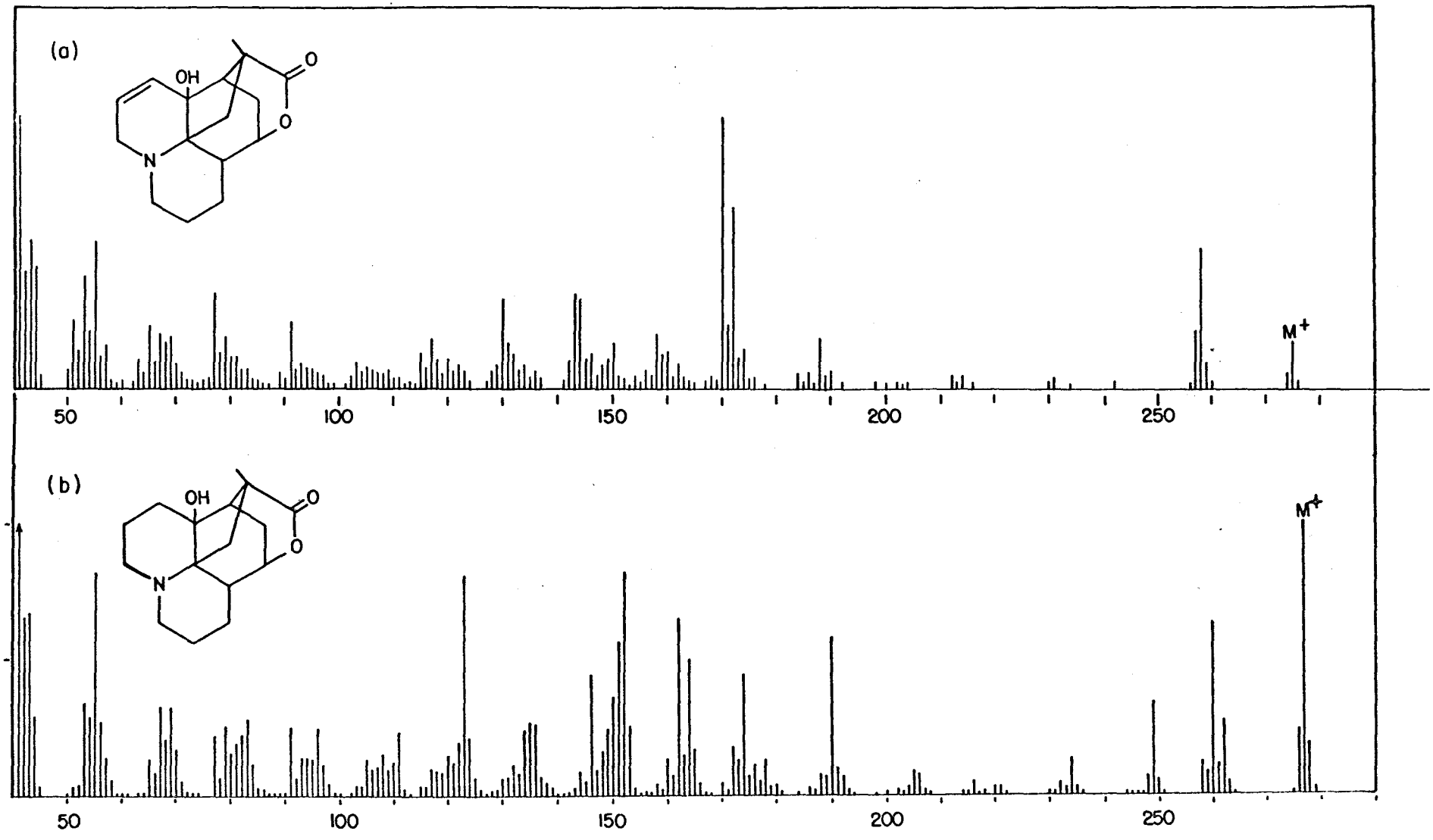
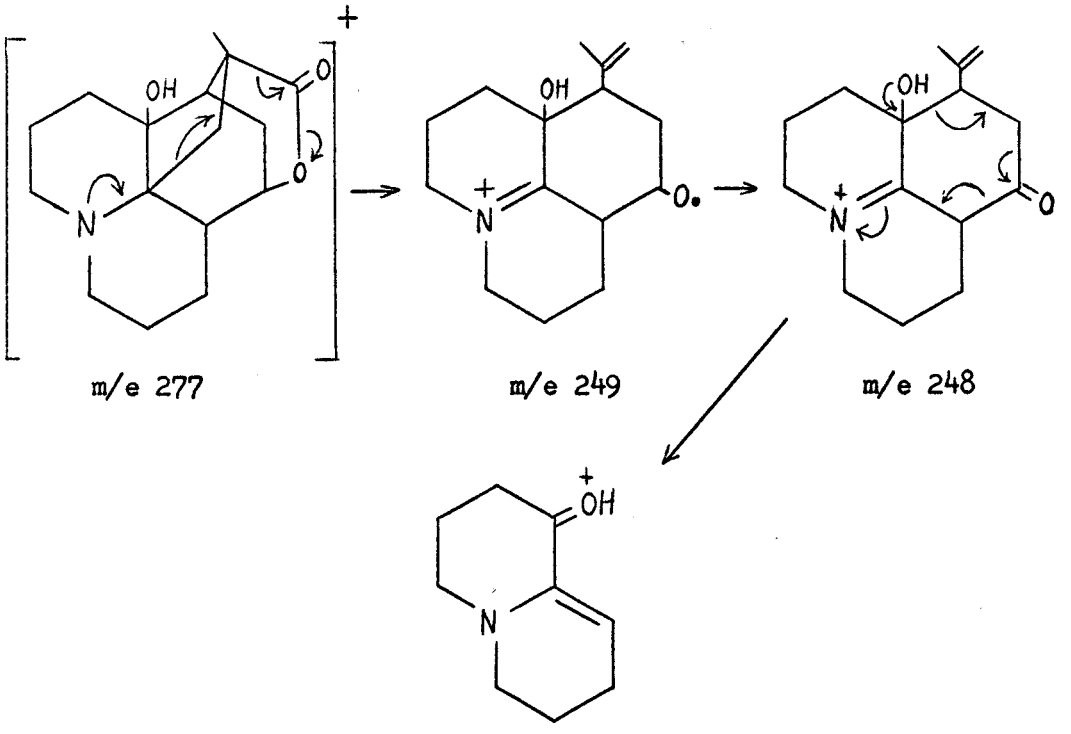


Figure 8. The Mass Spectrum of (a) Annotine (b) Dihydroannotine

A metastable peak is present at m/e 131.4 (m* calc. (276→190) = 130.8) in accord with the change formulated above.

There are differences, however, as well as similarities, in the mass spectra of the two compounds. These differences to a large extent reflect the diminished tendency of the molecular ion of dihydroannotine to lose the hydroxyl radical owing to the fact that such a process does not lead to a resonance stabilized carbonium ion, as in the case of annotine. The relative difficulty with which the hydroxyl radical is lost from the molecular ion is demonstrated by the intense peak corresponding to the latter; it is also shown by a comparison of the ratio of the intensity of the M-17 peak to the intensity of the M-1 peak in the spectra of annotine and dihydroannotine. The value of the ratio in the two spectra is 8.6 and 2.7, respectively.

As the molecular ion of dihydroannotine is not consumed to a large extent in the 277→260 transformation, other processes insignificant in the fragmentation of annotine, gain here considerable importance. Thus a relatively intense peak is present at m/e 262 presumably due to the loss of a methyl group; it provides evidence, independent from that available earlier, of the presence of such a group in the alkaloid. A peak at m/e 249 probably arises from the molecular ion by loss of carbon monoxide. The ion of mass 249 may give rise to the ions of mass 248 and 152:

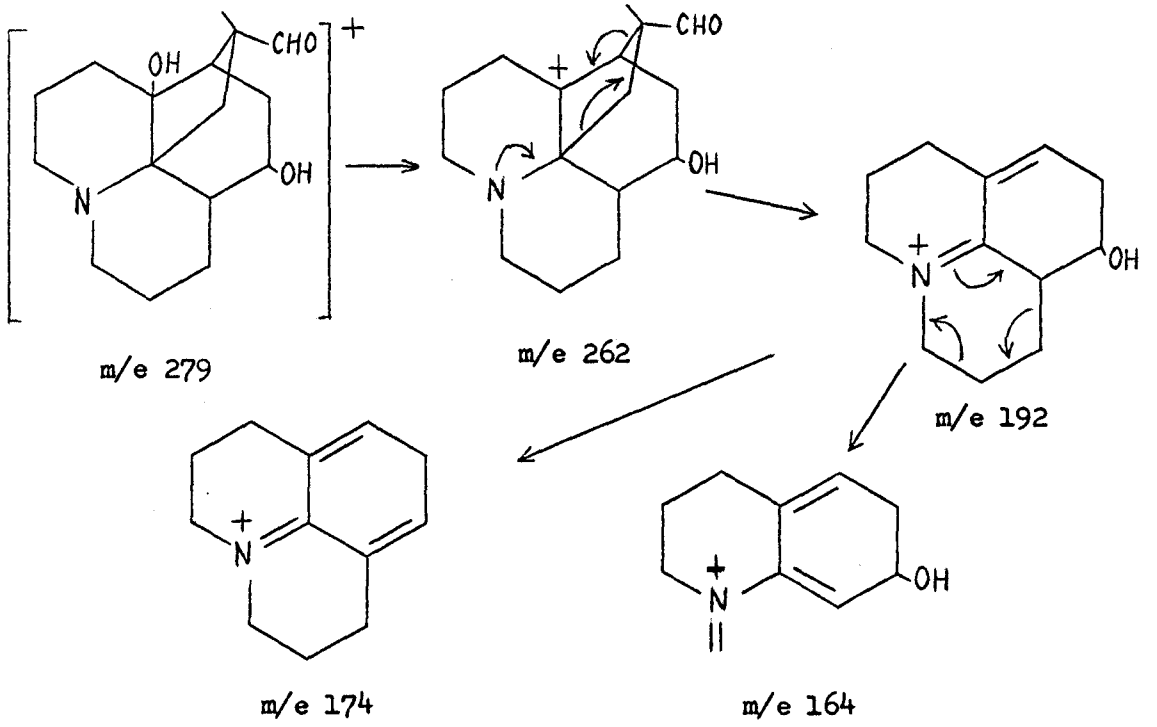


A metastable peak at 225.5 corresponds to the first step (m* calc. (277→249) = 223.8) of the sequence shown. The ions of mass 164 and 162 may arise from the fragment of mass 190 (see above) by loss of acetylene and ethylene, respectively.

The Mass Spectrum of Dihydroannotinol (Fig. 9(a))

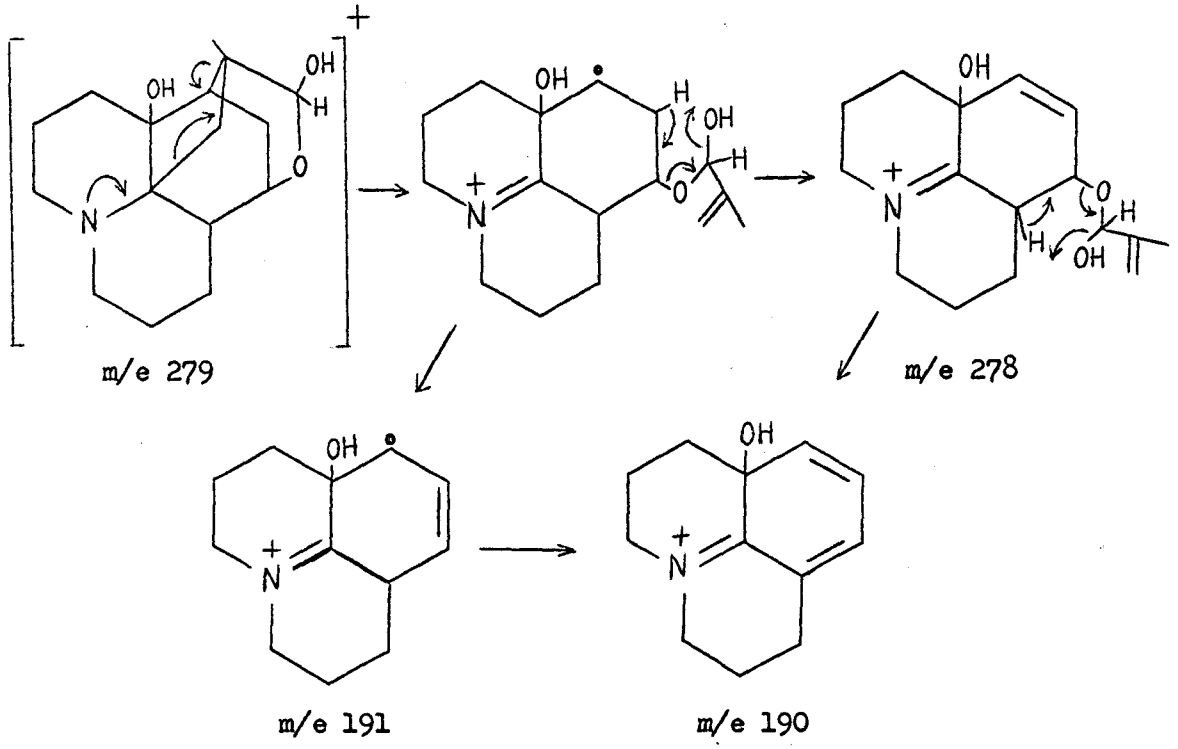
The infrared spectrum of dihydroannotinol shows that the compound in the solid state as well as in solution exists in a hemiacetal form. In the ion source of the mass spectrometer, however, dihydroannotinol is present in the gaseous phase, and probably consists of both the hemiacetal and the hydroxy aldehyde forms. We make use of the latter isomer in the proposed fragmentation mechanism.

A study of the mass spectrum of dihydroannotinol (IX) reveals, as expected, an analogy with the patterns present in the spectra of annotine and dihydroannotine. Here also, we find the two main fragmentation mechanisms advanced previously. The process initiating by the M→M-17 transformation is formulated below:



This mechanism is supported by the presence of metastable peaks at m/e 246.5 (m* calc. (279→262) = 246.0) and 159.0 (m* calc. (192→174) = 157.7). A metastable peak present at m/e 141.0 could be due to either one of the transformations: 262→192 (m* calc. = 140.7) and 192→164 (m* = 140.0).

The ions of mass 278, 191 and 190 may be accounted for by the following fragmentation scheme:



The ion-radical of mass 279 may lose methacrylaldehyde and water to form the species of mass 191 or it may be converted to the ion of mass 278 by loss of a hydrogen radical. As shown above, both processes may lead to the formation of the ion of mass 190. The ions of mass 261 and 260 probably arise by dehydration of the molecular ion and the ion of mass 278, respectively.

The Mass Spectrum of Compound X (Fig. 9(b))

Cleavage of the C-S bond is the dominant feature in the fragmentation of compound X. The peak at m/e 262 is by far the most intense in the spectrum. The peak at m/e 244 results from loss of water from ion of mass 262.

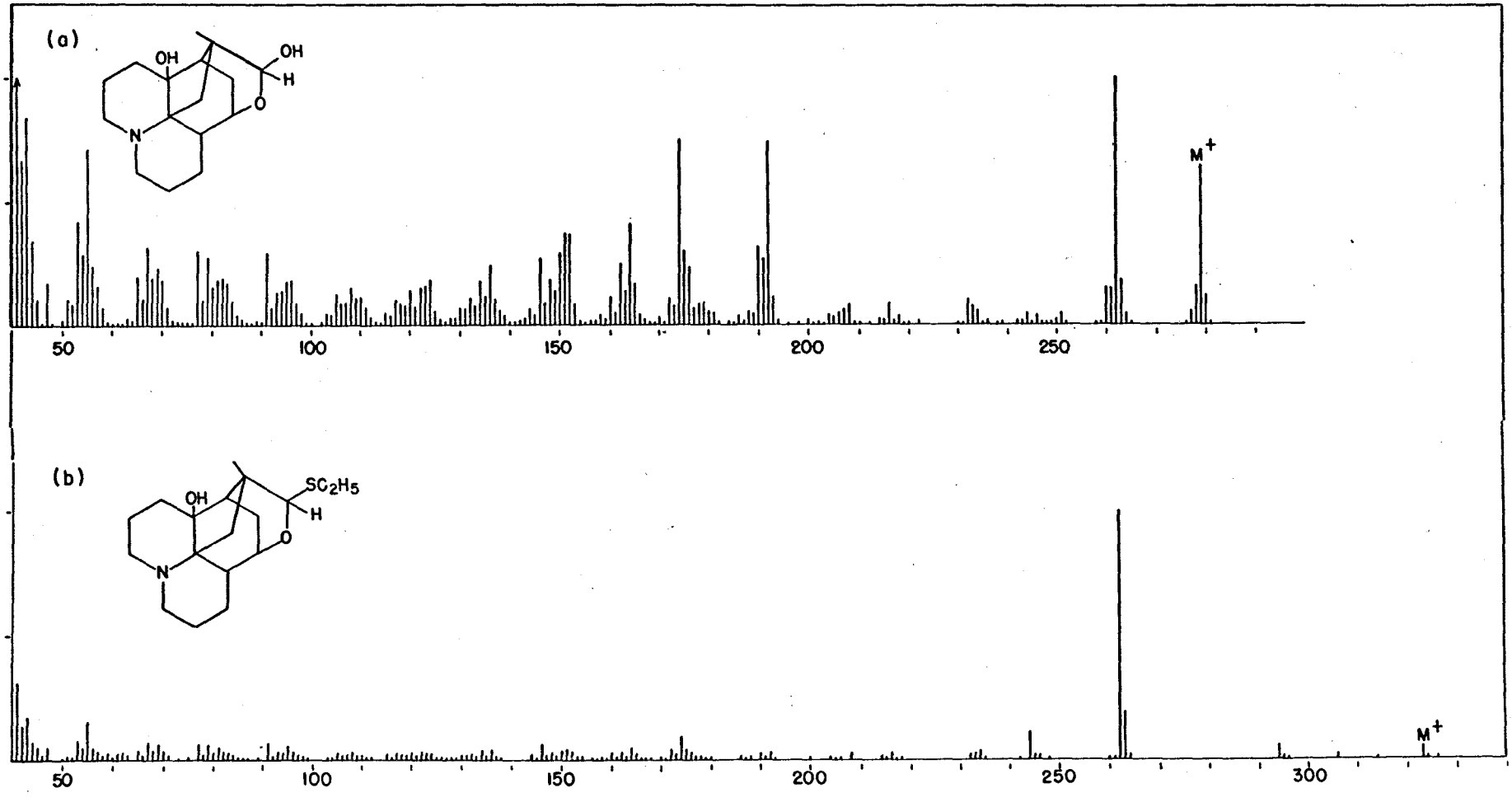
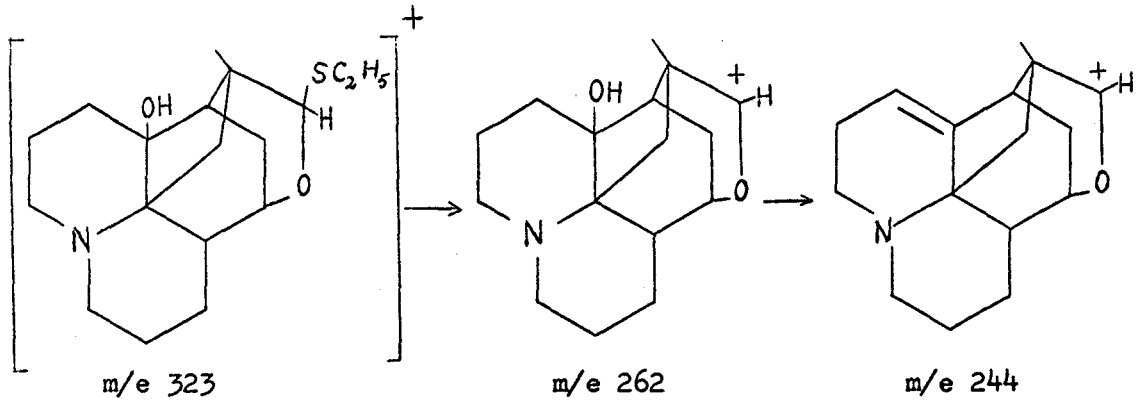
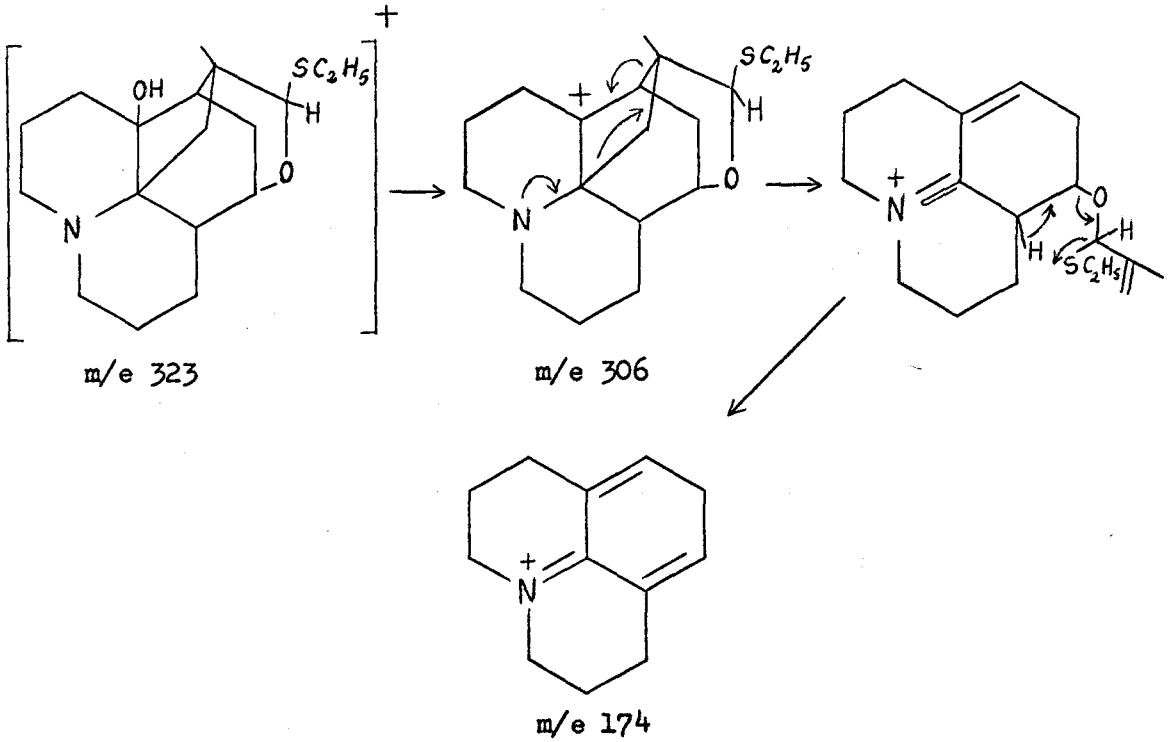


Figure 9. The Mass Spectrum of (a) Dihydroannotinol (b) Compound X



Metastable peaks are observed at m/e 213 (m* calc. (323→262) = 212.4) and 228 (m* calc. (262→244) = 227.2) in accord with these changes.

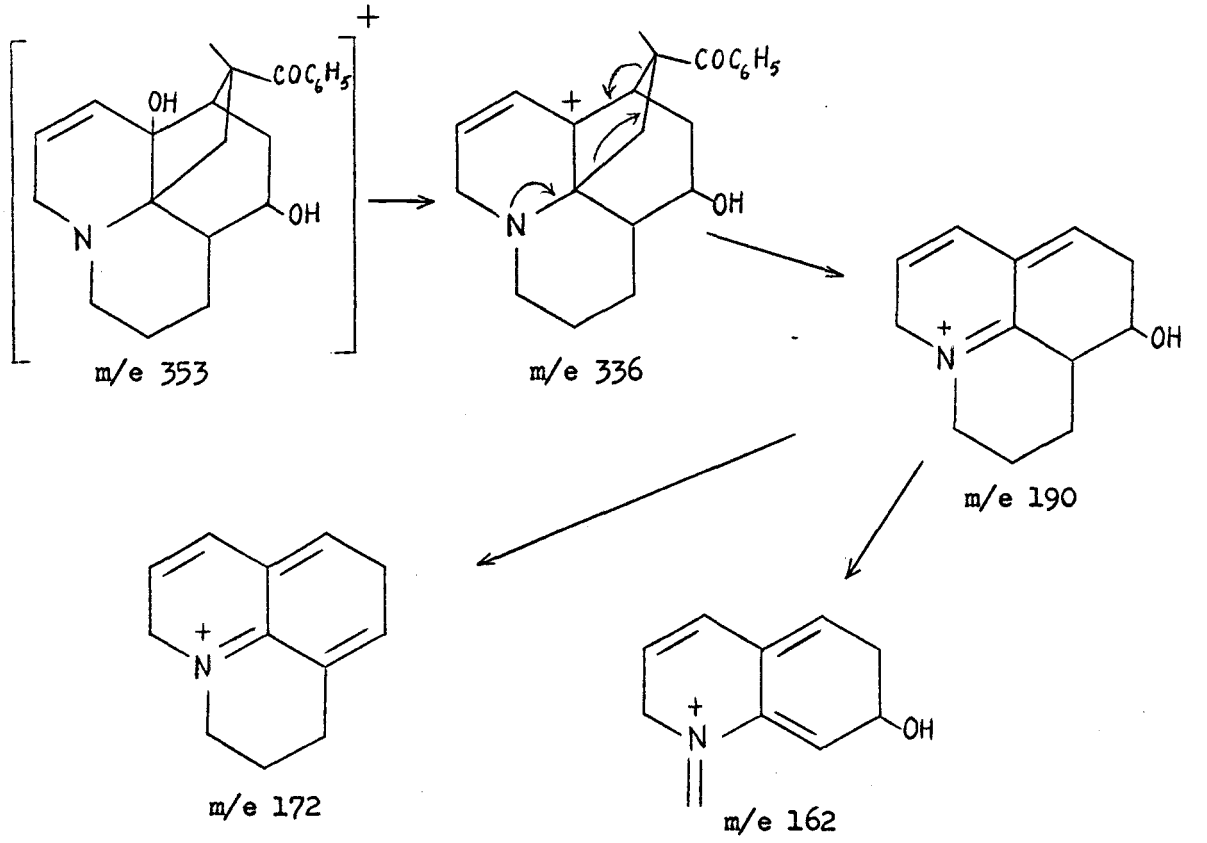
The signal at m/e 294 can be considered to arise by loss of the ethyl group from the molecular ion. Other peaks of low intensity are found at m/e 306 and 174; they probably arise by the already familiar process involving loss of the hydroxyl radical followed by expulsion of the bridge:



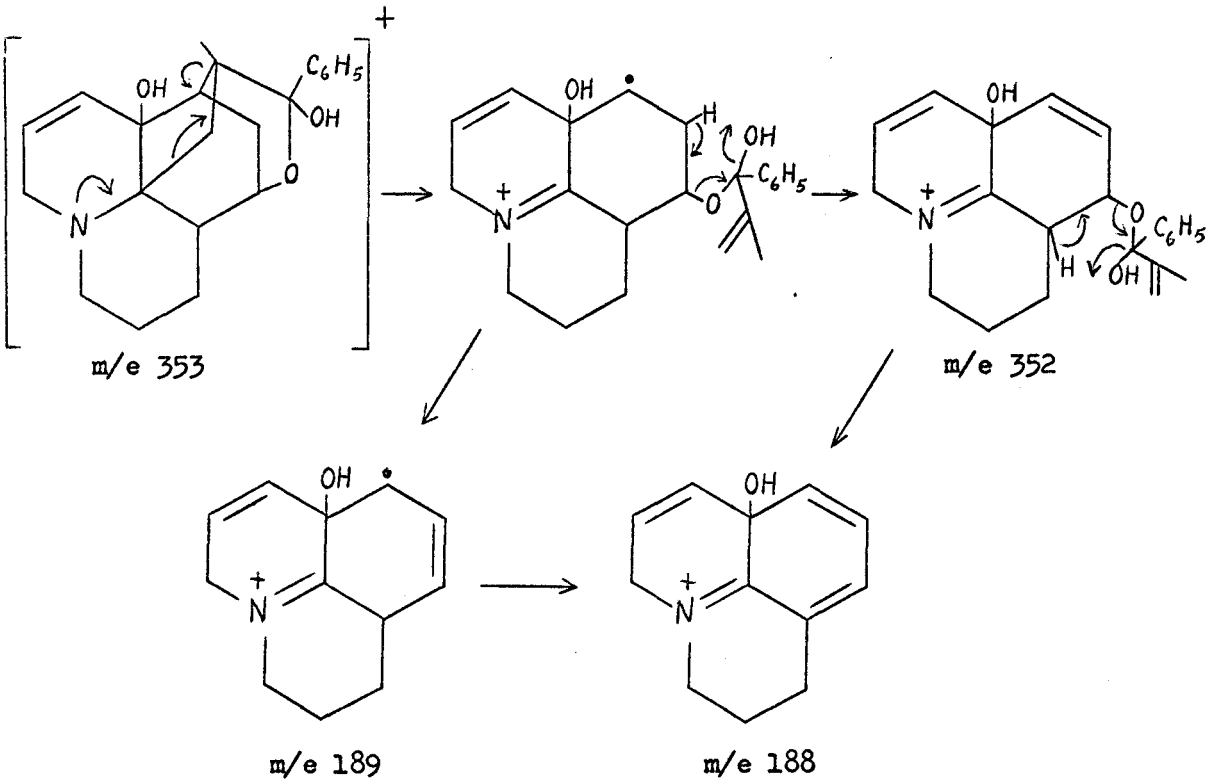
A metastable peak is observed at 290.5 corresponding to the process 323→306 (m* calc. = 289.8).

The Mass Spectrum of Phenylannotine (Fig. 10)

Phenylannotine, as shown by infrared spectroscopy, is a hemiketal in the solid state and in solution. In the ion source of the mass spectrometer, however, the compound is present in the gaseous state and probably consists of a mixture of hemiketal and hydroxy ketone. As expected phenylannotine undergoes a fragmentation which is very similar to that of dihydroannotinol:

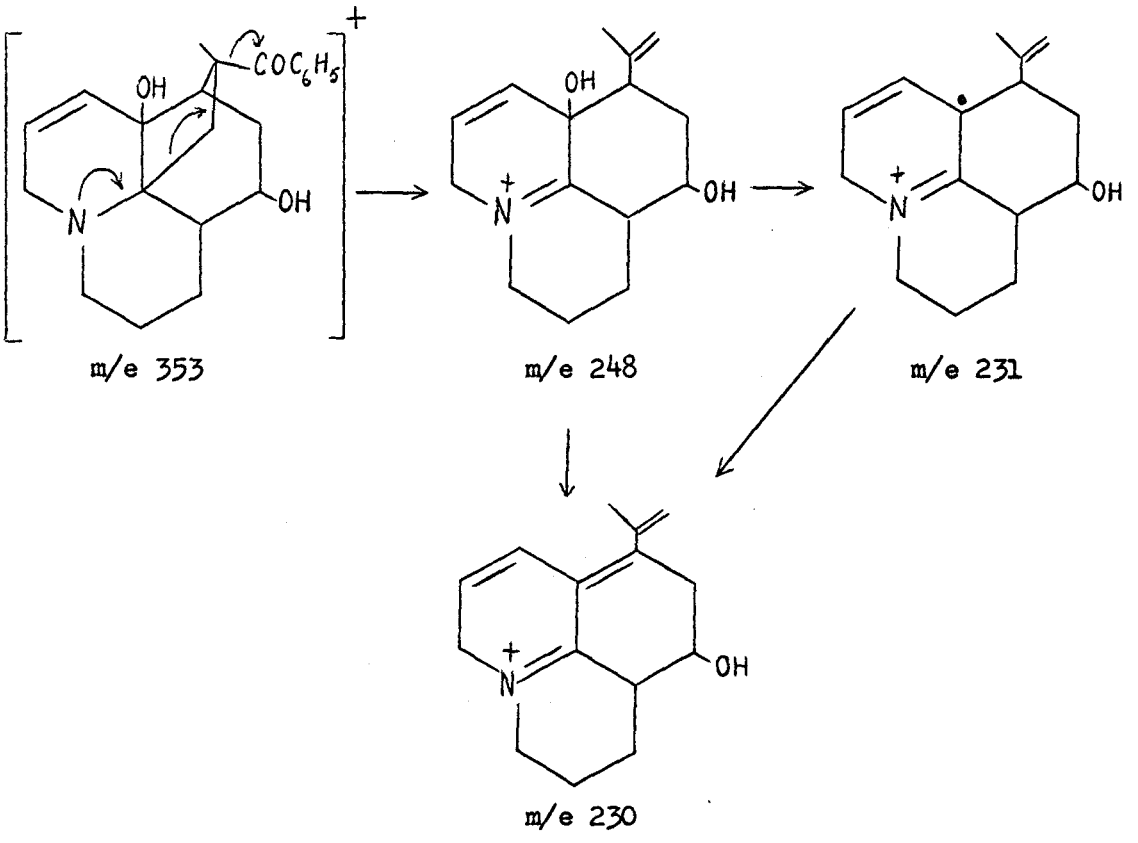


A metastable peak situated at m/e 320.3 is explained by the first step (m* calc. 353→336) = 319.8). The process starting with the M→M-1 transformation affords the species depicted below:



The process is completely analogous to the corresponding fragmentation of dihydroannotinol.

The formulated fragmentation schemes do not account for the formation of ions of mass 248, 231, 230 and 214. The first three fragments can be conceived to arise in the following manner:



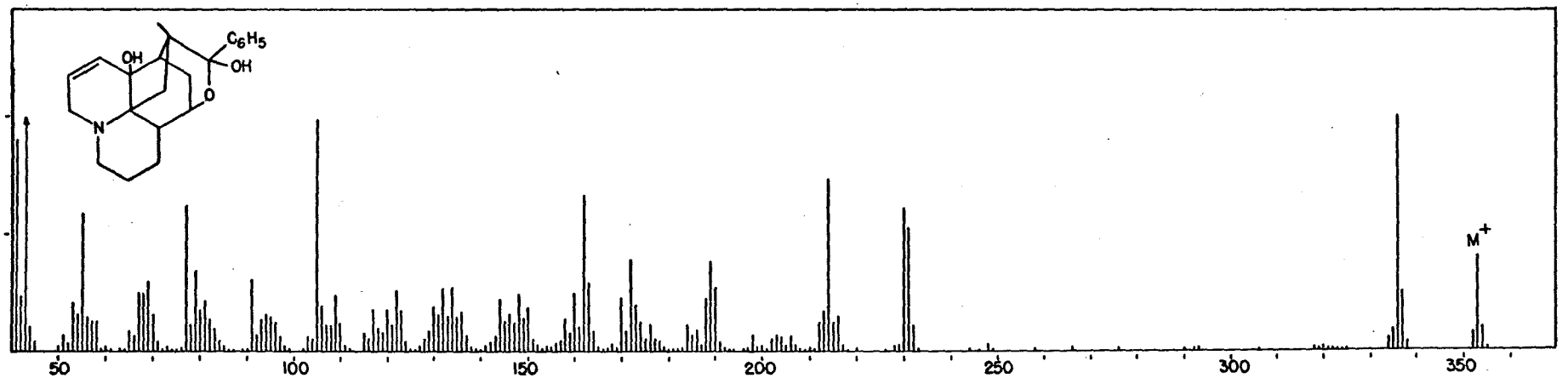
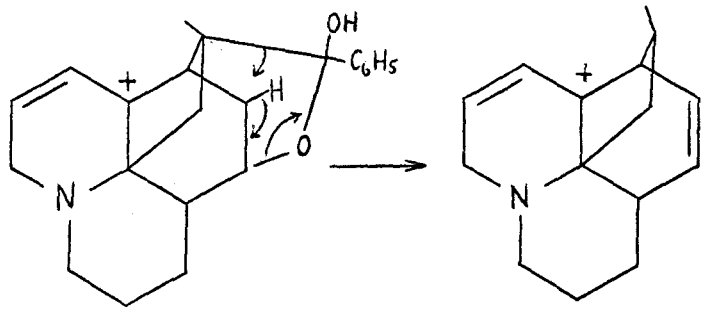


Figure 10. The Mass Spectrum of Phenylannotine

The m/e 214 peak could result from the process:



m/e 336 m/e 214

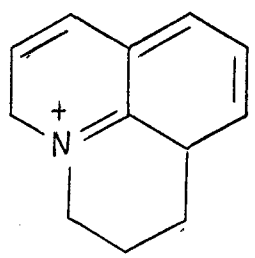
We should point out here that it is difficult to account for the high abundance of the ion of mass 214. The fragmentation mechanism above does not appear to be particularly favorable, but is offered in lieu of a better alternative.

Information on the Structure of Annotine Obtained by the Mass Spectrometric Studies

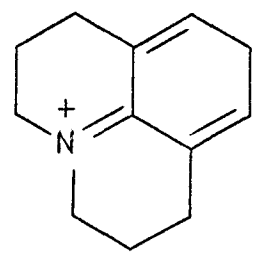
Structures IIA, IIB and IIC were proposed for annotine on the basis of the degradative work discussed in the introductory section and on the assumption that annotine should bear a close structural relationship to other Lycopodium alkaloids of known structure. The mass spectrometric studies which were discussed in the previous sections are compatible with the expressions IIA, IIB and IIC for annotine and lend further support to their validity.

In the present section we shall discuss the precise knowledge gained by the mass spectrometric studies. The evidence for the presence of a methyl group has been considered in the section dealing with the mass spectrum of dihydroannotine. Here we shall demonstrate that the mass spectra suggest strongly the presence of a two carbon bridge, bearing a methyl group and a carbonyl group, attached to a reduced julolidine system.

In the spectra of annotine and phenylannotine a peak is present at m/e 172, while in the spectra of the three derivatives in which the original double bond of annotine is reduced this peak appears at m/e 174. These two ions may be accommodated within a julolidine system as shown below.



m/e 172



m/e 174

Ions of this mass have been observed in the spectra of many other Lycopodium alkaloids of known structure which contain the reduced julolidine system. Loss of the bridge resulting in the formation of an ion corresponding to a simple julolidine derivative dominates the fragmentation process in these compounds.

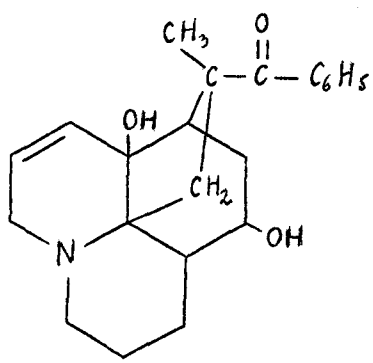
As shown by the presence of a metastable peak at m/e 115 in the mass spectrum of annotine, the ion of mass 172 is formed from the ion of mass 258 in a single step. This strongly suggests that the four carbon atoms of the fragment expelled ($C_4H_6O_2$) are present as a single unit in the bridge. One of them is necessarily the methyl group carbon; another

carbon atom is involved in the formation of a lactone carbonyl. Thus we are led to the conclusion that annotine contains a julolidine grouping to which a two carbon bridge is attached, bearing a methyl group and a lactone carbonyl.

The same line of argument can be applied on the basis of the spectra of dihydroannotine and dihydroannotinol. Though the transformations 306→174 and 352→189 in the fragmentation processes of dihydroannotine thioacetal and phenylannotine, respectively, probably occur in a single step, there are no metastable peaks present in the spectra to support this hypothesis.

The Pyrolysis of Phenylannotine

The conclusions, with regard to the structure of ring D, steming from the study of the mass spectra of annotine and its derivatives are corroborated by the investigation of the thermal degradation of phenylannotine. This compound, represented below in terms of structure IIA, was heated at 300° in the presence of zinc dust. The distillate collected during the



VIIIA

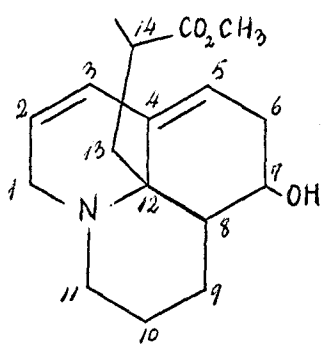
pyrolysis (see experimental section) was examined by gas-liquid partition chromatography. It was found to consist of two substances, one at least of which appeared to be a phenone on the basis of the ultraviolet spectrum of the mixture. The spectrum shows the characteristic absorption of such compounds, namely bands at 280 and 240 mμ. The identity of the compounds present in the distillate was revealed by comparison of their retention times with those of known phenones. A column of 5% SE-30 on celite was used. The pyrolysis product coming first through the column was found to have a retention time identical with that of propiophenone. The other pyrolysis product leaves the column simultaneously with both isobutyrophenone and α-methacrylophenone. In order to identify it with one of these two substances another column (5% carbowax 1500 on Chromosorb P) was used. In this case, the retention times of isobutyrophenone and α-methacrylophenone were found to be different; that of the latter compound being identical with the retention time of the pyrolysis product. The presence of propiophenone in the reaction mixture was confirmed by use of the "Carbowax column". In structure VIIIA the carbon atoms participating in the formation of α-methacrylophenone have been designated.

Relative Importance of Structures IIA, IIB and IIC

In the present section we shall consider whether any one of the three structures IIA, IIB and IIC, proposed for annotine, is favoured over the others. The conclusion, based on infrared spectroscopy, that dihydroannotinol and phenylannotine exist in the hemiacetal and hemiketal forms, respectively, is evidence against structure IIC. Examination of molecular models, based on structure IIC, of dihydroannotinol and phenylannotine reveal that considerable strain is released by opening the hemiacetal and hemiketal

rings, respectively. Thus, if structure IIC were correct one would expect dihydroannotinol to be a hydroxy aldehyde rather than a hemiacetal and phenylannotine to exist as the hydroxy ketone.

Structure IIA is favoured over both IIB and IIC 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 in which substitution is observed at C_{13}. This is a structural feature with which structure IIA alone is in agreement. Recently Professor Ayer, of the University of Alberta, informed us (54) that in collaboration with Professor Anet, of the University of Ottawa, and Professor Valenta, of the University of New Brunswick, he has arrived at structure XVII for a new alkaloid isolated from L. Annotinum. This structure bears a strikingly close relationship with IIA. The carbon skeleton of annotine is formally



XVII

derived from XVII by formation of a bond between C_5 and C_{14}. Thus it may be that annotine and this new alkaloid (XVII) are formed in the plant from a common precursor. The conversion of annotine to the carbon skeleton of XVII *in vitro* is readily visualized. Transformation of annotine to the hydroxy methyl ester, oxidation of the C_7 hydroxyl to the ketone followed by a β-elimination leads to the carbon skeleton of XVII.

EXPERIMENTAL

Melting points were taken on a Kofler Micro Hot Stage.

Infrared spectra were determined on a Perkin-Elmer Model 21 recording spectrophotometer or on a Beckman IR 5 infrared spectrophotometer with a sodium chloride prism. Ultraviolet spectra were measured in methanol on a Perkin-Elmer Model 4000 Spectrocord.

Nuclear magnetic resonance spectra were measured on a Varian V-4300B high-resolution spectrometer at a frequency of 60 Mc/sec. The NMR spectra were measured in concentrated deuterochloroform solutions with tetramethylsilane as an internal standard. Chemical shifts are expressed on the τ-scale of Tiers (55).

Gas chromatographic analyses were performed with a Research Specialties Co. gas chromatograph whereas gas chromatographic separations were carried out with an Aerograph A-700 Wilkens instrument.

The mass spectra were determined on a CEC-103C mass spectrometer. Samples were introduced directly into the ion source using a vacuum-lock system. All spectra were run through the courtesy of Professor K. Biemann of the Massachusetts Institute of Technology.

Microanalyses were performed by Dr. C. Daessle of Montreal and Dr. E. Thommen of Basel, Switzerland.

Isolation of Flabelliformine XII

The crude alkaloid mixture was obtained from L. Flabelliforme by the method of Manske and Marion (2). Lycopodine was isolated by column chromatography as described by Barclay and MacLean (56). The other, more strongly adsorbed, alkaloids were eluted with chloroform and combined. The mixture of bases was shaken with cold ether and the ether-insoluble residue crystallized from acetone-methanol. The material obtained was dissolved in acetone and treated with 48% hydrobromic acid. Flabelliformine hydrobromide separated immediately. From the mother liquors dihydrolycopodine was obtained. Recrystallization of the hydrobromide from acetone, containing a small quantity of water, gave crystals melting over 337° with decomposition. The alkaloid (XII) liberated from the hydrobromide salt melts at 210-211°. A change in crystalline form occurs at 150°. Calc. for $C_{16}H_{25}O_2N$:C, 72.96; H, 9.57; N, 5.32%. Found: C, 73.04; H, 9.68; N, 5.33%.

The infrared spectrum of flabelliformine, determined in chloroform, shows hydroxyl absorption at 3560 cm^{-1}, carbonyl absorption at 1705 cm^{-1}, and a band at 1415 cm^{-1} attributed to a methylene group adjacent to the carbonyl. The ultraviolet spectrum has λ_{max} at 303 mμ (ϵ, 50). The NMR spectrum of the alkaloid shows no absorption below a τ-value of 6.5, indicating the absence of the group $>\underline{C}H\text{-}OH$.

Flabelliformine, when treated with methyl iodide in acetone solution, gave a crystalline methiodide which melted over 335° with decomposition. Calc. for $C_{17}H_{28}O_2NI$:C, 50.37; H, 6.96; N, 3.45%. Found: C, 50.75; H, 7.08; N, 3.35%.

Preparation of Compound XIII

A solution of sodium borohydride (0.209) in ethanol was added slowly to a stirred ethanolic solution of flabelliformine (0.15 g). After addition of the hydride solution, the mixture was stirred for 2 hours and then left to stand overnight. Excess reagent was destroyed with acetone and the reaction mixture evaporated to dryness. Water was added to the residue. The mixture was acidified with hydrochloric acid and heated briefly on a steam bath. It was then basified with ammonia and extracted with chloroform. From the chloroform extract a crystalline product (compound XIII) was obtained (0.12 g) which, on recrystallization from ether, afforded crystals melting at 217 - 217.5°. Calc. for $C_{16}H_{27}O_2N$:C, 72.41; H, 10.26; N, 5.28%. Found: C, 72.41; H, 10.28; N, 5.28%.

The infrared spectrum (Nujol mull) of compound XIII shows no carbonyl-group absorption but has two bands in the hydroxyl region at 3610 and 3460 cm^{-1}.

Treatment of Flabelliformine with Chromium Trioxide

A solution of chromium trioxide (0.10 g) in water was added dropwise over a period of 1 hour to a stirred solution of flabelliformine (0.040 g) in 10% aqueous acetic acid. The temperature was maintained at -5° to -10° during the addition, and the mixture was stirred at this temperature for a further 5 hours. The oxidant was destroyed with methanol and the reaction mixture was kept in a refrigerator overnight. The solution was then concentrated, made strongly acidic with hydrochloric acid, and extracted with chloroform. It was then basified with ammonia and again extracted with chloroform. From the last extract flabelliformine (0.033 g) was recovered.

Attempted Oppenauer Oxidation of Flabelliformine

Flabelliformine (0.047g) and aluminum tertiary butoxide (0.3 g) were added to toluene (5 ml.) containing cyclohexanone (1 ml.). The mixture was heated under reflux for 27 hours. After the solution was cooled, it was poured into a dilute aqueous hydrochloric acid solution. The organic layer was separated from the aqueous layer and extracted with dilute aqueous hydrochloric acid. The combined aqueous solutions were extracted with ether, made basic by addition of ammonia and then extracted with chloroform. From the chloroform solution an oily product was obtained which was found to have an infrared spectrum identical with that of compound XIV (see below). The reaction product formed a crystalline methiodide; its melting point was not depressed on admixture with a sample of compound XV.

Treatment of Flabelliformine with Hydriodic Acid

Flabelliformine (0.05 g) was dissolved in hydriodic acid (4 ml., 47%). The solution was heated under reflux for 17 hours, then made basic with ammonia and extracted with chloroform. The compound isolated (0.03 g) from the chloroform extract was found to be identical with lycopodine: the melting point of the product was not depressed on admixture with an authentic sample of lycopodine and the infrared spectra of the two samples, measured both in Nujol mull and carbon tetrachloride were superimposable.

Dehydration of XII by Treatment with Phosphoric Acid

A solution of flabelliformine (0.11 g) in 88% aqueous phosphoric acid (7 ml.) was heated on a steam bath for 12 hours. The mixture was cooled, made basic with potassium hydroxide, and extracted with chloroform. Evaporation of the chloroform left an oil (XIV) (0.09 g) which could not be induced to crystallize.

The infrared spectrum (film) of compound XIV shows absorption at 1614 cm^{-1} (conjugated double bond) and 1678 cm^{-1} (conjugated carbonyl); the two bands are situated at 1617 and 1685 cm^{-1} when the spectrum is measured in carbon tetrachloride solution. Compound XIV has λ_{max} at 245 mμ. The NMR spectrum exhibits a triplet at a τ-value of 3.01.

Dissolution of compound XIV in acetone and treatment with an excess of methyl iodide afforded a crystalline methiodide, XV, which melted over 285° with decomposition. Calc. for $C_{17}H_{26}ONI$: C, 52.71; H, 6.77; N, 3.62%. Found: C, 52.40; H, 6.86; N, 3.55%.

The infrared spectrum (Nujol mull) of compound XV shows bands at 1620 and 1688 cm^{-1}.

Dehydration of XII by Treatment with p-Toluene-sulphonic Acid

A solution of flabelliformine and p-toluenesulphonic acid in o-xylene was heated under reflux for 12 hours. The reaction mixture was extracted with water. The extract was shaken with ether to remove completely the xylene, basified with ammonia, and extracted with chloroform. The product obtained from the chloroform solution was identical with compound XIV described in the preceding section.

Hydrogenation of Compound XIV

A solution of compound XIV in methanol was shaken with hydrogen (35 p.s.i.g.) and platinum oxide for 16 hours. The catalyst was removed by filtration and the solvent evaporated. The residue was dissolved in acetone and treated with methyl iodide. A crystalline methiodide separated which melted with decomposition at 315-318°. The methiodide was shown to be identical with lycopodine methiodide by a mixed melting point determination and comparison of infrared spectra.

Formation of Compound XVI

Methyl iodoacetate, prepared by reacting iodoacetic acid (1g) with diazomethane, was added to a solution of flabelliformine (0.059 g) in acetone. Crystallization yielded a small amount (0.013 g) of compound XVI. The mother liquors were taken to dryness and the residue was treated with ether (removal of excess methyl iodoacetate). The ether-insoluble part was dissolved in acetone; crystallization from acetone-ether gave an additional quantity (0.023 g) of product. Recrystallization afforded crystals melting at 300-304° with decomposition. Calc. for $C_{19}H_{30}O_4NI$:C, 49.25; H, 6.53%. Found: C, 48.86; H, 6.82%.

Isolation of Annotine

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 2M acetic acid and treated with a concentrated solution of sodium nitrate. The crystals of annotine nitrate were separated, dissolved in water and then 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 crystals which melted at 174.5 - 176°. The alkaloid obtained was identified by recording its melting point on admixture with an authentic sample of annotine.

The infrared spectrum of annotine (Nujol mull) shows 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.

From 23 Kg of dried plant material 1g of annotine was obtained.

Preparation of Dihydroannotine (III)

A solution of annotine (0.089 g) in 30 ml. of redistilled methanol containing 5 drops of concentrated hydrochloric acid and 0.050 g of Adams' catalyst was subjected to a hydrogen pressure of 50 p.s.i.g. for four hours. 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 the solvent was evaporated under reduced pressure to yield 0.080 g of product. Recrystallization from acetone-ether gave crystals melting at 203 - 204°. The melting point of the product was not depressed on admixture with an authentic sample of dihydroannotine and the infrared spectra of the two samples were superimposable.

The infrared spectrum(Nujol) of dihydroannotine shows hydroxyl absorption at 3810^{-1}, carbonyl absorption at 1728 cm^{-1} and strong absorption at 1118 cm^{-1}.

Dihydroannotinol IX

A sample of this compound, prepared in our laboratory by other workers, was used for the mass spectrometric studies.

Compound X

This compound was available in the laboratory having been prepared by other workers.

Preparation of Phenylannotine VIII

A three-necked flask was equipped with a dropping funnel, a mercury-sealed mechanical stirrer and a reflux condenser carrying a drying tube with calcium chloride. The apparatus was flushed with dry, oxygen-free nitrogen gas. Lithium shavings (0.02 g) were placed in the flask and then, a solution of 0.26 g of dry, redistilled bromobenzene in 3 ml. of anhydrous ether was added through the dropping funnel at a rate ensuring gentle reflux of the solvent. A solution of annotine (0.5 g) in anhydrous ether was then introduced and the resulting mixture boiled for 1 hour under nitrogen. The reaction mixture was cooled and then decomposed by pouring it onto ice containing glacial acetic acid. The organic layer was separated and the acidic aqueous solution washed with ether. The aqueous solution was then basified with 2N sodium hydroxide and extracted with chloroform. The chloroform solution was dried and the solvent removed under reduced pressure. A crystalline residue (0.04 g) was obtained which after recrystallization from acetone melted at 215 - 217°. The product was shown to be identical with phenylannotine (VIII) by a mixed melting point determination and comparison of infrared spectra.

The infrared spectrum (Nujol) of compound VIII shows an intense band in the hydroxyl region at 3470 cm^{-1}, and a weak band at 1640 cm^{-1} attributable to non-benzenoid unsaturation. Strong bands are present in the 600 - 1200 cm^{-1} region attributable to a benzene ring.

Pyrolysis of Phenylannotine VIII

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 hour. The material which distilled in the capillary was studied by vapour 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 identical retention times with propiophenone and α-methacrylophenone 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.

Preparation of α-Methacrylophenone

This compound was prepared from propiophenone, paraformaldehyde and dimethylamine hydrochloride by the method used by Burckhalter and Fuson (57).

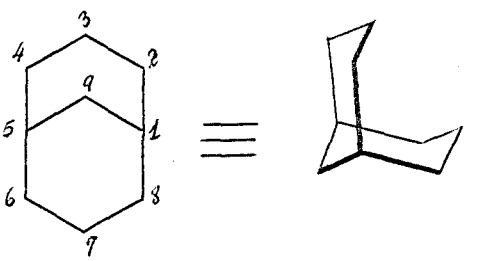
PART II

SYNTHETIC STUDIES

HISTORICAL INTRODUCTION

The Bicyclo[3.3.1]nonane System

With the exception of annotinine, the *Lycopodium* alkaloids of known structure contain a bicyclo[3.3.1]nonane grouping. This fact led us to consider a synthetic approach to the *Lycopodium* alkaloids involving

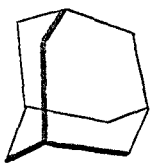


Bicyclo[3.3.1]nonane

construction of a bicyclo[3.3.1]nonane system carrying such substituents as would enable, at a later stage, its expansion to an alkaloid molecule. Accordingly we searched in the chemical literature to find available methods for the preparation of these bridged systems and in particular of substituted bicyclo [3.3.1]nonan-9-ones; a type of compound which was considered suitable as an intermediate in the synthesis of a *Lycopodium* alkaloid.

In the present introductory section we shall discuss those syntheses of substituted bicyclo[3.3.1]nonan-9-ones which have a relationship to our synthetic scheme and thus influenced its planning.

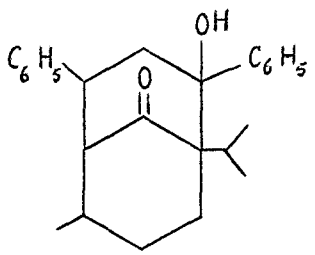
A synthesis of bicyclo [3.3.1] nonane was first achieved by Meerwein and his co-workers (58, 59). Adamantane, a related hydrocarbon shown below was prepared first by Prelog and Seiwerth (60); later, by a



Adamantane

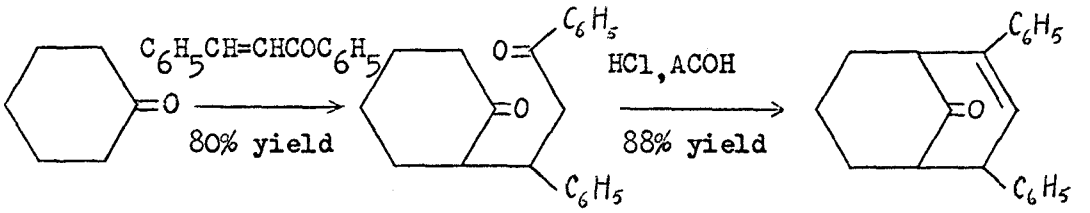
method affording the compound in much better yield, by Schleyer (61).

The synthesis of a substituted bicyclo [3.3.1] nonan-9-one was for the first time, described by Stobbe and Rosenberg (62). These workers prepared the cyclic keto alcohol shown, by the reaction of menthone with



benzalacetophenone followed by treatment of the intermediate monocyclic ketone with hydrogen chloride in warm alcohol.

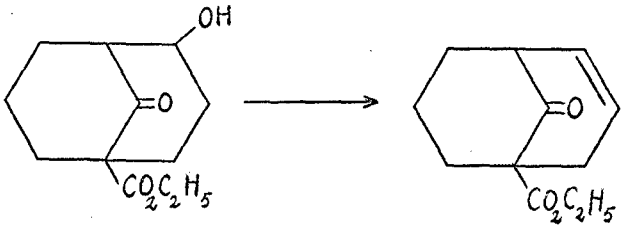
Later, Allen and Sallans (63) reported the preparation of 2,4-diphenylbicyclo [3.3.1] non-1-en-9-one by the Michael addition of benzalacetophenone to cyclohexanone, followed by cyclization through the action of concentrated sulphuric acid in absolute ethanol. Cope and his collaborators re-investigated (64) the reaction and provided direct evidence that the double bond in the product is not located at the bridgehead position, but at the 2,3 position in agreement with Bredt's rule. The same authors were able to improve the method for the cyclization step. They found that cyclization in the presence of acetic acid and hydrochloric acid gave a higher yield compared to that obtained when concentrated sulphuric acid was used.



In another publication (65) Cope and Hermann reported the synthesis of 2-phenylbicyclo [3.3.1] non-2-en-9-one. In the first step cyclohexanone was treated, in the presence of sodium hydroxide, with β-dimethylaminopropiophenone to give 2-(β-benzoylethyl)-cyclohexanone (50% yield). The diketone was then cyclized by an acid catalyzed internal aldol condensation (91%).

A synthesis of 1-carbethoxybicyclo [3.3.1] non-3-en-9-one XIX, reported by Cope and Synerholm (66), provided the basis for our synthetic scheme, and for this reason will be described here in some detail. The

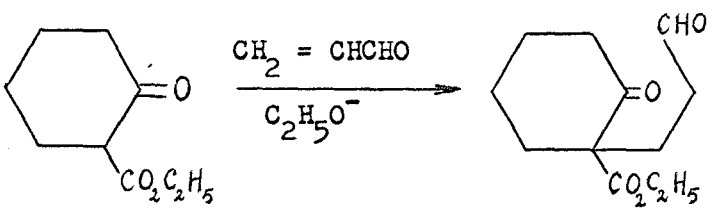
last step in the synthesis is the dehydration of the bicyclic alcohol XVIII by the action of concentrated sulphuric acid



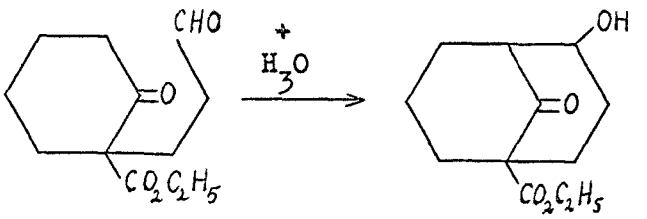
XVIII XIX

Compound XVIII was prepared by two routes. One of them involves alkylation of the potassium enolate of α-carbethoxycyclohexanone with β-chloropropionaldehyde diethyl acetal to produce β-(1-carbethoxy-2-keto-cyclohexyl)-propionaldehyde diethyl acetal in 31% yield. Hydrolysis of the acetal yielded the alcohol XVIII (71% yield), presumably through an intramolecular condensation of the liberated aldehyde.

The other method consists of the following transformations: reaction of α-carbethoxycyclohexanone with acrolein, in the presence of a small amount of ethoxide (at -70°), to give β-(1- carbethoxy-2-ketocyclo-hexyl)-propionaldehyde (65-70% yield); then conversion of the aldehyde to



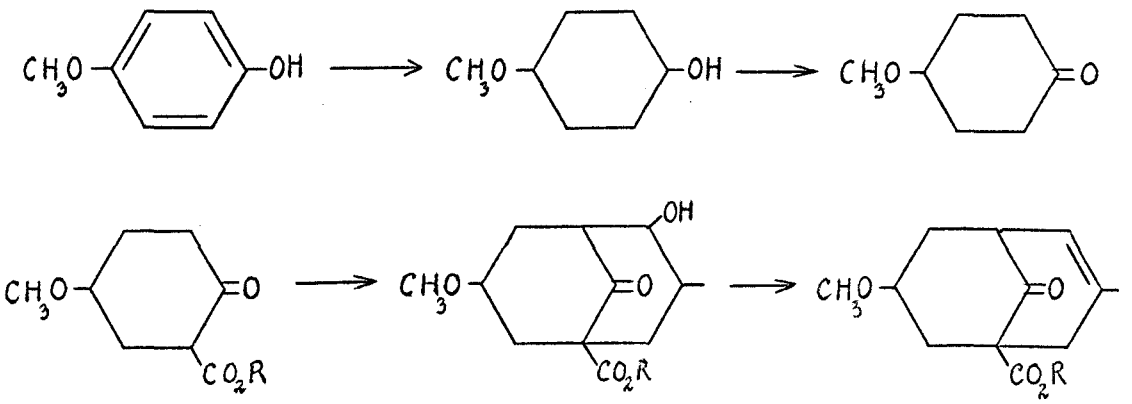
compound XVIII by an intramolecular aldol condensation, on treatment with a mixture of acetic acid, water and hydrochloric acid.



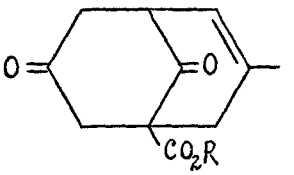
XVIII

DISCUSSION OF RESULTS

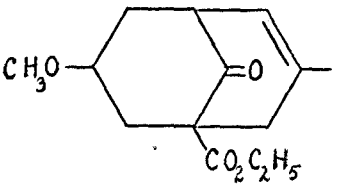
The work discussed here leads to the synthesis of a substituted bicyclo [3.3.1] nonan-9-one which was hoped to be a suitable intermediate for the synthesis of Lycopodium alkaloids containing the bicyclo [3.3.1] nonane system. The overall synthetic scheme employed in the work to be presented is outlined below:



An obvious disadvantage of this scheme is that the last two bicyclic compounds contain a number of asymmetric centers. The bicyclic alcohol contains five asymmetric centers and can exist in the form of eight enantiomeric pairs. The olefin, with three asymmetric centers, may occur in the form of two enantiomeric pairs. Thus, we expected from the outset that these two substances would be obtained as mixtures of several stereoisomers. We expected, however, to obtain the mixture of the two enantiomeric pairs corresponding to the bicyclic olefin in sufficient yield to permit its conversion to the molecule shown on the next page, which consists of one enantiomeric pair only.



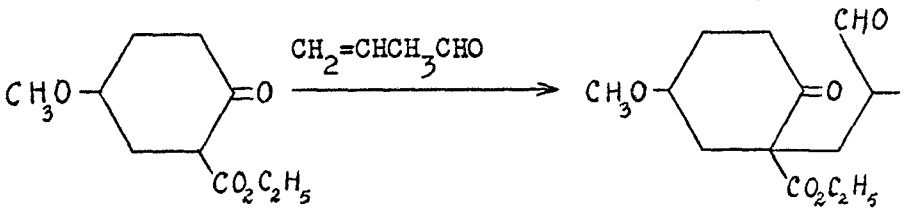
A reaction sequence similar to one of those reported by Cope and Synerholm (66) seemed appropriate for the preparation of 1-carbethoxy-3-methyl-7-methoxybicyclo[3.3.1]non-3-en-9-one (XXIII), a substance which



XXIII

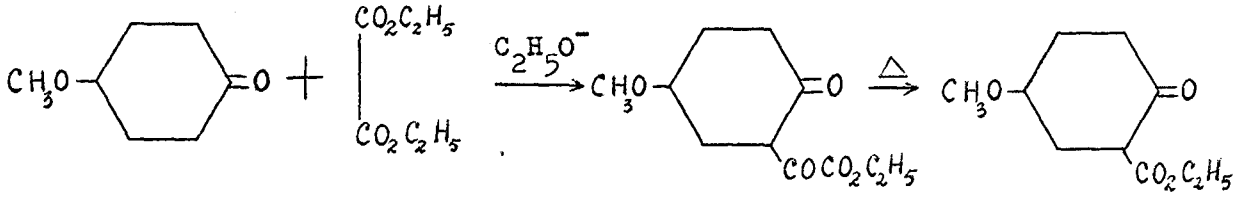
appeared suitable for further elaboration leading to the formation of a Lycopodium alkaloid.

The reaction corresponding to the initial step of one of Cope's syntheses is a Michael addition of methacrylaldehyde to 2-carbethoxy-4-methoxycyclohexanone, XX.



XX

The keto ester XX had been prepared by Cook and Lawrence (67) by the following reactions:



XX

4-Methoxycyclohexanone was condensed with ethyl oxalate to give 2-carbethoxyformyl-4-methoxycyclohexanone, which underwent thermal degradation to the desired compound XX. The yield of the product, in the overall conversion, was 25%.

The reaction sequence outlined above was repeated, in a slightly modified manner, by other investigators. McCasland and Bryce (68) carried out the decarbonylation by adding powdered glass and powdered iron to the

diketo ester, but did not achieve a better yield than that reported earlier. Hunziker et al (69) reported yields of 80% and 47% for the first and second steps, respectively. Decarbonylation in this case was effected by heating the diketo ester in the presence of boron-containing ground glass.

In the present investigation the preparation of compound XX by the reaction sequence discussed above was found to be unsatisfactory. Vapour phase chromatography showed the material obtained by this method to be a mixture containing besides the desired product unreacted 4-methoxycyclohexanone. Attempts to purify the reaction product XX were unsuccessful. The acidic properties of compound XX were reported to offer a means for its purification (69) Accordingly, the ethereal solution of the crude product was extracted with cold sodium hydroxide. The material isolated from the alkaline extract, however, was found to have approximately the same composition as the crude reaction product. The 4-methoxycyclohexanone probably arose by hydrolysis of the ester XX, in the alkaline medium, followed by decarboxylation on acidification of the aqueous solution. Separation of the components present in the reaction mixture by fractional distillation through a column with a heli-grid packing was also unsuccessful. After the distillation of some 4-methoxycyclohexanone (80°/5 mm) the boiling point rose to 108° and the pressure to 11 mm. The temperature then slowly dropped during the distillation though the pressure was maintained constant at 11 mm. The distillate collected over the range 108-87° (11 mm) was shown by vapour phase chromatography to be a mixture containing the keto ester XX and 4-methoxycyclohexanone. Apparently the latter compound was formed by the thermal decomposition of compound XX.

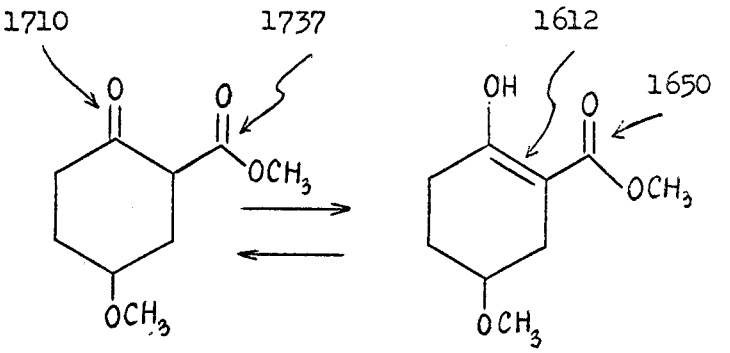
We were unable to account for this process by a plausible mechanism. Better results were obtained by a short path distillation through a Späth tube, but the product still contained some 4-methoxycyclohexanone as shown by vapour phase chromatography. In addition to the difficulties discussed above, compound XX was obtained in low yield, never exceeding 25% on the basis of 4-methoxycyclohexanone.

The starting material in the conversion described above was obtained by hydrogenation of p-methoxyphenol (70) followed by oxidation, with chromium trioxide, of the 4-methoxycyclohexanol produced. The products in the two consecutive steps were obtained in 75% and 60% yields, respectively.

The unsatisfactory manner in which the subsequent conversion to compound XX occurred was a serious limitation to our synthetic endeavour. Thus, it was necessary to consider other methods for the preparation of compound XX. The enamine acylation procedure, developed in recent years by Stork (71) was viewed as a promising possibility. Accordingly 4-methoxycyclohexanone was reacted with morpholine to yield the corresponding enamine (71). Interaction, however, of the latter with ethyl chloroformate failed to occur.

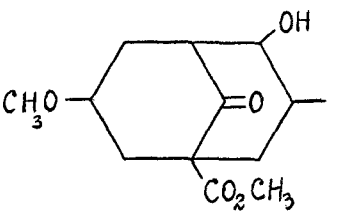
The difficulty encountered was resolved by finding a very good method for the preparation, not of compound XX, but of 2-carbomethoxy-4-methoxycyclohexanone XXI; a compound which is equally well suited for the planned synthesis. A reaction was used which has found previously application in the preparation of β-keto esters (72). 4-Methoxycyclohexanone was treated with a large excess of methyl carbonate in the pre-

sence of sodium hydride. In order to remove unreacted 4-methoxycyclohexanone from the product, a large volume of dry ether was added to the reaction mixture and the solid sodium enolate of the β-keto ester was filtered with suction and washed repeatedly with ether. Only a low yield (10%) of compound XXI was obtained from the enolate; most of the sodium enolate was dissolved in the filtrate. In subsequent reactions no attempt was made to remove unreacted 4-methoxycyclohexanone by washing the solid salt with ether. The enolate was converted to the β-keto ester by pouring the reaction mixture in aqueous acetic acid. Compound XXI, $C_9H_{14}O_4$, was obtained by this method in over 95% yield. As shown by vapour phase chromatography it was the sole product obtained from the reaction and contained only a trace of starting material. The infrared spectrum of compound XXI exhibits peaks at 1737, 1710, 1650 and 1612 cm^{-1}, which have been assigned as designated below:



XXI

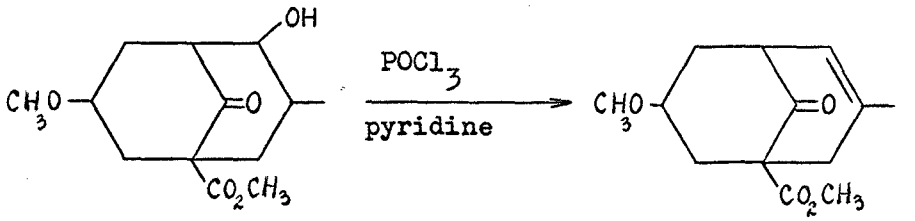
Having advanced a satisfactory method for the preparation of compound XXI, we turned our attention to the next step in the synthesis: the reaction of the keto ester with methacrylaldehyde. Examination of the reaction product by vapour phase chromatography indicated that it consisted of one major component containing a small amount of material of higher retention time. The bulk of the impurities was removed by preparative vapour phase chromatography. A study of the sample obtained indicates that the product is not an aldehyde, as was expected on the basis of the analogous reaction reported by Cope and Synerholm (66), but the alcohol depicted below



XXII

The infrared spectrum of compound XXII shows an intense hydroxyl band at 3380 cm^{-1} (film), and its NMR spectrum lacks absorption below a τ-value of 6.28. An aldehyde group proton usually gives rise to a signal in the region 0-1. The correctness of the structure assigned to compound XXII was demonstrated by its dehydration to compound XXIII.

Treatment of the bicyclic alcohol XXII with sulphuric acid, p-toluenesulphonic acid, tetraphosphoric acid or phosphorous oxychloride resulted, as shown by vapour phase chromatography, in the formation of a complex mixture. The desired dehydration reaction was found to occur on treatment of the alcohol (XXII) with phosphorous oxychloride in pyridine:

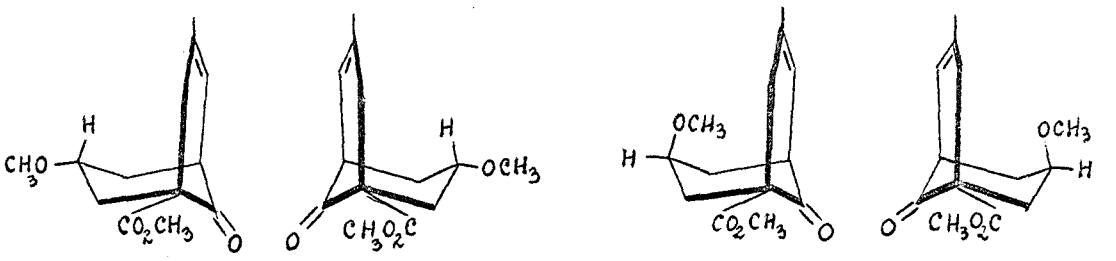


XXII XXIII

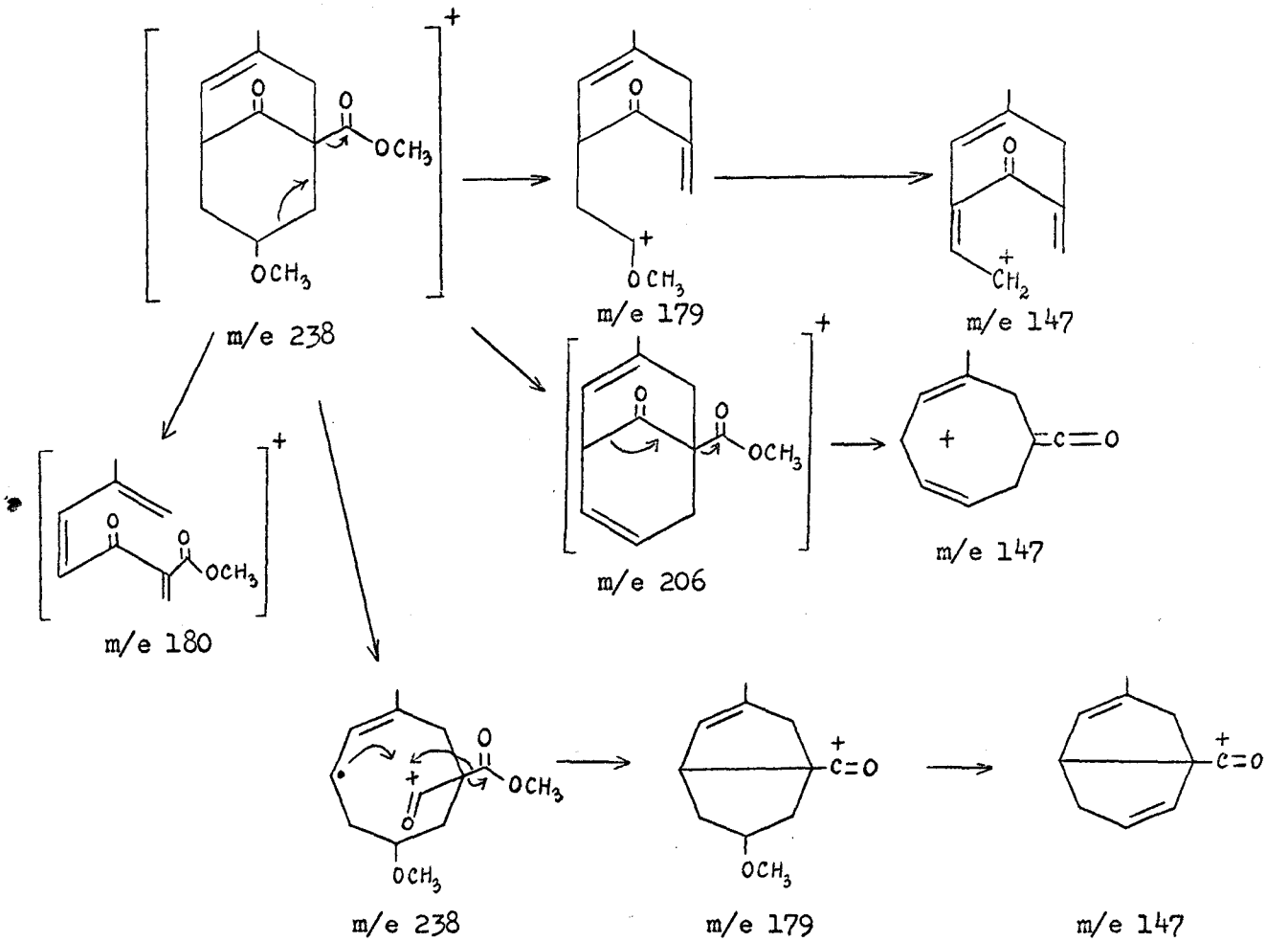
The crude product obtained in 42% yield was examined by vapour phase chromatography and was found to consist of one major component and several minor components. The major component was collected by preparative vapour phase chromatography and its properties examined.

The infrared spectrum of the bicyclic olefin XXIII, when measured in chloroform solution, shows a peak at 1680 cm^{-1} due to a trisubstituted double bond (73), and a broad band centered at 1720 cm^{-1} arising from the ketonic carbonyl and ester carbonyl groups. The NMR spectrum of compound XXIII exhibits a broad signal at 4.8 indicative of an olefinic proton. The protons of the groups - CO_2CH_3 and -OCH_3 give rise to two pairs of peaks. The two signals due to the carbomethoxy protons are situated at 6.27 and 6.32. The other two peaks are situated at 6.67 and 6.71. The occurrence of a pair of peaks attributable to each of the two methoxy groups

present in the molecule indicates the presence of the two enantiomeric pairs in which this molecule (XXIII) can exist:



The mass spectrum of compound XXIII, shown in Figure 11, is in agreement with the structure assigned to this substance. A possible fragmentation scheme is shown below:



Three processes have been offered to account for the presence of a peak at m/e 147. There is no way of distinguishing between them; however the two 179→147 processes are favored because of the presence of a metastable peak at m/e 121 (m* calc. (179→147) = 120.2). The ion of mass 165 apparently arises from the ion of mass 180 by loss of a methyl group. A metastable peak at m/e 152 is in accord with this process (m* calc. (180→165) = 151.2).

In conclusion we shall make an evaluation of this approach and discuss its feasibility to the total synthesis of a Lycopodium alkaloid. The main disadvantage in the present synthesis arose from the difficulty encountered in purifying compounds XXII and XXIII. These substances, obtained as liquids, were separated from the impurities present by a lengthy gas chromatographic procedure and were both recovered in low yield. Thus, to pursue the synthesis further seemed unprofitable.

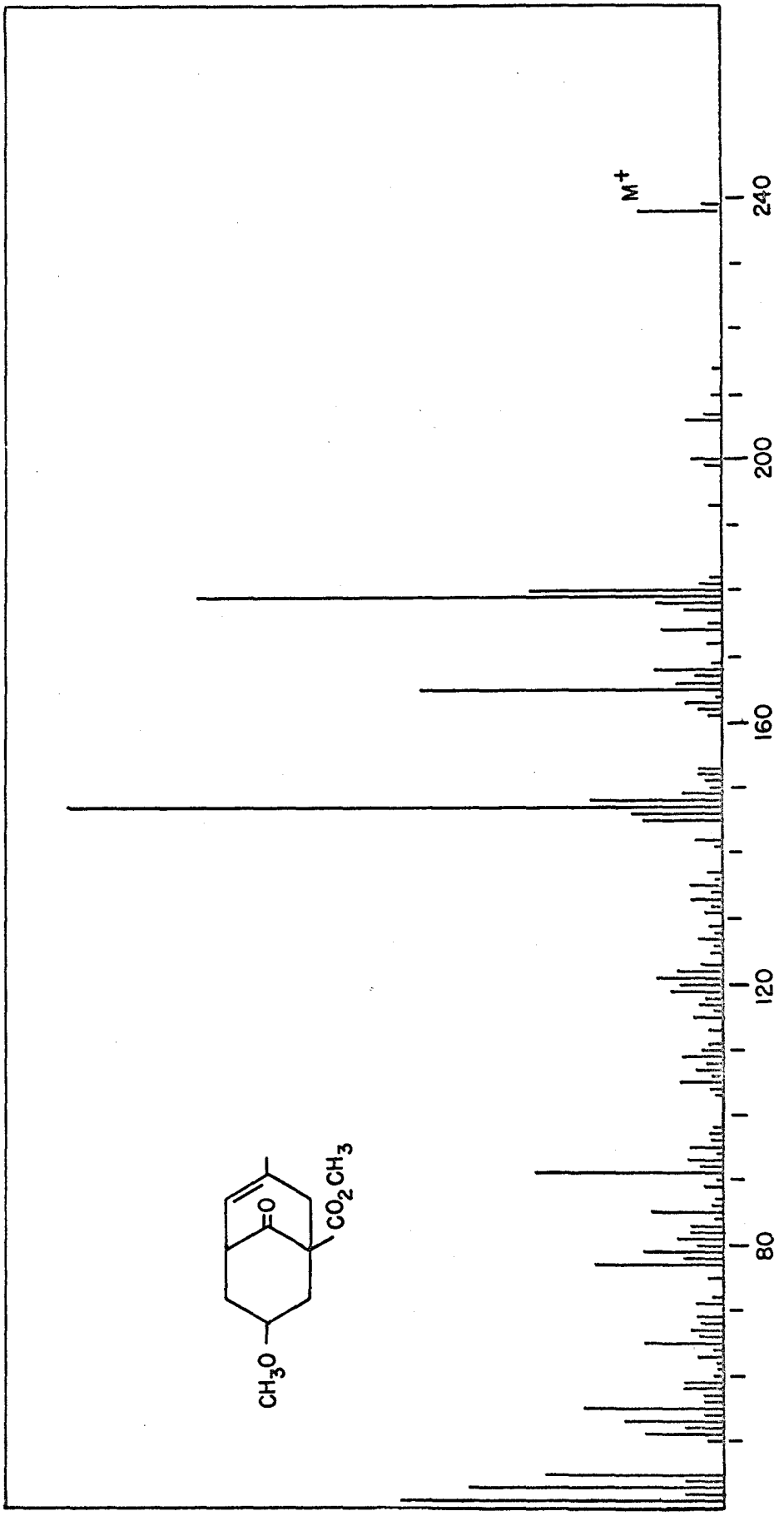


Figure 11. The Mass Spectrum of compound XXIII

EXPERIMENTAL

Hydrogenation of p-Methoxyphenol

p-Methoxyphenol (70 g) was dissolved in ethanol (300 ml.) and shaken with hydrogen at approximately 2600 p.s.i. over Raney nickel (10 g) for 15 hours. The hydrogenation was carried out at 160°. The solution was then filtered free of catalyst, and the alcohol was removed by distillation. The product was purified by fractional distillation through a heli-grid column, b.p. 113-114.5°/20 mm. The infrared spectrum (film) of 4-methoxycyclohexanol shows an intense peak at 3330 cm^{-1} due to the hydroxyl group, and a broad band centered at 1070 cm^{-1} attributable to the ether linkage.

Oxidation of 4-Methoxycyclohexanol

A solution of chromium trioxide (50 g) in aqueous acetic acid (90% acetic acid) was added dropwise over a period of 4 hours to a stirred solution of 4-methoxycyclohexanol (52 g) in water-acetic acid. The temperature of the reaction mixture rose to about 30° during the addition of the chromium trioxide, and was prevented from increasing further by placing the reaction flask in an ice bath. The reaction mixture was stirred at ice-bath temperature for a further 4 hours, then treated with methanol to destroy the excess of the oxidant and kept in a refrigerator overnight. The solution was diluted with water and extracted with chloroform. The chloroform and methanol were removed from the extract by distillation and

the 4-methoxycyclohexanone present in the residue was separated from acetic acid and other components by fractional distillation through a heli-grid column, b.p. 79°/4.5 mm. The product collected (30 g) was found to be pure by vapour phase chromatography. The identification of the reaction product was based on the formation of its semicarbazone, m.p. 177-178° (reported (74) 178°) and on infrared spectroscopy.

The infrared spectrum of 4-methoxycyclohexanone (film) shows strong absorption at 1700 cm^{-1} and no absorption in the hydroxyl region.

Preparation of 2-Carbethoxyformyl-4-methoxycyclohexanone

In a flask fitted with condenser, mercury sealed mechanical stirrer, and dropping funnel were placed 70 ml. of absolute ethanol. Sodium (3 g) was added to the ethanol and the resulting solution of sodium ethoxide in ethanol was cooled to -10°. A mixture of 4-methoxycyclohexanone (18 g) and ethyl oxalate (22 g) was added to the stirred solution of sodium ethoxide in a period of 1 hour. The mixture was stirred for a further 2 hours and left to stand at 0° overnight. The reaction mixture was acidified with cold dilute sulphuric acid and was then extracted with ether. The ether solution was extracted with a cooled aqueous solution of sodium hydroxide. The resulting alkaline solution was extracted with ether, then acidified with dilute hydrochloric acid and again extracted with ether. The crude ester remaining after the removal of the solvent was used for thermal degradation.

Preparation of Compound XX

The crude compound XX (26 g) was heated at 195°, under an atmosphere of nitrogen, in the presence of ground soft glass (0.2 g) and powdered iron (0.03 g) till gas (CO) ceased to evolve from the liquid (20 min). The reaction mixture was freed from powdered glass and iron and was examined by vapour phase chromatography. The chromatogram showed two peaks, one corresponding to 4-methoxycyclohexanone and another at a higher retention time which was attributed to product (2-carbethoxy-4-methoxycyclohexanone).

In order to purify compound XX, the crude product was dissolved in ether and the ethereal solution extracted with a cooled aqueous solution of sodium hydroxide. The resulting alkaline solution was washed with ether, then acidified with dilute hydrochloric acid and extracted with ether. The resulting solution was dried with sodium sulphate and the ether evaporated. The liquid remaining after removal of the ether was shown by vapour phase chromatography to have the same composition as the crude reaction product.

An attempt was made to purify compound XX by fractional distillation through a column with a heli-grid packing. The first fraction, obtained at 80°/5 mm, was 4-methoxycyclohexanone. When the temperature was raised to collect the keto ester XX neither the pressure nor the temperature of the distillate could be maintained constant, apparently owing to decomposition. The fractions which were collected were found to be mix-

tures of 4-methoxycyclohexanone and compound XX. The crude product from the decarbonylation reaction was then distilled under vacuum through a Späth tube. Some 4-methoxycyclohexanone distilled first and then a mixture (4.5 g) of 4-methoxycyclohexanone and the keto ester XX, consisting mostly of the latter. The product (XX) present in the last fraction was identified through its 2,4 dinitrophenylhydrazone, m.p. 128-131° (67).

The infrared spectrum (film) of the keto ester XX has bands at 1737 cm^{-1} (ester carbonyl), 1712 cm^{-1} (ketonic carbonyl), 1653 cm^{-1} (conjugated ester carbonyl) and 1612 cm^{-1} (olefinic double bond).

Preparation of the Morpholine Enamine of 4-Methoxycyclohexanone

This compound was prepared by the procedure of Stork et al (71). 4-Methoxycyclohexanone (5 g) was dissolved in dry toluene (15 ml). Morpholine (6.8 g) was added to the solution which was then refluxed under nitrogen for 24 hours. A water separator was used to remove the water generated during the reaction. The reaction flask was then connected with a condenser placed for distillation. The solvent was removed first; the enamine was distilled under reduced pressure, b.p. 122-123°/2 mm.

The infrared spectrum (film) of the enamine shows an intense peak at 1640 cm^{-1} (double bond).

Treatment of the Morpholine Enamine of 4-Methoxycyclohexanone with Ethyl Chloroformate

To a solution of 3.7 g of the morpholine enamine of 4-methoxycyclohexanone in 30 ml. of dry benzene ethyl chloroformate (1 g) was added under nitrogen while the enamine solution was being stirred rapidly. After re-

fluxing for about 8 hours the solution was cooled and filtered with suction, the precipitate of enamine hydrochloride being washed with dry ether. The combined filtrate and washings were returned to the reaction flask, 6 ml. of 10% aqueous hydrochloric acid was added, and the mixture was stirred vigorously for 1.5 hours. The layers were separated, the aqueous layer was extracted with benzene, and the combined organic layers were distilled at atmospheric pressure to remove solvent. The residue (3.7 g) was distilled under reduced pressure; it was found to be starting material by vapour phase chromatography and by comparison of its infrared spectrum with that of the enamine.

Preparation of 2-Carbomethoxy-4-methoxycyclohexanone XXI

Sodium hydride (3.5 g of 48% sodium hydride dispersed in mineral oil) was placed in a three-necked flask fitted with dropping funnel and drying tube containing calcium chloride. The mineral oil was removed from the sodium hydride by shaking repeatedly the solid with dry ether and removing the ether washings by decantation. The flask was then fitted with a mercury sealed mechanical stirrer and condenser. Methyl carbonate (50 g) was introduced through the dropping funnel, and then 4-methoxycyclohexanone (10 g) was added, with stirring, over a short period. Stirring was continued for a further 2 hours. The temperature was maintained throughout the reaction at 50-70°. As the condensation is very violent in the beginning, the reaction vessel had to be cooled by means of an ice bath. The reaction mixture was poured in ice-water covered with ether. The resulting aqueous solution was neutralized with acetic acid and extracted repeatedly with ether. The ether solution was extracted with aqueous sodium

bicarbonate to remove the acetic acid and then dried over sodium sulphate. Examination of the liquid obtained (13.8 g) from the ether solution, by vapour phase chromatography, showed that it consisted of product (XXI) containing a very small amount of starting material.

When the condensation was first carried out, an attempt was made to purify the keto ester by adding a large volume of dry ether to the reaction mixture and filtering with suction the sodium enolate present. However, not only did we obtain a small yield of product from the ether-washed solid salt, but also its purity was not higher than that obtained by the method described above. The method involving separation of the sodium enolate was found to be satisfactory when used on a small scale for the preparation of an analytical sample. Sodium hydride (0.5) was placed in an Erlenmeyer flask and washed free of mineral oil as described above. Compound XXI (3 g) was added in the flask and the resulting mixture was stirred with a magnetic stirrer for several hours. Ether was added to the mixture which was then filtered with suction. The sodium enolate of compound XXI was washed rapidly with ether and then dissolved in ice-cooled water covered with ether. The aqueous solution was neutralized with dilute hydrochloric acid and extracted with ether. The ether solution was washed with water and dried over sodium sulphate. From the ether solution a pure sample of compound XXI was obtained. Calculated for $C_9H_{14}O_4$: C, 58.05; H, 7.58%. Found: C, 58.43; H, 7.38%.

The infrared spectrum (film) of compound XXI exhibits bands at 1737 cm^{-1} (ester carbonyl), 1710 cm^{-1} (ketonic carbonyl), 1650 cm^{-1} (conjugated ester carbonyl) and 1612 cm^{-1} (olefinic double bond).

Preparation of the Bicyclic Alcohol XXII

Sodium (0.1 g) was added to absolute methanol (30 ml.) contained in a three necked flask fitted with stirrer, condenser and dropping funnel. The resulting solution was cooled to about -5° and a mixture of compound XXI (5 g) and freshly distilled methacrylaldehyde (2.4 g) was added to the stirred solution in a period of half an hour. The reaction flask was kept in an ice bath for a further 4 hours. The reaction mixture was then neutralized with dilute aqueous hydrochloric acid and extracted with ether. From the ether extract a viscous liquid was obtained (6.6 g), which on examination by vapour phase chromatography indicated that it consisted of one major component containing a small amount of material of higher retention time. The bulk of the impurities was removed by preparative vapour phase chromatography. The separation was carried out on a 6 ft., 15% SE-30 on Celite column at 208° and at a flow rate of 200 ml/min. Under these conditions a retention time of 10.8 min. was observed.

The infrared spectrum (film) of compound XXII shows strong hydroxyl absorption at 3380 cm^{-1} and a broad band in the carbonyl region centered at 1740 cm^{-1}. The NMR spectrum of compound XXII lacks absorption below a τ-value of 6.28.

Attempted Dehydration of Compound XXII

(a) with Sulphuric Acid

Compound XXII (1 g) was added drop-wise to concentrated sulphuric acid (2 ml.) cooled to 0°. The addition was completed in approximately 1 hour. The reaction mixture was left to stand at room temperature for 2 hours, it was then poured into ice-water and extracted with ether. The

ether extract was washed successively with aqueous sodium bicarbonate and water. Evaporation of the ether furnished a viscous residue (0.2 g) which was found to be a complex mixture by vapour phase chromatography.

(b) with p-Toluenesulphonic Acid

Compound XXII (0.2 g) was dissolved in o-xylene, treated with p-toluene sulphonic acid and the solution heated under reflux for 18 hours. The reaction mixture was then washed several times with aqueous sodium bicarbonate and water. It was then dried over sodium sulphate and the xylene distilled under reduced pressure. Vapour phase chromatography showed the residue to be a complex mixture.

(c) with Tetraphosphoric Acid

Compound XXII (0.15 g) was heated on a steam bath with tetraphosphoric acid (3 ml.) for 1 hour. The reaction mixture was then left to stand at room temperature overnight. It was then dissolved in ether and washed repeatedly with water. The material isolated from the ethereal solution was found by vapour phase chromatography to contain several components.

(d) with Phosphorous Oxychloride

A mixture of compound XXII (0.14 g) and freshly distilled phosphorous oxychloride was heated under reflux for 2 hours. The reaction mixture was poured carefully in ice-water covered with ether. The aqueous solution was repeatedly extracted with ether. The ether extract was washed with aqueous sodium bicarbonate and then water. From the ether solution a complex mixture was obtained. Chromatography on neutral alumina was unsuccessful in separating its components.

Preparation of Compound XXIII

Compound XXII (0.38 g), phosphorous oxychloride (2 ml.) and dry pyridine (8 ml.) were heated under reflux for 0.5 hours. The mixture was then left to stand at room temperature overnight. The reaction mixture was made acidic by careful addition of dilute hydrochloric acid and was then extracted with ether. The ether extract was washed successively with aqueous sodium bicarbonate and then water. Evaporation of the ether yielded 0.16 g of product. The crude product was examined by vapour phase chromatography and was found to consist of one major component which was collected by preparative vapour phase chromatography. A 6 ft. column of 15% SE-30 on Celite was used. At 208° and at a flow rate of 200 ml/min. the retention time of compound XXIII was found to be 7.2 min.

The infrared spectrum (chloroform) of compound XXIII shows a peak at 1680 cm^{-1} (trisubstituted double bond) and a broad band at 1720 cm^{-1} (ketonic and ester carbonyl). There is no absorption in the hydroxyl region. The NMR spectrum of compound XXIII shows signals at 4.8 (olefinic proton), 6.27, 6.32 ($-CO_2\underline{CH_3}$), 6.67, 6.71 ($-O\underline{CH_3}$) and 8.46 ($>C-\underline{CH_3}$). The mass spectrum shows a molecular ion peak at m/e 238. A possible fragmentation scheme has been given under Discussion of Results.

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