RE-EXAMINATION OF CYCLIZED PRODUCTS

FROM LYCOPODINE AND CLAVOLONINE

RE-EXAMINATION OF CYCLIZED PRODUCTS FROM LYCOPODINE AND CLAVOLONINE

By

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SCOPE AND CONTENTS:

Cyclized products derived from lycopodine and clavolonine through the Hofmann and von Braun reactions were prepared and examined. From evidence obtained new structures are proposed for the Hofmann products of lycopodine and clavolonine.

ACKNOWLEDGEMENTS

It is a pleasure to express my gratitude to Professor D. B. MacLean for his patience and guidance during the course of this research.

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GENERAL INTRODUCTION

The first isolation of an alkaloid from a species of *Lycopodium* was reported ninety years ago (1). The alkaloid is now known as lycopodine ($C_{16}H_{25}ON$) and it is the most widely distributed of the *Lycopodium* alkaloids. The structural investigation of this alkaloid was begun by Manske and Marion (2) in the early 1940's but its structure was not elucidated until 1960 by Harrison and MacLean (3). Since then the structures of many new *Lycopodium* alkaloids have been determined, among which is clavolonine ($C_{16}H_{25}O_2N$) whose structure was elucidated by Burnell and Taylor (4). Structurally, clavolonine was shown to be very similar to lycopodine, the only difference being the presence of a hydroxyl group in the equatorial position, on carbon eight.

During the investigation of the structures of lycopodine and clavolonine rearranged cyclized products were reported (4, 5). These rearranged products were formed by cleavage of a carbon-nitrogen bond and recyclization presumably at a carbon alpha to the carbonyl group. Because of the similarity of lycopodine to clavolonine one might expect that the cyclized products derived from these two alkaloids would be very similar.

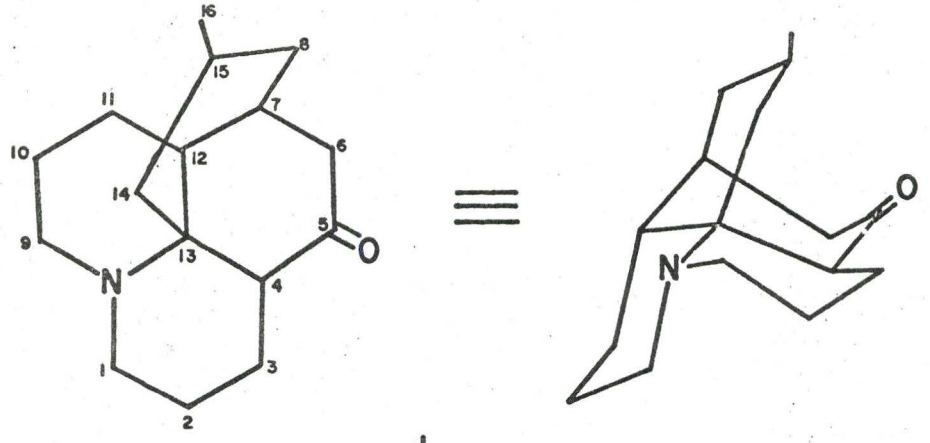
One objective of the investigation which will be described in this thesis was to establish whether the ring system present in the rearranged cyclized products of lycopodine and clavolonine were the same. We had hoped to establish the identity of the ring system by

removal of the -OH group on C-8 of clavolonine but because of the shortage of clavolonine the investigation was not completed. The cyclized products of lycopodine were examined more thoroughly, however. It was also an objective of this study to compare the cyclized products of clavolonine with paniculatine (6). Paniculatine ($C_{17}H_{27}O_2N$) was isolated in the laboratory from *L. paniculatum* obtained from Chile. This alkaloid is isomeric with the Hofmann product of clavolonine (4).

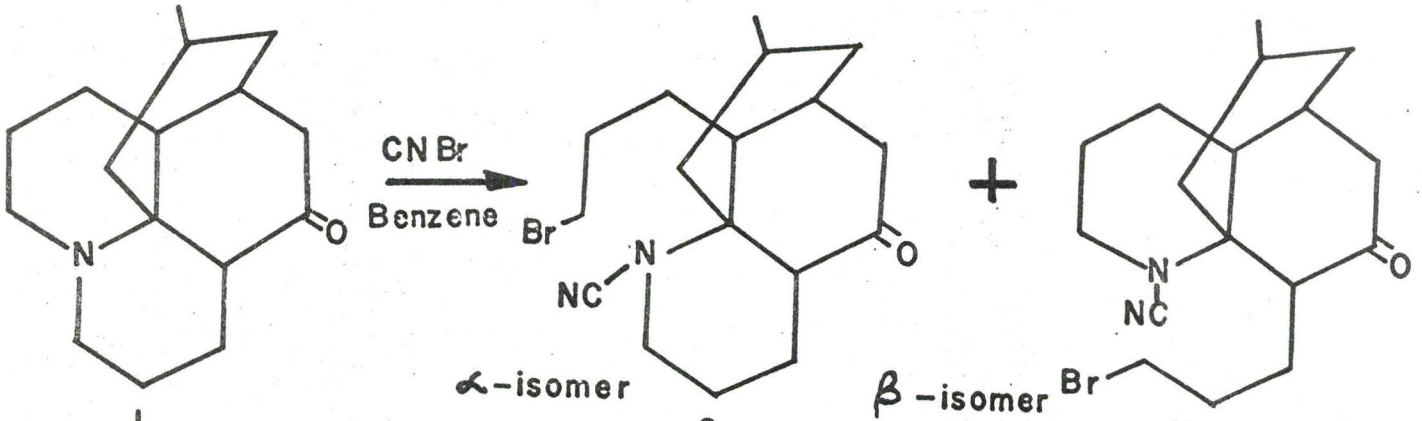
HISTORICAL INTRODUCTION

Lycopodine ($C_{16}H_{25}ON$) 1, was first isolated by Bödeker (1) in 1881 from *Lycopodium complanatum L.* MacLean, Manske and Marion (5) reported that attempts to degrade lycopodine through the N-oxide, the Emde or the Hofmann degradations had been unsuccessful. However, they found that reaction of the alkaloid with cyanogen bromide yielded two isomeric ring scission products. These were designated α- and β-cyano-bromolycopodine 2 and 3 ($C_{17}H_{25}ON_2Br$), respectively. A number of reactions of these bromocyanamides were reported at the same time, among which were those leading to the formation of two isomeric compounds of the formula ($C_{17}H_{24}ON_2$). Both isomers were formed in an elimination reaction with loss of hydrogen bromide. The α- isomer 4 was the product of the reaction of 2 with boiling methanolic potassium hydroxide. The β- isomer 5 was obtained by the action of boiling ethanolic potassium acetate on the β- bromide 3. From chemical and physical evidence, it was suggested that 4 and 5 were formed in cyclization reactions (5). The reactions are summarized in Scheme 1.

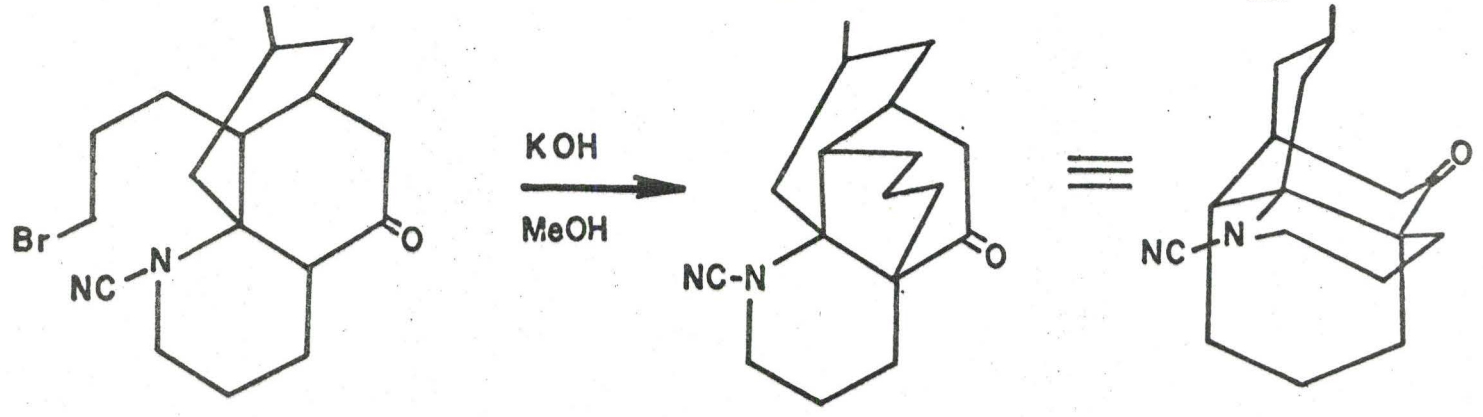
Barclay and MacLean (7) later showed that both bromide isomers are primary. Although the cyclized compounds 4 and 5 are isomeric, their properties differ in several ways. The infrared (i.r.) spectrum of 4 has a strong band near 1700 cm^{-1} in the carbonyl region. The presence of a carbonyl group in 4 was confirmed by the fact that 4 can be easily reduced with sodium borohydride to the alcohol. Compound 5



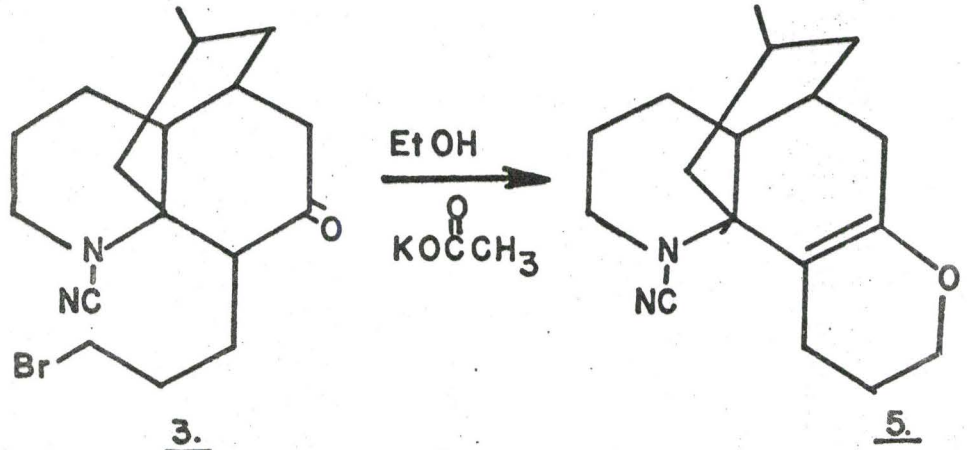
I.



I. 2. 3.



2. 4.



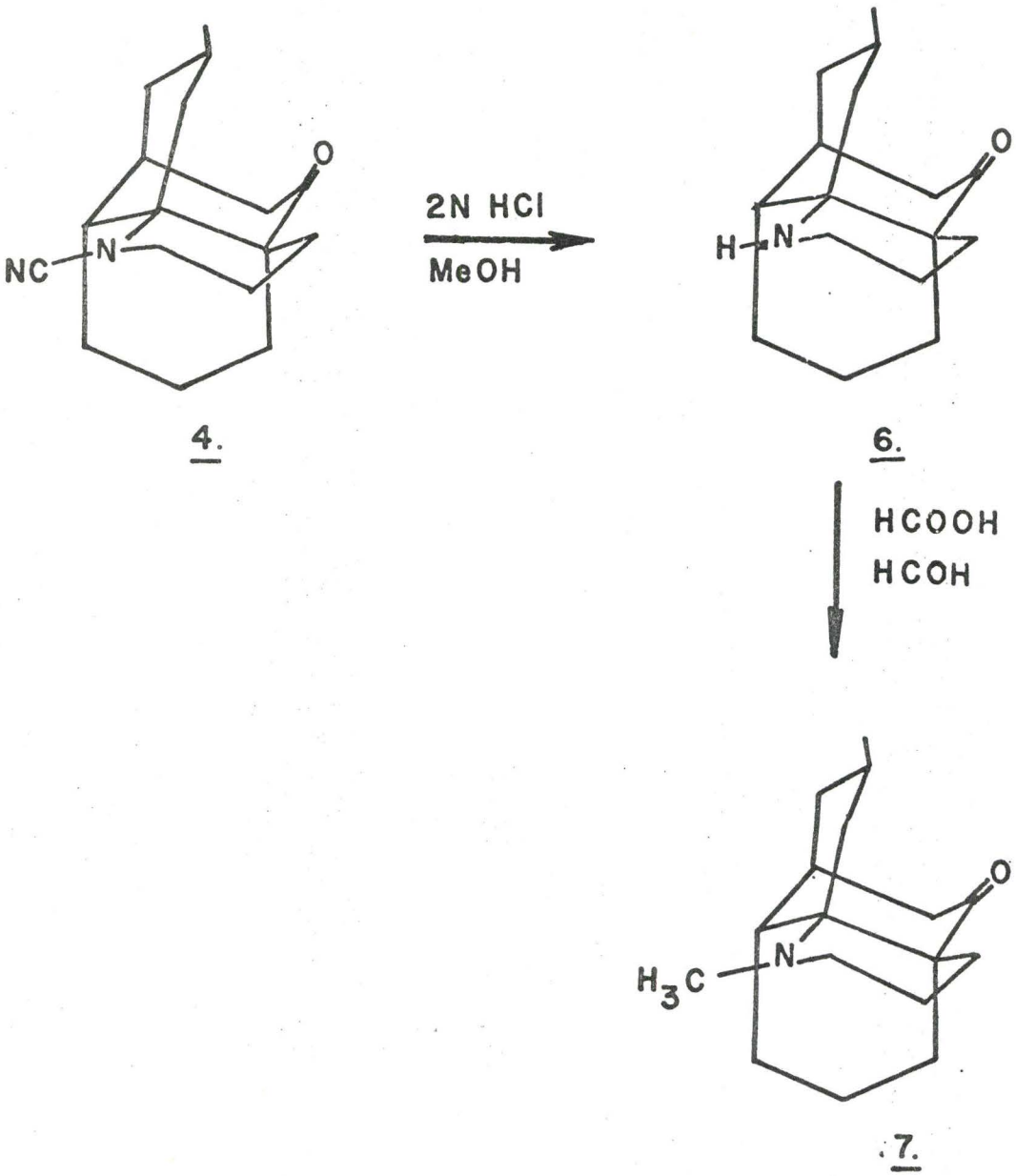
3. 5.

SCHEME 1

showed somewhat weaker absorption near 1675 cm^{-1} in the i.r. spectrum and gave no chemical indication of possessing a carbonyl group. Harrison et al. (8) later proposed the enol ether structure 5 (Scheme 1) for this isomer.

Indirect evidence that a cyclization reaction of 2 to 4 had occurred adjacent to the carbonyl group came from the following observations (9). Compound 4 failed to react with benzaldehyde when it was treated under the same conditions that led to reaction with α- cyanolycopodine (in α- cyanolycopodine the bromine atom of the bromocyanamide 2 has been replaced by hydrogen (5)). When 4 was hydrolysed to the secondary base and methylated to the tertiary base, Scheme 2, the latter failed to react with phenyl lithium. The first observation suggests that the methylene group which has been shown to be present in α-cyanolycopodine (7), is absent or more hindered in 4. The failure of 4 to form a benzylidene derivative cannot be taken as conclusive evidence for the absence of a methylene group adjacent to a carbonyl group for it has been reported that lycopodine does not react with benzaldehyde (9). The second observation indicates that the carbonyl group in 4 is more hindered than in lycopodine which reacts readily with phenyl lithium (5). Both observations lend support to a cyclization alpha to the carbonyl function. It has been shown (9) that in the absence of a carbonyl group cyclization does not occur.

Structures 4A and 4B of Fig. 1 were considered for the cyclized product 4. Harrison et al. (8) confirmed structure 4A through deuterium labelling experiments and i.r. spectroscopy. Compound 4 exchanged two

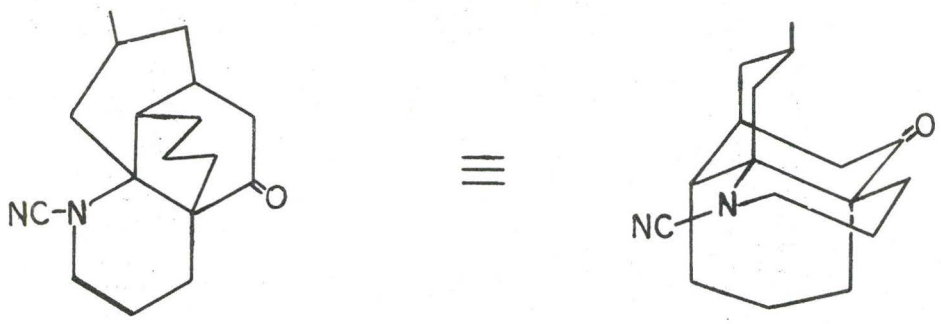


4.

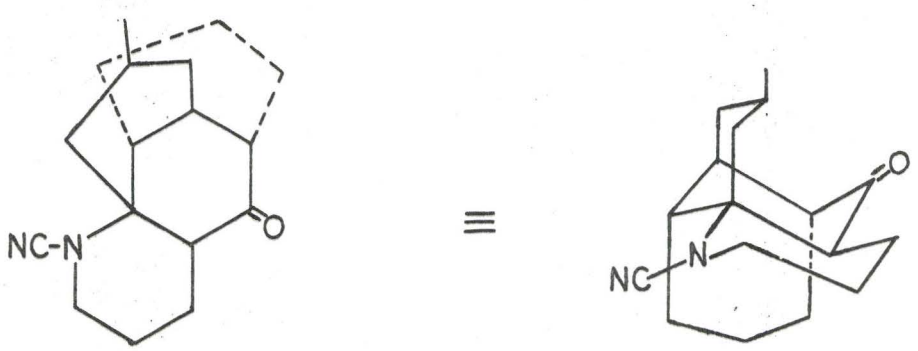
6.

7.

SCHEME 2



4A



4B

Figure 1

hydrogens for deuterium and this is consistent with structure 4A. Such a result is possible but unlikely for the bicyclo-(3,3,1)nonane system present in 4B. In the i.r. spectrum of 4 there is a band at 1413 cm^{-1} which is absent in deuterated 4. This behavior is in keeping with a ketone containing an α- methylene group (10, 11).

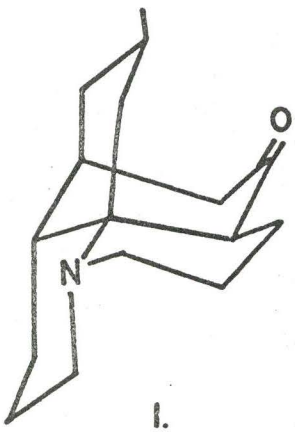
Compound 5 does not exchange hydrogen for deuterium (8) in keeping with the proposed structure 5, nor does it react with hydrides. Its i.r. absorption is characteristic of enol ethers. The reason for the unusual stability of the enol ether function of 5 becomes clear when an examination is made of a molecular model. The introduction of unsaturation at C-4 and C-5 relieves steric hindrance by eliminating the interaction of the C-4 hydrogen with the axial hydrogens of C-9 and C-11 as well as the interaction between the hydrogens of C-3 and C-14.

A third cyclized product 8 ($C_{17}H_{27}ON$), was derived from lycopodine methiodide by the action of potassium tertiary butoxide in tertiary butyl alcohol (8). On treatment of 8 with cyanogen bromide it yielded a cyanamide 9 ($C_{17}H_{24}ON_2$) which was isomeric but not identical with either of the two cyclized compounds 4 and 5.

This compound, 8 showed no double bond absorption in its i.r. spectrum and was inert to the action of ozone and hydrogen. Its nuclear magnetic resonance (n.m.r.) spectrum did not show peaks in the region of olefinic protons. The oxygen function of 8 and 9 was not affected by sodium borohydride in ethanol or by lithium aluminum hydride in ether or tetrahydrofuran. However, the i.r. spectrum of 8 and 9 showed a strong peak in the carbonyl region at 1695 cm^{-1} and also a band at

1410 cm^{-1} characteristic of a methylene group adjacent to a carbonyl function (10, 11). Compound 8 exchanged two hydrogens for deuterium and furthermore, the absorption in the i.r. spectrum at 1410 cm^{-1} was absent in the deuterated compound. Harrison et al. (8), therefore proposed the structure 8 of Scheme 3 for this cyclized compound. They noted that they were unable to reduce compounds 8 and 9. An attempt was made to establish the presence of a cyclobutane ring by chemical degradation (12). The compound was subjected to chromic acid oxidation (13) and the product of oxidation was examined for the presence of cyclobutane carboxylic acid. This experiment was repeated in this investigation, also without success.

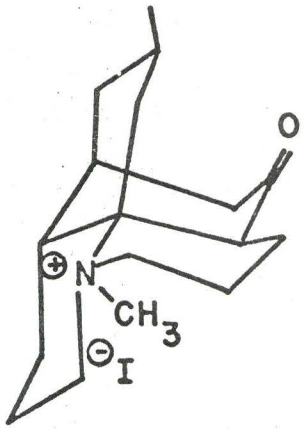
Clavolonine 10 ($C_{16}H_{25}O_2N$) was obtained as a major component of Jamaican *L. clavatum* (14). Its structural elucidation was done by Burnell and Taylor (4). They reported the reaction of clavolonine methiodide in tertiary butyl alcohol containing potassium tertiary butoxide. A crystalline product ($C_{17}H_{27}O_2N$) 11 was obtained which melted at 170-171°. This compound contained one N-CH_3 group, a carbonyl and a hydroxyl (i.r.). The n.m.r. spectrum showed no bands at low field, corresponding to olefinic protons and the i.r. spectrum contained no band characteristic of a terminal methylene group. Since the product contained an N-CH_3 group, cleavage of a carbon-nitrogen bond must have occurred. Both clavolonine and the Hofmann base show i.r. bands at 1410 cm^{-1} which suggests the presence of the grouping $CH_2\overset{O}{\overset{\|}{C}}-$. The optical rotatory dispersion of the Hofmann product shows a negative Cotton effect, as opposed to the positive effect in clavolonine,



1.

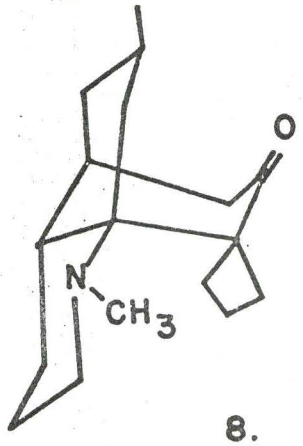
CH_3I

Acetone



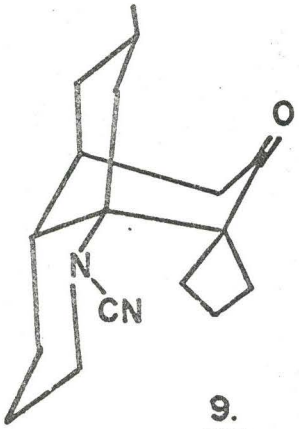
t- Bu OH

K^+ t-$^-$OBu



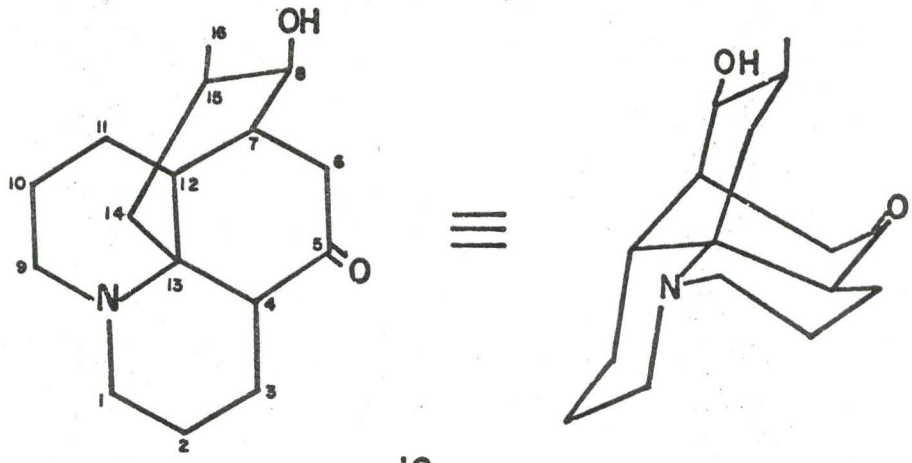
8.

CNBr

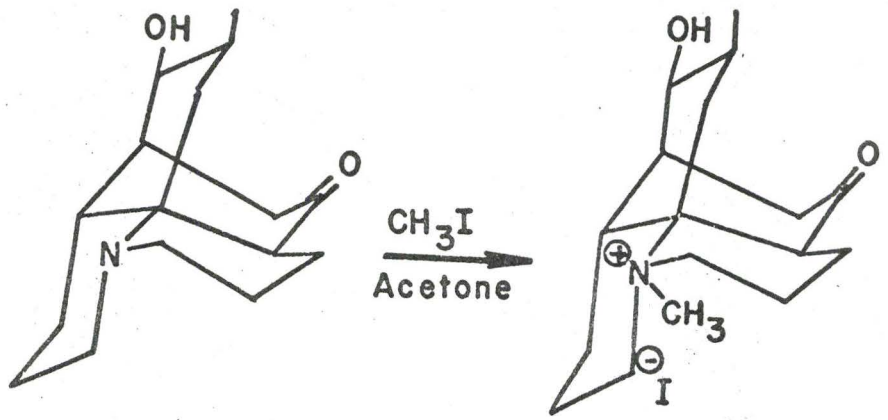


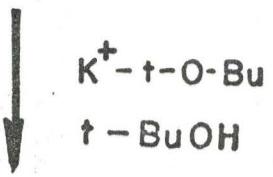
9.

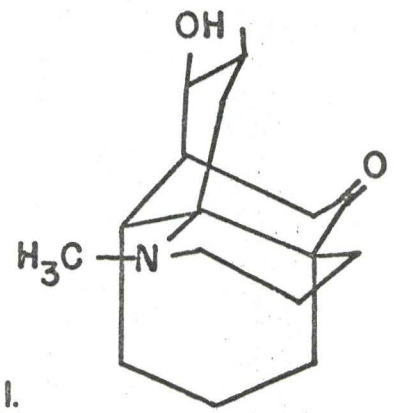
SCHEME 3



10.







11.

SCHEME 4

indicating the introduction of an axially disposed substituent adjacent to the carbonyl group. From the above evidence structure 11 of Scheme 4 was proposed for the Hofmann base.

Structure 11 proposed by Burnell and Taylor (4) for the Hofmann product of clavolonine is the same structure proposed by MacLean et al. for N-methyl α-cyclolycopodine 7. It was determined that the Hofmann product of lycopodine was not identical with N-methyl-α-cyclolycopodine and so structures 11 and 7 were suspect.

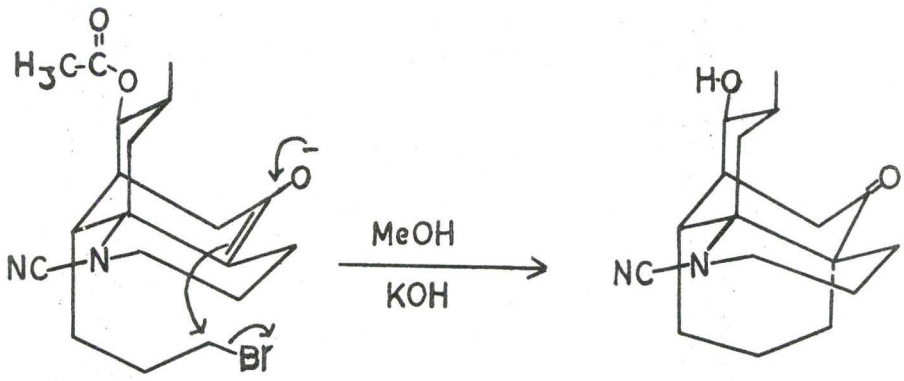
In the following disucssion, structure 18A based on new evidence, is proposed for the Hofmann product of lycopodine and on the basis of similar fragmentation on electron impact the Hofmann product of clavolonine is assigned structure 19.

DISCUSSION OF RESULTS

The reaction of lycopodine with cyanogen bromide and the subsequent cyclization of the bromocyanamides in basic media, reported by MacLean, Manske and Marion (5) has now been extended to clavolonine.

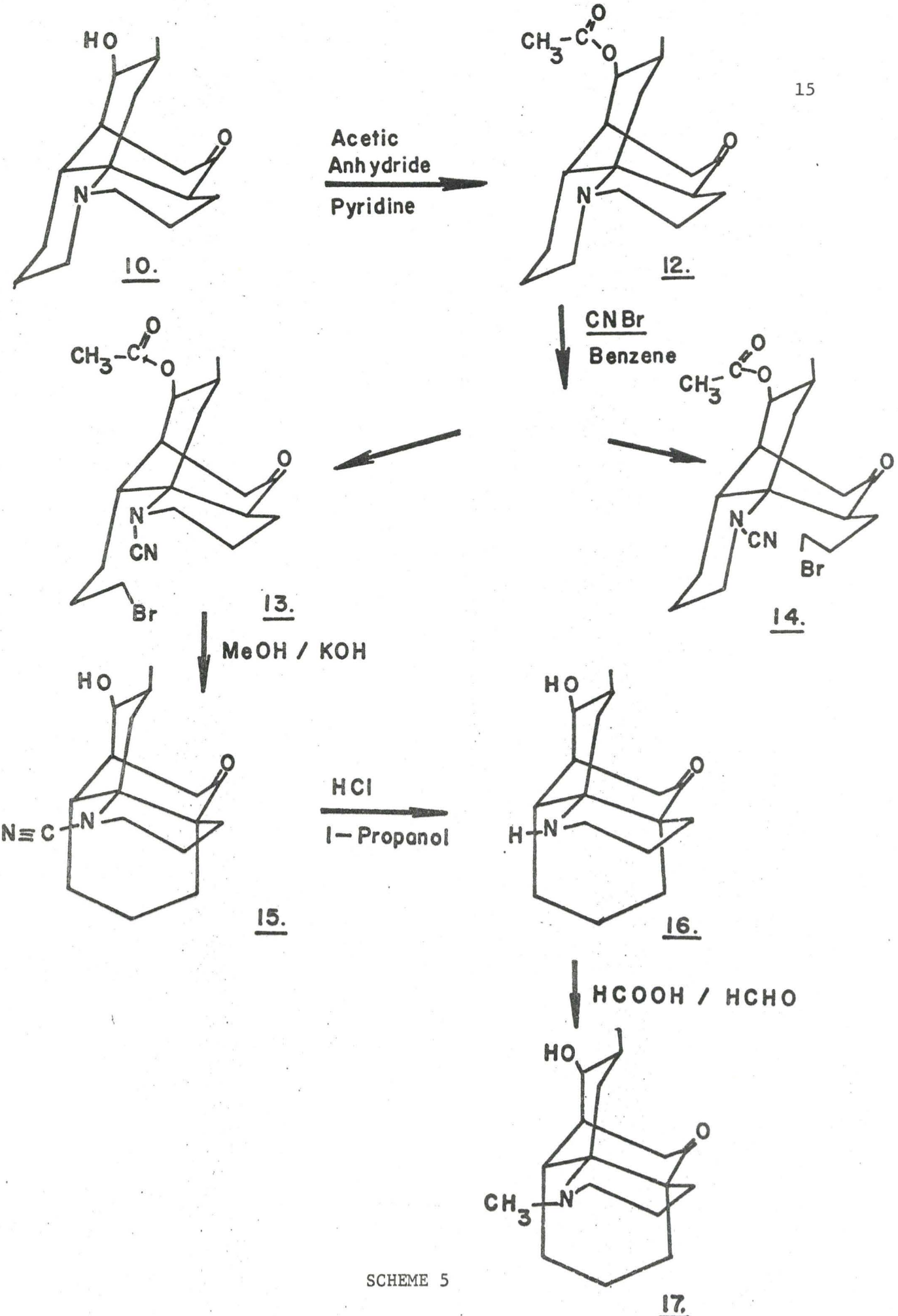
Clavolonine was acetylated, using acetic anhydride in pyridine, in order to protect the hydroxyl group on C-8. The reaction of O-acetylclavolonine 12 with cyanogen bromide yielded two neutral fractions 13 and 14. Separation of these fractions was accomplished over neutral alumina. The major component 13 had an i.r. spectrum showing strong absorption at 2210 cm^{-1} ($\rangle$N-CN), 1725 cm^{-1} ($CH_3\overset{O}{\overset{\|}{C}}$-O-) and 1700 cm^{-1} ($\rangle$C=O). The mass spectrum showed the molecular ion at m/e 410 with a definite bromine doublet. These results indicated that cleavage of a nitrogen-carbon bond had occurred with the incorporation of cyanogen bromide in the usual manner. This compound was designated O-acetyl-α-cyanobromoclavolonine 13. The minor fraction was not investigated but presumably has structure 14. Treatment of O-acetyl-α-cyanobromoclavolonine with methanolic potassium hydroxide gave a crystalline product 15 melting at 173-175°. The i.r. spectrum of the crystalline product had absorption at 1700 cm^{-1} ($\rangle$C=O), 2210 cm^{-1} ($\rangle$N-CN), and a band at 3400-3550 cm^{-1} (-OH). No bands attributable to $\rangle C{=}C\langle$ double bonds were detected. The mass spectrum showed the molecular ion at m/e 288. These results are compatible with a cyclized product, formed with the elimination of hydrogen bromide. In analogy with the

work of MacLean et al. (3, 5, 7, 8, 9) on lycopodine it was postulated that O-acetyl-α-cyanobromoclavolonine was obtained as a result of cleavage of the C-9-nitrogen bond. The cyclized product 15 designated α-cyclocyanoclavolonine is postulated to form in a manner similar to its analogue from lycopodine with formation of a bond between C-9 and C-4 shown below.



15

α-Cyclocyanoclavolonine 15 was hydrolysed to the secondary base 16 with dilute hydrochloric acid in 1-propanol. The mass spectrum of this base 16 gave the molecular ion at m/e 263 and the i.r. spectrum was void of a peak at 2210 cm^{-1} ($\rangle$N-CN). Methylation of this base with formic acid and formaldehyde yielded a crystalline product 17 ($C_{17}H_{27}O_2N$) melting at 198-199°. This product was designated N-methyl-α-cyclo-clavolonine. The mass spectrum gave the molecular ion at m/e 277. The n.m.r. spectrum had peaks attributable to an N-CH_3 group (3H singlet 2.11 δ) and a $CHCH_3$ group (3H doublet 0.88 δ). The i.r. spectrum in


HO
O
N
10.
Acetic
Anhydride
Pyridine
CH3-C(=O)-O
12.
CNBr
Benzene
13.
14.
Br
MeOH / KOH
N≡C
15.
HCl
I-Propanol
H
16.
HCOOH / HCHO
CH3
17.

SCHEME 5

deuterated chloroform had strong absorption at 1698 cm^{-1} ($\rangle C=O$), a band of medium intensity at 3610 cm^{-1} (-OH), and a peak at 1413 cm^{-1} attributed to a methylene group adjacent to a carbonyl function (10, 11). These reactions are summarized in Scheme 5.

N-methyl-α-cycloclavolonine was compared to the Hofmann degradation product of clavolonine (obtained from Dr. R. Burnell) and was found to be isomeric but not identical. The melting points of the two products were different. Thin layer chromatography gave different Rf values, and the mass spectra had major differences in their fragmentation patterns.

The Hofmann Product of Clavolonine

Refluxing clavolonine methiodide in tertiary butyl alcohol and potassium-t-butoxide gave a crystalline product, ($C_{17}H_{27}O_2N$) melting at 170-171° (4). This product was identical with the product obtained from Dr. Burnell. The melting points were the same. Thin layer chromatography gave the same Rf value and the mass spectra had the same fragmentation pattern. The compound contained one N-methyl group (n.m.r.), a carbonyl and a hydroxyl (i.r.). The n.m.r. spectrum showed no peaks at low field ascribable to olefinic protons and the i.r. spectrum contained no bands characteristic of olefins. Since the product contains a $N-CH_3$ group, cleavage of a carbon-nitrogen bond must have occurred but the potential alkene system must have cyclized intramolecularly. Both clavolonine and the Hofmann base show i.r. peaks at 1410 cm^{-1} which suggests the presence of the grouping $CH_2-\overset{O}{\overset{\|}{C}}-$ (10, 11). The optical rotatory dispersion of the Hofmann base reported by Burnell

shows a negative Cotton effect, as opposed to the positive effect in clavolonine, indicating the introduction of an axially disposed substituent adjacent to the carbonyl group (4).

The mass spectrum (Fig. 2) of the Hofmann base gave the molecular ion at m/e 277. The pattern of fragmentation of this base is very similar to that of the Hofmann product of lycopodine Fig. 3. In both cases the base peak was found at m/e 57. Similarity in the fragmentation patterns of the Hofmann products of lycopodine and clavolonine strongly suggests that the compounds are structurally similar.

Paniculatine ($C_{17}H_{27}O_2N$) was isolated in the laboratory from *L. paniculatum* by M. Castillo (6). He found that paniculatine contained one N-methyl and a $CHCH_3$ group (n.m.r.), a carbonyl and a hydroxyl (i.r.). Paniculatine, however, was not identical with the Hofmann product of clavolonine or with N-methyl-α-cycloclavolonine. The melting points of the compounds were different. Thin layer chromatography gave different Rf values for the three compounds and the mass spectra had different fragmentation patterns.

The α- cyclized products of lycopodine and clavolonine 7 and 17, respectively, arising from the cyanogen bromide sequence of reactions, both gave base peaks in their mass spectra which corresponded to the loss of the usual bridging atoms of lycopodine (15) and appear to have the same carbon skeleton. The removal of the hydroxyl group on C-8 of the cyclized product of clavolonine was considered but it was not accomplished because of a shortage of clavolonine. Only in this way would it be possible to compare directly the two systems.

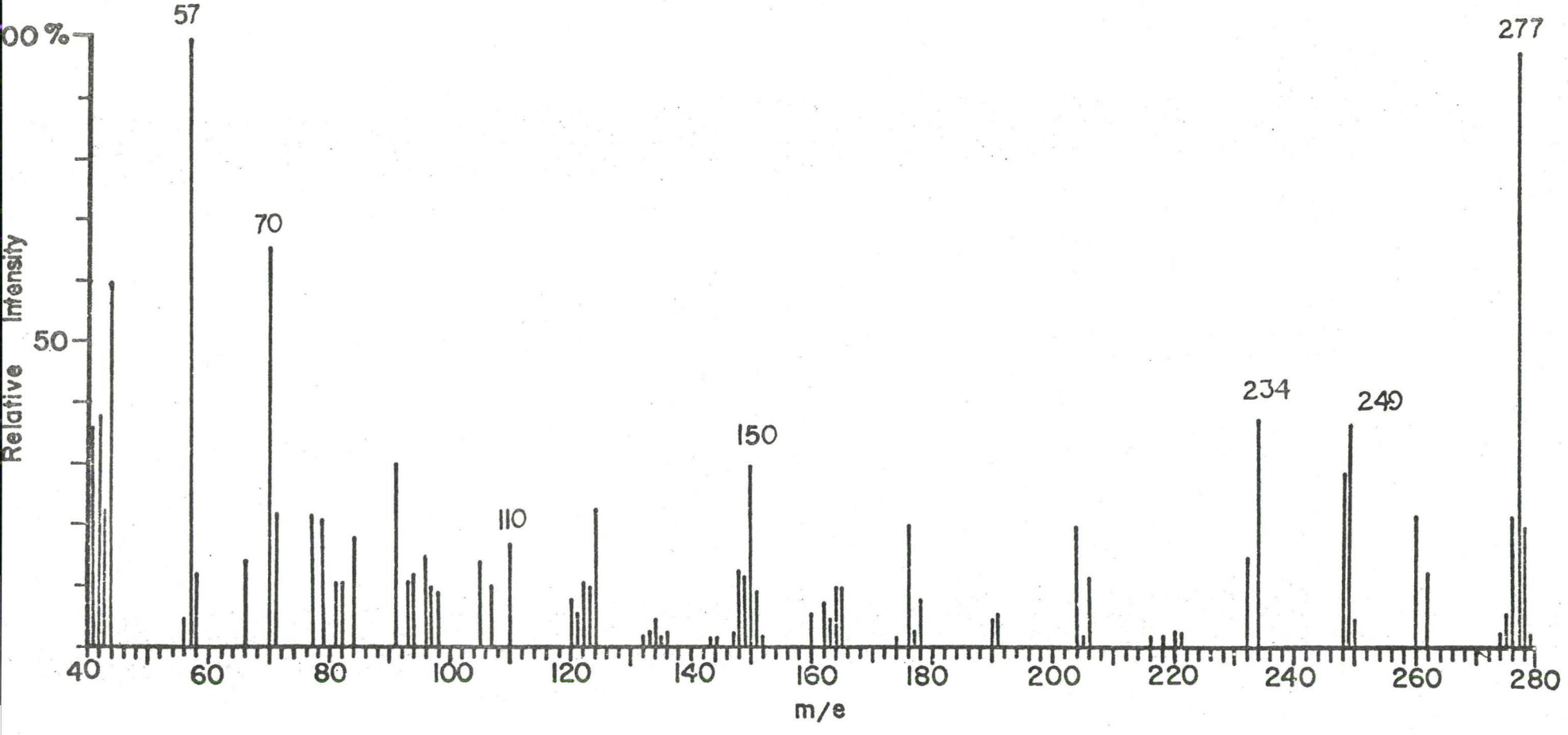


Figure 2. The Mass Spectrum of the Hofmann Product of Clavolonine

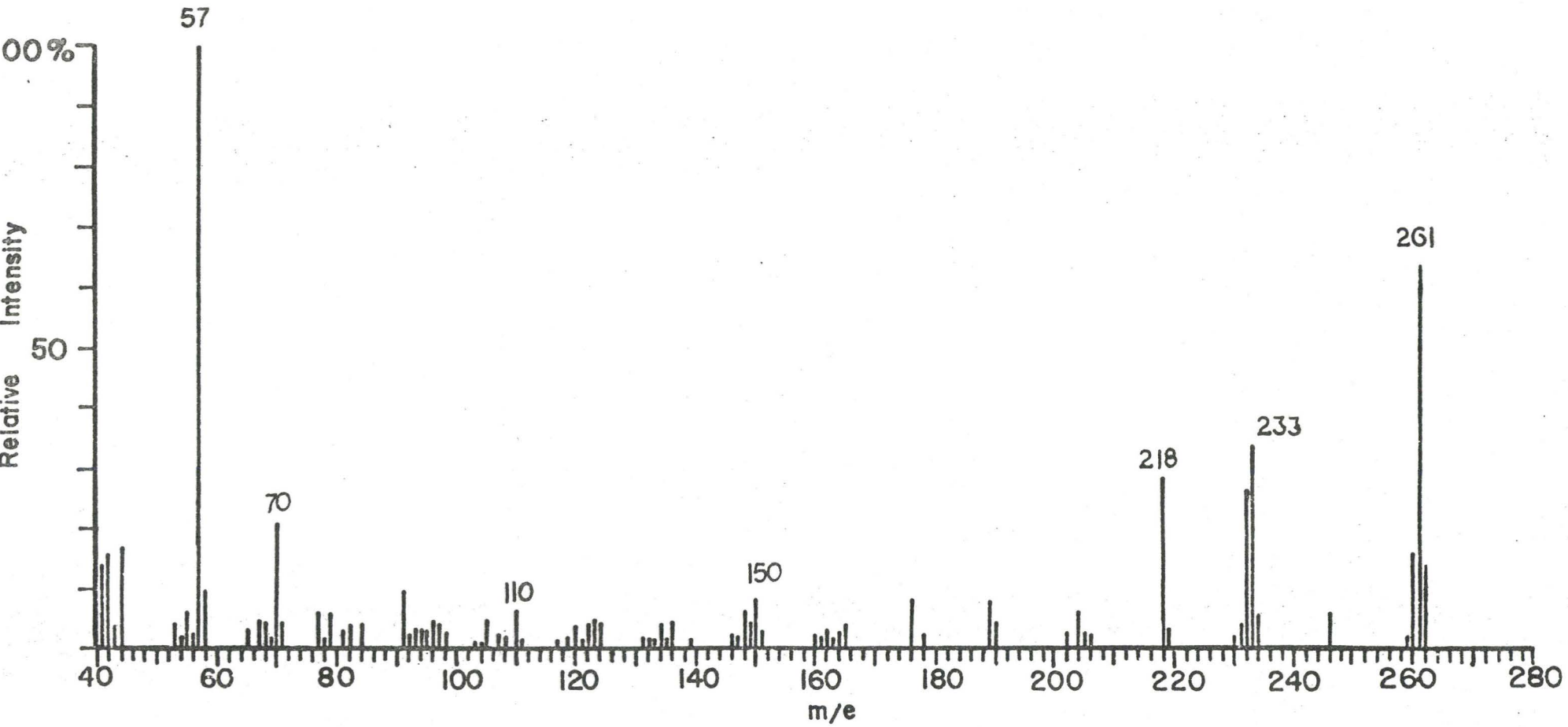


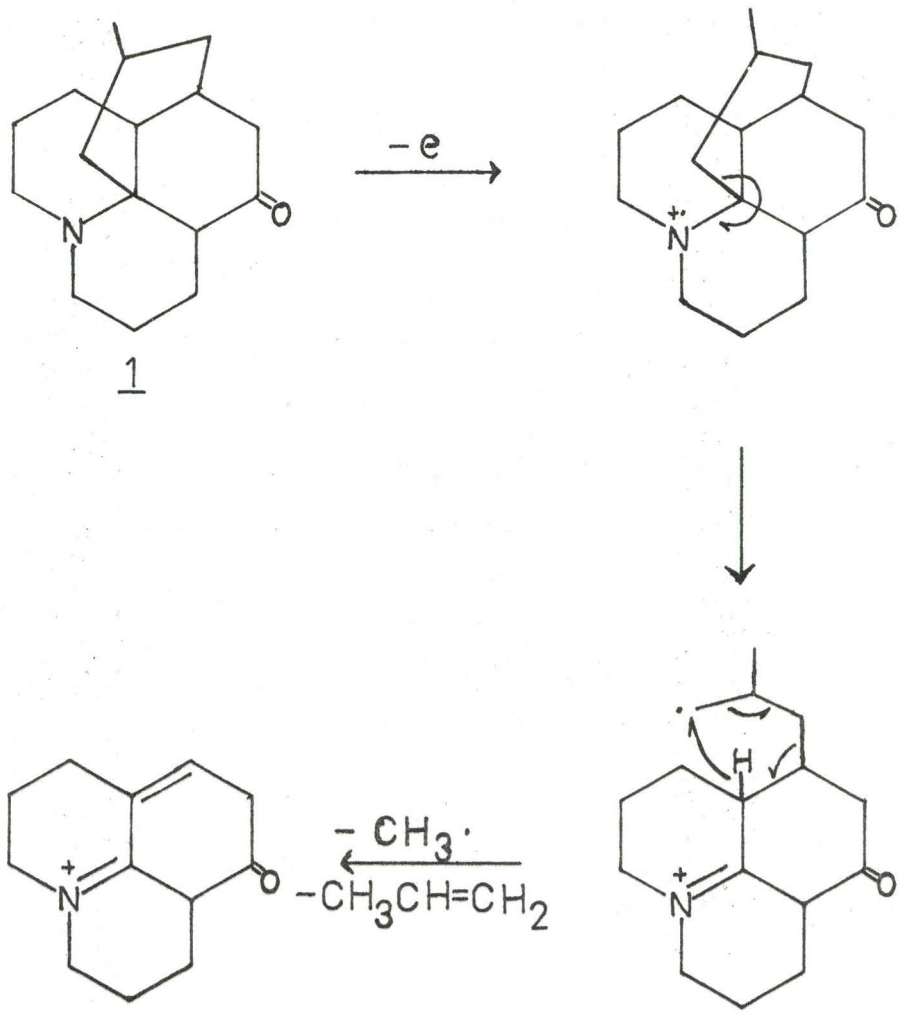
Figure 3. The Mass Spectrum of the Hofmann Product of Lycopodine

The evidence indicates that the Hofmann product of lycopodine and clavolonine have the same carbon skeleton but, it was not possible to remove the -OH group from the Hofmann product of clavolonine to make a direct comparison. It was decided therefore, to re-examine the cyclized products of lycopodine to see if further information could be gained. The two main areas of investigation which were used were n.m.r. and mass spectrometric analysis.

Mass Spectrometric Analysis

The mass spectra of *Lycopodium* alkaloids of known structure were recorded and reported by MacLean (15). A study of their fragmentation was made in order that the information so obtained might be applied to the study of alkaloids whose structures were yet unresolved. One group of *Lycopodium* alkaloids, which has been studied extensively by mass spectrometry are those related in skeletal structure to lycopodine. These alkaloids with the lycopodine skeleton undergo a fragmentation in which the elimination of C-8, C-14 and C-15, along with their attached substituents, shown in Scheme 6, gives a very intense peak and in most cases this peak is the base peak.

The mass spectra of the Hofmann products of lycopodine and clavolonine shown in Figs. 3 and 2, respectively, do not show an intense peak corresponding to the loss of the bridged system. This observation would indicate that the Hofmann products may not contain a bridged cyclohexylamine ring system similar to that in lycopodine. Another outstanding feature of the mass spectra of the Hofmann products of lycopodine and clavolonine is the presence of the base peak at m/e 57.



SCHEME 6

Through high resolution mass spectrometry the ion at m/e 57 was determined to be $C_3H_7N^+$. The presence of this ion $(C_3H_7N)^+$ in such abundance suggests that its formation is easy.

In order to gain greater insight into the fragmentation of the Hofmann product of lycopodine a number of specifically labelled compounds were prepared. The Hofmann product of lycopodine was treated with $CH_3OD-\bar{O}Me$ and from low voltage mass spectrometric analysis it was determined that a maximum of two hydrogens was exchanged. The only readily exchangeable hydrogens are those on the carbon alpha to the carbonyl function. This compound will now be referred to as HL-6. Lycopodine -9,9-d_2 was prepared and the Hofmann degradation reaction was performed on it to give the product referred to as HL-9. The Hofmann degradation reaction was performed on lycopodine methiodide N-methyl-d_3. The mass spectra of these compounds are shown in Figs. 4, 5 and 6, respectively.

The mass spectrum of the Hofmann product of lycopodine-N-methyl-d_3 Fig. 6 gave the molecular ion at m/e 264. The base peak of this compound was found at m/e 60. This is a clear indication that the ion $C_3H_7N^+$ does contain the N-methyl group. The mass spectrum of HL-9 Fig. 5 gave the molecular ion m/e 263. The base peak of this compound remained at m/e 57 although the peak at m/e 58 had increased considerably. Since the base peak in the spectrum of HL-9 remained at m/e 57, it appears that the original C-9 of lycopodine is not present in the $C_3H_7N^+$ fragment. To obtain the ion $C_3H_7N^+$ without involving C-9 from the proposed structure 8 is unlikely, since at least four carbon-carbon

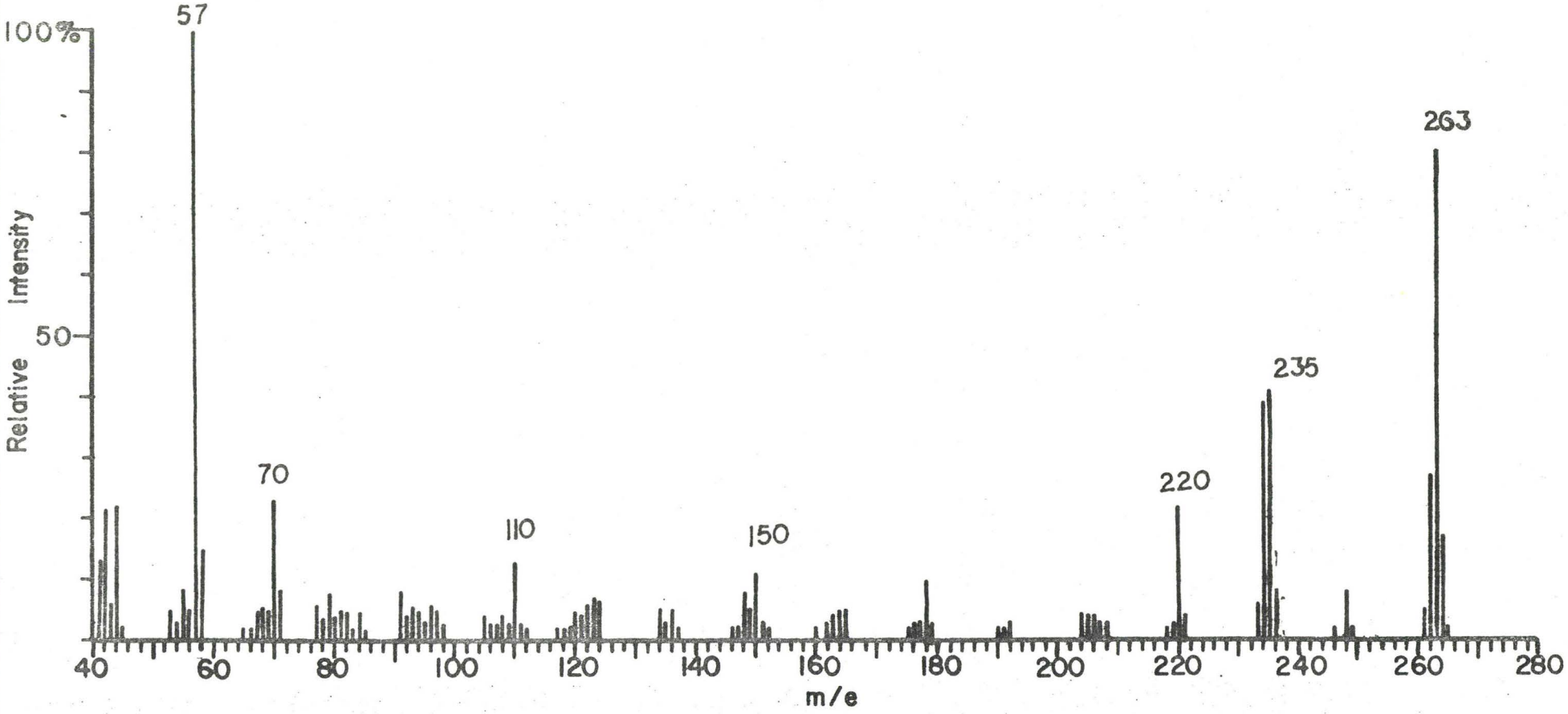


Figure 4. The Mass Spectrum of the Hofmann Product of Lycopodine-6,6-d_2

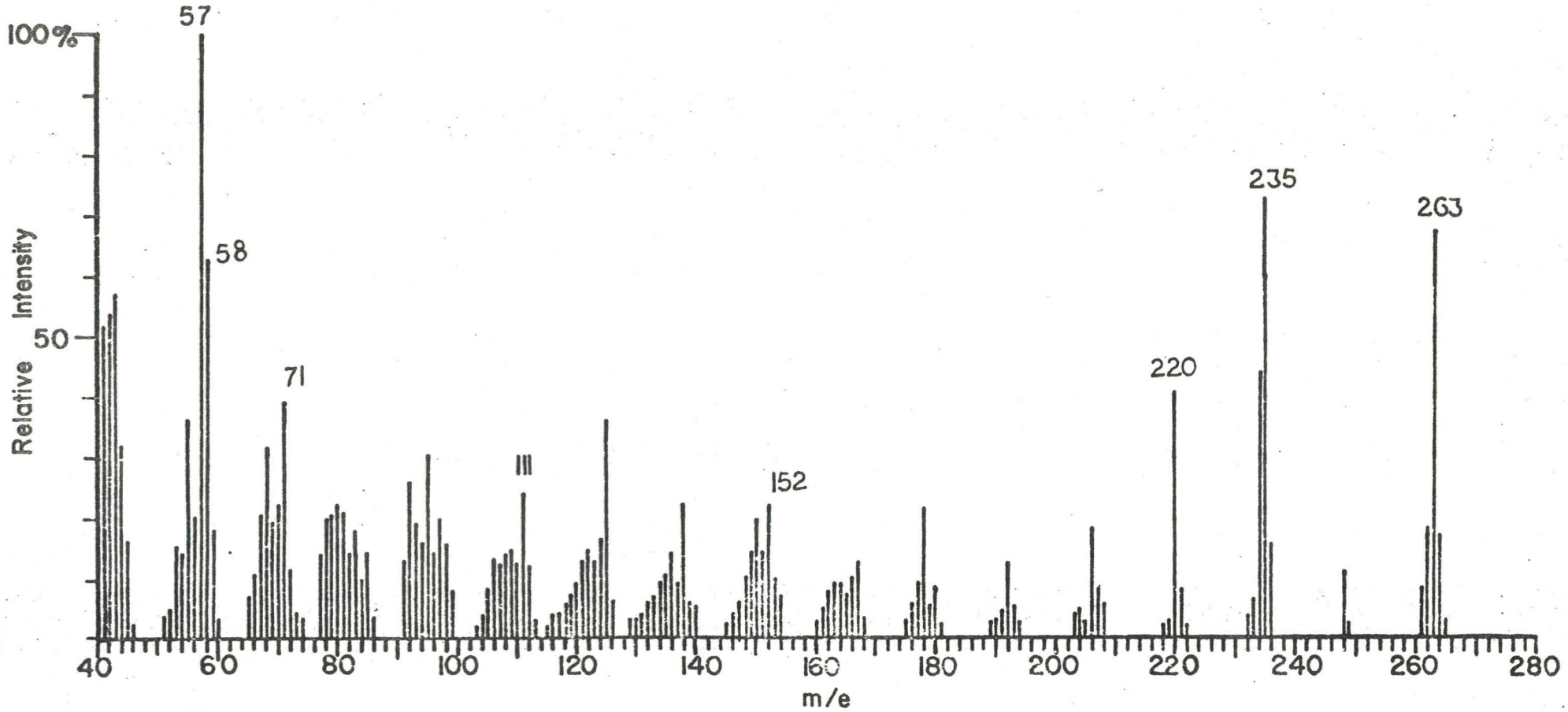


Figure 5. The Mass Spectrum of the Hofmann Product arising from Lycopodine-9,9-d_2

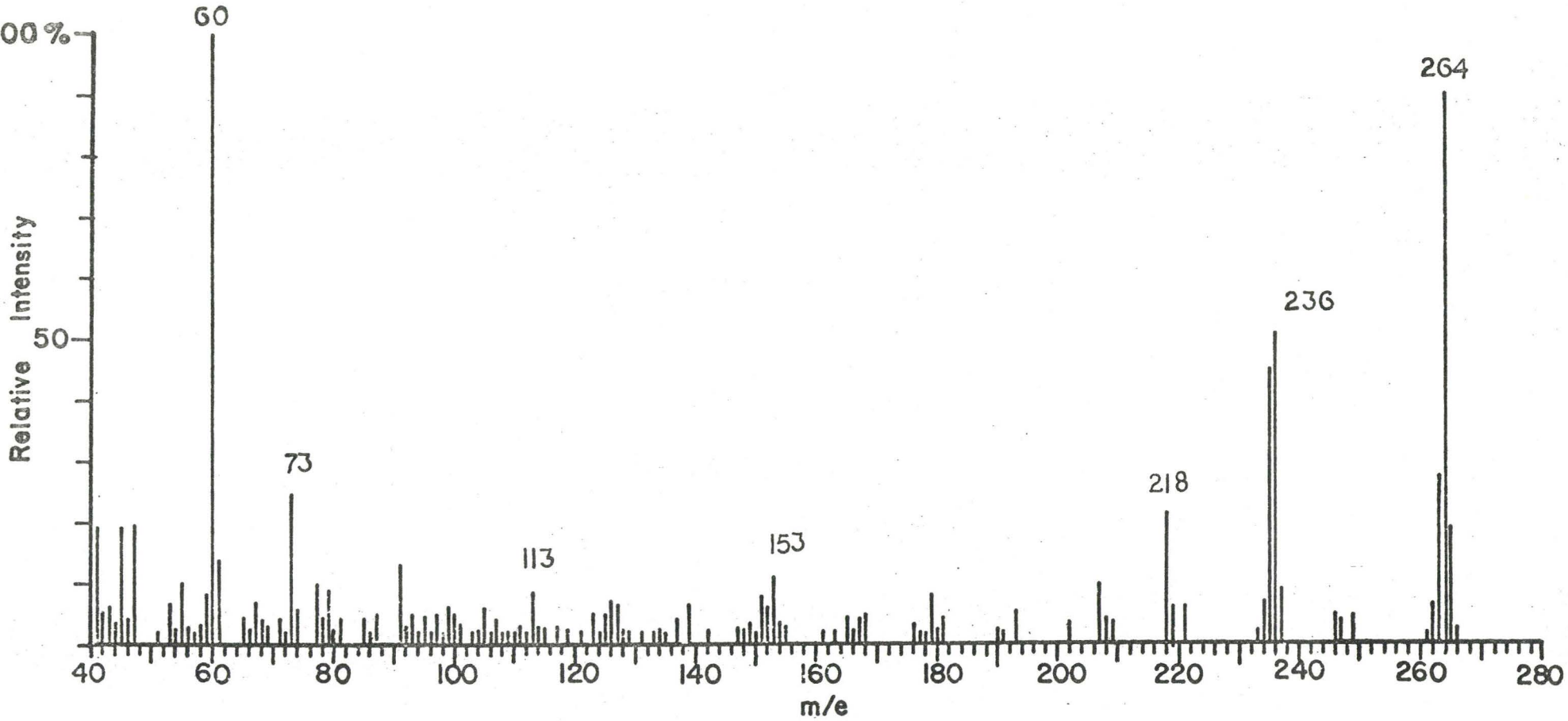


Figure 6. The Mass Spectrum of the Hofmann Product of Lycopodine-N-Me-d_3

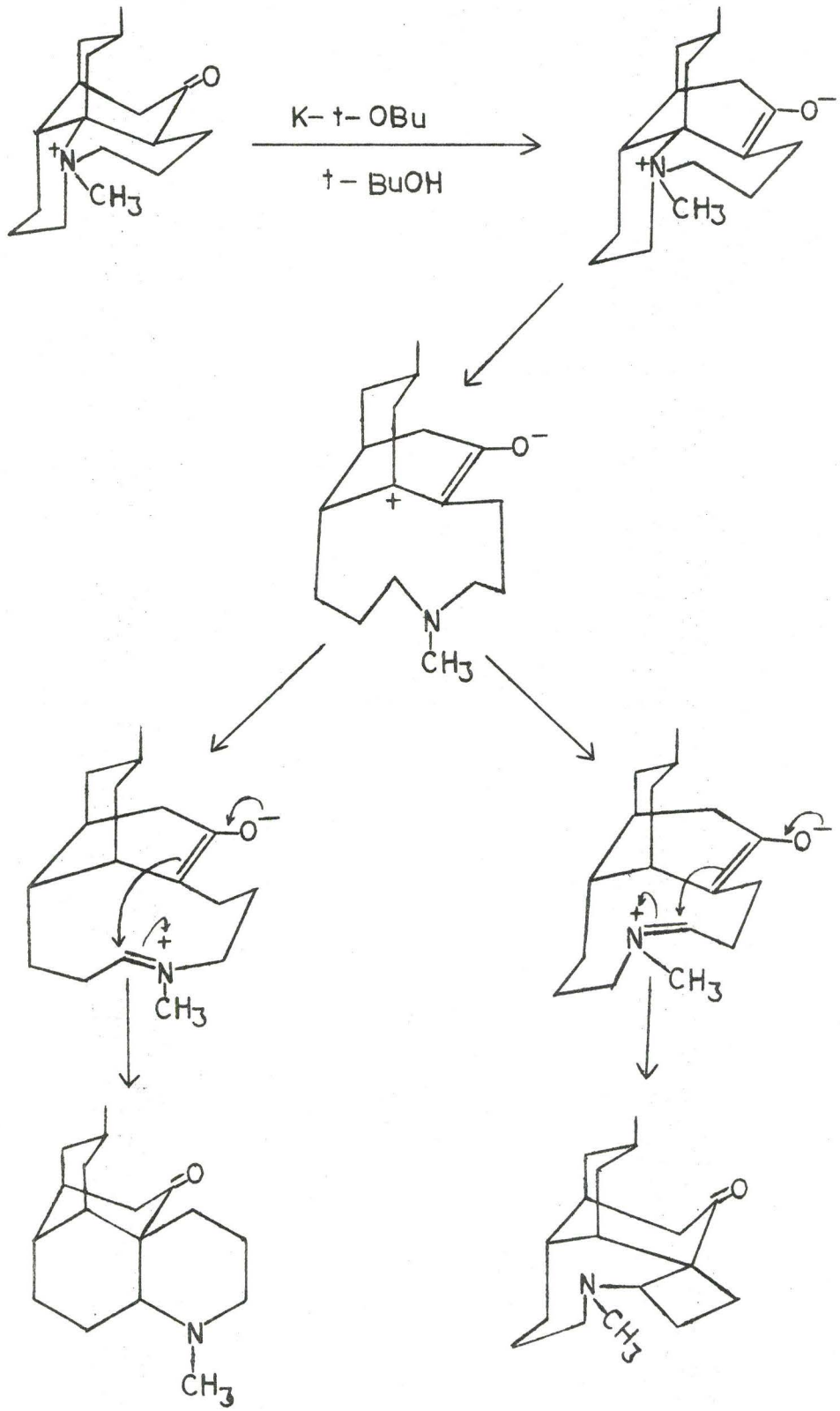
bonds must be broken. Therefore the mass spectrometric result makes the structure 8, for the Hofmann product of lycopodine, very unattractive. Because structure 8 was not compatible with the mass spectrometric result, it was decided to examine other possibilities.

An alternative structure for the Hofmann product of lycopodine is shown in Scheme 7. Lycopodine methiodide in the presence of a strong base will enolize. The resulting enolate ion will aid solvolysis and cleavage of C-13-nitrogen bond. The carbonium ion (16) can then undergo intramolecular hydride transfer from C-9 or C-1 to give a more stable immonium cation, which can then cyclize at the carbon adjacent to the carbonyl function. The two possible structures which can arise from such a mechanism are 18A and 18B shown in Scheme 7.

The mass spectrum agrees with the proposed structures 18A and 18B. These structures do not have the original bridged system, found in lycopodine, adjacent to the nitrogen atom. Therefore the loss of this bridged system would not be a favored process as in lycopodine. Moreover, to obtain an ion at m/e 57 $(C_3H_7N)^+$ without involving C-9 is not a problem.

Nuclear Magnetic Resonance Analysis

It should be possible to distinguish structures 18A and 18B from structure 8 by the use of n.m.r. Structures 18A and 18B have three protons on the carbons adjacent to the nitrogen, whereas structure 8 has two protons. Therefore by quaternizing the nitrogen the deshielding effect should affect those protons on the carbons adjacent to the nitrogen and cause them to shift downfield. The methiodide of the Hofmann



SCHEME 7

product of lycopodine was prepared and the n.m.r. spectrum was run at 220 MHz Fig. 7. The spectrum was compared with that of the Hofmann product of lycopodine Fig. 8. Three distinct peaks corresponding to three protons were observed downfield at 4.65δ, 4.08δ and 3.80δ, respectively. This seems to indicate that there are three protons on the carbons adjacent to the nitrogen in accord with the proposed structures 18A and 18B. This result would definitely rule out structures 7 and 8 for the Hofmann product of lycopodine.

If the three peaks, farthest downfield in the spectrum of the methiodide of Hofmann product of lycopodine correspond to the protons on the carbons adjacent to nitrogen, then they would consist of a methine and two methylene protons. The spectrum for the methiodide of the Hofmann product arising from lycopodine -9,9-d_2 should lack two of the three downfield peaks if structure 18B were correct. However, the spectrum Fig. 9 showed that only the peak at 4.65δ disappeared. This result is in agreement with the structure 18A only, since the peak farthest downfield should be the methine proton of C-9.

The peak at 4.65δ appears as a doublet of doublets with coupling constants of 9 and 4 cycles sec^{-1}, respectively. This result is in agreement with structure 18A since the methine proton on C-9 should be coupled to the two protons on C-10 to give a large and small coupling constant, as observed. Supporting evidence that the two peaks at 4.08δ and 3.80δ correspond to the methylene protons on the carbon adjacent to the nitrogen can be found in the works of Feltkamp (17). He studied trans-2,3-dimethylpiperidine which has two methylene protons on the

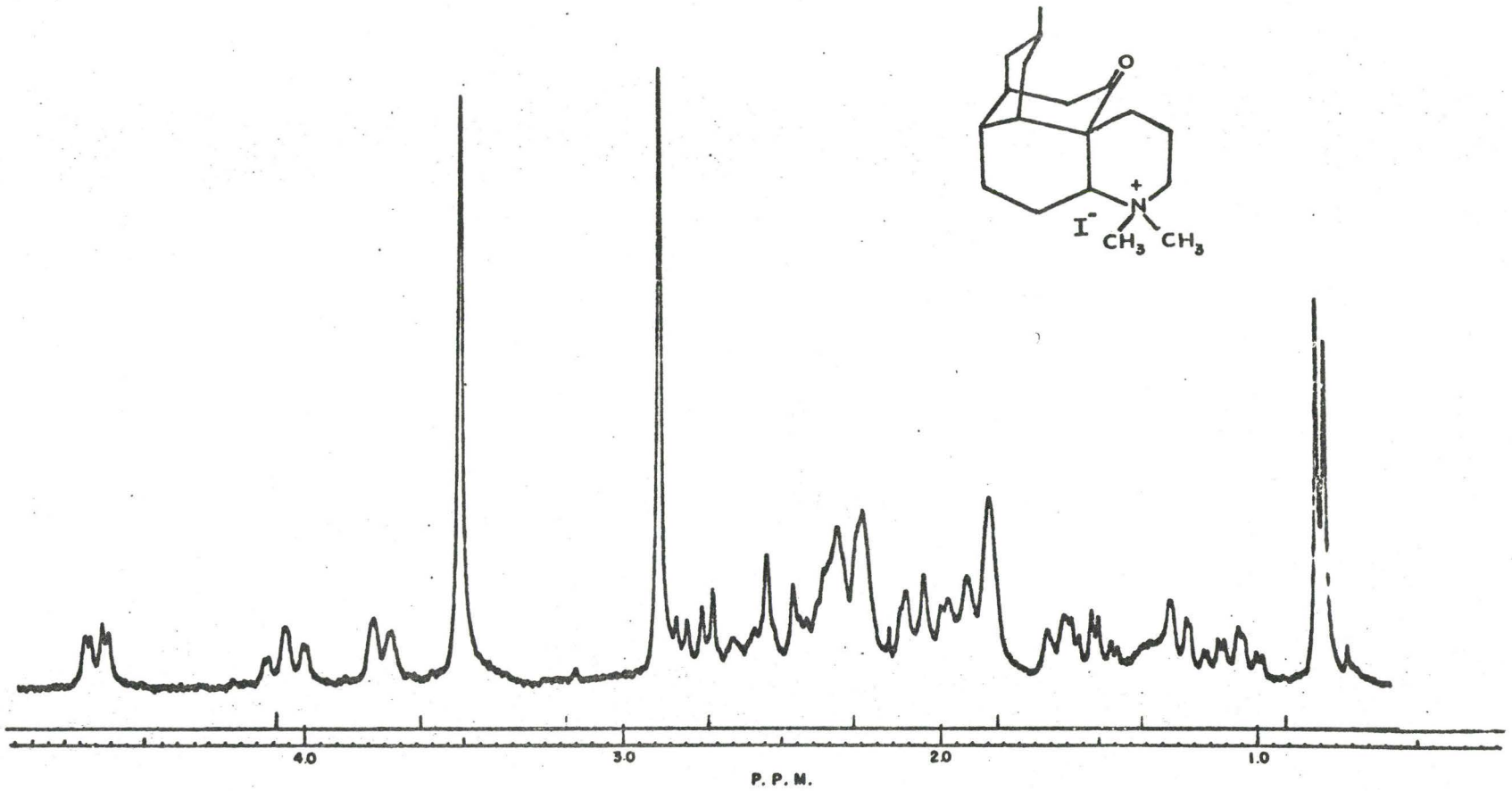


Figure 7. The N.m.r. Spectrum of the Methiodide of the Hofmann Product of Lycopodine

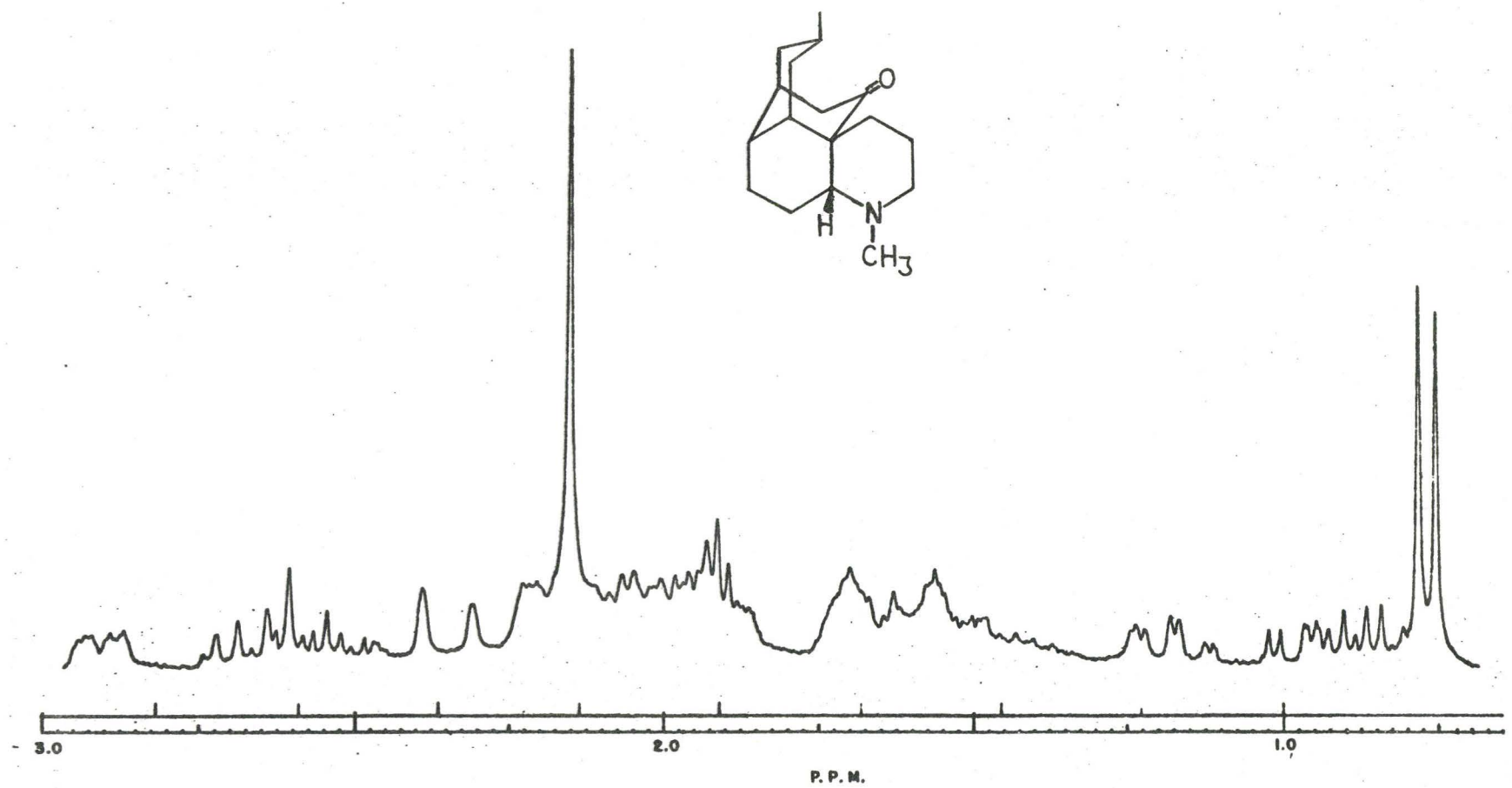


Figure 8. The N.m.r. Spectrum of the Hofmann Product of Lycopodine

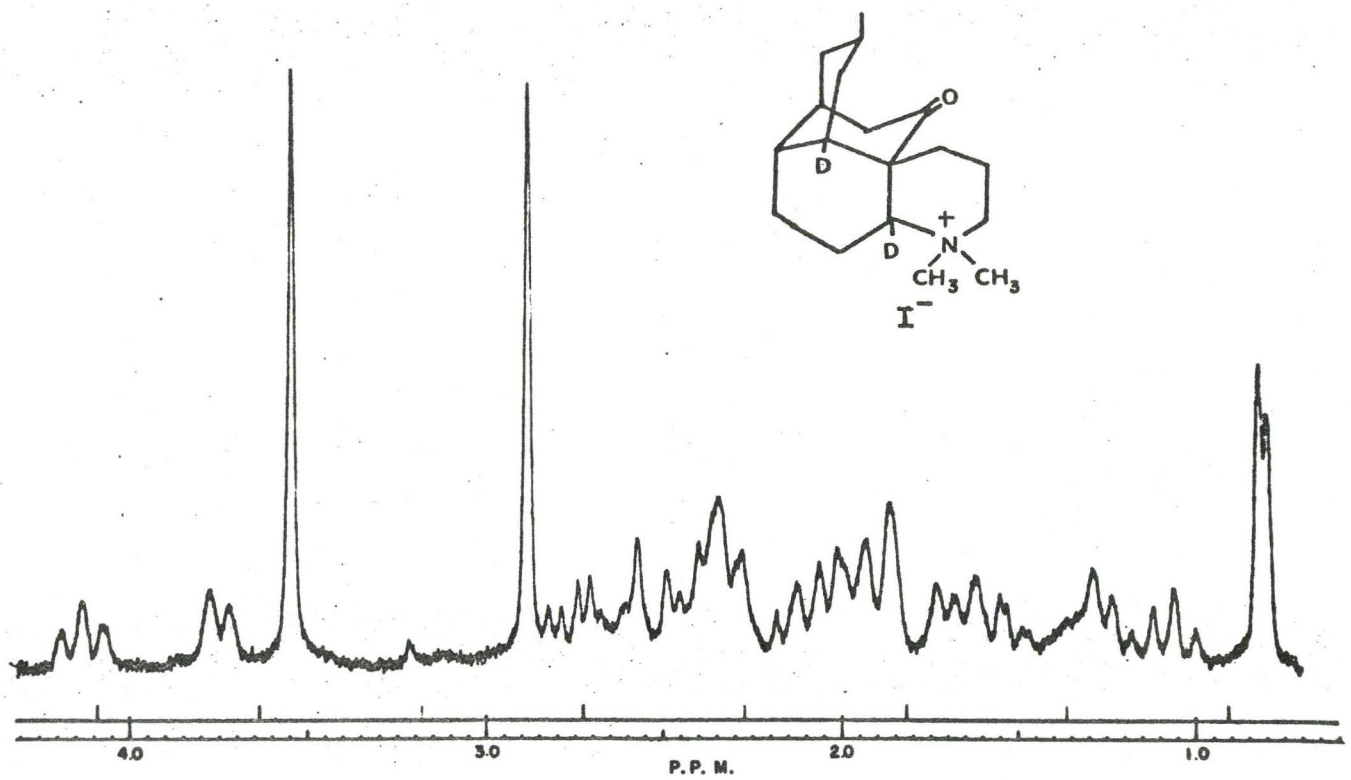


Figure 9. The N.m.r. Spectrum of the Methiodide of the Hofmann Product arising from Lycopodine-9,9-d_2

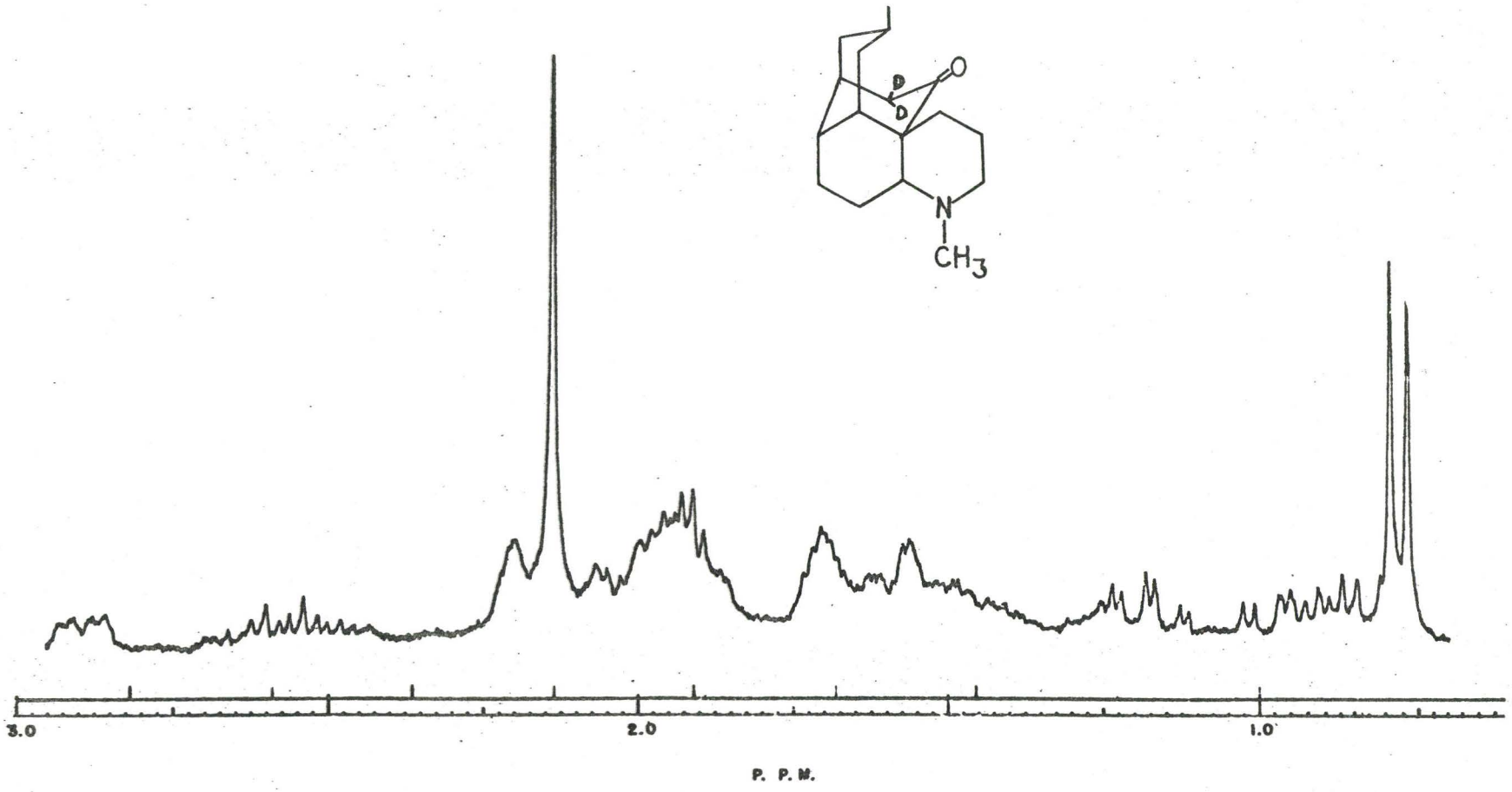


Figure 10. The N.m.r. Spectrum of the Hofmann Product of Lycopodine-6,6-d_2

carbon adjacent to nitrogen. The peaks corresponding to these methylene protons in the n.m.r. spectrum of trans-2,3-dimethylpiperidine are very similar to those obtained for the methiodide of the Hofmann product of lycopodine. The peak at 4.08δ and 3.80δ appear as a triplet and broad doublet, respectively.

Another significant feature of the spectrum of the methiodide of the Hofmann product of lycopodine is the presence of two separate peaks for the two N-CH_3 groups. The peaks were found at 3.50δ (3H singlet) and 2.90δ (3H singlet), respectively. This result seemed unusual at first, but on examination of a molecular model it was observed that in structure 18A, one of the N-CH_3 groups is located directly in the shielding cone of the carbonyl group and so would be shifted upfield.

The spectra of the Hofmann product of lycopodine and the Hofmann product of lycopodine-6,6-d_2 are shown in Figs. 8 and 10, respectively. Missing from the spectrum of the deuterated Hofmann product of lycopodine is a broad doublet at 2.35δ (one proton) and a quartet at 2.65δ (one proton). This is consistent with the ABX type system for the methylene protons on C-6 which are coupled to the methine proton on C-7.

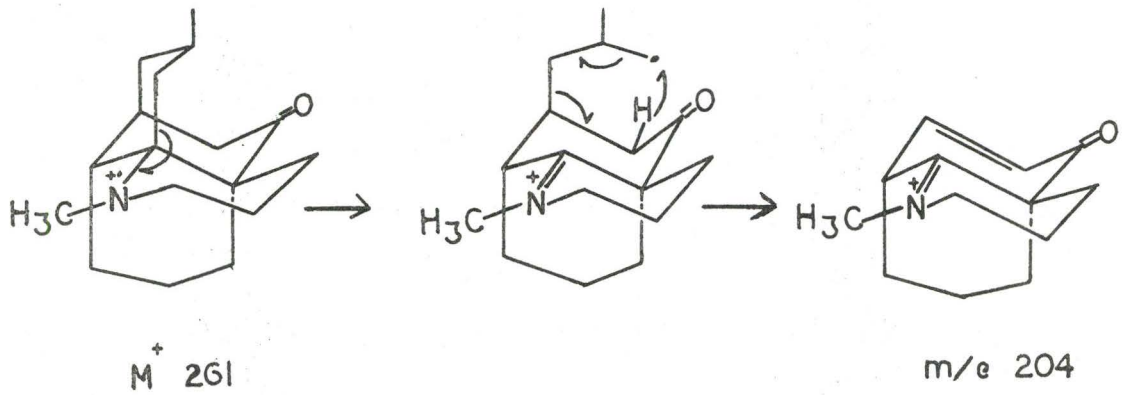
An ^{13}C n.m.r. spectrum of the Hofmann product of lycopodine was obtained through the courtesy of Dr. Bell and Mr. Easton. The spectrum gave sixteen carbons in the region of saturated carbons and one carbon in the region of carbonyl absorption (18).

Re-examination of N-Methyl-α-cyclolycopodine

N-Methyl-α-cyclolycopodine 7 was prepared and re-examined. The mass spectra of this compound and its deuterated analogue are shown in

Figs. 11 and 12, respectively. The n.m.r. spectra of these compounds were run at 220 MHz and are shown in Figs. 13 and 14, respectively. Some additional evidence was obtained which confirms structure 7 previously proposed (8).

The mass spectrum of N-methyl-α-cyclolycopodine gave the base peak at m/e 204. This ion corresponds to the loss of the original bridge of lycopodine plus hydrogen, which is expected since this compound retains the nitrogen adjacent to the bridgehead position. The composition of the ions was determined by high resolution mass spectrometry.



However, unlike lycopodine (15) the loss of the hydrogen atom does not occur from C-12, since a double bond between C-7 and C-13 would cause a severely strained system. In the mass spectrum of N-methyl-α-cyclolycopodine-6,6-d_2 the base peak was observed at m/e 205 showing that a deuterium and not a hydrogen was lost with the bridge atoms.

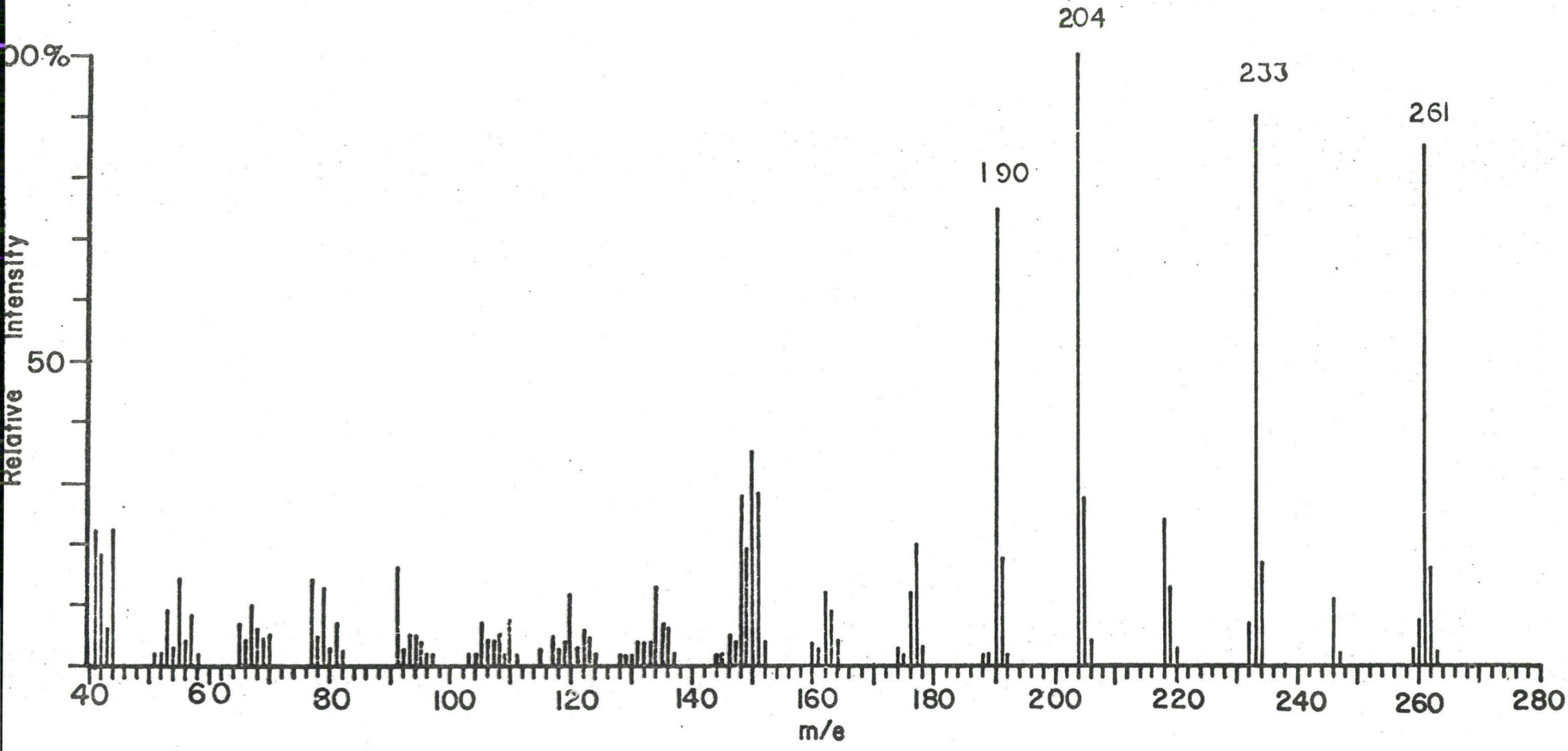


Figure 11. The Mass Spectrum of N-Me-α-cyclolycopodine

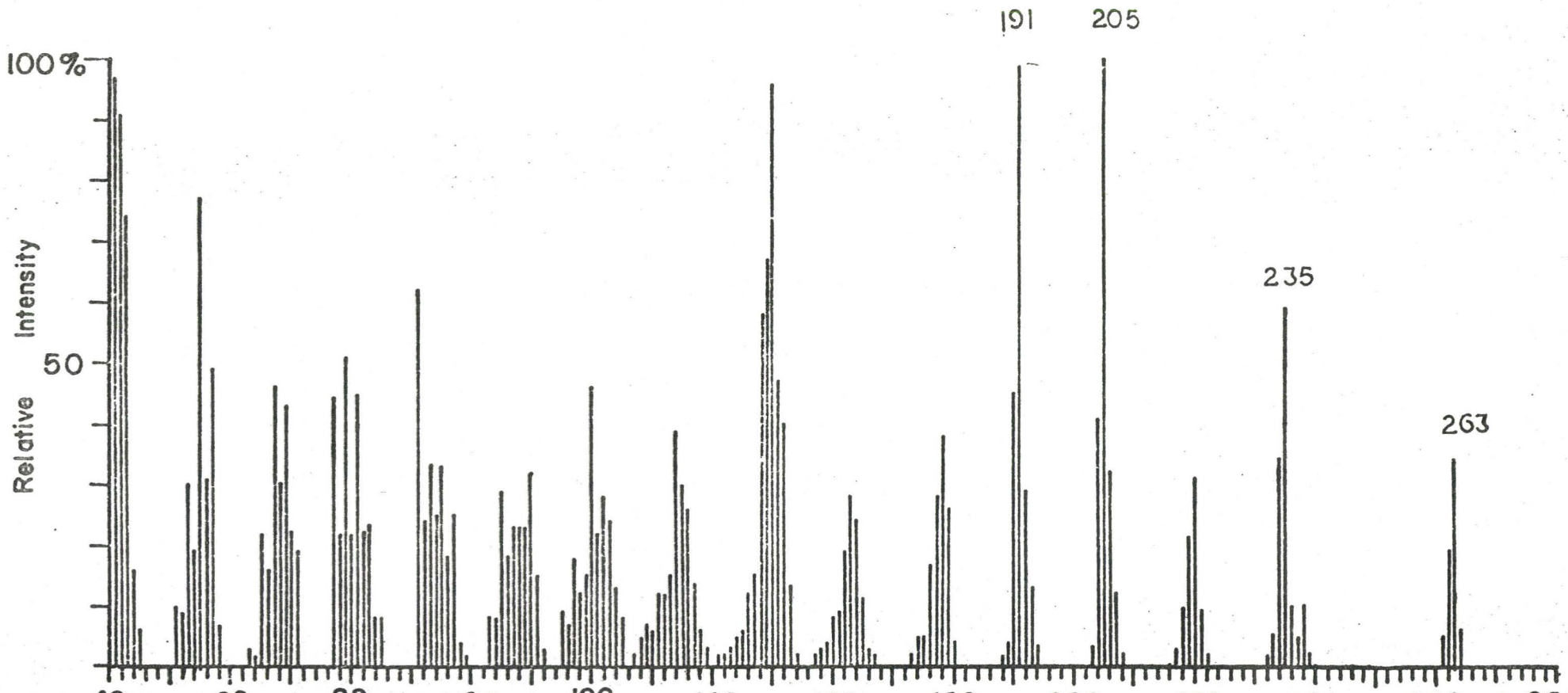
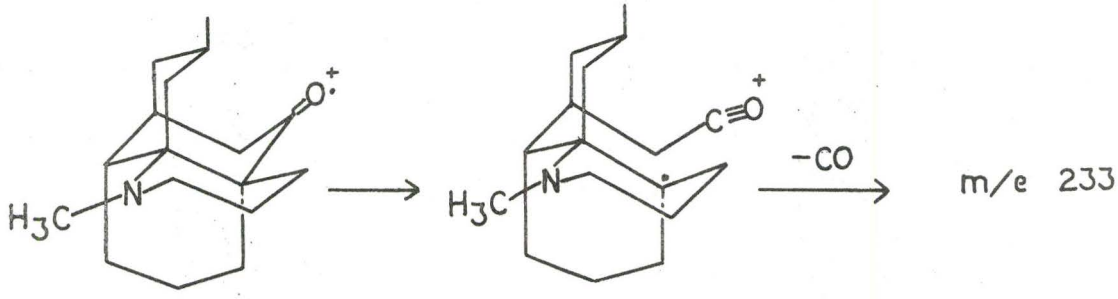


Figure 12. The Mass Spectrum of N-Me-α-cyclolycopodine-6,6-d_2

Presumably in 7 the hydrogen that is lost arises from C-6. This observation is in complete agreement with the proposed structure 7. Another feature of the mass spectrum of N-methyl-α-cyclolycopodine was an intense peak at m/e 233 corresponding to the loss of carbon monoxide. In lycopodine the peak corresponding to the loss of carbon monoxide is approximately two per cent of the base peak. The ease with which the N-methyl-α-cyclolycopodine loses carbon monoxide is probably associated with the presence of a quaternary carbon center adjacent to the carbonyl function (19). The tertiary radical center is more stable than the secondary center formed in lycopodine by similar cleavage thus promoting cleavage of the C-4, C-5 bond. Carbon monoxide can then be lost from the molecule.



The n.m.r. spectrum of N-methyl-α-cyclolycopodine-6,6-d_2, Fig. 14 differed from the spectrum of N-methyl-α-cyclolycopodine, Fig. 13

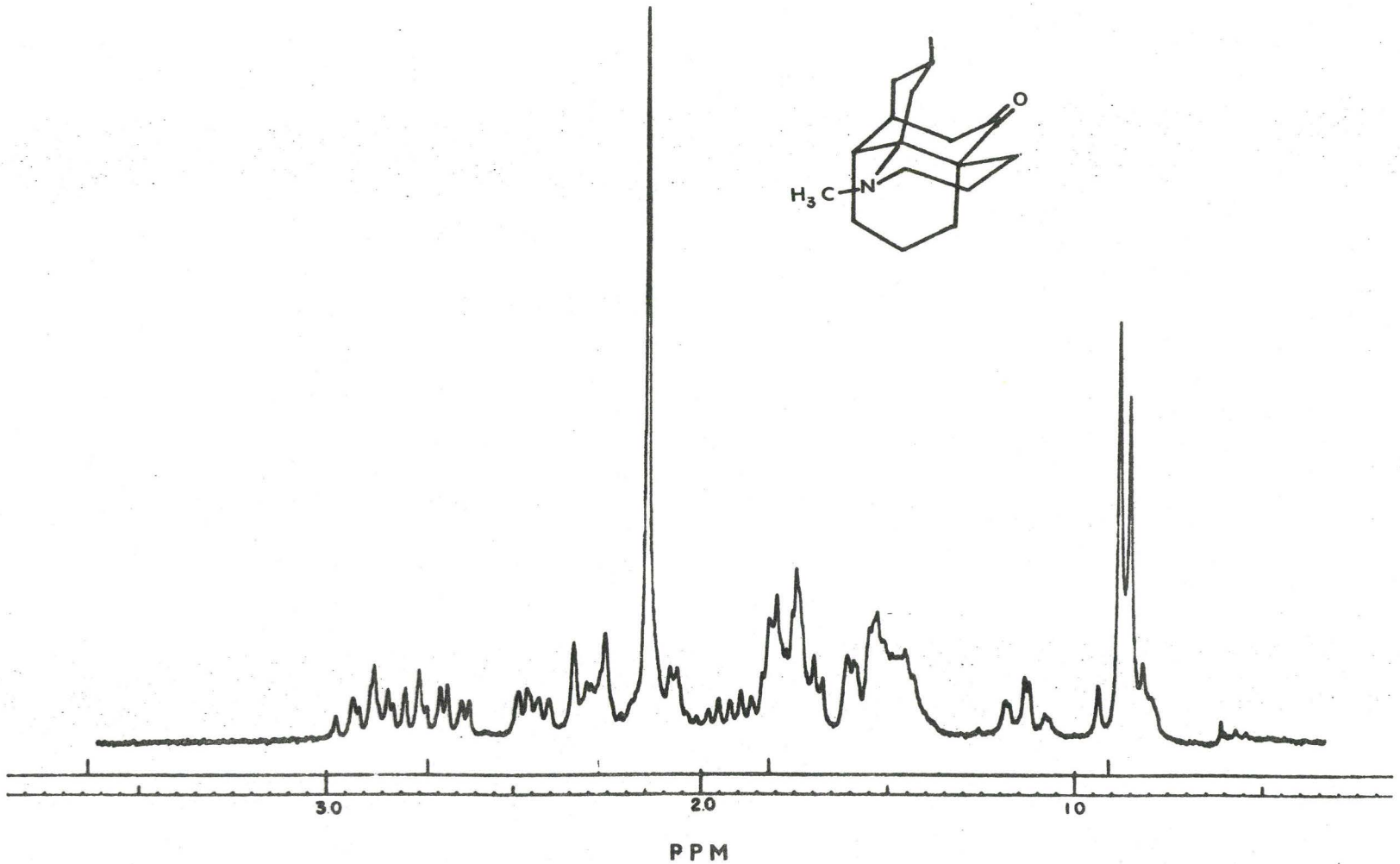


Figure 13. The N.m.r. Spectrum of N-Me-α-cyclolycopodine

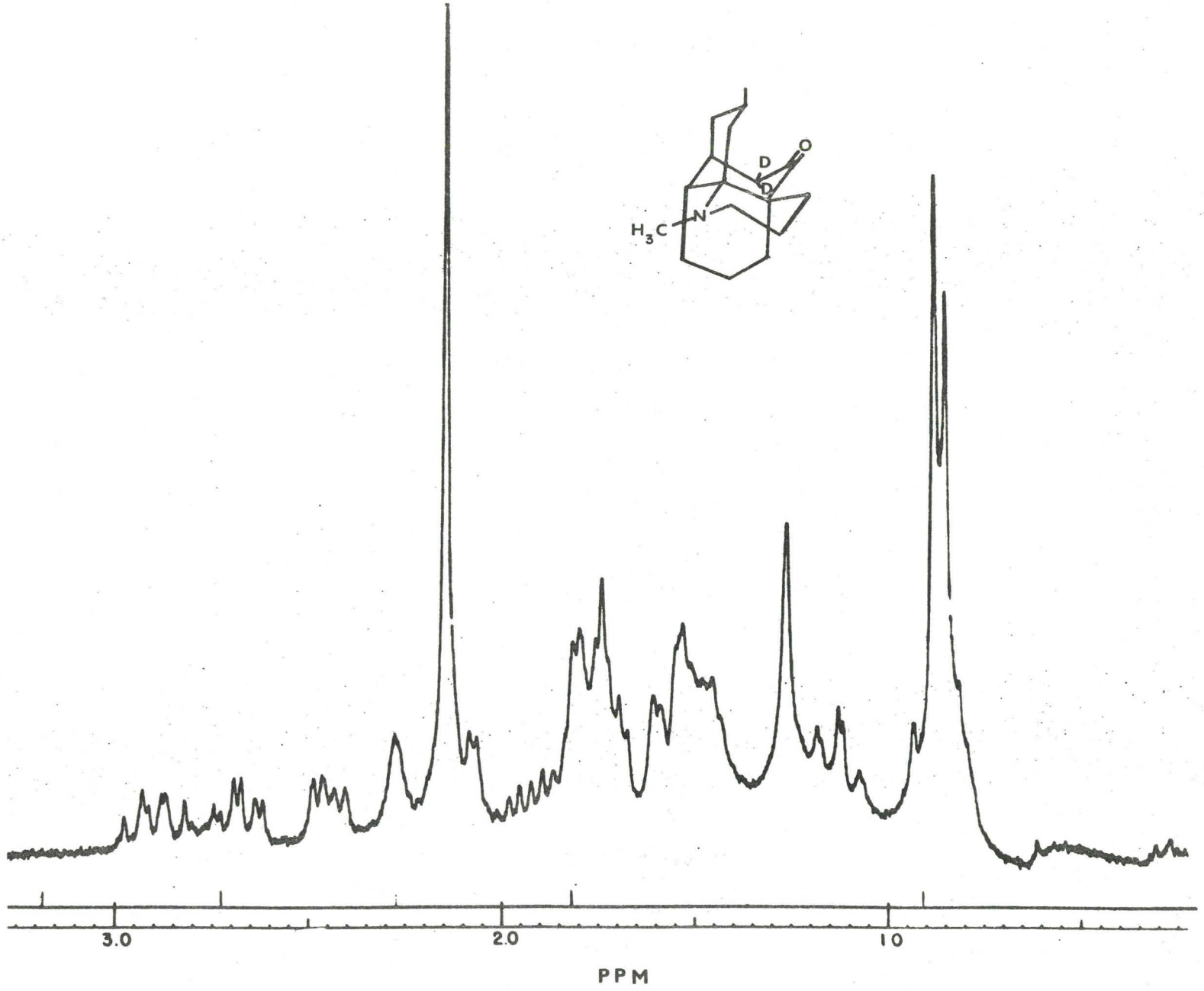
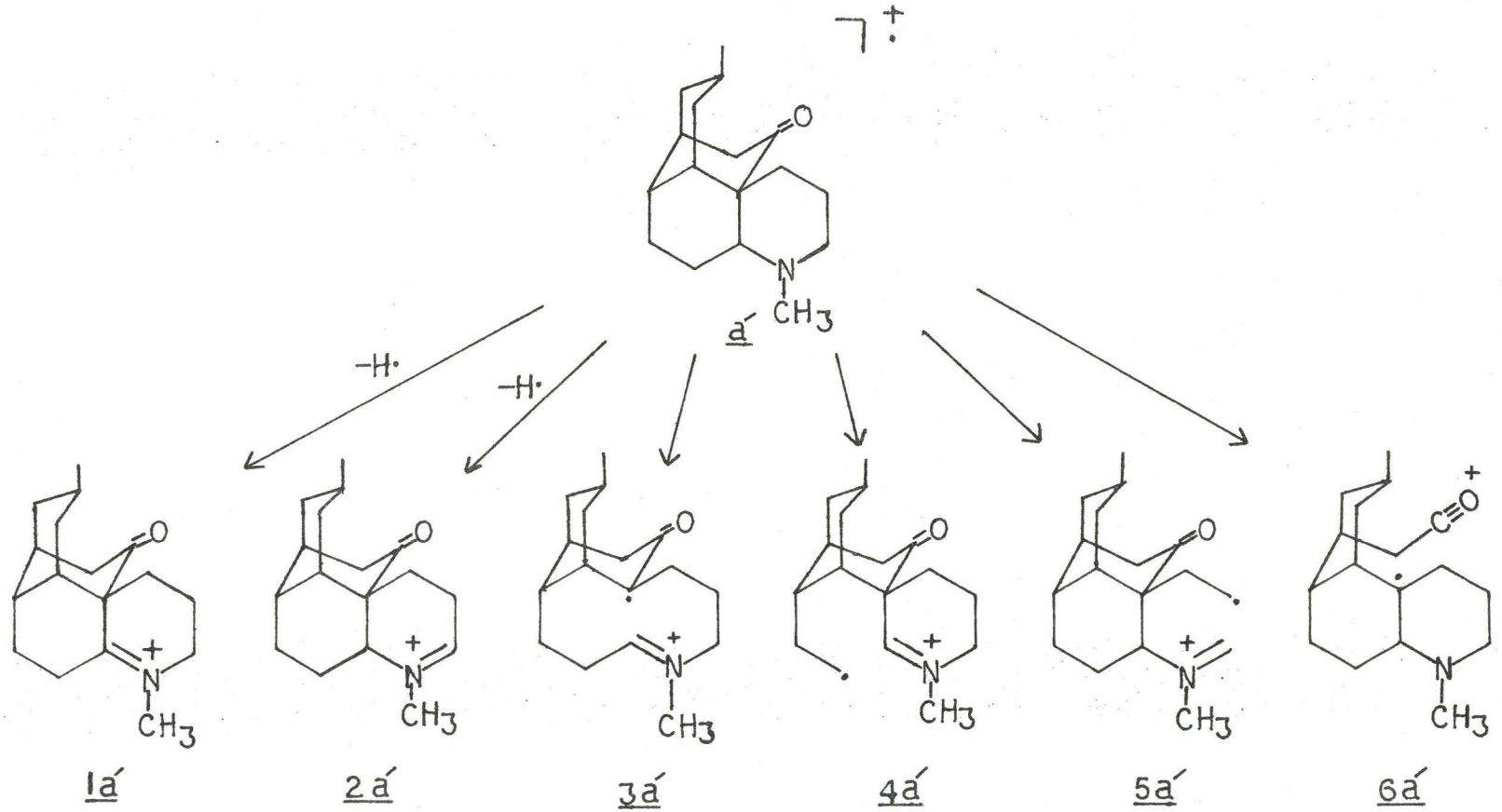


Figure 14. The N.m.r. Spectrum of N-Me-α-cyclolycopodine-6,6-d_2

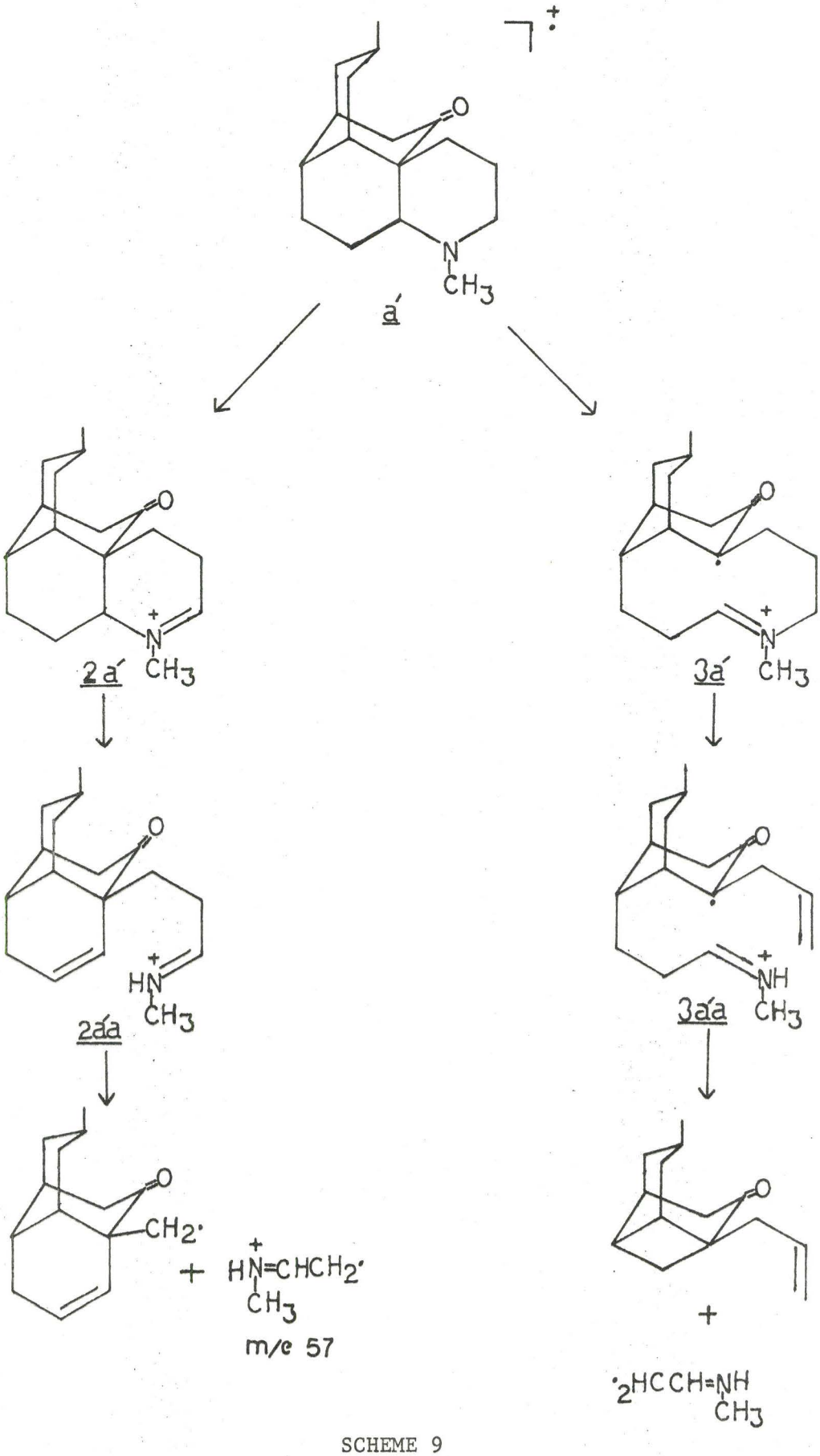
in that the spectrum of the deuterated compound lacked a doublet at 2.30δ and a quartet at 2.85δ corresponding to two protons. Such coupling is expected in the ABX type system present on the structure 7 for the geminal coupling of the protons on C-6 and then coupling to the methine at C-7.

Interpretation of the Mass Spectrum

The following Schemes are now proposed as likely fragmentation routes of the Hofmann product of lycopodine in the mass spectrometer. The composition of the ions was determined by high resolution mass spectrometry. Scheme 8 shows the radical ion a', formed as a result of the loss of an electron from the molecule. The radical ion a' may then lose an α-hydrogen atom to give ions 1a' or 2a' (20, 21). Alternatively the radical ion a' may undergo homolytic bond rupture to give the ions 3a', 4a', 5a', and 6a', respectively. The ion at m/e 57 $(C_3H_7N)^+$ can arise from the ion 2a' by first a hydrogen migration from the β-position (22) and then carbon-nitrogen bond rupture. The resulting ion 2a'a can then undergo allylic cleavage to give the ion m/e 57 shown in Scheme 9. Alternatively, rupture of C-4 and C-9 bond of the radical ion a' (23) furnishes tertiary radical ion 3a'. Hydrogen migration and carbon-nitrogen bond rupture will give ion 3a'a. This ion can then undergo allylic cleavage to give the ion m/e 57 $(C_3H_7N)^+$ shown in Scheme 9. Such fragmentation would explain the increased intensity of the ion m/e 58 in the spectrum of HL-9 (the Hofmann product arising from lycopodine-9,9-d_2).



SCHEME 8



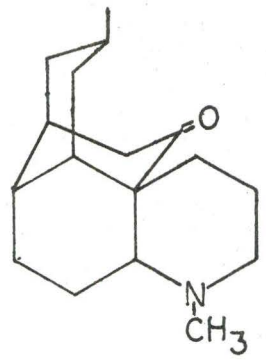
SCHEME 9

Scheme 10 shows the possible fragmentation route for the loss of carbon monoxide. The loss of carbon monoxide to give an intense peak is probably associated with the presence of a quaternary carbon center adjacent to the carbonyl function. The tertiary radical center at C-4 is fairly stable thus promoting cleavage of the C-4, C-5 bond. Carbon monoxide can then be lost from the molecule.

Scheme 11 shows two possible fragmentation routes for the formation of ion m/e 70. The first possibility is that the ion 2a'a can undergo a McLafferty type rearrangement (24) to give a stable conjugated ion m/e 70 $(C_4H_8N)^+$. An alternative fragmentation route is also shown in Scheme 11. First the radical ion 3a' undergoes allylic cleavage to give the fragment 3a'a. This fragment can then cleave at C-1, C-2 bond to give the ion m/e 70. This ion would explain the increased intensity of the peak at m/e 71 in the mass spectrum of HL-9.

Scheme 12 shows a possible fragmentation route for the ion m/e 110 $(C_7H_{12}N)^+$. The ion 3a'b may first undergo hydrogen transfer and then cleavage of C-11, C-12 bond to give the ion m/e 110 $(C_7H_{12}N)^+$. The mass spectrum of HL-9 also showed increase of intensity of the peak at m/e 111 which is compatible with this ion.

Scheme 13 shows a possible fragmentation route for the ion m/e 150 $(C_{10}H_{16}N)^+$. The mass spectrum of HL-9 gave a peak at m/e 152 while the spectrum of HL-6 gave a peak at m/e 150. The mass spectral evidence indicates that the protons on C-9 and C-13 remain on the ion $C_{10}H_{16}N^+$ while those on C-6 are lost. The ion 6a'a loses C-6, C-7, C-8, C-14, C-15 and C-16 along with their attached substituents to give the ion m/e 150 $(C_{10}H_{10}N)^+$.



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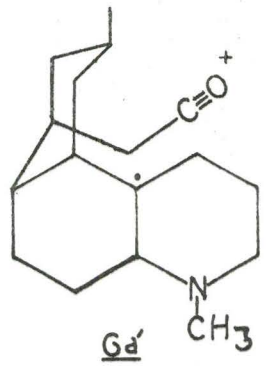
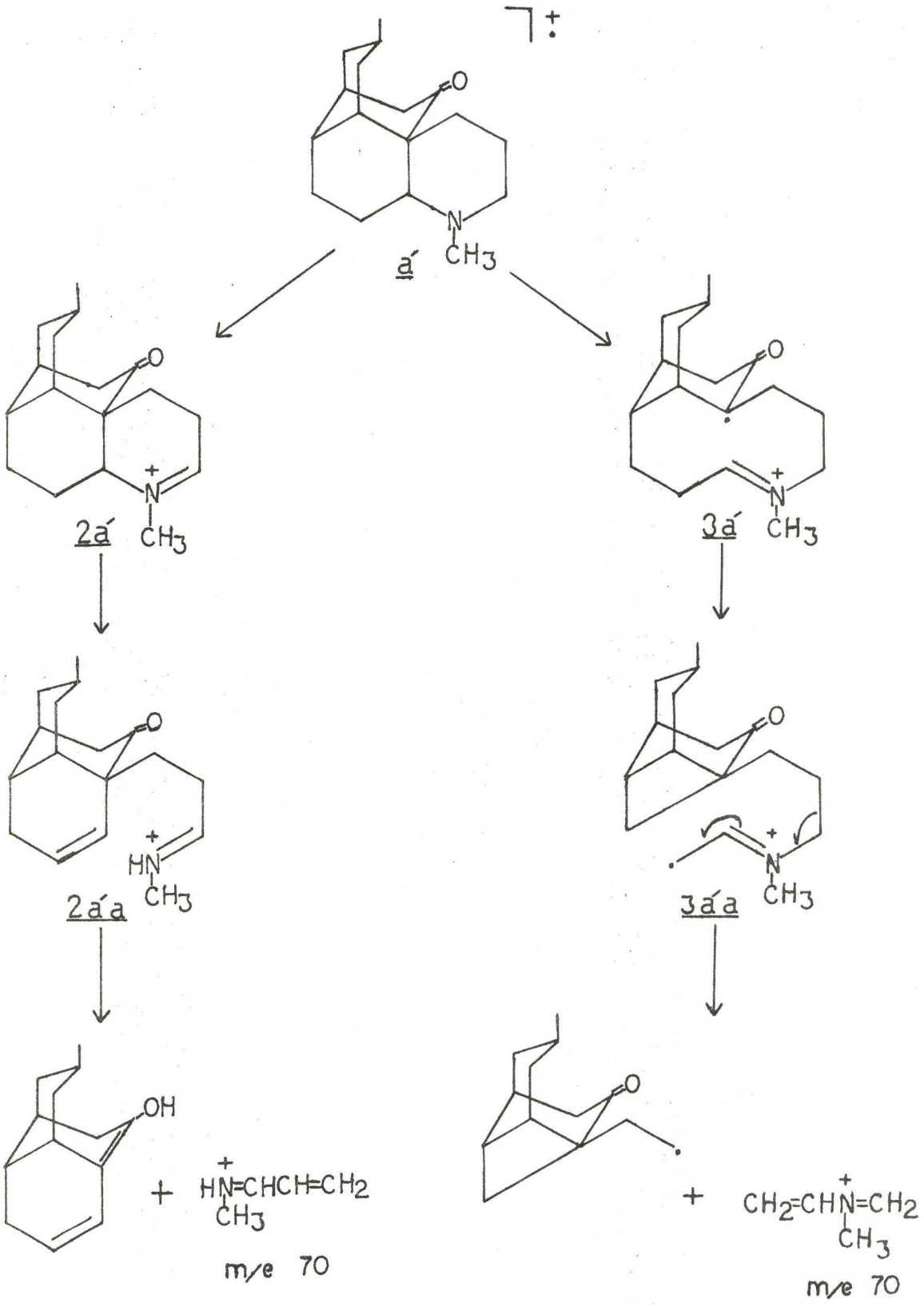


Gd'

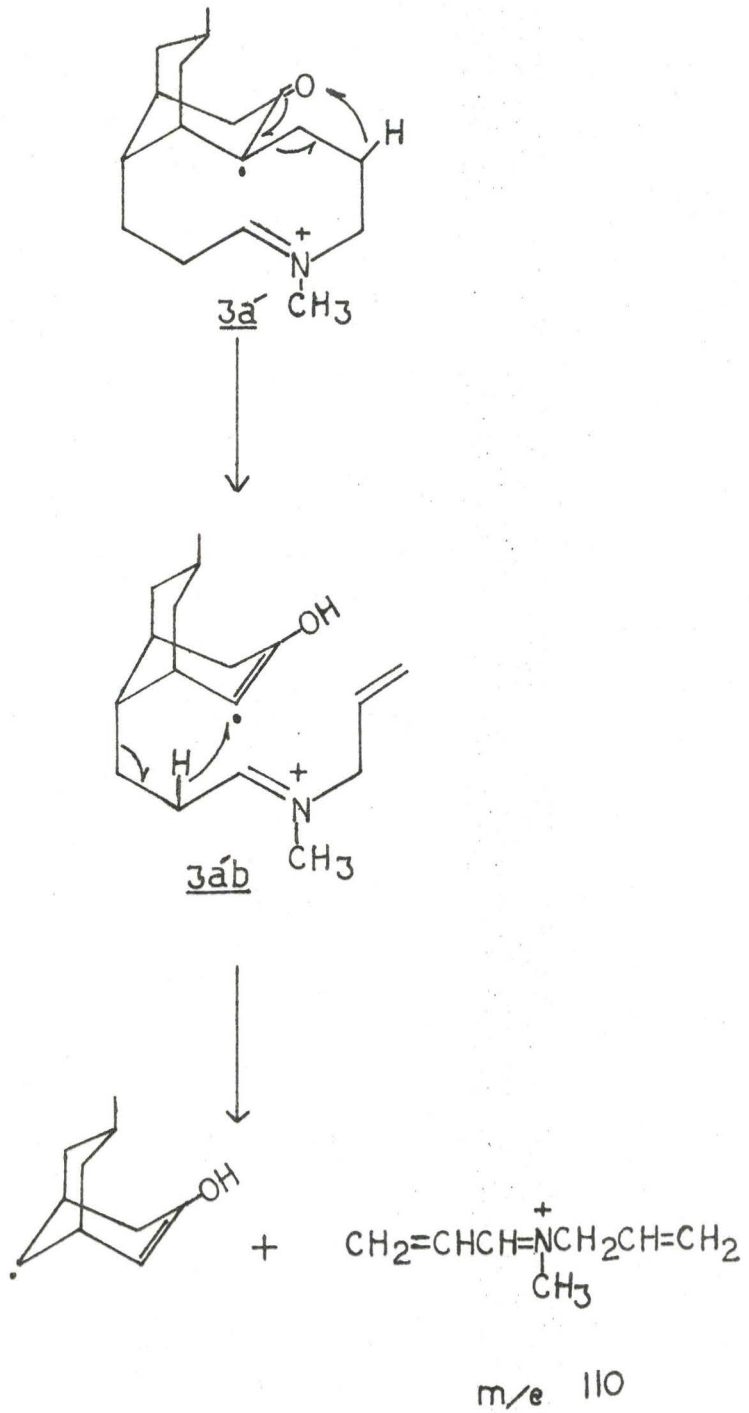
-CO

m/e 233

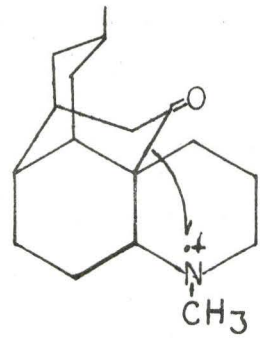
SCHEME 10



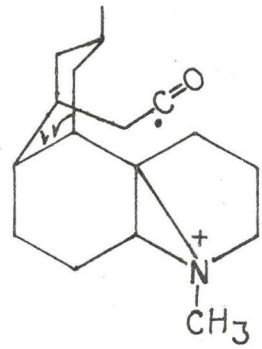
SCHEME 11



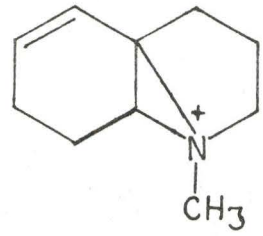
SCHEME 12



a´



Gáa



m/e 150

SCHEME 13

The mass spectrum of the Hofmann product of clavolonine was determined by high resolution mass spectrometry. Ions at m/e 57, 70, 110 and 150 were also observed with the composition of $C_3H_7N^+$, $C_4H_8N^+$, $C_7H_{12}N^+$ and $C_{10}H_{16}N^+$, respectively. The formation of these ions from clavolonine can be rationalized in exactly the same way as the corresponding ions from lycopodine.

EXPERIMENTAL

Apparatus, Methods and Materials

Mass spectra were recorded on a CEC 21-110B double-focusing mass spectrometer at an ionization potential of 70 eV and an ionization current of 100 μA. Spectra are plotted in terms of relative abundance, with the most intense peak (base peak) being taken as 100%.

The n.m.r. spectra were recorded using the frequency sweep mode of a Varian HA-100 spectrometer and for detailed analysis the frequency sweep mode of a Varian HR-220 spectrometer was used. Samples were dissolved in $CDCl_3$ using added TMS as the internal locking signal.

A Perkin-Elmer 337 i.r. spectrometer was used to record infrared spectra of samples dissolved in $CHCl_3$ unless otherwise stated. All melting points were determined on a Kofler Micro Hot Stage.

Thin layer chromatography was carried out on silica gel plates with chloroform-methanol in appropriate ratio as eluant.

Separation of Clavolonine from *L. clavatum*

The residues from an extract of *L. clavatum* from which lycopodine had been removed were used as starting material. This residue was subjected to counter-current distribution using ten separatory funnels (25). The crude residue (10 g) was dissolved in 300 ml. of chloroform and the chloroform solution was shaken with 300 ml. of a citrate-phosphate buffer solution (pH 6.95) (26) for five minutes in a one litre

separatory funnel. The chloroform phase, after separation was complete, was transferred to a second separatory funnel containing 300 ml. of fresh buffer solution and 300 ml. of fresh chloroform was added to the first separatory funnel. Both funnels were then shaken for five minutes each. This procedure was continued until ten transfers had been completed.

The resulting extract was worked up as outlined below. The chloroform fractions were separated from their respective fractions and each washed with water and the water washings were added to their respective buffer fractions. The chloroform extracts were then dried over anhydrous sodium sulphate and evaporated to dryness in tared flasks. The buffer fractions were basified with ammonium hydroxide and extracted with chloroform.

The chloroform extracts were then dried over anhydrous sodium sulphate and evaporated to dryness in tared flasks.

Flask No.	Buffer fractions weight in grams	Chloroform fractions weight in grams
1	0.9554	0.0357
2	0.6788	0.0530
3	0.5051	0.0841
4	0.2197	0.0858
5	0.2582	0.1310
6	0.2623	0.3140
7	0.2351	0.3077
8	0.2522	0.3300
9	0.1238	1.1100
10	0.2214	2.7623

The first buffer fractions contained the strong bases (2.76 g.) and the last two chloroform fractions the weak bases (3.87 g.). The crude alkaloid in flask number one of the buffer fraction was dissolved in acetone and when this solution was allowed to stand at room temperature white crystals were obtained. Recrystallization from acetone gave clavolonine (0.22 g) melting at 237-238°, identified by comparison with an authentic sample. The crude alkaloids in flask numbers 2 and 3 of the buffer fractions were combined and eluted on an alumina column using chloroform as eluant. From this column 0.12 g. of clavolonine was obtained. Alkaloids from flasks numbers 4 and 5 were combined and eluted on an alumina column using chloroform as eluant yielding 0.09 g. of clavolonine. Other crystalline products were obtained but were not further investigated.

Acetylation of Clavolonine

Freshly distilled acetic anhydride (2 ml.) was added to a mixture of 50 mg. of clavolonine in 1 ml. of pyridine (anhydrous). The mixture was then heated under reflux for twenty four hours (4). After evaporation of the reaction mixture under reduced pressure, the residue was taken up in ether (5 ml.) and water (5 ml.) and was transferred into a separatory funnel. The reaction flask was washed with more ether-water mixture and the washings were again added to the separatory funnel. The ether-water layers were made basic with a solution of saturated sodium carbonate. After vigorous shaking the ether layer was drawn off and the aqueous layer washed twice with fresh ether. The ether extract was dried over potassium carbonate and evaporated to an oil. The oily

product was then passed through an alumina column pre-treated with ethyl acetate, using benzene as eluant.

The mass spectrum of the slightly coloured oil which was eluted showed the expected peak at m/e 305 for O-acetyl clavolonine together with a peak at m/e 347 which has been attributed to the enol acetate of O-acetyl clavolonine. Thin layer chromatography showed virtually one spot with a slight head. The i.r. spectrum of this compound in chloroform had strong maxima at 1700 cm^{-1} (ketone) and 1725 cm^{-1} (O-acetyl) but the presence of a band attributable to an enol acetate was not clearly detectable but may be hidden under the strong absorption of the alcoholic O-acetate.

Reaction of Acetyl Clavolonine with Cyanogen Bromide

Acetyl clavolonine (80 mg.) dissolved in dry benzene (2 ml.) was added dropwise over a period of two hours at room temperature with stirring to a solution of freshly distilled cyanogen bromide (0.6 g.) in dry benzene (3 ml.) (5). Stirring was continued for about six hours after addition was completed and the reaction mixture was allowed to stand overnight at room temperature. The benzene and excess cyanogen bromide were distilled off under reduced pressure. The residue was dissolved in chloroform and washed with water, dilute hydrochloric acid and dilute bicarbonate solution. The chloroform solution was dried over sodium sulphate and was taken to dryness. The residue, an oil, was chromatographed on a column of neutral alumina with chloroform as eluant. Fractions of 10 ml. each were collected from the column. The first two fractions which were eluted showed similar single spots on thin layer

chromatography and had i.r. bands at 2210 cm^{-1} (N-CN); 1725 cm^{-1} ($CH_3-\overset{O}{\overset{\|}{C}}-O$); 1700 cm^{-1} (C=O). Crystallization was attempted using various solvents and on slow evaporation of ether a semi-crystalline product was obtained in 41% yield. This compound is designated α-cyanobromoclavolonine.

Its mass spectrum showed the presence of a molecular ion at m/e 410 and a small peak at m/e 452 attributed to the enol acetate.

Preparation of α-Cyclocyanoclavolonine

α-Cyanobromoclavolonine (80 mg.) was dissolved in methanol (3.5 ml.), potassium hydroxide (0.3 g.) was added, and the solution was heated under reflux for three hours (5). The methanol was evaporated, water was added and the residue was extracted with chloroform. The chloroform extract was taken to dryness to yield crude rosette-like crystals which melted at 173-175°.

The i.r. spectrum of the cyclized compound had carbonyl absorption at 1700 cm^{-1} and peaks at 2210 cm^{-1} ($\geq$N-CN); 3400-3550 cm^{-1} (OH). No peaks attributable to vinyl carbon were detected. The mass spectrum showed the expected molecular ion at m/e 288 together with a small peak at m/e 320 attributed to α-cyclocyanoacetylclavolonine.

Hydrolysis of α-Cyclocyanoclavolonine

α-Cyclocyanoclavolonine (0.08 g.) was dissolved in n-propanol (5 ml.) and 2N HCl (1.5 ml.) was added (9). The solution was heated under reflux on a steam cone for four hours and the solvent was distilled off. The residue was dissolved in water and filtered. The

filtrate was made basic with ammonium hydroxide and extracted with chloroform. The crude product was dissolved in acetone and acidified with perchloric acid but did not yield a crystalline salt. The mixture was again basified with ammonium hydroxide and extracted with ether. Evaporation of the ether left an oil which was purified on an alumina column. The i.r. spectrum of the base showed absorption at 1698 cm^{-1} (C=O) and no absorption at 2210 cm^{-1} (N-CN). The mass spectrum gave the expected molecular ion at m/e 263.

Alkylation of the α-Cycloclavolonine

α-Cycloclavolonine (0.06 g.) was dissolved in a mixture of 40% formaldehyde (1.5 ml.) and 90% formic acid (2 ml.) (9). After the reaction mixture was heated under reflux for thirty hours, it was poured into water, made basic with ammonium hydroxide and extracted with ether. The dried ether extract on slow evaporation gave long needle-like crystals (0.04 g.). The crystals were again dissolved in ether, washed with dilute hydrochloric acid solution and the acid layer basified and extracted with ether. The dried ether solution was evaporated and the residue recrystallized from ether-petroleum ether yielding crystals which melted at 198-199°.

Mass spectrum of the crystalline product gave the expected molecular ion at m/e 277. The n.m.r. spectrum confirmed the presence of a $-CH-CH_3$ group at 0.88δ (doublet), also the presence of $N-CH_3$ group (singlet at 2.11δ). The i.r. spectrum showed strong absorption at 1698 cm^{-1} (C=O), 1413 cm^{-1} ($CH_2C=O$). The molecular composition was determined by high resolution mass spectrometry.

The Hofmann Degradation Product of Clavolonine

Clavolonine (0.2 g.) was dissolved in a mixture of methanol and acetone and excess methyl iodide was added. The reaction mixture was heated under reflux for a few minutes until crystals appeared. The reaction flask was allowed to stand overnight at room temperature to complete crystallization. The excess solvent was evaporated yielding white crystals which were recrystallized from methanol and a few drops of acetone. Total yield was 0.16 g. of clavolonine methiodide which melted at 325-327°.

Clavolonine methiodide (0.16 g.) was added to a solution of potassium (0.6 g.) in t-butyl alcohol (8 ml.) and the mixture was heated under reflux for sixty-four hours (4). After evaporation of the solvent, the residue was taken up in water and extracted with chloroform. The dried chloroform extract was evaporated to yield a slightly brown crystalline product which was recrystallized from acetone. The crystals melted at 170-171° and were identical with a sample supplied by Dr. R.H. Burnell. The mass spectrum showed the molecular ion at m/e 277. The molecular composition was determined by high resolution mass spectrometry.

Reaction of Lycopodine with Cyanogen Bromide

Lycopodine (0.47 g.) dissolved in dry benzene (6 ml.) was added dropwise over a period of three hours at room temperature with stirring to a solution of cyanogen bromide (2.6 g.) in dry benzene (6 ml.) (5). Stirring was continued for about eight hours after addition was completed and the reaction mixture was allowed to stand at room temperature overnight. The benzene and excess cyanogen bromide were distilled

off under reduced pressure. The residue was dissolved in chloroform and washed with water, dilute hydrochloric acid and dilute sodium bicarbonate solution. From the solution of water washings and acid washing 0.06 g. of unreacted lycopodine was recovered. The chloroform solution was dried over sodium sulphate and was evaporated to dryness. The residue was chromatographed on an alumina column with chloroform as eluant. The first portion of eluant from the column was a mixture of α- and β-cyanobromolycopodine.

The mixture of α- and β-cyanobromolycopodine was recrystallized from ether. The first crop of crystals gave α-cyanobromolycopodine (0.19 g.) which melted at 140-141°. The mass spectrum gave a molecular ion at m/e 352. Further crystallization of the mother liquor yielded a mixture of α- and β-cyanobromolycopodine which could be separated manually. The β- compound melted at 106-107°.

Preparation of α-cyclocyanolycopodine

α-Cyanobromolycopodine (0.19 g.) was dissolved in methanol (7 ml.), potassium hydroxide (0.6 g.) added, and the solution was heated under reflux for three hours (5). The methanol was evaporated, water was added and the residue was extracted with chloroform. The chloroform extract was taken to dryness to yield a crude residue in 90% yield. This residue was recrystallized from methanol and a few drops of ether to yield a compound which melted at 142-144°.

The i.r. spectrum of the cyclized compound showed bands at 2210 cm^{-1} (N-CN); and 1700 cm^{-1} (C=O). The mass spectrum showed a molecular ion at m/e 272.

Hydrolysis of α-Cyclocyanolycopodine

α-Cyclocyanolycopodine (0.07 g.) was dissolved in n-propyl alcohol (4 ml.) and 2 N hydrochloric acid (1.5 ml.) was added (9). The solution was heated under reflux on a steam cone for thirty hours and the solvent was evaporated. The residue was dissolved in water and filtered. The filtrate was made basic with ammonium hydroxide and extracted with chloroform. Evaporation of the chloroform left a crude solid product which was dissolved in acetone and acidified with perchloric acid. A crystalline solid separated which melted at 299-300°.

The free base was liberated from the perchlorate salt and was recrystallized from petroleum-ether. It melted at 105-106°.

The i.r. spectrum did not show a band at 2210 cm^{-1} but showed bands at 1695 cm^{-1} (C=O) and at 3330 cm^{-1} (N-H). The mass spectrum showed the molecular ion at m/e 247.

Alkylation of α-Cyclolycopodine

α-Cyclolycopodine (0.08 g.) was dissolved in a mixture of 40% formaldehyde (3 ml.) and 90% formic acid (5 ml.). After the reaction mixture was heated under reflux for thirty-three hours, it was poured into water, made basic with ammonium hydroxide and extracted with ether (9). The ether extract was taken to dryness and the residue was chromatographed on an alumina column using chloroform as eluant. The tertiary base was eluted in the first fraction. After evaporation of the solvent the residue was recrystallized from an ether-petroleum ether mixture. The crystals melted at 86-88°.

The mass spectrum showed the expected molecular ion at m/e 261. The n.m.r. spectrum gave three proton signals at 2.1δ ($N-CH_3$ group) and at 0.88δ attributed to $CH-CH_3$ group.

The Hofmann Degradation Product of Lycopodine

Lycopodine (0.63 g.) was dissolved in acetone (10 ml.) and excess methyl iodide added. The methiodide salt separated in crystalline form immediately and quantitatively. The methiodide was recrystallized from a methanol-acetone mixture. The salt melted at 295-296°.

Lycopodine methiodide (0.6 g.) was added to a solution of potassium (0.5 g.) in t-butyl alcohol (30 ml.) and the mixture was heated under reflux for forty-eight hours (8). The reaction mixture was taken to dryness and water added to the residue. The aqueous solution was extracted with ether. The ether extract was dried over sodium sulphate and evaporated to dryness leaving a crystalline residue. The residue was dissolved in acetone and the base was precipitated as its hydrochloride by addition of concentrated hydrochloric acid. The hydrochloride was dissolved in water and the solution was made basic with ammonium hydroxide and then extracted with ether. Evaporation of the solution gave a crystalline product which was recrystallized from petroleum-ether. The crystals melted at 80-81°.

The n.m.r. spectrum gave three proton signals at 2.1δ ($N-CH_3$ group) and at 0.88δ attributed to $CH-CH_3$ group. The mass spectrum gave the molecular ion at m/e 261.

Treatment of the Hofmann Product of Lycopodine with Cyanogen Bromide

The Hofmann product of lycopodine (0.21 g.) was dissolved in dry benzene (5 ml.) and treated with an excess of cyanogen bromide (0.4 g.) in dry benzene (8 ml.) and the mixture was allowed to stand overnight in the refrigerator (8). The cyanogen bromide and benzene were removed under reduced pressure and the residue was taken up in chloroform, washed with dilute hydrochloric acid, dilute sodium hydroxide solution and water. The chloroform solution was dried over sodium sulphate and then evaporated to dryness. The residue formed a crystalline solid which was recrystallized from ether and a few drops of acetone. The crystals obtained (0.04 g.) melted at 162-164°.

Chromic Acid Oxidation of the Hofmann Degradation Product of Lycopodine

In a control experiment 20% sulphuric acid (5 ml.) was added to cyclobutane carboxylic acid (0.10 g.) and chromium trioxide (0.5 g.) in a 25 ml. flask. The reaction mixture was heated under reflux for twenty-four hours (27). Excess chromium trioxide was destroyed by the addition of a few drops of methanol and the product extracted with ether. The ether layer was washed with water, dried and evaporated to an oil. Thin layer chromatography showed one spot which was identical with the starting material.

The above procedure was performed on the Hofmann degradation product of lycopodine (0.10 g.). The oxidation product was examined by G.C. and T.L.C. but show no evidence of cyclobutanecarboxylic acid.

Deuterium Exchange on the Hofmann Product of Lycopodine

A solution of sodium methoxide in deuteromethanol was prepared by dissolving sodium (0.05 g.) in deuteromethanol (1 ml.). The Hofmann product of lycopodine was added to the solution of sodium methoxide and the mixture was heated under reflux for four hours (28). The deuteromethanol was removed under reduced pressure and the residue treated with 1 ml. of D_2O and extracted with ether. The ether solution was evaporated and the exchange process was repeated twice more. The final crude residue was sublimed and the sublimed product recrystallized from petroleum ether. The crystals melted at 80-81°.

The composition of the product determined by low voltage mass spectrometry was: $d_0 = 0\%$, $d_1 = 20\%$, $d_2 = 80\%$.

The n.m.r. spectrum was taken on the Varian HR-220 spectrometer and shown in Fig. 8.

Oxidation of Lycopodine to the α-Lactam

Lycopodine (0.90 g.) was dissolved in 60 ml. of acetone which had been distilled over potassium permanganate, and to the above solution potassium permanganate (0.60 g.) was added at room temperature over a period of three hours (29). The mixture was left standing at room temperature for one hour and then filtered and the filtrate was evaporated to dryness. The above procedures were repeated on the residue obtained above. Finally the excess potassium permanganate was destroyed by the addition of methanol. The mixture was again filtered and the filtrate evaporated to dryness. The crude residue was taken up in dilute hydrochloric acid, the pH adjusted to 4 with ammonium

hydroxide and extracted twice with methylene chloride.

The methylene chloride extract was evaporated to dryness and the residue was eluted from an alumina column using chloroform as eluant. The eluted fractions were evaporated and the residue obtained was dissolved in ether. (Chloroform solutions give poor G.C. readings.) G.C. showed a very small amount of lycopodine in the sample which was mainly the α-lactam of lycopodine (comparison to an authentic sample). Evaporation of the ether left a crystalline residue which was sublimed and recrystallized from ether. The crystals melted at 163-164°. The mass spectrum showed the molecular ion at m/e 261. The i.r. spectrum had strong absorption at 1700 cm^{-1} (C=O) and 1626 cm^{-1} (lactam carbonyl) using KBr pellets.

Reduction of the α-Lactam with Lithium Aluminum Deuteride

The α-lactam of lycopodine (0.19 g.) dissolved in dry tetrahydrofuran (3 ml.) was added dropwise to a solution of lithium aluminum deuteride (0.25 g.) in THF. The reaction mixture was heated under reflux for eighteen hours and the excess deuteride was destroyed by careful addition of wet ether. Before filtering the ether layer sodium sulphate (anhydrous) was added to facilitate the coagulation of the aluminum salt.

Evaporation of the dried ether solution left a crystalline product and comparative G.C. showed one peak, which is identical with dihydrolycopodine. Recrystallization from ether gave crystals melting at 167-169°. Total yield of crystalline product was 60%. The mass spectrum showed the molecular ion at m/e 252 and the incorporation of deuterium was determined by low voltage mass spectrometry. The

composition of the product was: d_1 2%, d_2 3%, d_3 95%.

Oxidation of Dihydrolycopodine-5,9,9-d_3

Dihydrolycopodine-5,9,9-d_3 (0.11 g.) was dissolved in acetone (10 ml.) and Jones' reagent (13) (0.5 ml.) was added dropwise over a period of two hours with continuous stirring. The reaction mixture was left standing at room temperature for an additional two hours and worked up as follows: excess solvent was removed under reduced pressure and water was added. The aqueous product was basified with ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and on evaporation a brown solid remained which was purified on an alumina column. The column was eluted with 10% chloroform in benzene. Evaporation of the solvent left a crystalline residue which was recrystallized from hexane. The crystals melted at 116°. The mass spectrum showed the molecular ion at m/e 249. Deuterium incorporation determined by low voltage mass spectrometry was: d_1 9%, d_2 91%.

The Hofmann Product of Lycopodine-9,9-d_2

The Hofmann degradation reaction was performed on lycopodine-9, 9-d_2. The procedures were as for lycopodine on page 58. The mass spectrum showed the molecular ion at m/e 263, and the incorporation of deuterium was determined by low voltage mass spectrometry to be d_0 0%, d_1 10%, d_2 90%.

The Hofmann Product of Lycopodine-N-Methyl-d_3

The Hofmann product of lycopodine was prepared from lycopodine methiodide N-methyl-d_3 as described before. The mass spectrum of the Hofmann product of lycopodine N-methyl-d_3 gave a molecular ion at m/e 264. The composition determined by low voltage mass spectrometry was: d_2 2%, d_3 98%.

SUMMARY

The cyclized product arising from α-cyanobromolycopodine was re-examined and supporting evidence for the proposed structure was obtained.

The Hofmann products of lycopodine and clavolonine were prepared and studied. It is now proposed that methiodides of the alklaloids in alkaline media undergo solvolysis which causes cleavage of the C-13-nitrogen bond. The resulting carbonium ion is then stabilized by hydride transfer from C-9. The immonium salt so formed then cyclizes between C-9 and C-4.

Structure 18A for the Hofmann product of lycopodine is strongly supported by mass spectrometric and nuclear magnetic resonance evidence. By analogy we conclude that the Hofmann product of clavolonine has structure 19.

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