MASS SPECTROMETRY OF ORGANIC COMPOUNDS

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PART I. OXYGENATED QUINOLINES AND FUROQUINOLINE ALKALOIDS

PART II. LOBINALINE AND ITS HOFMANN DEGRADATION PRODUCTS

BY

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Part II. Lobinaline and its Hofmann Degradation Products

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SCOPE AND CONTENTS:

Part I

The mass spectra of the seven isomeric monomethoxyquinolines, the seven monohydroxyquinolines, the two N-methylquinolones and six furoquinoline alkaloids have been recorded. Fragmentation mechanisms are proposed to account for the major peaks in all the spectra. Use has been made of intense metastable peaks found in most of the spectra to aid the interpretation of the fragmentation routes. In addition, deuterium and carbon-13 labelling have been employed in analyzing the fragmentation routes of the methoxyquinolines.

Part II

The Hofmann degradation of lobinaline is described. Examination of lobinaline, its derivatives and its Hofmann degradation products by mass spectrometry and other physical and chemical techniques has allowed a structure to be assigned to the alkaloid.

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PART I

OXYGENATED QUINOLINES AND FUROQUINOLINE ALKALOIDS

GENERAL INTRODUCTION

Although the electron impact induced fragmentation of a large number of organic compounds has been studied in recent years very little work has been reported on the fragmentation of quinoline derivatives. The work reported in the first part of this thesis is devoted to a study of the fragmentation upon electron impact of monooxygenated quinolines, the methoxyquinolines, hydroxyquinolines and N-methyl-quinolones. The work has been extended to some furoquinoline alkaloids which have a furan ring fused in the 2,3-positions of a quinoline ring bearing oxygenated substituents.

Intense metastable peaks in the spectra of all the compounds studied were used in the interpretation of the fragmentation processes operating in the various compounds. In addition, deuterium labelling techniques were employed in the analysis of the fragmentation of some of the methoxyquinolines.

It has been possible to differentiate among the isomeric compounds in each group by mass spectrometry, although in some cases differences among the isomers would not be sufficient to allow unambiguous assignment of a functional group to a definite position in an unknown compound. A characteristic fragmentation pattern has been observed in those quinoline systems which have a methoxyl group in the 2-, 3- or 8-position.

The work reported in the second part of this thesis is concerned with the application of mass spectrometry, in conjunction with chemical and other spectral techniques, in the structural elucidation of lobinaline, an alkaloid containing a reduced quinoline system.

HISTORICAL INTRODUCTION

Theory and Instrumentation of Mass Spectrometry

The observation that atoms of different masses can be separated by deflection of the corresponding positive ions in electric and magnetic fields was made by Wien (1) in 1898. Thirteen years later Thomson (2) used this knowledge to demonstrate the existence of two isotopes of neon. Aston's (3) development of a practical mass spectrograph, and subsequent improvements on his design (4,5,6), enabled very accurate measurement of atomic masses. For a review on the development and applications of mass spectroscopy the reader is referred to the review published by Duckworth (7) in 1958.

The mass spectrograph focused the ion beams on a photographic plate, giving a spectrum composed of a series of lines on the plate. Early workers in the field were limited in their attempts to determine relative abundances of the various ions by their inability to measure accurately the relative densities of the lines obtained on the photographic record. For this reason the mass spectrometer designed by Dempster (8) in 1918 and modified by Nier (9,10,11,12) has proved more useful to the organic chemist. In Dempster's design the ion beams are focused in turn on an electrical detector which measures the ion current. Early instruments of Dempster's design allowed easy measurement of the relative intensities of ion beams, but did not allow determination of the accurate masses of the ions. Modern instruments

based either on Dempster's or Aston's design are capable of measuring both properties accurately. Most organic mass spectrometry is still carried out on instruments designed on Dempster's model.

A commonly used instrument design is diagrammed in Figure 1. The molecules, of mass M, are ionized by an electron beam in the source. The ion source is maintained at a positive potential, V, which gives the ions a potential energy eV, where e is the charge on the ions. After acceleration through the voltage drop V the kinetic energy of the ions will be equal to the potential energy before acceleration,

$$eV = \tfrac{1}{2} Mv^2. \qquad (1)$$

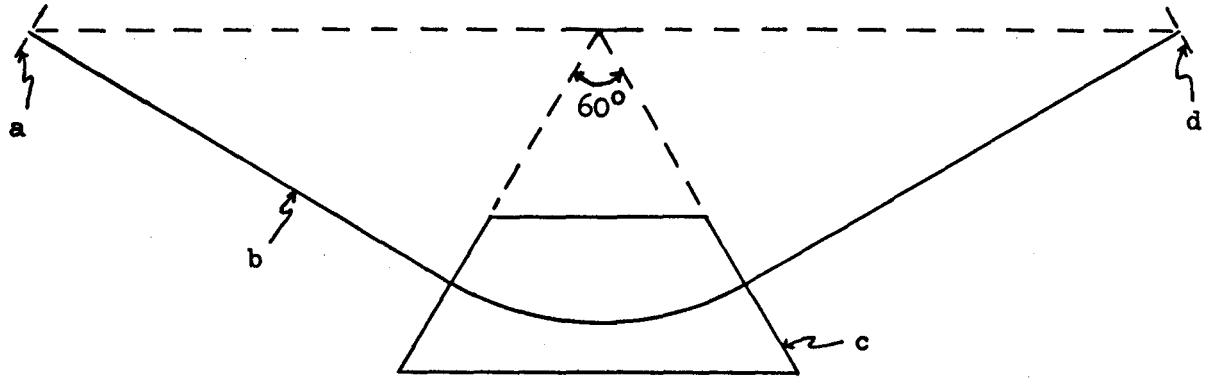
Upon entering the magnetic field, H, the ions will experience a centripetal force Hev which is counterbalanced by a centrifugal force $\frac{Mv^2}{r}$,

$$Hev = \frac{Mv^2}{r} . \qquad (2)$$

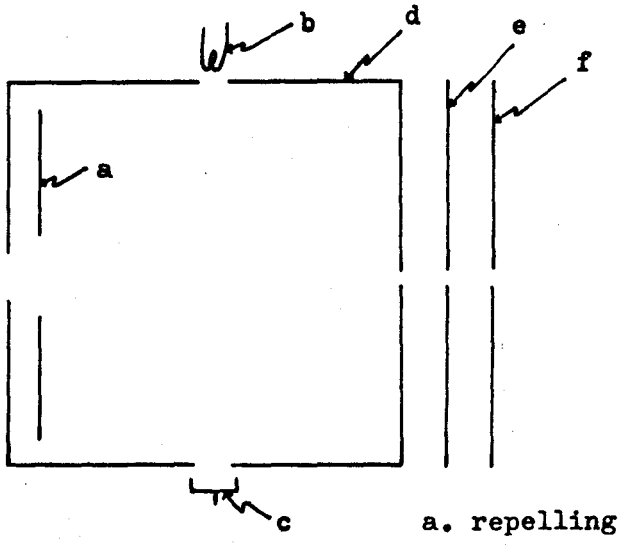
Elimination of v from equations (1) and (2) results in the mass spectrometer equation,

$$\frac{M}{e} = \frac{H^2 r^2}{2V} . \qquad (3)$$

From equation (3) it is seen that the mass of the ions collected will vary directly with the square of the magnetic field strength and inversely with the accelerating voltage. Thus, instruments equipped with magnetic scanning will be able to cover a much larger range of masses in one sweep, but electric scanning is often employed despite its relatively restricted range because it is easier to achieve electronically and because the magnetic field in the source (of 180° instruments) can then be kept constant.



a. source slit
b. ion beam
c. magnet
d. detector slit



a. repelling electrodes
b. electron source
c. electron target
d. chamber
e. lens electrodes
f. accelerating electrodes

Figure 1. A 60° sector mass spectrometer and ion source.

In addition to peaks corresponding to molecular ions and peaks corresponding to fragment ions many mass spectra contain broad peaks of relatively low intensity which often do not correspond to integral mass numbers. These so-called metastable peaks are due to metastable ions which arise when a parent ion fragments to a daughter ion and a neutral fragment after acceleration through the voltage drop V but before passing through the analyzing field H. Thus it is seen that the species detected has been accelerated as one ion and deflected in the magnetic field as another ion of lower mass and will be observed at a mass different from that of either ion. If it is assumed that the parent ion is fully accelerated before decomposition, but that fragmentation occurs before the ion reaches the magnetic field, it can be shown in the manner outlined below that the metastable ion will be observed at an apparent mass $m^* = \frac{M_2^2}{M_1}$, where M_1 and M_2 are the masses of the parent and daughter ions, respectively.

The kinetic energy of the parent ion is given by equation (1). After fragmentation, the kinetic energy of the daughter ion will be related to the potential energy of the parent ion by equation (4).

$$\tfrac{1}{2} M_2 v_2^2 = \frac{M_2}{M_1} eV \qquad (4)$$

Upon entering the magnetic field the daughter ion will be subjected to equal centrifugal and centripetal forces as given in equation (5).

$$Hev_2 = \frac{M_2 v_2^2}{r^2} \qquad (5)$$

Eliminating v_2 from equations (4) and (5) we get equation (7).

$$H^2e^2 = \frac{M_2^2\ 2eV}{M_1r^2} \qquad (6)$$

$$\text{i.e.,}\ \frac{M_2^2}{eM_1} = \frac{H^2r^2}{2V} = m^* \qquad (7)$$

This derivation ignores those ions which fragment in other than the region between acceleration and deflection. While a given ion may fragment at any point in its path from formation to detection, those fragments formed during the periods of rapid acceleration or deflection will only contribute to the general background of the instrument, so such an assumption is valid for our purposes.

The presence of metastable peaks in a mass spectrum provides a useful tool in analyzing the fragmentation of the molecule. Thus, a metastable peak is found only if the daughter ion arises from the parent ion in one step. While the presence of a metastable peak supporting a proposed fragmentation is evidence that the fragmentation occurs in one step, the absence of a metastable peak does not necessarily mean that the proposed transformation does not take place.

High resolution mass spectrometry, developed by Beynon (13,14) and refined with application of computer techniques by Biemann (15,16), has greatly facilitated the analysis of the fragmentations of complex organic molecules. Modern high resolution instruments are capable of measuring the mass of a given ion sufficiently accurately to limit the actual composition of the ion to a few possibilities. With this information a proposed fragment ion can be checked against the accurate

mass of the observed ion to verify its elemental composition.

Applications of Mass Spectrometry in Organic Chemistry

The first major application of mass spectrometry to organic compounds was found in the petroleum industry (17) where the technique provided an accurate and fast analysis of complex hydrocarbon mixtures. Although the hydrocarbons encountered in petroleum mixtures undergo some unpredictable rearrangements upon electron impact, the spectrum obtained from a given compound is very reproducible and can usually be discerned in the spectrum of a mixture containing the compound. The non-specificity of hydrocarbon rearrangements has limited to some extent the usefulness of the mass spectrometer in determining the nature of unknown hydrocarbons.

In recent years mass spectrometry has been applied to a wide variety of problems in organic chemistry. The technique has proved particularly useful when only very small quantities of a compound are available for investigation. When used in conjunction with purification by vapour phase chromatography, mass spectrometry enables detailed study of microgram quantities of material. In contrast to the unpredictable rearrangements of some hydrocarbons, compounds containing heteroatoms usually undergo specific fragmentation processes which facilitate interpretation of their mass spectra (18).

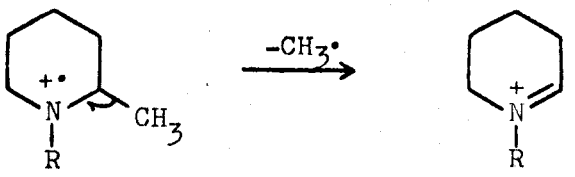
Perhaps the most obvious information to be obtained from the mass spectrum of a compound is its molecular weight. If the molecular ion survives long enough to pass through the analyzing field and be detected a peak will be found in the spectrum corresponding to the molecular weight. With modern high resolution instruments the mass of

the ion can be determined with sufficient accuracy to limit the composition of the ion to a very small number of combinations of atoms, thus facilitating determination of the molecular formula. Unfortunately not all compounds give a molecular ion in their mass spectra, and in other cases it is difficult to determine whether the peak at highest mass is in fact due to a molecular ion or whether it is due to a fragment formed from a compound of still higher molecular weight or to an impurity. Biemann (19) has discussed indirect methods of determining molecular weight from a low resolution mass spectrum, and in a recent publication (21) he has described a computer program which facilitates determination of molecular weights from a high resolution mass spectrum.

A great deal of information can be obtained from the fragmentation pattern of a molecule upon electron impact. The application of mass spectrometry to the determination of structures of organic molecules is based upon the interpretation of the fragmentation pattern determined from the mass spectrum. Biemann has attempted to rationalize the fragmentation of organic compounds in terms of carbonium ion chemistry and has classified the normal modes of fragmentation into eight types of simple cleavage and five types of rearrangement (18).

Many methods have been used to analyze the fragmentation patterns of organic compounds. First of all, experience has shown that fragmentation usually involves processes which are energetically the most favourable from consideration of the ground state. In many cases an obviously stable ion can be formed by straightforward bond

cleavage, for example I ⟶ II*, while in other cases rearrangement



I II

may give rise to a more stable ion. Elimination of stable molecules, where the number of bonds broken is equalled by the number of new bonds formed in the fragmentation, is also a favourable process.

Heavy atom labelling has proved to be a useful technique in analyzing fragmentation patterns. In particular deuterium labelling has been widely used in the structural elucidation of compounds into which the deuterium can be introduced specifically by a reaction of known mechanism. Biemann (22) and Djerassi (23) have thoroughly discussed methods of introducing heavy atom labels and of analyzing the resulting mass spectra.

It is not the intention in this thesis to give a comprehensive review of the compounds studied by mass spectrometry. For recent reviews the reader is referred to the books by Budzikiewicz, Djerassi and Williams (23,24) and references therein. The following discussion, therefore, is devoted to a consideration of previous work which bears

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* In depicting a fragmentation mechanism a double headed arrow is used to depict a two electron shift while a single headed arrow depicts a one electron shift (20). It is important to note that "structures" are drawn for fragment ions and "mechanisms" proposed for their formation only for convenience in depicting proposed fragmentation routes. The physical evidence for the existence of such "structures" and "mechanisms" is, at best, very tenuous. The mechanisms of several elimination reactions have been found to be considerably different in the mass spectrometer than was originally proposed on the basis of similar reactions in solution (21).

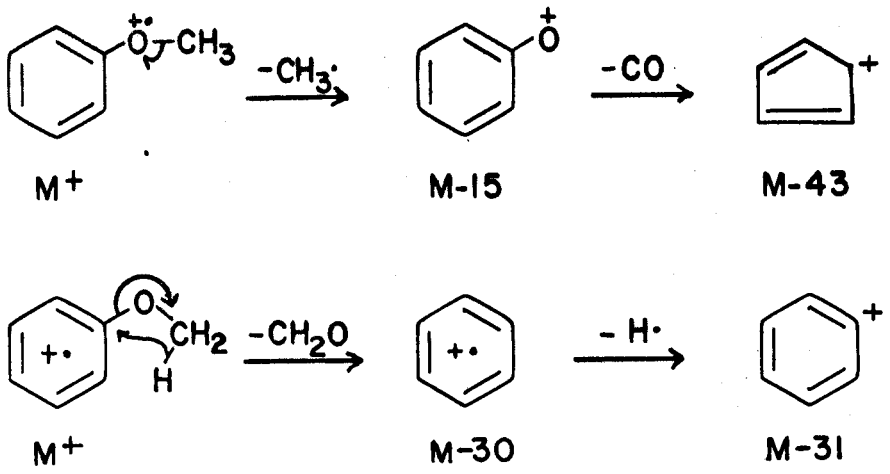
directly on the problems studied in this investigation. In particular, the known behaviour upon electron impact of quinoline, methoxybenzene and furan derivatives is outlined.

The mass spectra of quinoline and isoquinoline have been recorded (25). Both show intense molecular ion peaks and the only significant fragmentation is the loss of twenty-seven mass units (HCN) from the molecular ion. These results are entirely analogous to those which have previously been observed for pyridine (26). Other simple nitrogen heterocyclics have also been examined (27). They include pyrrole, indole, imidazole, etc. An intense molecular ion peak was found in most cases with loss of HCN again being a significant fragmentation.

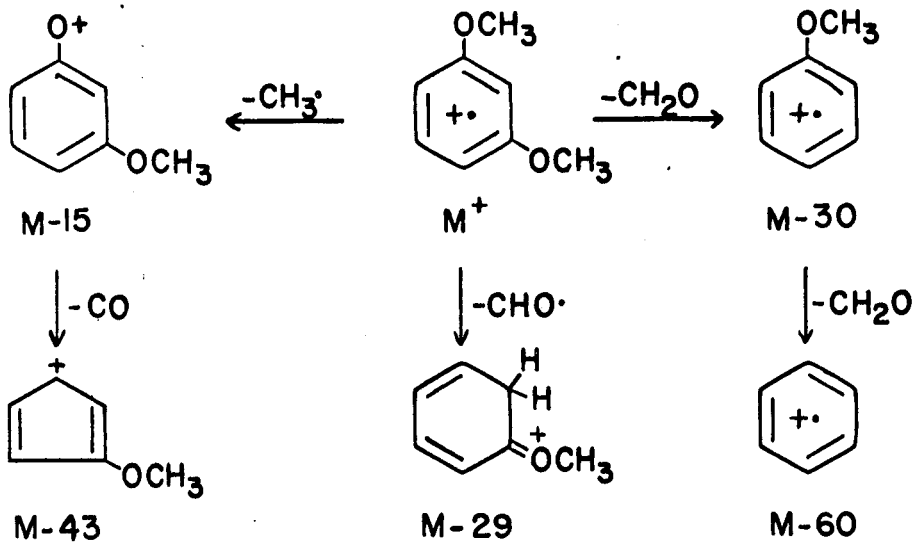
The fragmentation of methoxybenzenes upon electron impact has been studied in considerable detail (28,29). Methoxybenzene undergoes two types of fragmentation, both involving the methoxyl group as shown in Scheme 1.

Substitution of a second methoxyl group on the benzene ring of methoxybenzene increases the complexity of the spectrum considerably (28). Moreover, significant differences are found among the three isomeric dimethoxy compounds. Thus, m-dimethoxybenzene has been shown to fragment according to scheme 2, while o- and p-dimethoxybenzenes fragment according to scheme 3. Marked differences in the fragmentation patterns of positional isomers of aromatic compounds have been observed for a wide variety of different substituents (30,31,32).

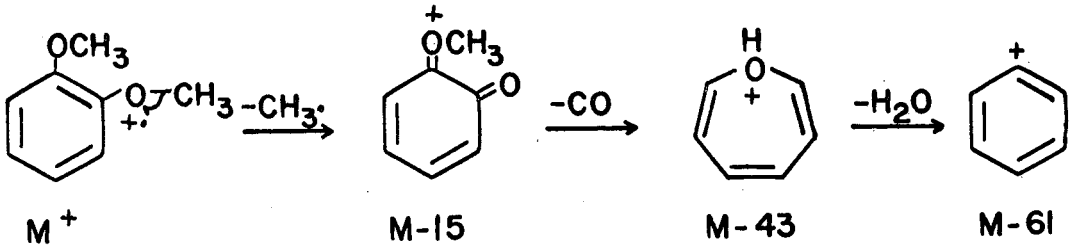
The mass spectral fragmentation of some furans (33) and benzofurans (34,35,36,37) has been shown to involve the heterocyclic



Scheme 1



Scheme 2



Scheme 3

atom in the primary step. Thus, benzofuran loses twenty-eight or twenty-nine mass units (CO or CHO•) to give the only significant fragment ion peaks in its mass spectrum. Furans bearing alkyl substituents undergo cleavage beta to the aromatic ring in the manner observed for many aromatic compounds (38).

DISCUSSION OF RESULTS

The mass spectra of the seven isomeric monomethoxyquinolines, the seven monohydroxyquinolines, the two N-methylquinolones and six furoquinoline alkaloids have been recorded. Fragmentation schemes are offered to explain the major peaks found in the spectra and the mechanisms proposed in the case of the methoxyquinolines have been verified by examining the spectra of suitably labelled compounds.

Monomethoxyquinolines

The spectra of the seven monomethoxyquinolines are recorded in Figure 2. In every case a very intense molecular ion peak is observed which is characteristic of the spectra of aromatic compounds. The spectra may be divided into two main groups on the basis of the relative intensities of the M-1 and M-43 fragment ion peaks. Thus, 2- and 8-methoxyquinoline show intense M-1 peaks while the remaining isomers show intense M-43 peaks. Considerable variation does, however, exist among the isomers in each group. The major peaks in every spectrum, except that of the 3-methoxy compound, may be explained on the basis of the two fragmentation mechanisms outlined in Schemes 4 and 5. Both of these mechanisms involve the methoxyl group in the initial fragmentation.

Scheme 4 depicts the mechanism proposed for the formation of ions appearing at M-1, M-29, M-30, M-57 and M-83. Loss of one of the methyl hydrogen atoms gives rise to a carbonium ion stabilized by the

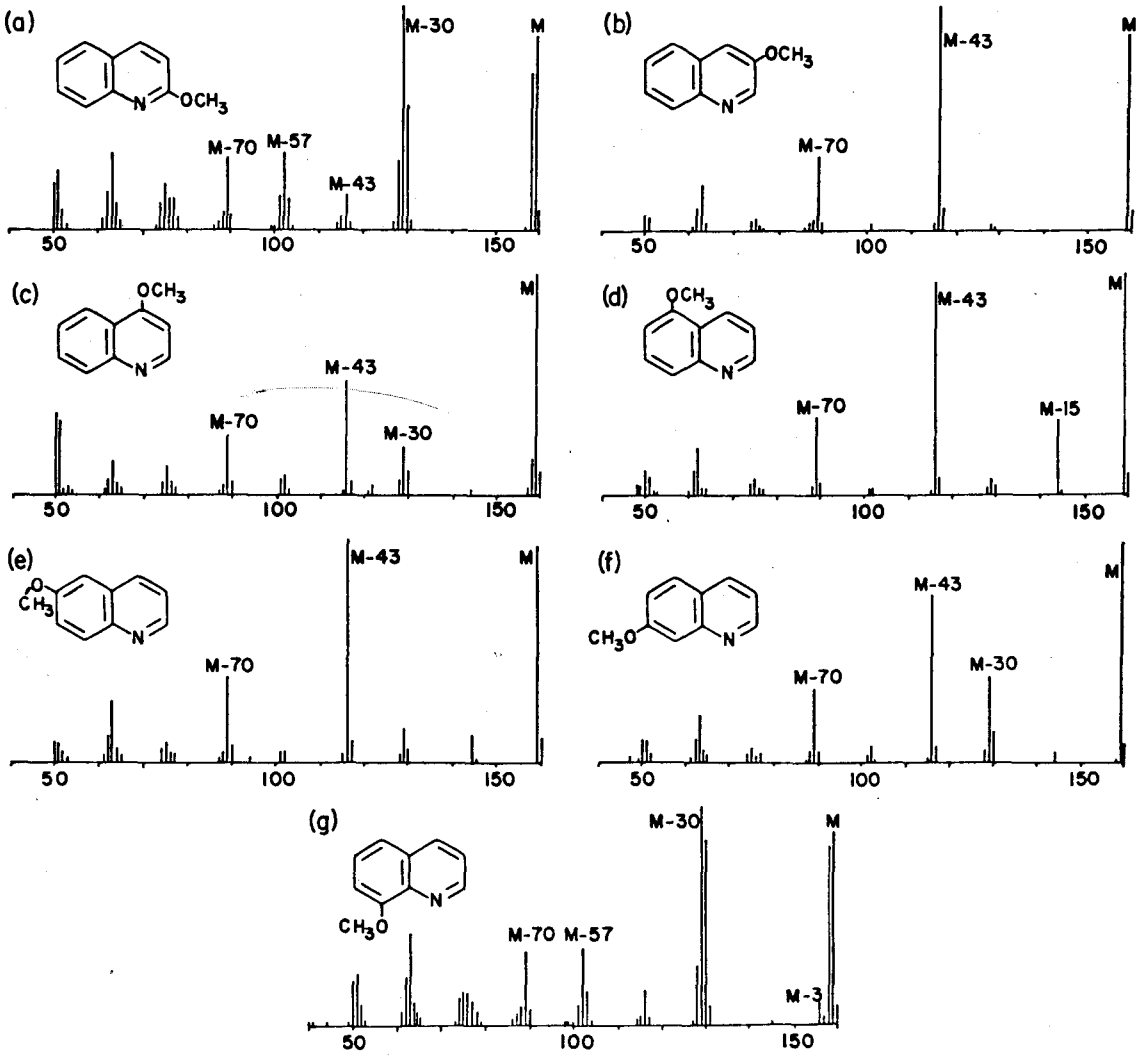
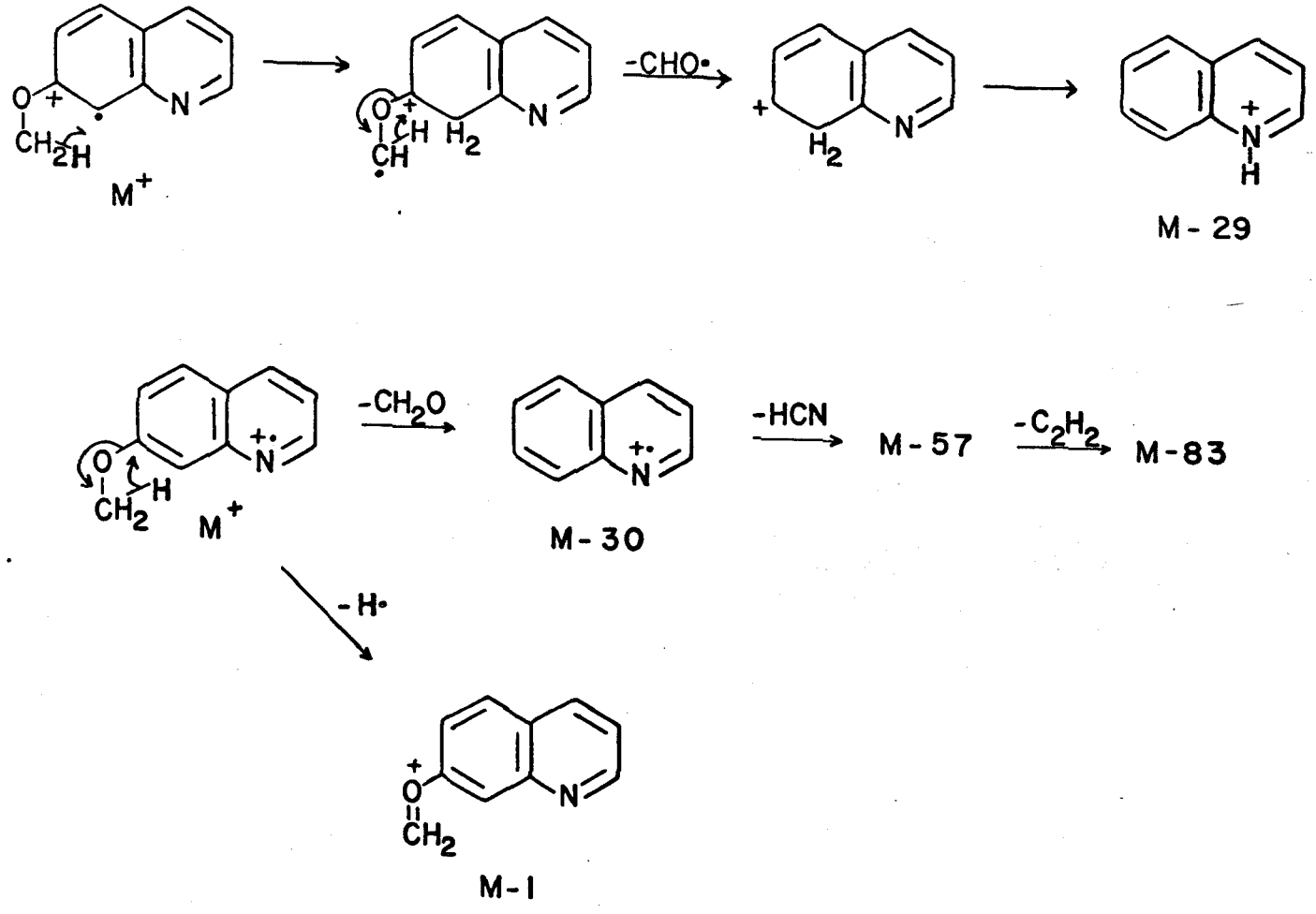
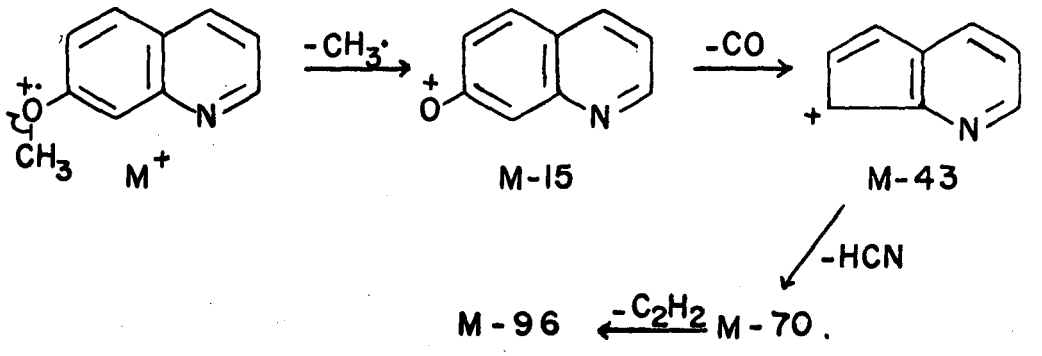


Figure 2. Mass spectra of (a) 2-methoxyquinoline, (b) 3-methoxyquinoline, (c) 4-methoxyquinoline, (d) 5-methoxyquinoline, (e) 6-methoxyquinoline, (f) 7-methoxyquinoline, (g) 8-methoxyquinoline.



Scheme 4



Scheme 5

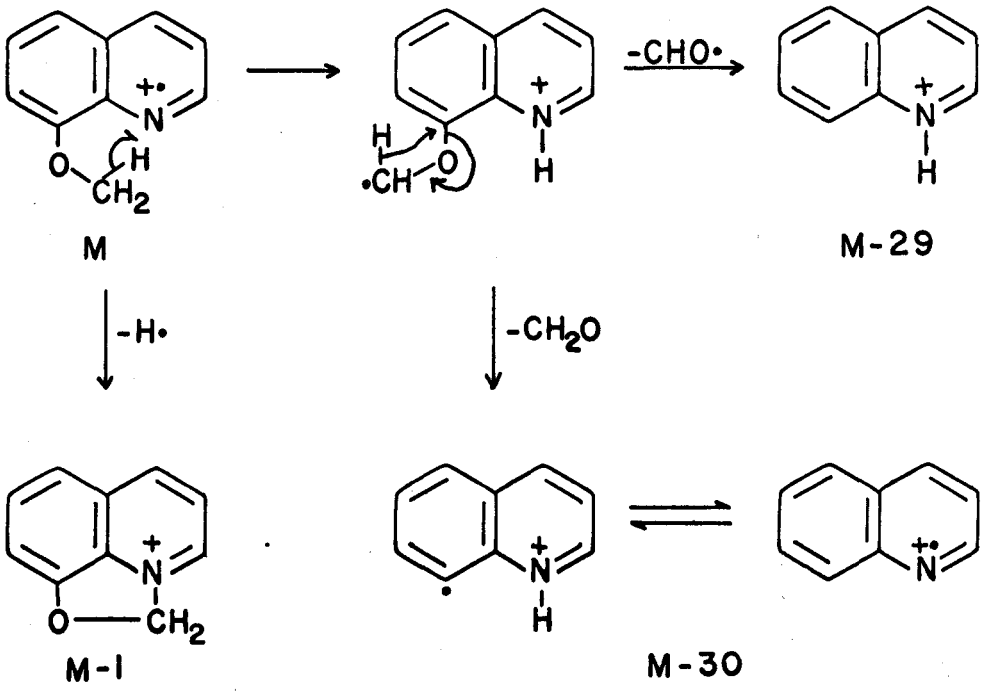
unshared electrons on the neighbouring oxygen atom. Elimination of a formyl radical or a formaldehyde molecule can occur by the mechanism advanced previously for the fragmentation of methoxybenzenes (29). Further fragmentation of the quinoline radical ion, formed by elimination of formaldehyde from the molecular ion, proceeds by consecutive elimination of HCN and acetylene as in the spectrum of quinoline itself (25). Table I lists the metastable peaks in the various spectra which support the transformations outlined in Scheme 4.

TABLE I

Metastable Peaks for Transformations of Scheme 4

Transformation	Calcd.	Observed						
		2-	3-	4-	5-	6-	7-	8-
M ⟶ M-1	157.0	157.0	-	157.0	157.0	-	157.0	157.0
M ⟶ M-29	106.3	106.5	-	106.6	-	-	106.4	106.5
M ⟶ M-30	104.7	-	-	104.9	104.8	104.8	104.9	-
M-30 ⟶ M-57	80.7	80.9	-	80.8	-	-	80.8	80.8
M-57 ⟶ M-83	56.6	56.7	-	56.7	-	56.7	-	56.6

The fact that the M-1, M-29 and M-30 peaks attain their greatest relative intensities in the spectra of 2- and 8-methoxyquinoline suggests that the neighbouring nitrogen atom exerts some influence favouring the formation of these fragment ions. One way in which the nitrogen atom may affect the fragmentation of 8-methoxyquinoline is shown in Scheme 6. The carbonium ion formed by loss of one of the methyl hydrogens can be stabilized by the unshared pair of electrons on the nitrogen atom, while the loss of CHO• can be facilitated by



Scheme 6

transfer of hydrogen to the nitrogen atom instead of to a carbon atom (cf. Scheme 4). Loss of CH_2O may also proceed in this fashion. A similar scheme can also be applied to the 2-methoxy compound.

The mass spectra of the O-CD_3 analogues of 2- and 8-methoxyquinolines, Figure 3(a), (c), support the fragmentation mechanisms proposed in Schemes 4 and 6. The M-1, M-29 and M-30 peaks of the unlabelled compounds are found at M-2, M-30 and M-32, respectively, in the deuterated analogues. The fragments lost are, therefore, D•, CDO• and CD_2O; proving that only the hydrogens of the methyl group are lost in these processes. Evidence that it is the methyl carbon atom which is lost in the CHO• and CH_2O fragments is obtained from the spectrum of 8-methoxyquinoline labelled in the methyl carbon with ^{13}C. It is seen from Figure 3(d) that all the label is lost in the M-29 and M-30 fragment ions.

In Scheme 5 a mechanism is proposed for the formation of ions appearing at M-15, M-43, M-70 and M-96 in the mass spectra of the methoxyquinolines. Loss of the methoxyl methyl group followed by expulsion of carbon monoxide is the usual mode of fragmentation of phenylmethyl ethers (29) and it is not surprising that it is observed for the methoxyquinolines. Subsequent loss of HCN and acetylene occurs in the same manner as outlined in Scheme 4. Table II lists the metastable peaks which support the transformations outlined in Scheme 5.

Fragmentation according to Scheme 5 dominates the spectra of 4-, 5-, 6- and 7-methoxyquinolines and is observed to a lesser degree in the spectra of the 2- and 8-isomers. The reverse situation applies to fragmentation according to Scheme 4.

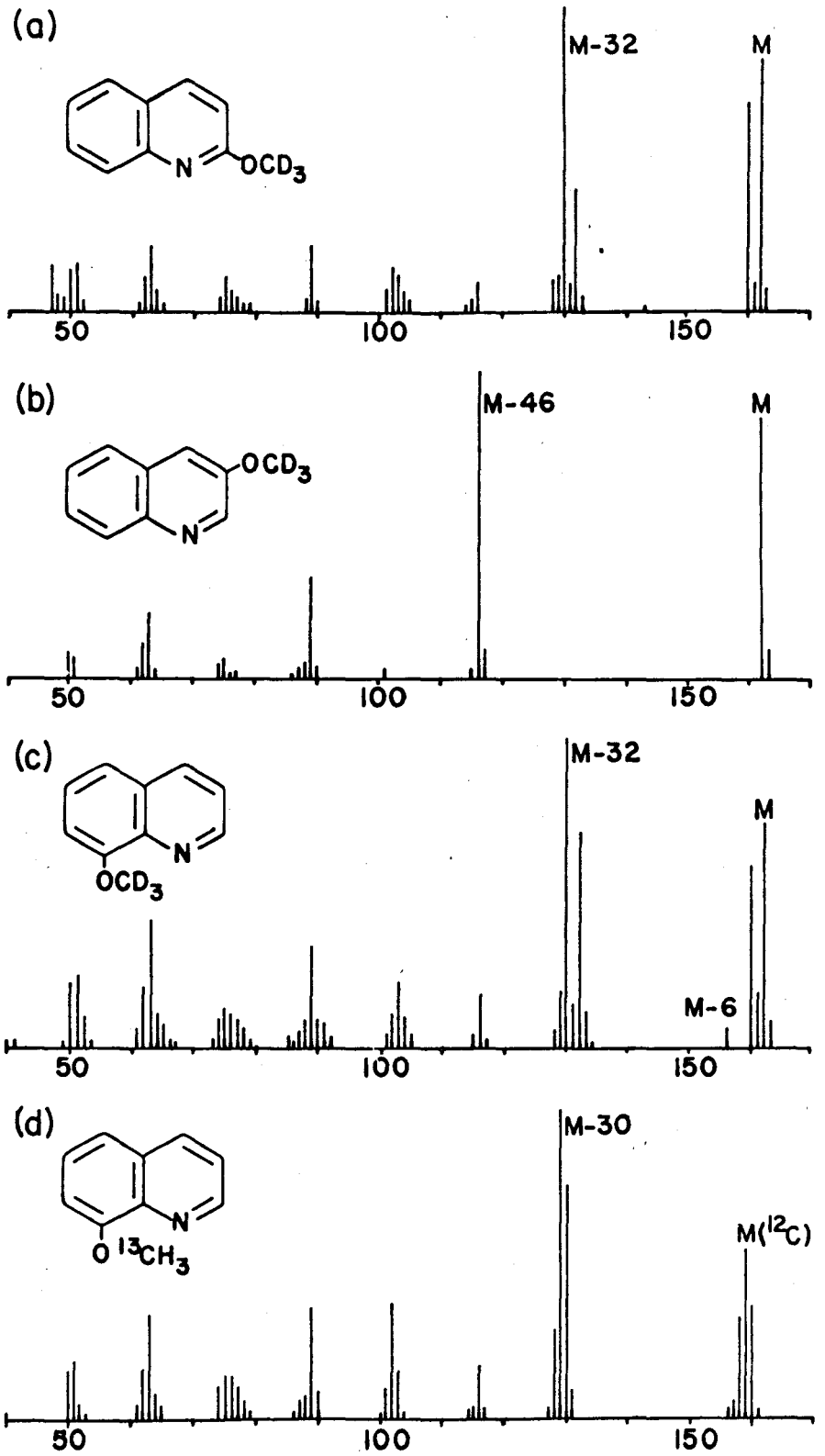


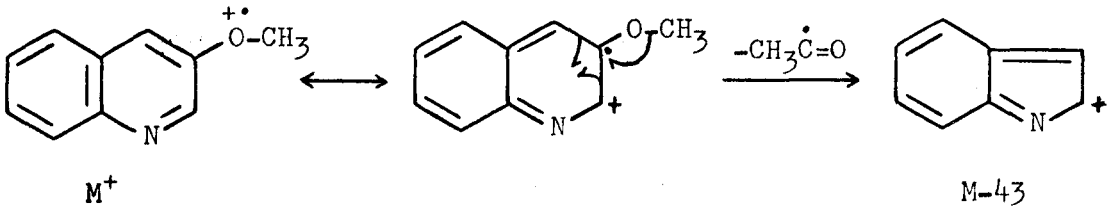
Figure 3. Mass spectra of (a) 2-methoxyquinoline-d_3, (b) 3-methoxyquinoline-d_3, (c) 8-methoxyquinoline-d_3, (d) 8-methoxyquinoline-^{13}C.

TABLE II

Metastable Peaks for Transformations of Scheme 5

Transformation	Calcd.	Observed						
		2-	3-	4-	5-	6-	7-	8-
M ⟶ M-15	130.4	-	130.5	-	130.5	-	130.5	-
M-15 ⟶ M-43	93.4	-	-	93.7	93.7	93.8	93.8	-
M-43 ⟶ M-70	68.3	68.4	68.4	68.4	68.3	68.4	68.3	68.5
M-70 ⟶ M-96	44.6	44.7	44.6	44.7	44.7	44.7	44.7	44.7

3-Methoxyquinoline appears to be unique in the series. There is little evidence that fragmentation according to either Scheme 4 or 5 occurs to any appreciable extent. The peak at M-43 is the most intense in its mass spectrum while that at M-15 is too small to be plotted in Figure 2. A metastable peak at m/e 85.0 corresponds to the transformation M ⟶ M-43 (m* calcd. 84.6). While fragmentation according to Scheme 5 may make some contribution to the intensity of the M-43 fragment ion peak, it is obvious that some mechanism such as that shown below must operate in order to lose 43 mass units in one step from the molecular ion. The spectrum of 3-methoxyquinoline-d_3,



M^+ M-43

Figure 3(b), confirms that the methoxyl methyl group is lost in forming the ion appearing at M-43. The deuterated compound shows a large peak at M-46 with a metastable peak at m/e 83.4 corresponding

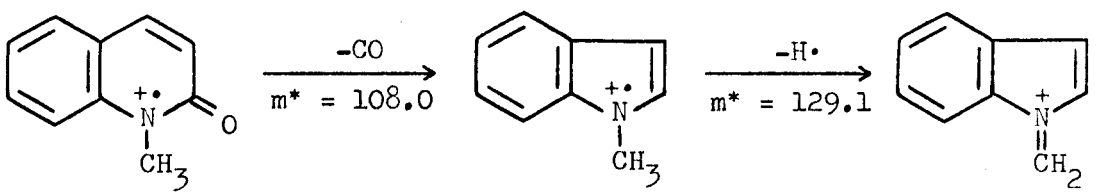
to the transformation M $\longrightarrow$ M-46 (m* calcd. = 83.1).

8-Methoxyquinoline undergoes a unique fragmentation wherein all three hydrogens are lost from the methoxyl methyl group to give rise to a peak at M-3 in its mass spectrum. Metastable peaks at m/e 157.0 and m/e 154.3 indicate that the fragmentation occurs in two steps with initial loss of one hydrogen atom followed by simultaneous expulsion of two hydrogen atoms. The spectrum of 8-methoxyquinoline-d_3, Figure 3(c), contains a peak at M-6 which arises by loss of one deuterium atom followed by simultaneous expulsion of two deuterium atoms. Thus, it is seen that only the methyl hydrogens are lost in formation of the ion at M-3.

From the above results it is seen that 2- and 8-methoxyquinoline can be differentiated from the remaining isomers by the presence of an intense M-1 peak in the mass spectra of the former. Furthermore, 8-methoxyquinoline can be differentiated from the 2-isomer by the fact that the 8-substituted compound shows a peak at M-3 in its mass spectrum. Loss of the three methyl hydrogens may be a general fragmentation of compounds containing an 8-methoxyl group in a quinoline system since a similar process has been observed in the furoquinoline alkaloids which contain an 8-methoxyl substituent. Of the 3-, 4-, 5-, 6- and 7-isomers, the 3-substituted compound can be distinguished by its loss of forty-three mass units in one step. The remaining compounds show significant, but not definitive, differences in the relative intensities of some peaks in the spectrum, particularly the M-15 and M-30 peaks.

N-methylquinolones

2- and 4-Methoxyquinolines are known to undergo thermal rearrangement to the corresponding N-methylquinolones (39,40). While it is now thought that such rearrangements are largely intermolecular reactions (41) it was necessary to examine the mass spectra of N-methyl-2- and -4-quinolones to eliminate the possibility that 2- and 4-methoxyquinolines were undergoing rearrangement either in the inlet system or upon electron impact. The mass spectra of the quinolones are recorded in Figure 4. It is seen that the major fragment ion peak appears at M-28, corresponding to expulsion of a molecule of CO. This behaviour is entirely analogous to that observed previously for 2-pyridone (32). 4-Pyridone has been observed to lose CO or HCN with almost equal ease (32); however, N-methyl-4-quinolone loses only CO from the molecular ion. The radical ion at M-28 then loses a hydrogen atom to give the ion appearing at M-29 as shown below. Metastable peaks in the spectra of both compounds



M^+ M-28 M-29

support the proposed fragmentation route.

Hydroxyquinolines

The mass spectra of the seven monohydroxyquinolines are recorded in Figure 5. The most intense peak in the spectrum of each

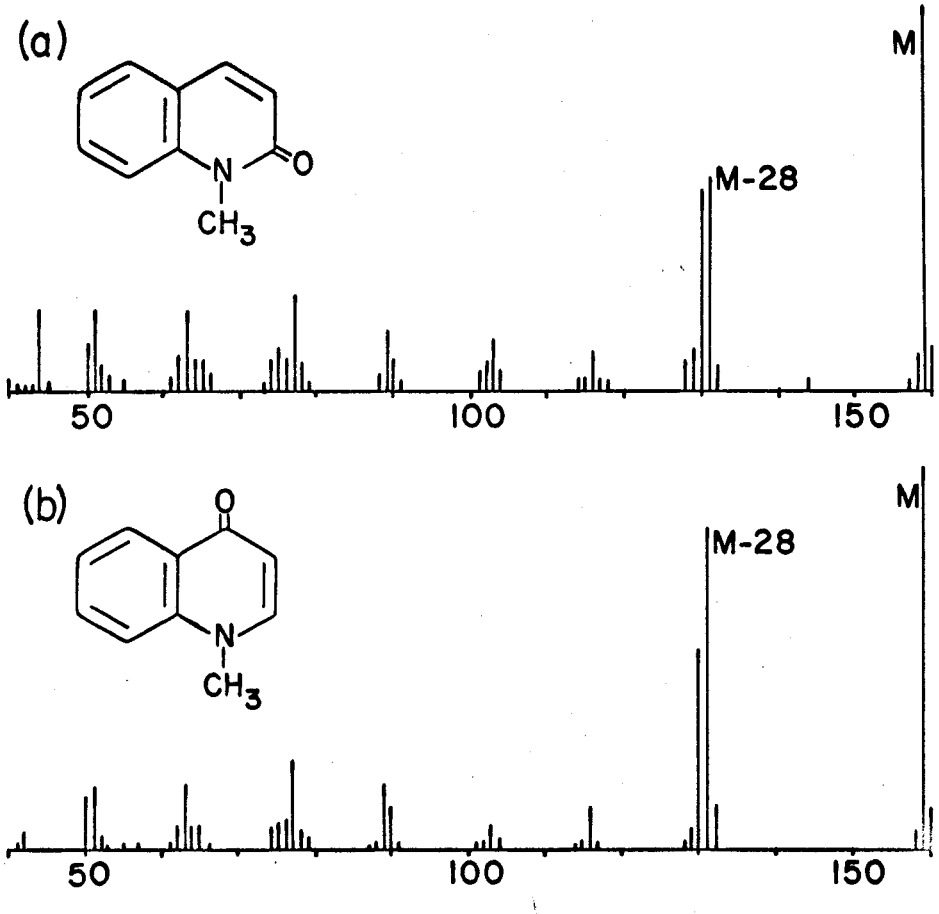


Figure 4: Mass Spectra of a) N-methyl-2-quinolone and b) N-methyl-4-quinolone

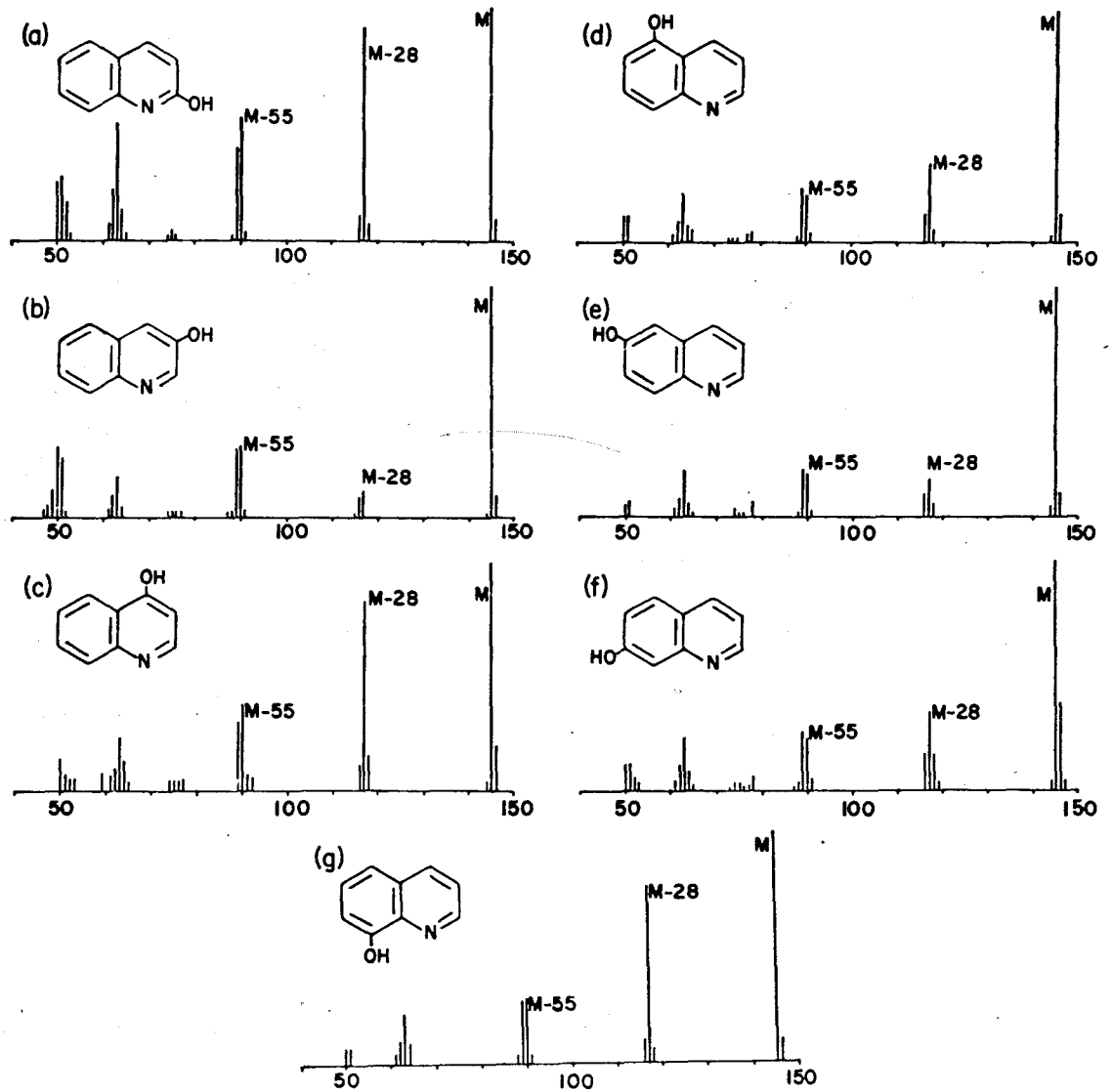


Figure 5. Mass spectra of (a) 2-hydroxyquinoline, (b) 3-hydroxyquinoline, (c) 4-hydroxyquinoline, (d) 5-hydroxyquinoline, (e) 6-hydroxyquinoline, (f) 7-hydroxyquinoline, (g) 8-hydroxyquinoline.

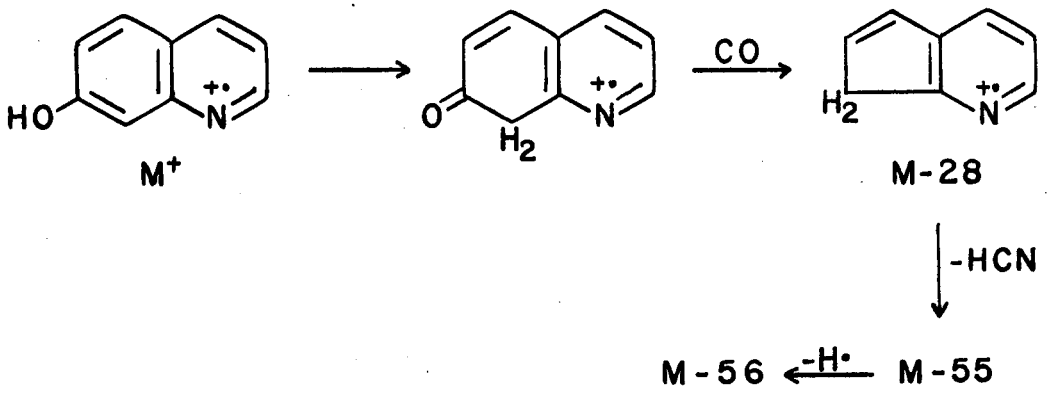
isomer is that due to the molecular ion. The major fragment ion peaks in all the spectra may be accounted for by the mechanism outlined in Scheme 7. The initial fragmentation proceeds by loss of a carbon monoxide molecule in the same way that has been observed for phenol and its derivatives (31) yielding the radical ion appearing at M-28. The M-28 radical ion then loses HCN followed by loss of a hydrogen atom to give the ions observed at M-55 and M-56. Table III lists the metastable peaks for the transformations proposed in Scheme 7.

TABLE III

Metastable Peaks for Transformations of Scheme 7

Transformation	Calcd.	Observed						
		2-	3-	4-	5-	6-	7-	8-
M ⟶ M-28	94.4	94.6	94.6	94.6	94.5	94.6	94.6	94.6
M-28 ⟶ M-55	69.2	69.2	69.3	69.2	69.3	69.2	69.3	69.2
M-55 ⟶ M-56	88.0	88.1	88.1	88.1	88.1	88.1	88.1	88.1

That the ease of fragmentation of the various isomers differs to a considerable degree is indicated by an examination of the intensity of the molecular ion peak relative to the fragment ion peaks in the different spectra. Thus, 3-, 5-, 6- and 7-hydroxyquinolines do not fragment to any great extent while the 2-, 4- and 8-isomers yield relatively intense fragment ion peaks. Facile loss of CO from 2- and 4-hydroxyquinolines is understandable on the basis of participation of the tautomeric quinolone structures. While a tautomeric structure is not possible for 8-hydroxyquinoline, loss of CO may be facilitated by



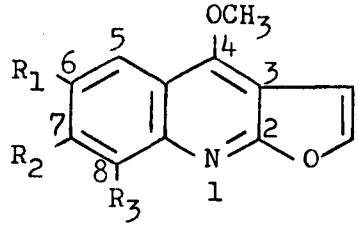
Scheme 7

the ability of the nearby nitrogen atom to accept the hydroxyl hydrogen atom.

Furoquinoline Alkaloids

The furoquinoline alkaloids are a group of natural products characterized by the presence of a quinoline ring system fused in the 2,3-positions to a furan ring and bearing a methoxyl group in the 4-position. In addition, most of the alkaloids contain one or more oxygenated substituents on the benzenoid ring. The isolation and structural elucidation of these alkaloids has been summarized in recent reviews (43,44).

The mass spectra of dictamnine I, maculine II, kokusaginine III, skimmianine IV, maculosidine V and flindersiamine VI are recorded in Figure 6. Every compound shows the intense molecular ion



I. $R_1 = R_2 = R_3 = H$

II. $R_1 = R_2 = -OCH_2O-$, $R_3 = H$

III. $R_1 = R_2 = OCH_3$, $R_3 = H$

IV. $R_1 = H$, $R_2 = R_3 = OCH_3$

V. $R_1 = R_3 = OCH_3$, $R_2 = H$

VI. $R_1 = R_2 = -OCH_2O-$, $R_3 = OCH_3$

peak typical of aromatic compounds. In addition, every spectrum contains fragment ion peaks at M-15, M-43 and M-71 which can be

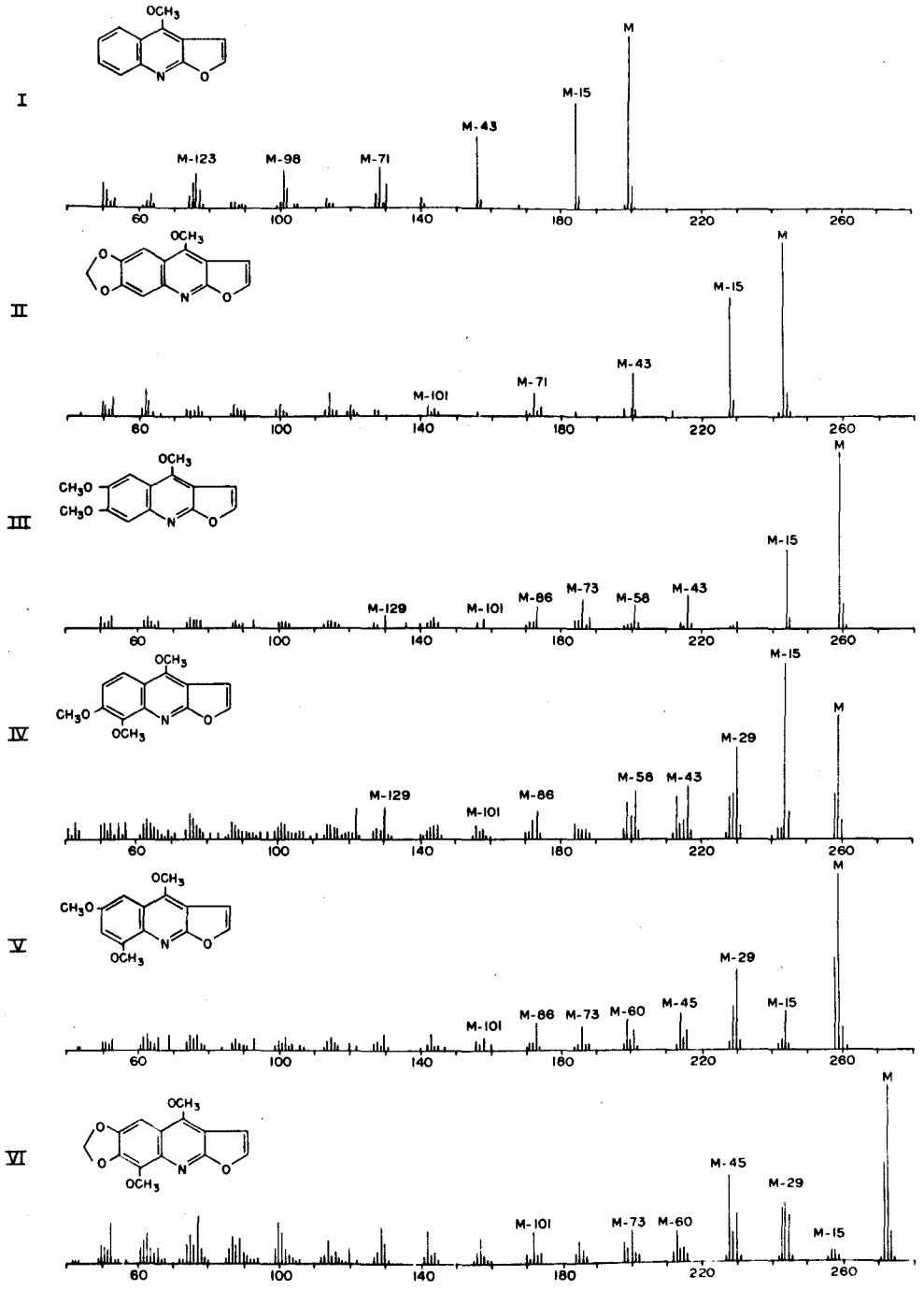
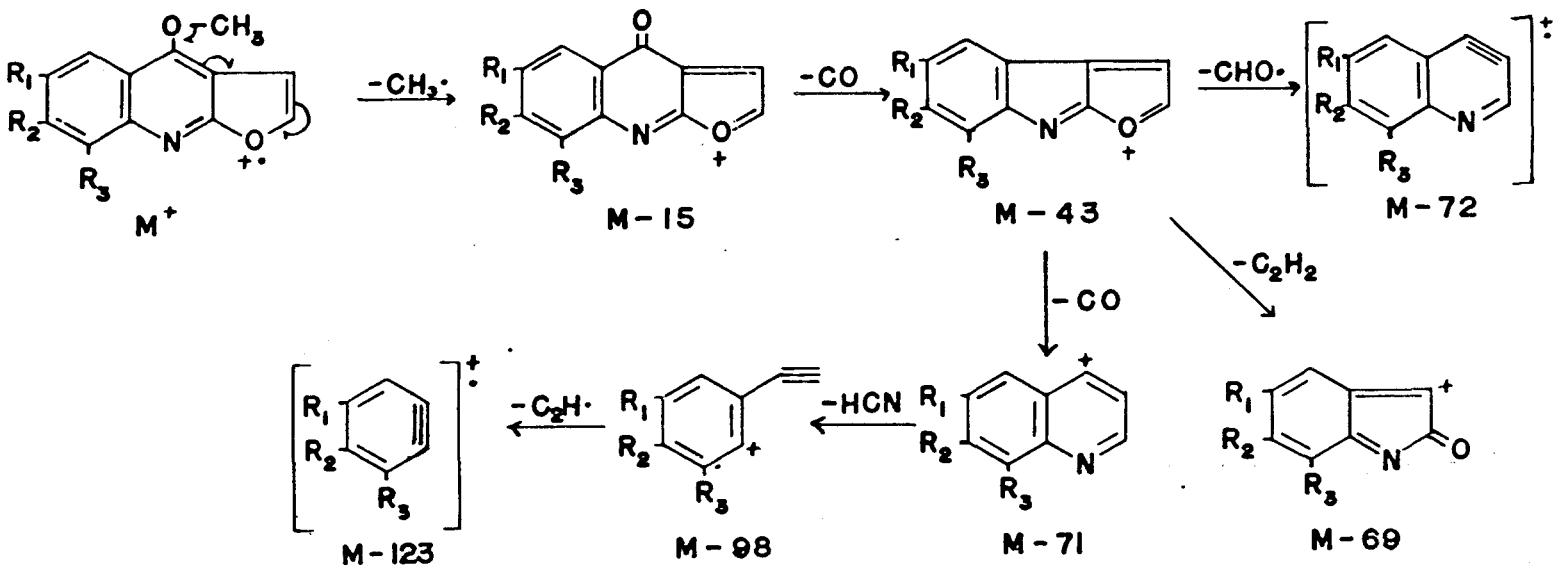


Figure 6. Mass spectra of dictamnine I, maculine II, kokusaginine III, skimmianine IV, maculosidine V, and flindersiamine VI.

rationalized as outlined in scheme 8. The fragmentation mechanism proposed in Scheme 8 also explains the formation of ions appearing at M-69, M-72, M-98 and M-123. The latter peaks are found to a significant extent only in the spectrum of dictamnine. The initial fragmentation of dictamnine is similar to that proposed in Scheme 5 for the methoxyquinolines. Thus, loss of the methoxyl methyl group followed by expulsion of carbon monoxide from the quinoline ring gives rise to the ion observed at M-43. Ions appearing at M-69, M-71 and M-72 arise from that appearing at M-43 by loss of C_2H_2, CO and CHO•, respectively. Elimination of CO and CHO• from furan rings upon electron impact has been observed previously (42).

Some evidence that the ion appearing at M-15 expels a molecule of carbon monoxide from the 4-position of the quinoline ring rather than from the furan ring is provided by the mass spectrum of dictamnine. If the furan ring fragmented first some loss of CHO• as well as CO would be expected (42), thereby giving rise to a peak at M-44 as well as at M-43. Since no peak is found at M-44 no CHO• is lost from the M-15 ion. Peaks at M-72 and M-71 indicate, however, that CHO• and CO are lost from the M-43 ion. Thus, the furan ring in dictamnine is apparently cleaved after expulsion of CO from the quinoline system. In the alkaloids which contain a second methoxyl group the 4-methoxyl function may not be involved in the initial cleavage. For the sake of convenience, and in the absence of evidence to the contrary, it is assumed that the M-15 and M-43 peaks arise by loss of the 4-methoxyl group in all cases.

The ion appearing at M-71 in the spectrum of dictamnine under-



Scheme 8

goes further fragmentation in a manner similar to that observed for the methoxyquinolines with loss of HCN and C_2H_2 moieties. Table IV lists the metastable peaks for the transformations depicted in Scheme 8.

TABLE IV

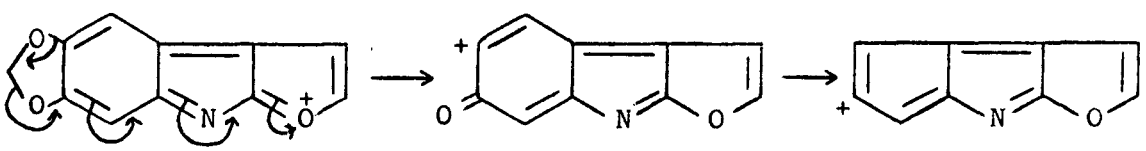
Metastable Peaks for Transformations of Scheme 8

Transformation	Alkaloid	Calcd.	Observed
M ⟶ M-15	I	170.1	170.2
	II	213.9	214.0
	III,IV,V	229.9	230.0
	VI	243.8	244.5
M-15 ⟶ M-43	I	132.3	132.5
	II	175.4	176.0
	III,IV,V	191.2	191.5
	VI	-	-
M-43 ⟶ M-69	I	108.3	108.5
	II	151.4	151.5
	III,IV,V	-	-
	VI	-	-
M-43 ⟶ M-71	I	105.0	105.3
	II	147.9	148.1
	III,IV,V	163.6	164.0 (III only)
	VI	-	-

Continued . . . .

Transformation	Alkaloid	Calcd.	Observed
M-43 ⟶ M-72	I	103.4	-
	II	146.2	-
	III,IV,V	-	-
	VI	-	-
M-71 ⟶ M-98	I	79.7	80.0
	II	122.2	122.5
	III,IV,V	-	-
	VI	-	-

All six alkaloids show at least some of the fragment ion peaks arising by the mechanism shown in Scheme 8. Alkaloids II, III, IV, V and VI, however, give additional peaks because of the additional substituents present in these compounds. For example, the M-43 ion derived from maculine, II, may eliminate CH_2O followed by CO from the methylenedioxy function as shown below. Peaks are present at M-129



M-43 M-73 M-101

and M-156 for the subsequent loss of CO and HCN from the ion appearing at M-101.

Kokusaginine, III, has methoxyl groups in the six and seven positions in addition to the 4-methoxyl function. Peaks due to fragmentation via Scheme 8 are found in its mass spectrum, but additional

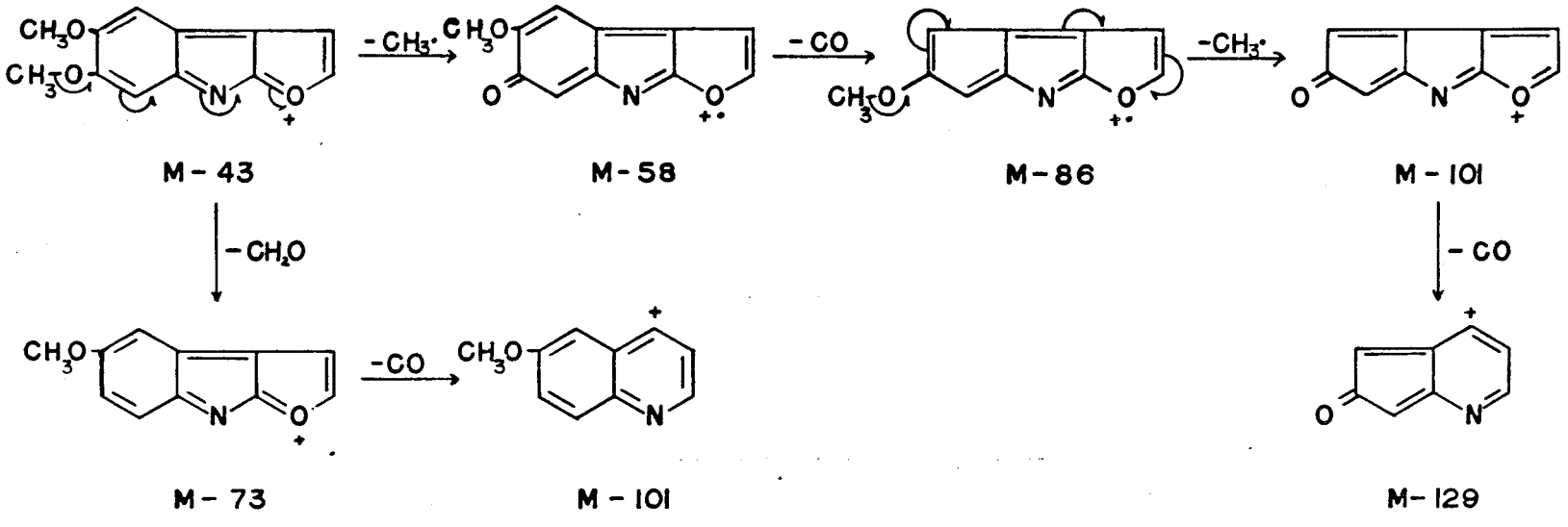
peaks at M-58, M-73, M-86, M-101 and M-129 are also present. A possible fragmentation pattern involving the six and seven methoxyl groups is outlined in Scheme 9, although several variations of the basic pattern may be involved in formation of the observed ions. It is assumed that the M-43 ion is formed in the usual way whereupon it may fragment by several different pathways. Ions appearing at M-58, M-86, M-101 and M-129 may be accounted for by consecutive loss of methyl radicals and carbon monoxide molecules from the methoxyl groups. Alternatively, a molecule of formaldehyde may be eliminated followed by carbon monoxide to give rise to ions appearing at M-73 and M-101. With slight variation this Scheme may be applied in its entirety to IV and V and partially to VI. Table V lists the metastable peaks for the transformations proposed in Scheme 9.

TABLE V

Metastable Peaks for Transformations of Scheme 9

Transformation	Alkaloid	Calcd.	Observed
M-43 ⟶ M-58	III,IV,V	187.0	187.2-187.5
	VI	201.0	-
M-43 ⟶ M-73	III,IV,V	160.2	160.4 (III,V)
	VI	173.9	-
M-58 ⟶ M-86	III,IV,V	148.9	-
	VI	162.6	-
M-73 ⟶ M-101	III,IV,V	134.2	134.2 (V)
	VI	147.9	148.0

Continued . . . . .


CH3O
CH3O
N
O
+
-CH3·
CH3O
O
N
O
+·
-CO
CH3O
N
O
+·
-CH3·
O
N
O
+
M-43
M-58
M-86
M-101
-CH2O
-CO
M-73
M-101
M-129

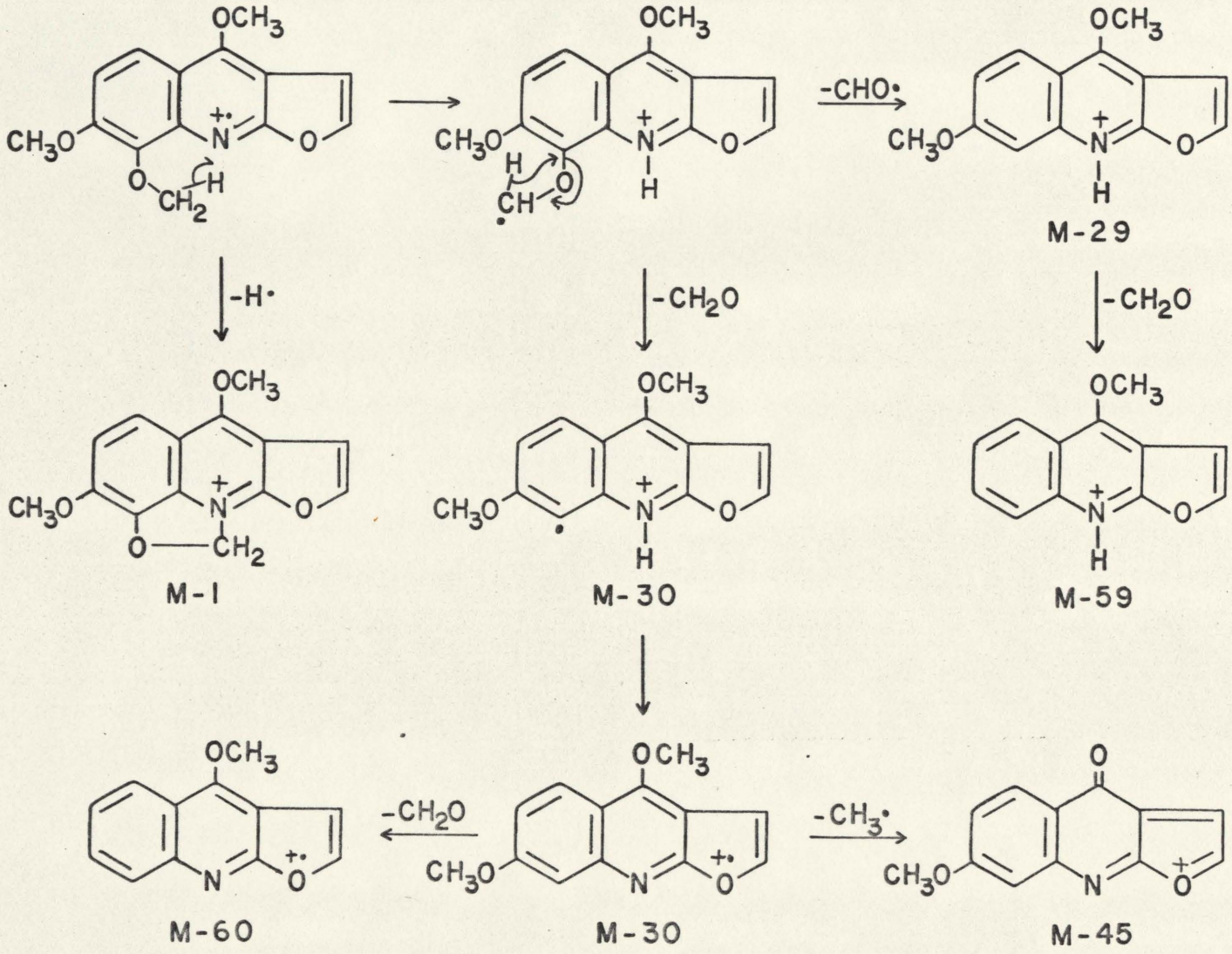
Scheme 9

Transformation	Alkaloid	Calcd.	Observed
M-86 ⟶ M-101	III,IV,V	144.3	-
	VI	158.2	-
M-101 ⟶ M-129	III,IV,V	107.0	107.5 (III)
	VI	120.6	-

The presence of an 8-methoxyl group in IV, V and VI causes their mass spectra to differ considerably from the spectra of alkaloids I, II and III. As in the case of 8-methoxyquinoline itself, loss of one hydrogen atom from the 8-methoxyl group occurs very readily giving an intense peak at M-1. The 8-methoxyl group readily loses a formyl radical or a formaldehyde molecule to give peaks at M-29 and M-30 as outlined in Scheme 10. A possible mechanism for the formation of ions appearing at M-45, M-59 and M-60 from those at M-29 and M-30 is also formulated in Scheme 10. Only a few of the transformations proposed in Scheme 10 are supported by metastable peaks in the spectra. The M ⟶ M-29 transformation is supported by metastable peaks at m/e 204.5 and 204.4 in IV and V, respectively; that of M-30 ⟶ M-45 by a metastable peak at m/e 200.2 in V; that of M-30 ⟶ M-60 by metastable peaks at m/e 172.9 in V and m/e 186.9 in VI.

Alkaloids IV, V and VI all show a peak of low intensity at M-3 in their mass spectra. Metastable peaks in every case indicate that the sequence M ⟶ M-1 ⟶ M-3 is being followed in the manner observed in 8-methoxyquinoline.

Although mass spectrometry has not proven as useful in



Scheme 10

differentiating among the furoquinoline alkaloids as it has in other groups of alkaloids, some conclusions may be drawn from the above results. In the first place, the number and nature of the substituents on the aromatic nucleus can be determined from the molecular weight of the compound. The presence of intense M-1 and M-29 peaks appears to be characteristic of those alkaloids which contain a methoxyl group in the 8-position of the quinoline ring system. Skimmianine, IV, is the only one of the alkaloids studied to give a fragment ion peak of greater intensity than the molecular ion peak. This very intense peak at M-15 is not due only to the presence of ortho methoxyl groups (cf. kokusaginine, III) but appears to be a function of the 7,8-dimethoxy-furoquinoline system.

EXPERIMENTAL

Mass spectra were recorded on a Hitachi Perkin-Elmer RMU-6A mass spectrometer equipped with an all-glass inlet system maintained at 200°C. An ionizing potential of 80 eV and an ionizing current of about 50 μA were used. All spectra have been plotted in terms of relative abundance with the most intense peak in the spectrum being taken as 100%. Only those peaks with an intensity equal to or greater than 2% of the most intense peak have been recorded.

2-, 4- and 8-Hydroxyquinolines and 6-methoxyquinoline were obtained commercially. CD_3OH (99 atom % D), CD_3I (99 atom % D) and $^{13}CH_3I$ (53 atom % ^{13}C) were obtained from Merck Sharp and Dohme of Canada Ltd. All methoxyquinoline, hydroxyquinoline and quinolone samples were purified by vapour phase chromatography on a 6 ft. column of 3% SE-30 silicone gum rubber on Chromosorb P. The furoquinoline alkaloids were used directly as obtained from Dr. E. Ritchie of the University of Sydney, Australia.

2-methoxyquinoline, 4-methoxyquinoline

The hydroxyquinoline was converted to the corresponding chloroquinoline by treatment with excess $POCl_3$ as described by Abramovitch (45). The chloro compound was heated under reflux with sodium methoxide in methanol for 3 hours (46). The methanol was removed, water added and the mixture extracted with chloroform. The

chloroform extract was dried and evaporated to yield the methoxyquinoline contaminated with chloroquinoline. The methoxyquinoline was purified by vapour phase chromatography.

2-methoxyquinoline-d_3

The procedure used was identical with that above except that sodium methoxide-d_3 and CD_3OH were substituted for their undeuterated analogues.

3-hydroxyquinoline

The diazonium chloride, obtained from 3-aminoquinoline by the method of Abramovitch (45), was slowly added to boiling water. The resulting solution was cooled, made basic with $NaHCO_3$ and the hydroxyquinoline filtered off. The product was recrystallized from ethanol-water.

3-methoxyquinoline

3-Hydroxyquinoline was converted to 3-methoxyquinoline with diazomethane in ether.

3-methoxyquinoline-d_3

3-Hydroxyquinoline was heated under reflux with excess CD_3I and NaOH in methanol solution for 1 hour. Evaporation of the solvent and partition of the residue between water and chloroform yielded 3-methoxyquinoline-d_3 in the chloroform extract.

5-hydroxyquinoline

5-Nitroquinoline was reduced to 5-aminoquinoline which in turn was converted to the hydroxy compound by treatment with hydroxide ion

according to the procedure used by Kochańska and Brobański (47).

5-methoxyquinoline

5-Methoxyquinoline was prepared by the following sequence of reactions described by Bradford, Elliott and Rowe (48). 3-Nitro-p-anisidine was diazotized and treated with cuprous cyanide to yield 3-nitro-4-cyanoanisole which was reduced to 4-cyano-m-anisidine with tin and hydrochloric acid. Formation of 5-methoxyquinoline-8-carboxylic acid under Skraup conditions, followed by decarboxylation of the silver salt yielded 5-methoxyquinoline.

6-hydroxyquinoline

6-Methoxyquinoline was heated under reflux with excess 47% HI for 5 hours. The acidic solution was neutralized and the 6-hydroxyquinoline recovered by extraction with chloroform in the usual manner.

7-methoxyquinoline

m-Anisidine (6 g), nitrobenzene (6 g), glycerol (45 g) and powdered $FeSO_4$ (4 g) were stirred together while conc. H_2SO_4 (35 g) was added slowly. The resulting mixture was brought to a boil, let stand for ½ hour and then heated under reflux for 4 hours. Nitrobenzene was removed by steam distillation and primary amine destroyed by addition of excess $NaNO_2$. The resulting solution was made basic with KOH and steam distilled until the distillate was clear. The distillate was extracted with chloroform and the oil (7 g) obtained from the chloroform extract was distilled at 12 mm. A fraction collected up to 140° was discarded while a second fraction collected

from 140-145° was further purified by vapour phase chromatography.

7-hydroxyquinoline

7-Methoxyquinoline was converted to 7-hydroxyquinoline with HI in the same manner as in the preparation of 6-hydroxyquinoline.

8-methoxyquinoline

8-Hydroxyquinoline, excess methyl iodide and sodium hydroxide were dissolved in methanol-water. The solution was heated in a sealed tube at 100° for ½ hour. The reaction mixture was poured into water and extracted with chloroform. The dried chloroform extract yielded a red resin on evaporation to dryness. 8-Methoxyquinoline was isolated from the residue by vapour phase chromatography.

8-methoxyquinoline-d_3

The procedure used was the same as described above except that CD_3I was substituted for CH_3I.

8-methoxyquinoline-^{13}C

The procedure used was the same as for 8-methoxyquinoline except that $^{13}CH_3I$ was substituted for CH_3I.

N-methyl-2-quinolone and N-methyl-4-quinolone

The quinolones were prepared by N-methylation of the corresponding hydroxyquinoline with methyl iodide in sodium hydroxide solution as described previously (49,50).

SUMMARY

The mass spectra of the seven isomeric monomethoxyquinolines, the seven monohydroxyquinolines, the two N-methylquinolones and six furoquinoline alkaloids have been examined. It has been possible to differentiate among the isomeric compounds of each group by mass spectrometry.

The monomethoxyquinolines all show an intense peak for the molecular ion. The 2- and 8-isomers are differentiated from the other isomers by the presence of intense peaks at M-1 and M-29 in their mass spectra. The formation of ions appearing at these masses has been explained on the basis of the ability of the unshared pair of electrons on the nearby nitrogen atom to stabilize the fragment ion. This stabilization may result from ring closure (in the M-1 ion) or from transfer of a hydrogen atom to the nitrogen atom (in the M-29 ion). 8-Methoxyquinoline has been found to undergo an unusual fragmentation wherein all three hydrogens are lost from the methyl group. A fragmentation of this type has not been reported before. 3-Methoxyquinoline has also been found to undergo a unique, and previously unknown, fragmentation wherein the 3-methoxyl group plus one carbon atom is lost in one step to give an ion appearing at M-43 in the mass spectrum. The fragmentation mechanisms proposed for the 2-, 3- and 8-isomers have been supported by deuterium labelling experiments. In addition,

the spectrum of 8-methoxyquinoline labelled in the methyl group with ^{13}C has lent further support to the fragmentation mechanism proposed for this compound. The spectra of the 4-, 5-, 6- and 7-isomers contain major fragment ion peaks at M-15, M-43 and M-70. While the differences in the spectra allow unequivocal differentiation among these isomers, they would not be sufficient to allow assignment of a methoxyl group to a definite position in an unknown quinoline.

The mass spectra of N-methyl-2- and -4-quinolone are considerably different from those of their O-methyl analogues, showing that O to N methyl migration does not occur in 2- and 4-methoxyquinoline upon electron impact.

The monohydroxyquinolines all fragment in a similar manner upon electron impact. The 2-, 4- and 8-isomers are distinguished from the remainder by the greater intensities of fragment ion peaks relative to the molecular ion peak in their spectra. The relative ease of fragmentation of the 2- and 4-isomers has been explained on the basis of participation of the corresponding quinolone tautomers while the ease of fragmentation of 8-hydroxyquinoline has been explained by the ability of the neighbouring nitrogen atom to accept the hydrogen from the hydroxyl group.

The number and nature of the substituents on the aromatic nucleus of a furoquinoline alkaloid can be determined from the molecular weight of the compound. Furthermore, the isomeric furoquinoline alkaloids studied can be unambiguously differentiated by examination of their mass spectra. Those alkaloids containing an 8-methoxyl group show intense peaks at M-1 and M-29 and a peak of

lower intensity at M-3 like 8-methoxyquinoline itself. Skimmianine, containing methoxyl groups in the 7- and 8-positions, can be differentiated from maculosidine, which has methoxyl groups in the 6- and 8-positions, by the presence of a very intense peak at M-15 in the mass spectrum of the former.

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PART II

LOBINALINE AND ITS HOFMANN DEGRADATION PRODUCTS

GENERAL INTRODUCTION

Proctor (1) reported the presence of alkaloids in Lobelia inflata in 1836. Since then many alkaloids have been isolated from Lobelia species and their structures determined. Until 1938, however, all known Lobelia alkaloids contained only one nitrogen atom and were simple mono- or disubstituted piperidines or tetrahydropyridines. In 1938 Manske (2) reported that the major alkaloid of Lobelia cardinalis is a binitrogenous base which he named lobinaline. Since Manske's original paper a few other binitrogenous bases have been found in Lobelia species.

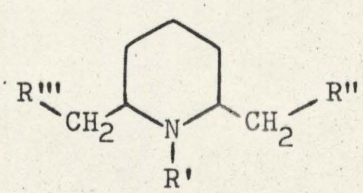
At the time the work reported in this thesis was begun the structures of none of the binitrogenous Lobelia alkaloids had been elucidated. During the course of this work, however, the structures of syphilobine A and syphilobine F (3) and of lobinaline (4) were established by other workers. Our results on lobinaline agree almost completely with the structure proposed by Robison, et al. (4).

The experiments which were carried out in the course of the present work were directed towards a chemical degradation of the alkaloid. Hofmann exhaustive methylation has been found to be the most useful approach. Mass spectral studies on lobinaline and its Hofmann degradation products allowed an assignment of structure to the alkaloid independently of Robison's results.

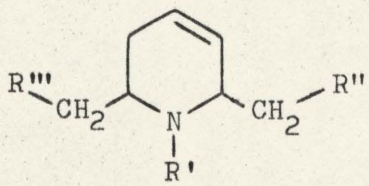
HISTORICAL INTRODUCTION

The marked physiological activity of the North American species Lobelia inflata L. (Indian Tobacco) has long been recognized as evidenced by the nature and variety of local names (gagroot, emetic weed, asthma weed, etc.) ascribed to the plant (5). As early as 1836 Proctor (1) recorded the presence of alkaloids in L. inflata while Lewis (6) isolated lobeline as an oil in 1877. Siebert (7) prepared and analyzed several crystalline derivatives of Lewis's lobeline and, in 1891, proposed the formula $C_{18}H_{23}NO_2$. In 1915 Wieland (8) reported on the pharmacology of a crystalline base from L. inflata which he also called lobeline.

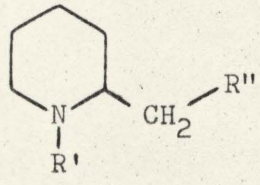
Since 1921 the alkaloids of L. inflata have been extensively investigated, particularly by Wieland and his group (9-18) and more recently by Schöpf and his group (19-25). Altogether the presence of forty-five alkaloids has been indicated in this plant and the structures of many of them have been determined. All the alkaloids whose structures were elucidated before 1964 may be described by one of the three structural types I, II or III.



I



II

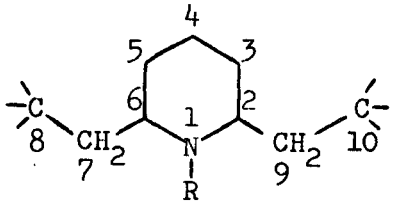


III

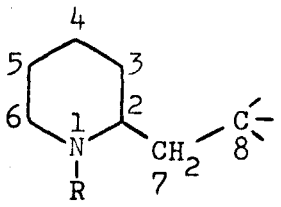
where R' = H or CH_3
R'' and R''' = RCO or RCHOH
R = phenyl or alkyl

Alkaloids of types I and III have been synthesized while those of type II have been converted to known compounds of type I by hydrogenation. A summary of the methods used for structural elucidation and synthesis of the Lobelia alkaloids with a single nitrogen atom may be found in The Alkaloids, Volume 1 (26).

In 1957 Schöpf, et al. (22) proposed a systematic nomenclature for the Lobelia alkaloids. In their system the molecules are numbered as shown in IV and V. The basic name lobeli- is assigned to structure IV

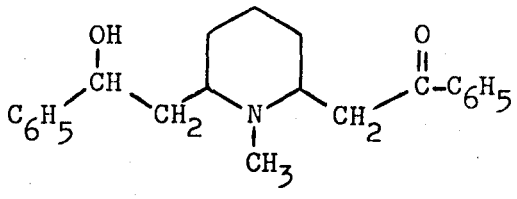


IV



V

and the name lobel- to structure V. The nature of the oxygen substituents at C-8 and C-10 is indicated by a suffix; -ol for a hydroxyl group, -one for a carbonyl function. Lobeline, VI, is named 8,10-diphenyllobelionol under this system. Many of the minor Lobelia alkaloids have



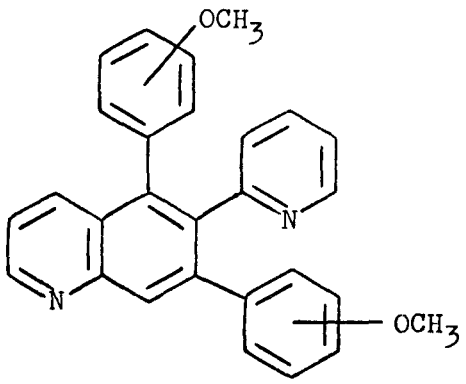
VI

not been assigned trivial names but are known only by their systematic names.

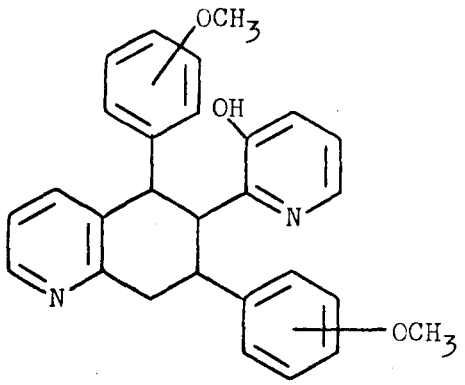
Several species in addition to L. inflata have been examined for their alkaloid content. Except for a few binitrogenous bases, the

majority of alkaloids found in all the species examined are also found in L. inflata. The Lobelia species which have been examined and their contained alkaloids are listed in Table I. Table II contains the systematic names of those alkaloids originally assigned a trivial name.

All the alkaloids isolated from L. inflata, and from most other Lobelia species contain only one nitrogen atom and have been shown to be mono- or disubstituted piperidine or tetrahydropyridine derivatives. In 1938 Manske (2) reported the major, and possibly only alkaloid in Lobelia cardinalis L. to be lobinaline, a binitrogenous base of empirical formula $C_{28}H_{36}N_2O$. More recently Steinegger and Egger (38) have isolated lophiline, $C_{28}H_{38}N_2O_3$ or $C_{27}H_{36}N_2O_3$, from L. syphilitica. In 1964 Tschesche, Kloeden and Fehlhaber (3) isolated and elucidated the structures of syphilobine A, VII, and syphilobine F, VIII, from L. syphilitica. Other workers have detected lobinaline in



VII



VIII

L. inflata (29), L. Kalmii L. (48), L. anceps L. (48) and L. elongata Small (43) by paper chromatography.

Absolute Configuration of Lobeline

In 1965 Schöpf and Müller (25) established the absolute

Table I

Alkaloids of *Lobelia* Species

Species	Alkaloids	References
L. inflata L.	l-lobeline	1,6,7
	dl-lobeline (formerly lobelidine)	10
	lobelanine	12
	norlobelanine (formerly isolobelanine)	12
	lobelanidine	12
	norlobelanidine	15
	dl-lelobanidine-I	18
	l-lelobanidine-I	18
	l-lelobanidine-II	18
	d-norlelobanidine	18
	lobinine	16
	isolobinine	18,27,28
	lobinanidine	18
	isolobinanidine	18
	8,10-diethyllobelidiol	22
	(+)-8-methyl-10-phenyllobelidiol	22
	8-methyl-10-ethyllobelidiol	22
	(-)-8-phenyllobelol-I	22
	(+)-8-phenylnorlobelol-I	22
	(+)-8-ethylnorlobelol-I	22
	$C_{18}H_{27-29}NO_3$	22
	lobinaline (paper chromatography)	29

Table I (Cont.)

Species	Alkaloids	References
L. inflata L. (Cont.)	23 unidentified bases (paper chromatography)	22
L. sessilifolia Lamb	lobeline	30
L. cardinalis L.	lobinaline	2
	cardinalis alkaloid 2	31
L. decurrens	lobeline	32
L. urens L.	l-lobeline	33
	lobelanidine	33
	lurenine	34
	norlobelanine	35
L. tupa G. Don	lobeline	36
	lobelanidine	36
	5 unidentified bases (paper chromatography)	36
var. mucronata	lobeline	37
L. syphilitica L.	lophilacrine	38
	lophiline	38
	(-)-3-dehydro-trans-8,10-diethyllobelidiol	39
	(-)-3(or 4)-dehydro-trans-8-methyl-10-ethyllobelidiol	39
	(-)-cis-8,10-diethyllobelionol	39
	8,10-diethyllobelidione	39
	cis-8,10-diethylnorlobelionol	39
	syphilobine A	3
	syphilobine F	3
	$C_{14}H_{27}NO_2$	39

Table I (Cont.)

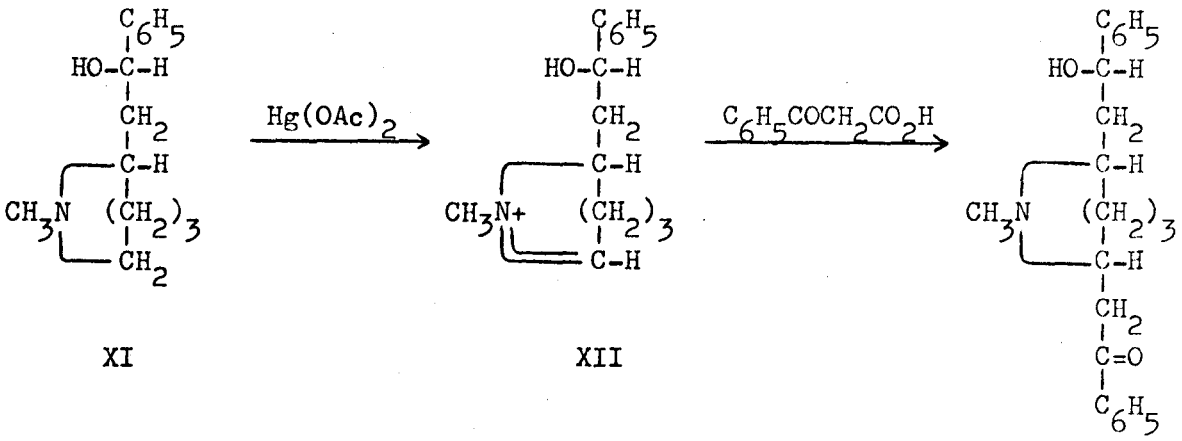
Species	Alkaloids	References
L. nicotianaefolia Heyne	lobelanidine	40
	l-lelobanidine-I	40
	l-lelobanidine-II	41
	l-lelobanidine-III	41
	d-norlobelanidine	41
L. vadicans	lobeline	42
	lobelanine	42
L. elongata Small	lobinaline (paper chromatography)	43
L. salicifolia G. Don	dl-lobeline	44
	norlobelanine	44
	salicilobine	45
	norlobelanidine	45
	lobelanine	45
	d-lobelanine	45
	l-lobelanine	45
	unidentified base (paper chromatography)	45
L. langeana	l-lobeline	46
	lobelanine	46
	lobelanidine	46
	unidentified base (paper chromatography)	46
L. pyramadalis	lobeline	47
	2 unidentified bases (paper chromatography)	47

Table II

Systematic Names of *Lobelia* Alkaloids

Trivial Name	Systematic Name
l-lobeline	(-)-*cis*-8,10-diphenyllobelionol
dl-lobeline	racem-*cis*-8,10-diphenyllobelionol
lobelanine	*cis*-8,10-diphenyllobelidione
norlobelanine	*cis*-8,10-diphenylnorlobelidione
lobelanidine	*cis*-8,10-diphenyllobelidiol
norlobelanidine	*cis*-8,10-diphenylnorlobelidiol
dl-lelobanidine	racem-*cis*-8-ethyl-10-phenyllobelidiol
l-lelobanidine-I	(-)-*cis*-8-ethyl-10-phenyllobelidiol-I
l-lelobanidine-II	(-)-*cis*-8-ethyl-10-phenyllobelidiol-II
l-lelobanidine-III	(-)-*cis*-8-ethyl-10-phenyllobelidiol-III
d-norlelobanidine	(+)-*cis*-8-ethyl-10-phenylnorlobelidiol
lobinine	(-)-3(or 4)-dehydro-*trans*-8-ethyl-10-phenyllobelionol
isolobinine	(-)-3(or 4)-dehydro-*cis*-8-ethyl-10-phenyllobelionol
lobinanidine	(-)-3(or 4)-dehydro-*trans*-8-ethyl-10-phenyllobelidiol
isolobinanidine	(-)-3(or 4)-dehydro-*cis*-8-ethyl-10-phenyllobelidiol

configuration of lobeline and some of its derivatives. (-)-Lobeline was shown to have the absolute configuration shown in structure XIII on the basis of its synthesis from (-)-sedamine, XI. The absolute configuration of (-)-sedamine had been established earlier by Schöpf et al. (49). When XI was heated with mercuric acetate it yielded the



XI XII XIII

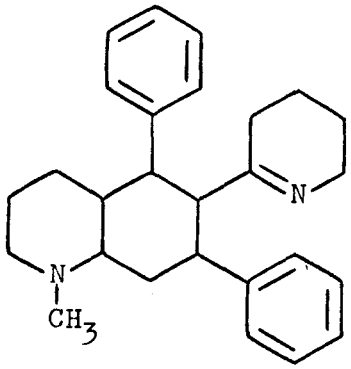
immonium compound XII which in turn condensed with benzoylacetic acid to yield XIII. The cis relationship of the substituents on the piperidine ring in lobeline had long been known (15). This synthesis, therefore, established the absolute configuration of (-)-lobeline. Alkaloids which can be formed from lobeline by oxidation or reduction also have their absolute configurations established by this synthesis.

Lobinaline

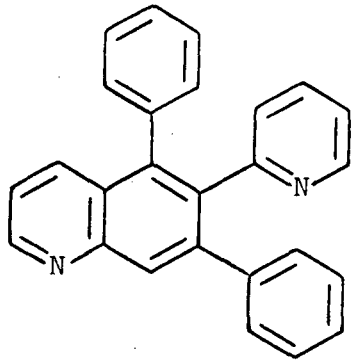
Preliminary chemical studies on lobinaline were carried out by Manske (2). He found that the alkaloid crystallized with difficulty even after purification as the monohydrochloride. Permanganate oxidation yielded less than one equivalent of benzoic acid which he

interpreted to mean that only one monosubstituted benzene ring was present in the molecule.

Between 1938 and 1964 very little further work was reported on lobinaline. Manske (50) revised the empirical formula to $C_{28}H_{36}N_2$ and also reached the conclusion that the compound contains two monosubstituted benzene rings. In 1964 Robison, et al. (4) proposed structure IX for lobinaline on the basis of its conversion to the



IX



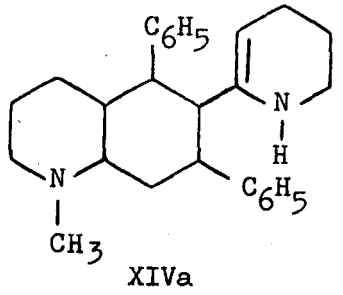
X

wholly aromatic compound X and synthesis of the latter for direct comparison.

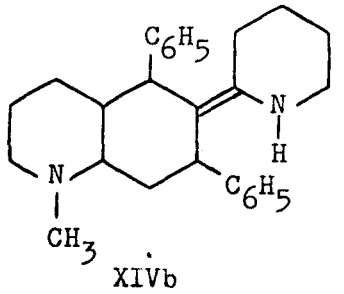
DISCUSSION OF RESULTS

When this study of the structure of lobinaline was begun all that was known about the compound was the work reported by Manske in 1938 (2) and his later revision of the molecular formula to $C_{28}H_{36}N_2$ (50). No simple relationship appeared to exist between lobinaline and the Lobelia alkaloids whose structures were known at that time.

The original purpose of the present work was the complete structural elucidation of lobinaline. Before this aim had been accomplished, however, other workers (4) established that lobinaline has structure IX. Since our results corroborate structure IX for the alkaloid, the reactions reported in this thesis will be discussed in terms of the complete structure. The only minor difference indicated by our data is that the alkaloid appears to exist as a mixture containing variable amounts of tautomer XIVa or XIVb as well as IX.



XIVa



XIVb

Isolation

The dried, above-ground parts of Lobelia cardinalis collected

while the plant was flowering yielded lobinaline in about 0.3% yield. The crude alkaloid obtained by methanol extraction was subjected to a preliminary purification by chromatography on alumina. The semi-crystalline material obtained from chromatography was converted to the monohydrochloride as described by Manske (2) and the salt was purified by recrystallization. Regeneration of the free base from the monohydrochloride yielded crystalline material which still showed a small amount of a second compound on thin layer chromatography. The minor component was removed by repeated chromatography on alumina or by vapour phase chromatography.

Characterization

Analyses of the free base and several derivatives agreed with the molecular formula $C_{27}H_{34}N_2$. This assignment is supported by a molecular ion peak at m/e 386 in the mass spectrum of lobinaline. Contrary to another report (4), we found no distinguishable difference between the pK_a's of the two nitrogen atoms on titration with dilute acid.

The nuclear magnetic resonance (NMR) spectrum of lobinaline contains peaks at 2.3 δ* and 7.3 δ corresponding to three N-methyl and ten phenyl protons respectively. In addition, a highly purified sample of the alkaloid in a degassed solution shows a broad peak at 4.5 δ. This peak integrates for about one-half a proton and shifts to 5.7 δ with considerable broadening upon addition of a trace of anhydrous

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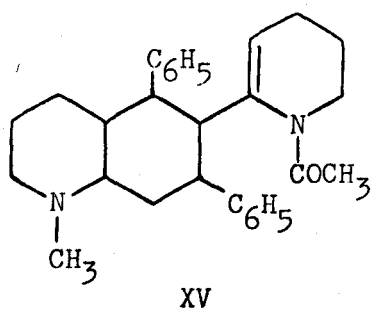
* δ = parts per million from tetramethylsilane as internal standard.

hydrogen chloride. We have assigned the peak at 4.5 δ to the amino hydrogen of tautomer XIVa or XIVb. If tautomer XIVa were present in the solution a peak due to its ethylenic hydrogen atom would be expected to show in the region of 4.5 δ also (52). Thus, any vinyl hydrogen absorption in the free base might be hidden under the N-H absorption. When acid is added to the sample and the N-H absorption shifted to lower field by exchange, the vinyl hydrogen peak should become visible. However, protonation of enamines has been shown to cause them to tautomerize to imines (53). This would tend to decrease the intensity of any vinyl hydrogen absorption. In addition, the tautomer XIVb, while contributing to the N-H peak, does not contribute to the vinyl hydrogen absorption. This combination of factors will decrease the intensity of vinyl absorption relative to N-H absorption to a large extent. Thus, the fact that a distinct ethylenic hydrogen absorption is not observed in the NMR spectrum of lobinaline does not eliminate the possibility that a significant proportion of the alkaloid exists as tautomer XIVa or XIVb.

Acetyllobinaline, XV, does show a peak at 5.0 δ for the ethylenic proton (54) indicating that in this compound the double bond is endocyclic as in tautomer XIVa for lobinaline.



XV

The infrared spectrum of lobinaline contains a band of low intensity at 3320 cm^{-1} providing further evidence for the presence of tautomers XIVa or XIVb both of which contain a N-H bond. A strong band at 1660 cm^{-1} corresponds to a C=C or C=N linkage and a series of four weak bands between 1700 and 2000 cm^{-1} are indicative of mono-substituted benzene rings (51).

Dehydrogenation of lobinaline with selenium, platinum or palladium metal yields benzene, toluene, pyridine, α-picoline and β-picoline identified by vapour phase chromatography. No characterizable products were isolated from the involatile residue in any case. Others (4) have experienced similar difficulty in obtaining dehydrogenation products of higher molecular weight from lobinaline; they found, however, that de-N-methyllobinaline dehydrogenated to yield the aromatic compound X.

The mass spectrum of lobinaline is shown in Figure 1(a). In addition to a molecular ion peak of low intensity at m/e 386, the spectrum contains intense peaks at m/e 186 and 200. In the mass spectrum of lobinaline which has been treated with CH_3OD, Figure 1(b), the relative intensities of the peaks at m/e 387 and 187 are enhanced while the intensity of the peak at m/e 201 is unchanged relative to that at m/e 200. The only easily exchangeable hydrogen in the molecule is that attached to the piperideine nitrogen in tautomers XIVa and b. For this reason it appears that the fragments of m/e 186 and 200 from lobinaline differ in composition only by a N-methyl group. Such a fragmentation pattern can be rationalized as shown in Scheme 1. The molecular ion is written in two forms to depict the proposed fragment-

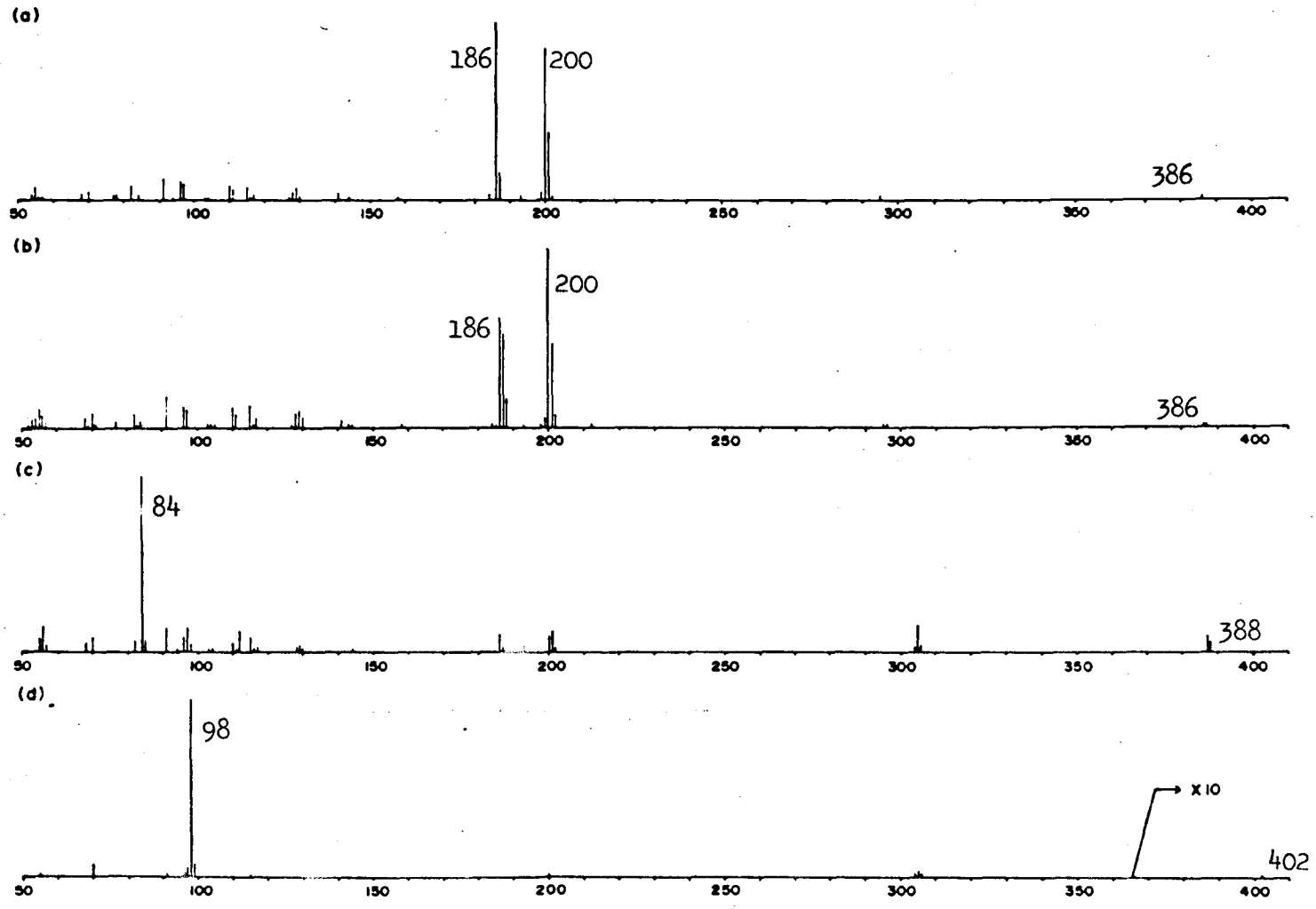
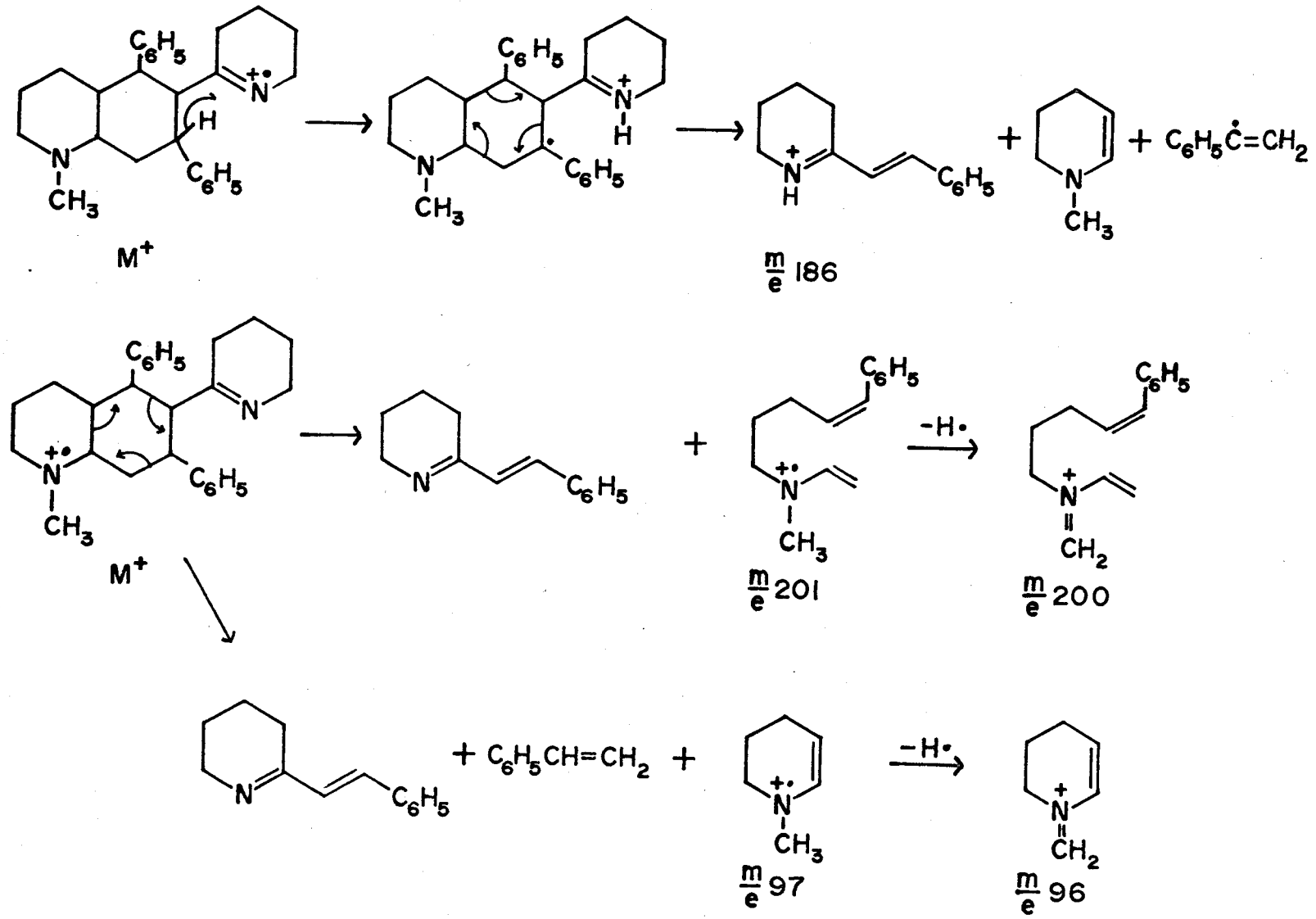


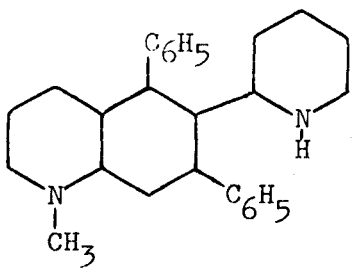
Figure 1. Mass spectra of (a) lobinaline, (b) lobinaline-d_1, (c) dihydrolobinaline, (d) N-methyldihydrolobinaline.



Scheme 1

ation pattern. If ionization occurs by removal of one of the unshared electrons from the piperideine nitrogen, hydrogen transfer from a benzylic carbon to the nitrogen atom followed by ring cleavage will yield the ion observed at m/e 186 with elimination of a molecule of N-methylpiperideine and a styryl radical. On the other hand, if ionization occurs by loss of an unshared electron from the decahydroquinoline nitrogen, ring cleavage will produce either the N-methylpiperideine radical ion observed at m/e 97 or the radical ion observed at m/e 201. Both of these radical ions will readily lose a hydrogen atom to give the ions observed at m/e 96 and m/e 200.

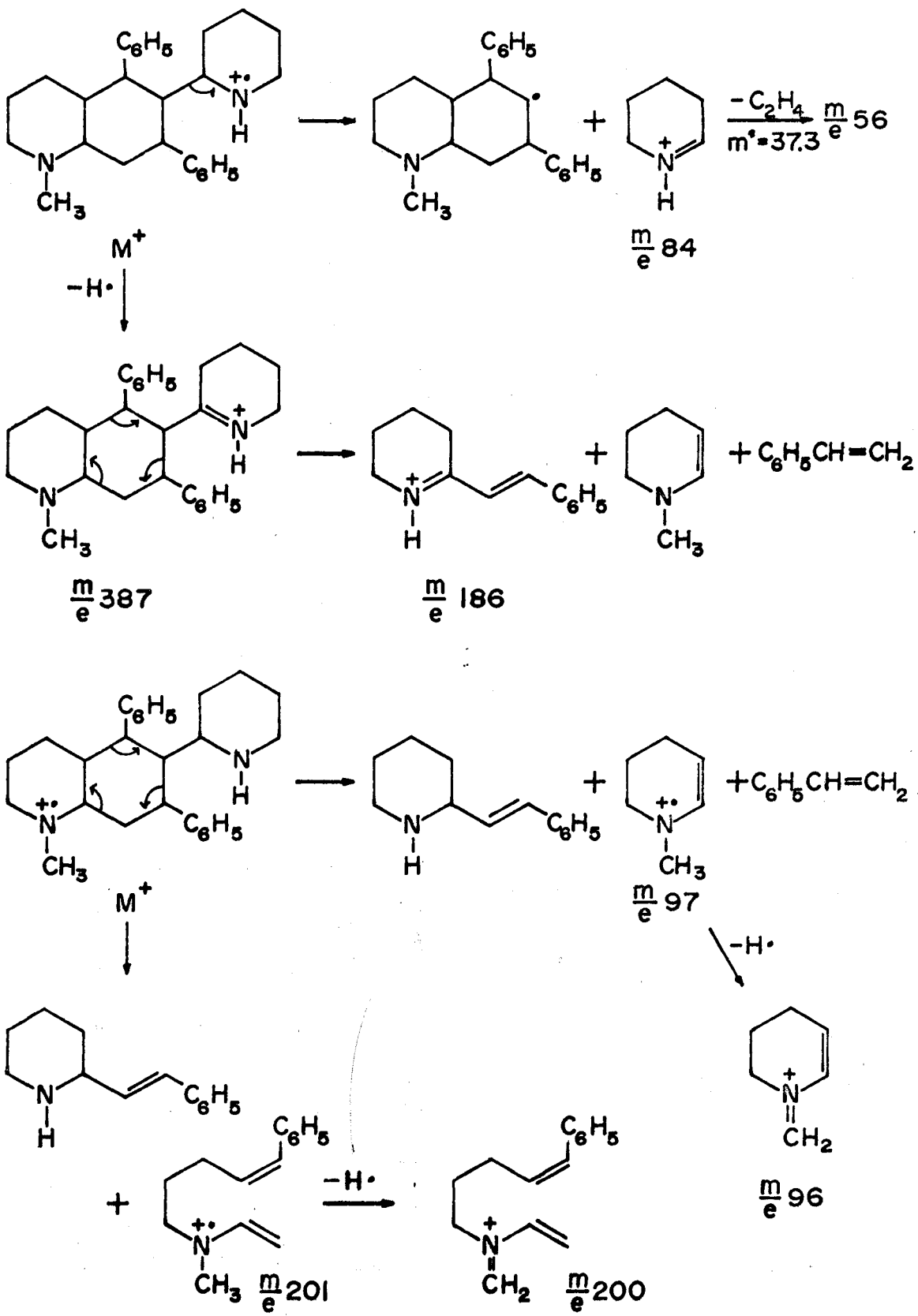
Dihydrolobinaline XVI is prepared by hydrogenation of lobinaline. The infrared spectrum of XVI contains intense N-H



XVI

absorption at 3270 cm^{-1} but no double bond absorption at 1660 cm^{-1}.

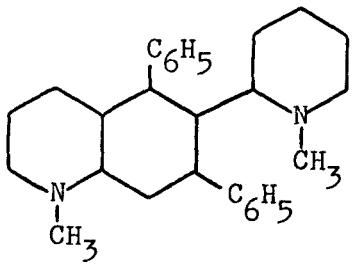
The mass spectrum of dihydrolobinaline is shown in Figure 1(c). The most striking feature of the spectrum is the very intense peak at m/e 84. Other peaks at m/e 388 (molecular ion), 387, 305, 201, 200 and 186 are of much lower intensity. A possible mechanism for the formation of the major peaks in the mass spectrum of XVI is outlined in Scheme 2. The ion which appears at m/e 84 readily forms from the free piperidine ring. This fragmentation was blocked by the double


C6H5
N
H
CH3
M+
-H·
-C2H4
m*=37.3
m/e 56
m/e 84
m/e 387
m/e 186
+C6H5CH=CH2
m/e 97
-H·
CH2
m/e 96
m/e 201
m/e 200

Scheme 2

bond in lobinaline, but in the reduced compound it will be very favourable. The piperidinium ion eliminates an ethylene molecule to give the peak at m/e 56. Another fragmentation path involves loss of a hydrogen atom from the molecular ion followed by ring cleavage to form the ions appearing at m/e 387 and m/e 186 in turn. If the molecular ion arises by loss of an unshared electron from the decahydroquinoline nitrogen atom cleavage of the alicyclic ring will yield the radical ions appearing at m/e 201 and 97. Each of these radical ions will lose a hydrogen atom to give the ions appearing at m/e 200 and 96. The peak at m/e 305 is probably caused by an ion formed by expulsion of the free piperidine ring as a piperideine molecule.

Eschweiler-Clarke methylation (56) of dihydrolobinaline yields N-methyldihydrolobinaline, XVII, a compound which has no absorption



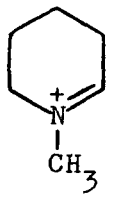
XVII

near 3400 cm^{-1} in the infrared.

The NMR spectrum of XVII is interesting in that it contains peaks at 1.8 and 2.3 δ, each corresponding to three N-methyl protons. The N-methyl group in lobinaline absorbs at 2.3 δ, so that the higher field peak is due to the N-methyl group on the piperidine ring. The unusually high field of the latter is probably due to the shielding

effect of the two adjacent benzene rings. A similar effect will be noted in several degradation products containing N-methyl groups.

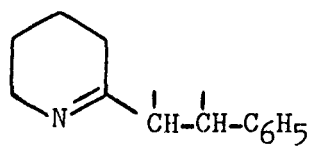
The mass spectrum of XVII, Figure 1(d), is dominated by a peak at m/e 98 due to the N-methylpiperidinium ion A. A similar



A

fragmentation mechanism to that proposed in Scheme 2 for dihydrolobinaline can be used to account for the peaks found in the mass spectrum of the N-methyl derivative. Thus, the peaks at m/e 56, 84, 186, 387 and 388 in the dihydrolobinaline spectrum are all shifted fourteen mass units higher on introduction of the N-methyl group.

The mass spectra of lobinaline and of its dihydro- and N-methyldihydro- derivatives have established unequivocally the presence of a monosubstituted tetrahydropyridine ring in the alkaloid. The substitution of the piperidine ring in the reduced compounds must be in the 2-position to explain the intensity of the peaks at m/e 84 in XVI and m/e 98 in XVII. The formation of ions at m/e 186 from IX and XVI but not from XVII means that these ions contain the monosubstituted piperidine ring and indicate that the structural feature XVIII is present in the molecule. Ions appearing at m/e 201, 200, 97



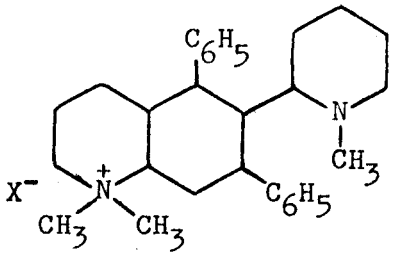
XVIII

and 96 must not involve the piperidine ring but rather be formed from the decahydroquinoline system in the manner shown. Some indication of the substitution pattern on the alicylic ring is therefore obtained since no fragment ion of high intensity containing both benzene rings or both nitrogen atoms is formed.

Hofmann Degradation

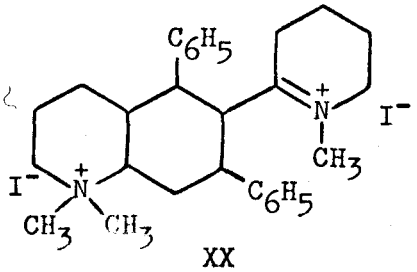
Long heating of the mono- or diquaternary salt of N-methyldihydrolobinaline with potassium tert-butoxide in tert-butanol yields ring opened products. Examination of the products from the Hofmann reaction by mass spectrometry has allowed further insight into the structure of lobinaline.

The monoquaternary salt, XIX, of N-methyldihydrolobinaline can



XIX

be prepared in two ways. Reduction of lobinaline dimethiodide, XX, with sodium borohydride has proved to be the more convenient method. Compound XX is prepared by reaction of lobinaline with methyl iodide at room temperature. Compound XIX can also be obtained by treatment of XVII with methyl iodide in acetone. The product, which has taken

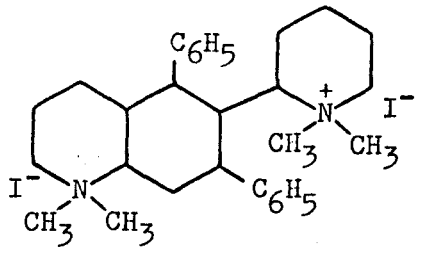


XX

up only one equivalent of methyl iodide, gives an infrared spectrum which is nearly identical with that of XIX obtained by borohydride reduction of XX.

The NMR spectrum of XIX contains a broad double peak at 3.2 δ and a sharp peak at 2.5 δ corresponding to six and three protons respectively. The low field peak is assigned to the quaternary N-methyl protons while that at 2.5 δ is assigned to the tertiary N-methyl protons.

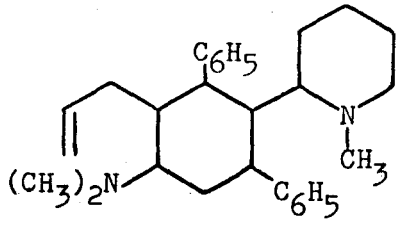
N-methyldihydrolobinaline dimethiodide, XXI, is prepared by



XXI

heating the monoquaternary salt with methyl iodide in acetone at 100°C in a sealed tube.

Long heating of XIX in a concentrated solution of potassium tert-butoxide in tert-butanol yields the methine XXII along with a small amount of XVII. A band at 1640 cm^{-1} in the infrared spectrum

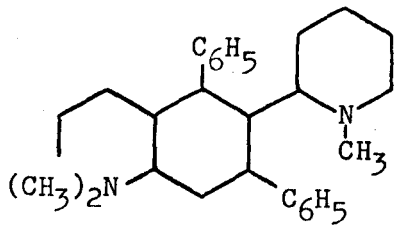


XXII

of XXII can be assigned to the C=C linkage, while bands at 912 and 1000 cm^{-1} are indicative of a terminal methylene group attached to a carbon bearing one hydrogen (51).

A multiplet at 5.0 δ in the NMR spectrum of XXII corresponds to two hydrogens and is assigned to the protons of the terminal methylene group. A highly split one-proton multiplet at 5.5 δ is assigned to the third ethylenic proton; the very complex splitting is caused by the four hydrogens on adjacent carbon atoms.

Catalytic hydrogenation of XXII gives XXIII, a compound showing



XXIII

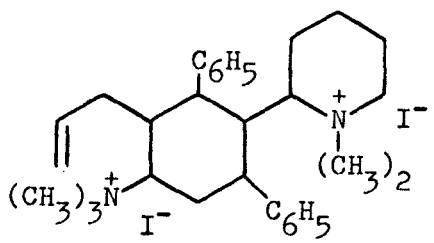
no absorption between 4 and 7 δ in its NMR spectrum and no absorption at 1640 cm^{-1} in the infrared. XXIII does, however, show a triplet centred at 0.9 δ in its NMR spectrum which is indicative of a methyl group attached to a methylene group. Both XXII and XXIII give a nine proton peak at 2.2 δ in their NMR spectra for the three N-methyl groups.

Modified Kuhn-Roth oxidation (57) of XXIII and analysis of the acids formed by vapour phase chromatography of their methyl esters indicates the presence of at least a two carbon side chain. Acetic and propionic acids are formed by the oxidation and can only arise from a CH_3-CH_2-C grouping. The absence of butyric acid from the oxidation product is presumably caused by hindrance to the approach

of the oxidizing agent to the ring carbon atom. The difficulty of oxidation of the carbocyclic ring was demonstrated earlier by Manske's (2) failure to recover two equivalents of benzoic acid from the oxidation of lobinaline.

The mass spectrum of XXII, Figure 2(a), is again dominated by a peak at m/e 98 corresponding to the N-methylpiperidinium ion, A. In addition, peaks of low intensity are found at m/e 416 (molecular ion), 320, 318, 200 and 160. The ions appearing at these masses undoubtedly arise by breakdown of the alicyclic ring as suggested in Scheme 3; however, such processes play a minor role in the total fragmentation of the molecular ion. A peak of very low intensity at m/e 375 is due to the ion formed by loss of the three carbon side chain in XXII as an allyl radical.

Treatment of XXII with methyl iodide yields the dimethiodide XXIV. It was expected that the Hofmann elimination on XXIV would



XXIV

yield trimethylamine and a methine containing only one nitrogen atom. Indeed, when the dimethiodide was heated under reflux with potassium tert-butoxide in tert-butanol an amine odour was detected above the reflux condenser and a very small amount of trimethylamine picrate was formed by sweeping the reaction mixture with nitrogen and passing the

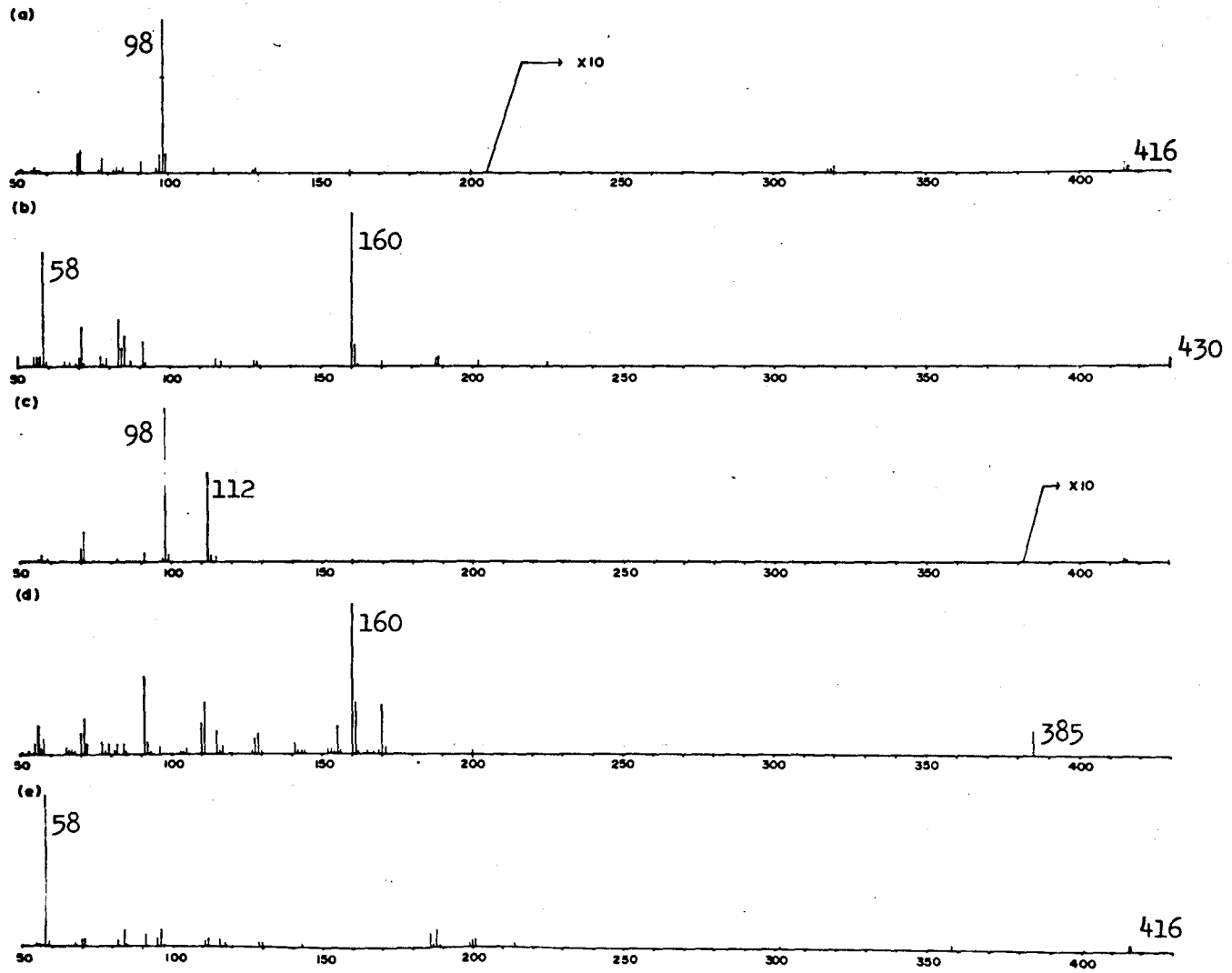
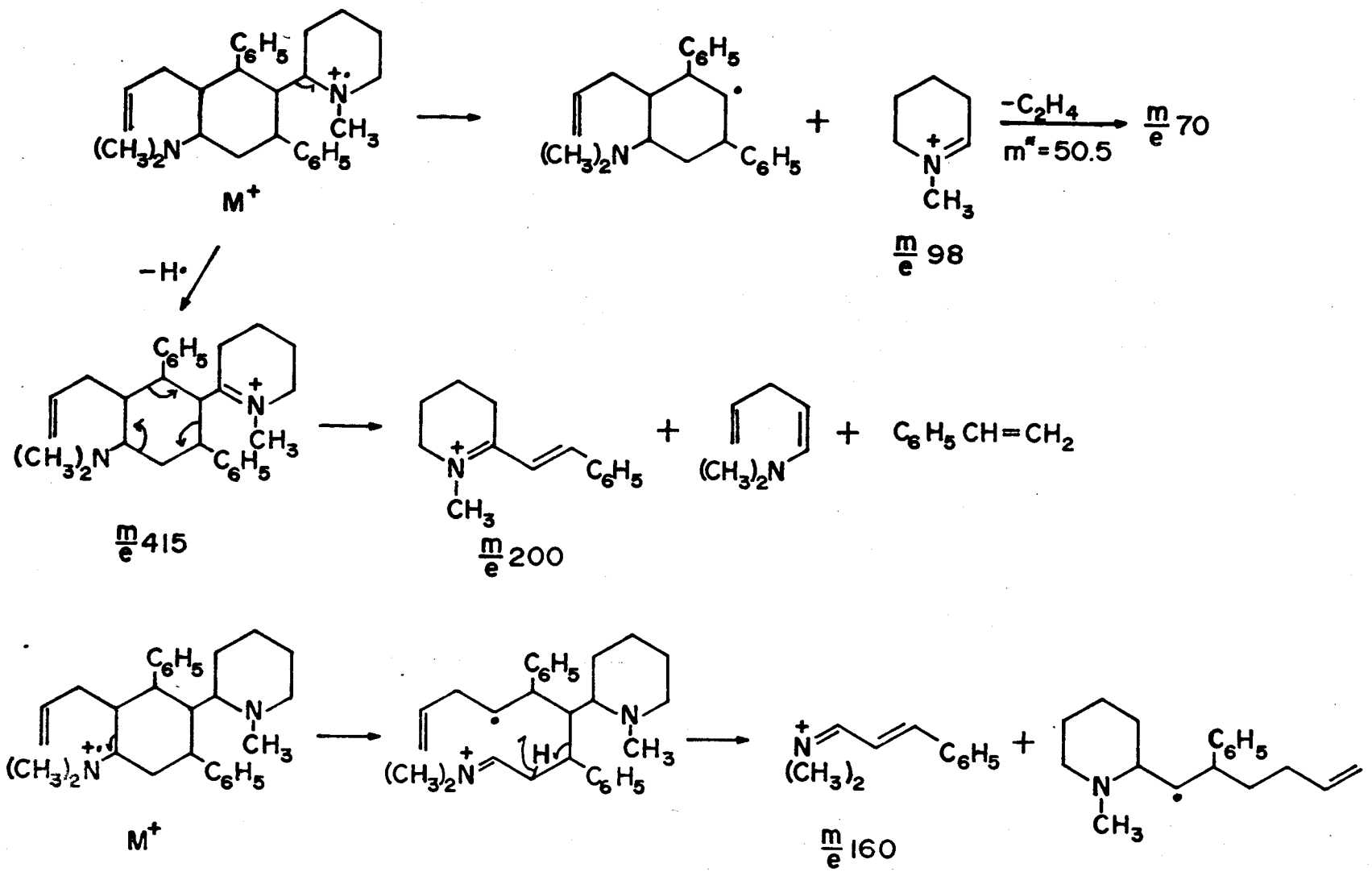
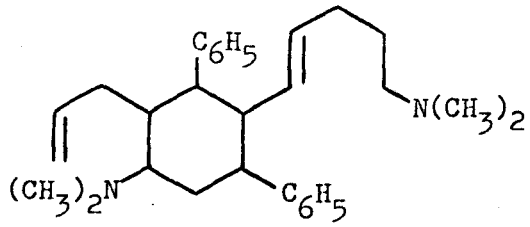


Figure 2. Mass spectra of Hofmann degradation products (a) XXII, (b) XXV, (c) XXX, (d) XXXII and (e) XXXIII.


C6H5
(CH3)2N
CH3
M+
-H·
m/e 415
m/e 200
-C2H4
m* = 50.5
m/e 70
m/e 98
C6H5CH=CH2
m/e 160

Scheme 3

gas stream through a picric acid solution as it left the reaction vessel. However, the amount of trimethylamine formed never corresponded to a significant percentage of the amount of XXIV used in the reaction and the only methines isolated contained two nitrogen atoms. The major product, XXV, shows a series of peaks which integrate for a



XXV

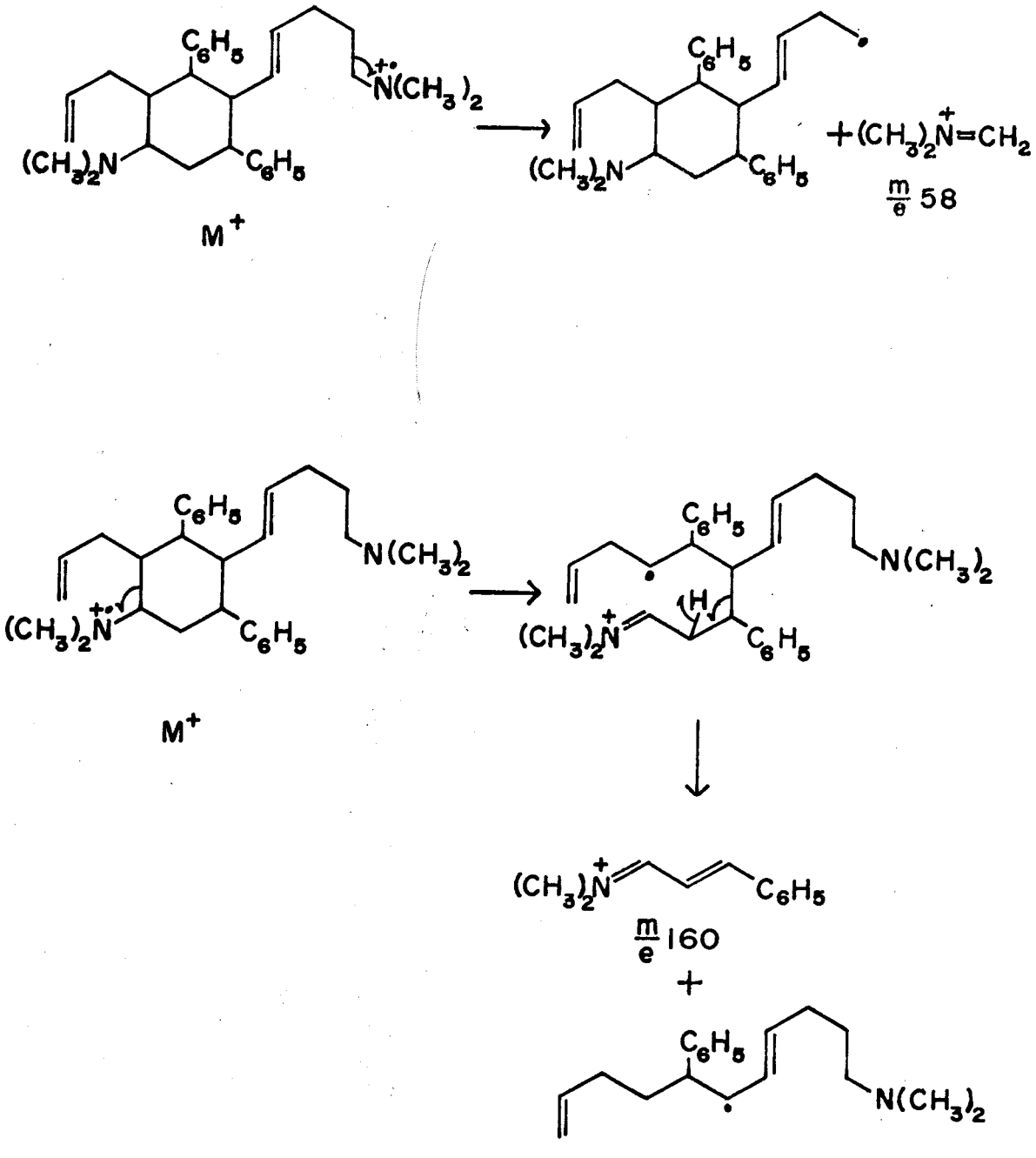
total of five ethylenic protons between 4.5 and 6.0 δ in its NMR spectrum. In addition, the NMR spectrum contains two singlets at 2.0 and 2.2 δ corresponding to six N-methyl protons each.

The mass spectrum of XXV is shown in Figure 2(b). It contains intense peaks at m/e 160 and 58 and a much less intense peak at m/e 430 for the molecular ion. Scheme 4 depicts the mechanism by which the major fragment ions are believed to arise. The ion, B, appearing at m/e 58 is formed from the terminal dimethylamino group. The ion

$$(CH_3)_2\overset{+}{N}=CH_2$$

B

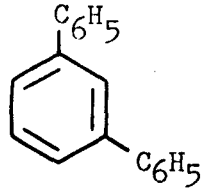
appearing at m/e 160 is postulated to form in the same way as outlined in Scheme 3. In the spectrum of XXV, however, the peak at m/e 160 is much more intense than it was in the spectrum of XXII because of the lack of other favourable ways to open the alicyclic ring. Small peaks


C6H5
N(CH3)2
(CH3)2N
C6H5
M+
+(CH3)2N=CH2
m/e 58
N(CH3)2
(CH3)2N
H
(CH3)2N
C6H5
m/e 160
+
N(CH3)2

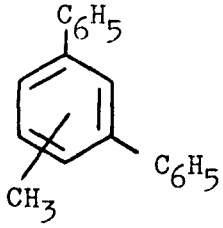
Scheme 4

at m/e 225, 202, 189, 188 and 170 must arise by less obvious routes.

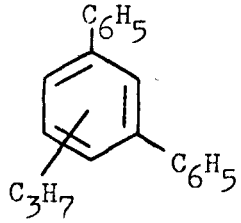
Zinc dust distillation of XXV by Biemann's semimicro technique (58) yields a mixture of compounds which have been partially separated by vapour phase chromatography. All the high molecular weight products appear to be terphenyls. The first fraction collected from vapour phase chromatography (fraction A) shows a very broad maximum at 250 mμ in the ultraviolet. A similar absorption is found for biphenyl and m-terphenyl but not for o- or p-terphenyl (59). The mass spectrum of fraction A, Figure 3(a), indicates that it is a mixture for there are peaks at m/e 230 and 244 in the high mass region which correspond in mass to terphenyl, XXVI, and methylterphenyl, XXVII,



XXVI

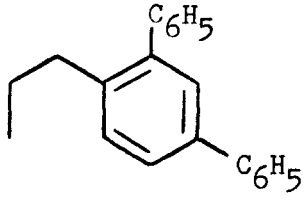


XXVII



XXVIII

respectively. The second fraction from vapour phase chromatography, Fraction B, shows a molecular ion peak at m/e 272 in its mass spectrum, Figure 3(b), with a fragment ion peak at m/e 243 corresponding to the loss of 29 mass units. A molecular weight of 272 corresponds to propylterphenyl XXVIII and the fact that the molecular ion loses twenty-nine mass units gives some indication that XXVIII contains a n-propyl side chain as in XXIX.



XXIX

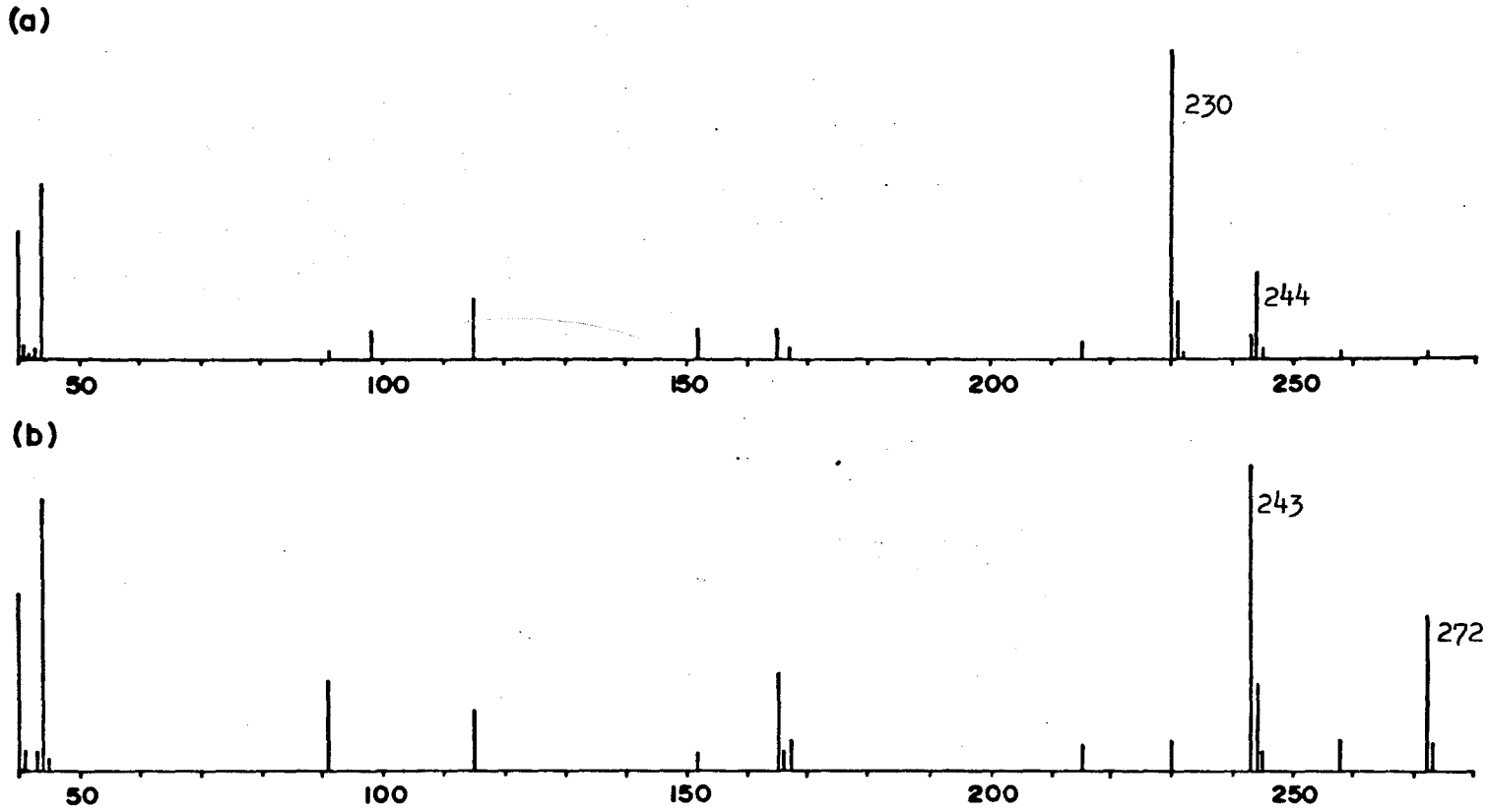
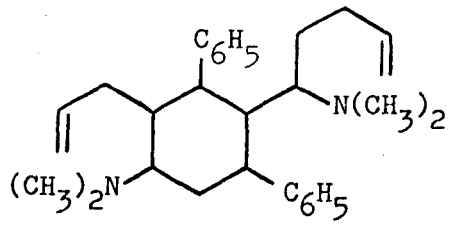


Figure 3. Mass spectra of products from Zinc distillation of XXV; (a) Fraction A and (b) Fraction B.

A second fraction is obtained from the Hofmann reaction on XXIV. Although this fraction is a mixture, mass spectral evidence indicates the presence of a binitrogenous Hofmann product different from XXV. Formula XXX is proposed on the basis of an intense peak at m/e 112 in its mass spectrum, Figure 2(c). The fragment of m/e 112,



XXX

C, arises from the $C_7H_{12}N$ side chain of XXX. Absence of a peak at

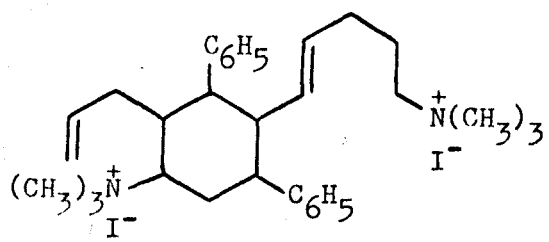
$$(CH_3)_2\overset{+}{N}=CHCH_2CH_2CH=CH_2$$

C

m/e 58 demonstrates the absence of a terminal dimethylamino group.

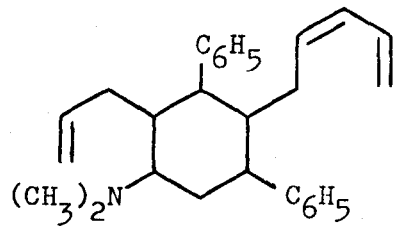
Compound XXX was obtained in small yield and was not pure. A peak at m/e 98 in its mass spectrum is most probably caused by ion A from some starting material, XXII, which is present. A molecular ion peak at m/e 430 indicates, however, that the ring opened compound is present.

A further Hofmann elimination on the dimethiodide, XXXI, obtained from XXV by treatment with methyl iodide, again failed to



XXXI

remove the dimethylamino group attached to the alicyclic ring. The only product from the reaction was basic and showed a molecular ion peak at m/e 385 in its mass spectrum. The compound has been assigned structure XXXII on the basis of spectral evidence.



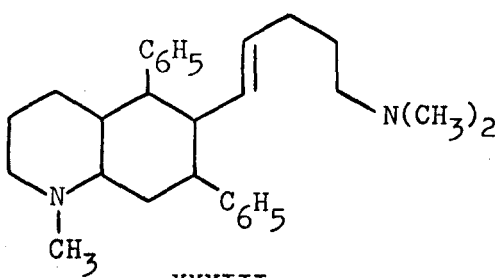
XXXII

Compound XXXII gives a broad maximum at 235 mμ in its ultraviolet spectrum indicating that the two double bonds in the five-carbon side chain are probably conjugated. Such a shift of one double bond into conjugation with another is not unexpected under the strongly basic conditions of the Hofmann reaction. That the terminal double bond has not shifted is indicated by the absence of any C-methyl absorption in the region of 1 δ in the NMR spectrum. The NMR spectrum of XXXII contains N-methyl absorption at 2.3 δ corresponding to six protons. The vinylic proton absorption is a very broad multiplet between 4.8 and 6.2 δ and integrates for seven protons. Structure XXXII requires eight ethylenic hydrogens; however, the discrepancy in the observed integration may be caused by the very broad nature of the multiplet.

The mass spectrum of XXXII is shown in Figure 2(d). The most intense peak in the spectrum is found at m/e 160. The molecular ion peak at m/e 385 is relatively more intense than that found for any of

the other Hofmann products because of the lack of favourable fragmentation processes. The ion appearing at m/e 160 is postulated to arise by the rearrangement outlined in Scheme 4 for XXV, a process which is certainly not as favourable as the simple single bond cleavages found in all of the other compounds studied except lobinaline. Peaks at m/e 170 and 111 can arise from cleavage of the carbocyclic ring as shown in Scheme 5. Either the fragment of mass 111 or that of mass 170 can carry off the charge so both radical ions give peaks in the mass spectrum. Loss of a hydrogen atom from the radical ion appearing at m/e 111 gives rise to the ion at m/e 110. Loss of a methyl radical from the radical ion appearing at m/e 170 gives rise to the ion at m/e 155.

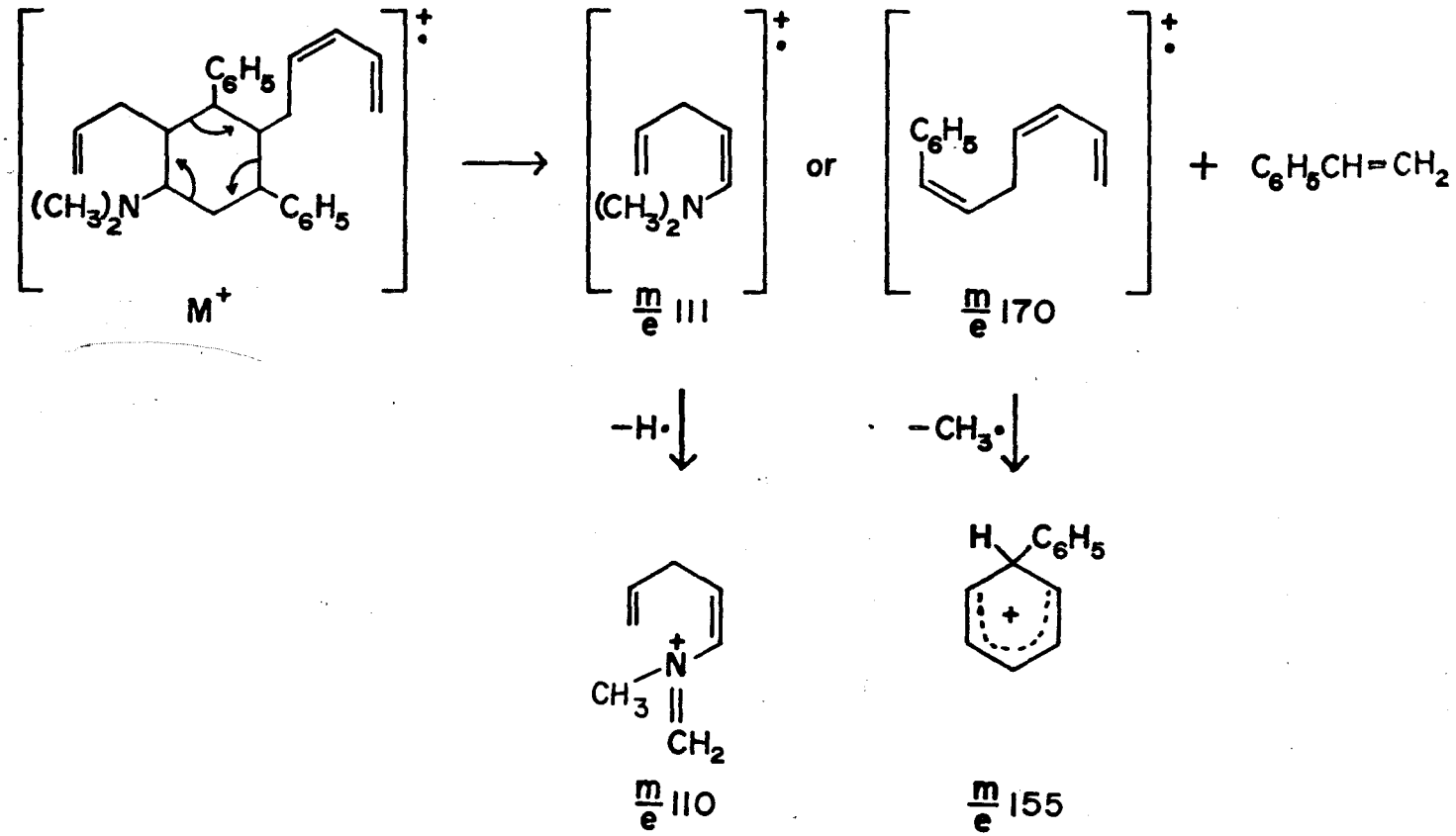
N-methyldihydrolobinaline dimethiodide XXI has also been used as the starting material for a Hofmann degradation sequence. Treatment of XXI with potassium tert-butoxide in tert-butanol yields a mixture of two compounds which are formed in almost equal amounts. One product is identical with XXV while the other is assigned structure XXXIII in which the decahydroquinoline system is intact and the piperidine ring



XXXIII

has been opened.

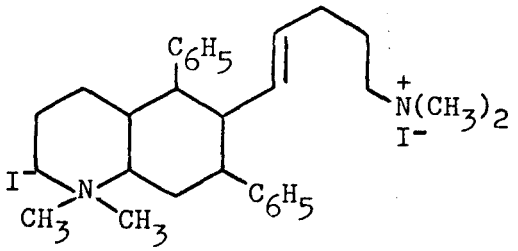
The NMR spectrum of XXXIII contains a multiplet centred at 4.6 δ corresponding to two ethylenic hydrogens, a singlet at 2.2 δ


C6H5
(CH3)2N
C6H5
M+
(CH3)2N
m/e 111
or
C6H5
m/e 170
+ C6H5CH=CH2
-H·
-CH3·
CH3
N
CH2
m/e 110
H
C6H5
m/e 155

Scheme 5

corresponding to three N-methyl hydrogens and a singlet at 2.0 δ corresponding to six N-methyl hydrogens. A peak at m/e 58 corresponding to ion B is the most intense in the mass spectrum, Figure 2(e), as would be expected for a compound containing a terminal dimethylamino group. The absence of a peak at m/e 160 supports the view that the decahydroquinoline system is intact since in such a system no fragmentation of the type depicted in Scheme 4 can occur. The molecular ion peak is found at m/e 416 with very small peaks at m/e 401 ($M-\dot{C}H_3$) and 358 ($M-(CH_3)_2NCH_2\cdot$). Peaks of low intensity at m/e 200 and 201 arise in the manner depicted in Scheme 1 while ions appearing at m/e 186 and 188 must be formed by less obvious routes.

Treatment of XXXIII with methyl iodide yields the dimethiodide XXXIV which, under the conditions of the Hofmann reaction yields



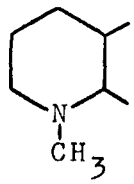
XXXIV

exclusively XXXII.

Examination of the mass spectra of lobinaline, its derivatives and its Hofmann degradation products enabled the assignment of structure IX to the alkaloid prior to the publication of the results of Robison, et al. (4). The presence of a piperideine ring monosubstituted at position 2 was established by the spectra of IX, XVI and XVII. The profound difference in the mass spectra of lobinaline

and dihydrolobinaline shows that the 2-carbon atom of the piperideine ring is involved in the vinylic linkage.

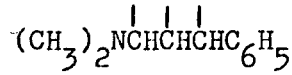
Peaks at m/e 97 and 96 in the mass spectra of IX, XVI, XVII and XXXIII indicate the presence of partial structure XXXV in these compounds. When the disubstituted piperidine ring in XXXV is opened,



XXXV

as in XXII, XXV and XXX, the peaks at m/e 97 and 96 are no longer present. The radical ion at m/e 201 which is found in the spectra of IX, XVI and XXXIII but not in the spectra of XXII, XXV and XXX must also contain structural unit XXXV. From mass considerations alone the radical ion appearing at m/e 201 must contain a benzene ring, thereby establishing the presence of one of the benzene rings within two carbon atoms of the disubstituted piperidine ring. These results in conjunction with biogenetic considerations strongly suggest structure IX for the alkaloid.

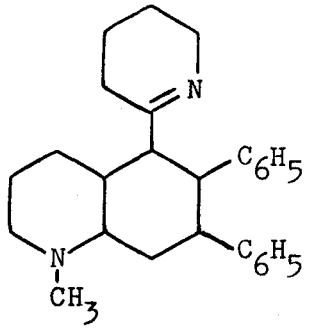
The presence of a peak at m/e 160 in the mass spectrum of every Hofmann product in which the disubstituted piperidine ring is opened supports the presence of partial structure XXXVI in these compounds.



XXXVI

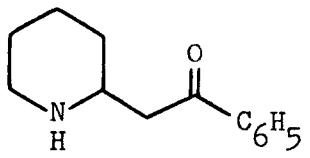
Evidence that the disubstituted piperidine ring is in fact involved in a decahydroquinoline ring system, as opposed to an isoquinoline derivative, is also provided by the mass spectra. Opening a decahydroisoquinoline ring in either direction would produce a dimethylamino group attached to a methylene group. A structure of this type would be expected to give an intense peak at m/e 58 in the mass spectrum, whereas a peak at m/e 58 is only observed in those compounds where it is derived from the monosubstituted piperidine ring.

The substitution pattern about the carbocyclic ring assigned to lobinaline in structure IX is supported by the fact that m-terphenyl is obtained on dehydrogenation of XXV. The only other possible structure for lobinaline on the basis of the mass spectral evidence presented above is XXXVII which would yield o-terphenyl on dehydrogenation.



XXXVII

Structure XXXVII is unfavourable on biogenetic grounds as well. It is apparent from either structure IX or XXXVII that lobinaline can be formally derived from two phenylethylpiperidine moieties. The alkaloid may arise in the plant by condensation of two molecules of a simple base such as XXXVIII. Such a condensation would yield structure IX



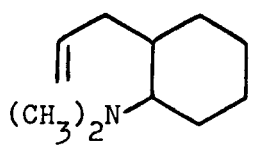
XXXVIII

in preference to structure XXXVII. No one has yet succeeded in isolating a precursor to lobinaline such as XXXVIII from Lobelia cardinalis, although lobinaline has been detected in L. inflata (29), which also contains alkaloids similar in structure to XXXVIII.

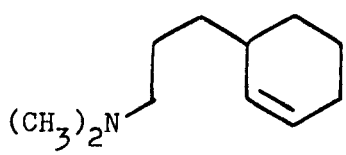
Stereochemistry of lobinaline

Neither the results reported in this thesis nor those published by Robison, et al. (4) allow the stereochemistry of lobinaline to be assigned. The alkaloid contains five asymmetric centres which will make its relative configuration difficult to determine. Some tentative conclusions may, however, be drawn from the observations recorded in this thesis.

McKenna and Tulley (60) have investigated the course of Hofmann ring opening in a rigid decahydroquinoline system, the 4-azasteroids. They found that only those compounds in which the nitrogen atom occupied an equatorial position on the carbocyclic ring yielded methines of type XXXIX. Compounds in which the nitrogen



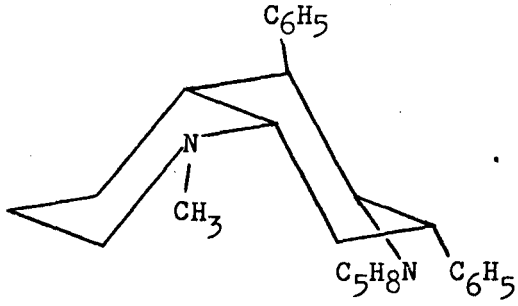
XXXIX



XL

occupied an axial position yielded methines of type XL. They also found that in systems where the nitrogen atom could become equatorial (cis-1-methyldecahydroquinolines) methine XXXIX was formed in the Hofmann reaction.

Lobinaline with three bulky groups in adjacent positions on the carbocyclic ring should approximate a rigid decahydroquinoline system. Since methine XXXIX is formed from lobinaline the nitrogen atom very likely occupies an equatorial position on the carbocyclic ring. This result is compatible with a trans ring juncture in lobinaline. The same result could, however, be obtained from configuration XLI with a cis ring fusion.



XLI

It has been shown that methine XXXIX from acetyllobinaline, XV, is cyclized in glacial acetic acid to a 2-methyloctahydroindole system (54). An analogous reaction has been observed by McKenna and Tulley in their study of the A,B-trans-4-azasteroids. Although this result is compatible with a trans ring fusion in lobinaline, the evidence is not conclusive. The studies reported here do not allow any conclusions to be drawn regarding the configuration of the three substituents on the carbocyclic ring of lobinaline.

EXPERIMENTAL

Infrared spectra were recorded with a Beckman model IR-5 spectrophotometer on liquid films unless otherwise stated. Ultraviolet spectra were measured in methanol solution on a Perkin-Elmer Spectracord 4000. NMR spectra were measured in concentrated solution on a Varian V-4300B or a Varian A-60 spectrometer. Chemical shifts were determined in parts per million (δ) from tetramethylsilane used as an internal standard.

Mass spectra were determined on a CEC21-103C or a Hitachi-Perkin-Elmer RMU-6A mass spectrometer. The CEC instrument was equipped with a metal inlet system and was operated at an ionizing potential of 70 eV and an ionizing current of 50 μA. The Hitachi instrument was equipped with an all glass inlet system and was operated at an ionizing potential of 80 eV and an ionizing current of approximately 50 μA. The inlet systems of both instruments were maintained at 200°C.

Vapour phase chromatography was carried out on SE-30 silicone gum rubber supported on celite or chromosorb in glass columns. All compounds were purified by vapour phase chromatography for mass spectrometric examination.

Lobinaline

Lobinaline was isolated from Lobelia cardinalis L. collected in Grey County in Ontario. The dried, ground stems, leaves and flowers

were extracted with methanol by the procedure of Manske and Marion (61). The crude alkaloid was purified by chromatography on alumina with 5% chloroform in benzene as the eluant. The base obtained from chromatography was taken up in ether and 6 N hydrochloric acid added until no further precipitate formed. The monohydrochloride obtained in this way was recrystallized from acetone-methanol to a constant melting point of 203-205°. After long drying at 80° the salt analyzed for a sesquihydrate.

Calcd. for $C_{27}H_{34}N_2 \cdot HCl \cdot 1.5\ H_2O$: C, 72.1; H, 8.5; N, 6.2; Cl, 7.9%.

Found: C, 71.4; H, 8.6; N, 6.2; Cl, 7.8%.

The free base obtained from the recrystallized monohydrochloride showed one major and one minor component on thin layer chromatography. Careful chromatography of the base on alumina with benzene as the eluant followed by repeated recrystallizations from ether yielded crystals which melted at 116-118°C after long drying at 60° and showed only a trace of impurity on thin layer chromatography.

Calcd. for $C_{27}H_{34}N_2$: C, 83.9; H, 8.8; N, 7.3; 1 $N\text{-}CH_3$, 3.9%.

Found: C, 83.6; H, 9.1; N, 7.7; $N\text{-}CH_3$, 3.9%.

The mass spectrum showed a molecular ion peak at m/e 386, while the mass spectrum of a sample of lobinaline enriched in impurity showed an additional molecular ion peak at m/e 400.

Lobinaline was converted to the dipicrate upon mixing of hot, concentrated methanolic solutions of the alkaloid and of picric acid. The product which crystallized on cooling the reaction mixture was recrystallized from 50% ethanol-water. After drying at 80° under

vacuum the dipicrate melted at 230-233°.

Calcd. for $C_{39}H_{40}N_8O_{14}$: C, 55.5; H, 4.7; N, 13.3%.

Found: C, 55.5; H, 5.0; N, 13.2%.

Conversion of lobinaline to the distyphnate was accomplished in the same way as the preparation of the dipicrate. The product was recrystallized from 95% ethanol and melted, with decomposition, at 225°.

Calcd. for $C_{39}H_{40}N_8O_{16}$: C, 53.4; H, 4.6; N, 12.8%.

Found: C, 53.6; H, 4.9; N, 12.6%.

Dehydrogenation of Lobinaline

Lobinaline (1.0 g) was mixed with Pd black (0.5 g) in a vessel equipped with a cold trap and through which a slow stream of nitrogen was passed. The reaction vessel was immersed in a bath at 300° and maintained at this temperature for one hour. The volatile components were analyzed by vapour phase chromatography on an Apiezon N on Celite column at 85°. Peaks were obtained at retention times corresponding to benzene, toluene, pyridine, α-picoline and ethylbenzene or β-picoline. The latter peak was collected by bubbling the column effluent through methanol. Its ultraviolet spectrum was superimposable on that of β-picoline.

Lobinaline dimethiodide XX

Lobinaline (1.25 g) was treated with excess methyl iodide (2 ml) in dry acetone (30 ml) at 25° for one hour. The solvent and excess reagent were slowly evaporated under water pump vacuum without warming until no more crystals formed. The yield of crystalline dimethiodide was 2.05 g. A small amount of tarry material was recovered upon

complete evaporation of the solvent.

Recrystallization of the dimethiodide from dry acetone followed by long drying yielded an analytical sample which melted at 164-166° with decomposition.

Calcd. for $C_{29}H_{40}N_2I_2$: C, 52.0; H, 6.0; N, 4.2%.

Found: C, 51.8; H, 6.0; N, 4.0%.

Dihydrolobinaline XVI

Lobinaline monohydrochloride (1.1 g) dissolved in glacial acetic acid (40 ml) was shaken at 50° for 24 hours with hydrogen gas at 200 psig in the presence of Adams' catalyst. The resulting mixture was filtered to remove the platinum metal and the filtrate was evaporated to dryness. The residue was taken up in water, basified with concentrated ammonia and extracted with chloroform. The chloroform solution was dried over sodium sulfate and evaporated to yield a yellow oil. The product was chromatographed on alumina with benzene as eluant to yield a colourless oil (1.0 g). The oil obtained in this manner showed a strong band at 3400 cm^{-1} but no band at 1650 cm^{-1} in its infrared spectrum. The mass spectrum had a molecular ion peak at m/e 388 with a small peak at m/e 394 presumably due to the presence of a species containing one reduced benzene ring. Hydrogenation of lobinaline monohydrochloride in methanol solution proceeded much more slowly but the product obtained was not contaminated by products of higher molecular weight than dihydrolobinaline.

Dihydrolobinaline (0.1 g) was converted to N-methyldihydrolobinaline monomethiodide monohydriodide by treatment with methyl

iodide (1 ml) in acetone (15 ml). The solution was heated under reflux for twenty minutes and most of the solvent was then allowed to evaporate. The crystals which separated on cooling were collected. The dried sample melted at 223-224°C.

Calcd. for $C_{29}H_{42}N_2I_2$: C, 51.8; H, 6.3; N, 4.2%.

Found: C, 51.8; H, 6.0; N, 4.0%.

N-Methyldihydrolobinaline XVII

Dihydrolobinaline, XVI, (0.5 g) was heated under reflux with 40% formaldehyde solution (4 ml) and 90% formic acid (6 ml) for 12 hours. The resulting solution was poured into excess cold sodium carbonate solution and extracted with chloroform. The chloroform solution was dried over sodium sulfate and the solvent evaporated to yield a yellow oil. Chromatography of the product on alumina with benzene as eluant yielded a colourless oil (0.45 g) which showed no bands in the region of 3400 cm^{-1} in its infrared spectrum. The mass spectrum had a molecular ion peak at m/e 402.

N-Methyldihydrolobinaline mono quaternary salt XIX

Compound XVII (0.11 g) was heated under reflux with methyl iodide (0.5 ml) in dry acetone (1 ml) for 1.5 hours. During reflux a yellow oil collected on the sides of the flask. The mixture was evaporated to dryness to yield a yellow powder in theoretical yield. The NMR spectrum of the product showed peaks at 2.6 δ and 3.2 δ corresponding to three and six protons respectively.

Alternatively, XX (0.5 g) was dissolved in 95% ethanol (5 ml) and excess sodium borohydride added in portions over 0.5 hour. After

the reaction had stood at room temperature for an hour the excess borohydride was destroyed with acetic acid and the solution evaporated to dryness under reduced pressure. The oily residue was taken up in water, made basic with concentrated ammonia and extracted with chloroform. The chloroform solution was dried and evaporated to yield an oil which turned to a powder on trituration with ether. The infrared spectrum of the product in nujol was the same as that of the product from treatment of XVII with methyl iodide except for the presence of a strong band at 3300 cm^{-1}.

N-Methyldihydrolobinaline dimethiodide XXI

XIX (0.04 g) was placed in a sealed tube with methyl iodide (1 ml) and acetone (1 ml) and heated on the steam bath for 5 hours. The solution became cloudy almost immediately and a dark oil slowly precipitated. Crystals formed at the interphase and filtration of the cooled reaction mixture yielded white needles (0.05 g) which melted at 251-252°. A dried sample analyzed for the dihydrate.

Calcd. for $C_{30}H_{44}N_2I_2 \cdot 2\ H_2O$: C, 49.8; H, 6.6; N, 3.9%.

Found: C, 49.5; H, 6.4; N, 4.0%.

Methine XXII

XIX (1.74 g) was heated under reflux in a solution of potassium (1.8 g) in dry tert-butanol (100 ml) for 16 hours. A few ml of water were added to the cooled reaction mixture and the butanol removed under vacuum. The residue was taken up in chloroform and extracted with water. The chloroform solution was dried over sodium sulfate and evaporated to yield a yellow oil (1.3 g). Chromatography

on alumina yielded a colourless oil (0.47 g) eluted with petroleum ether and a second band (0.15 g) eluted with benzene. The latter gave an infrared spectrum superimposable on that of XVII.

The oil eluted with petroleum ether showed peaks at 1640, 1000 and 912 cm^{-1} in its infrared spectrum and at 2.2 δ (9 protons), 5.0 δ (2 protons) and 5.5 δ (1 proton) in its NMR spectrum. A sample was purified by VPC on a 4-foot column of 2.5% SE-30 silicon gum rubber on celite at 250° for mass spectrometry. The mass spectrum has a molecular ion peak at m/e 416.

XXII was converted to its dipicrate with picric acid in methanol. The product was recrystallized from 95% ethanol and dried at 60° under vacuum whereupon it melted at 188-190°.

Calcd. for $C_{41}H_{46}N_8O_{14}$: C, 56.3; H, 5.3; N, 12.8%.

Found: C, 56.0; H, 5.5; N, 12.6%.

Dimethiodide of Methine XXII, XXIV

XXII (0.3 g) was heated under reflux with methyl iodide (1 ml) in acetone (10 ml) for two hours. During the heating a crystalline compound precipitated. The solvent was evaporated to a small volume and XXIV (0.35 g) filtered off. The dried crystals melted at 263-265°C.

Hydrogenation of Methine XXII

Methine XXII (0.1 g) was dissolved in methanol (10 ml) containing 10% Pd on carbon catalyst and shaken overnight under hydrogen (45 psig). The reaction mixture was filtered and the solvent evaporated to yield a yellow oil (0.1 g) which was purified by chromatography on alumina

with benzene as eluant. The oil, XXIII, obtained showed no bands at 1640, 1000 or 912 cm^{-1} in the infrared and no peaks between 4 and 7 δ in its NMR spectrum. The mass spectrum showed a molecular ion peak at m/e 418.

Modified Kuhn-Roth oxidation of XXIII

The reduction product was subjected to modified Kuhn-Roth oxidation (57) and the acids collected converted to their methyl esters with diazomethane in ether. Vapour phase chromatography on a didecylphthalate column at 75° gave peaks at retention times corresponding to methyl acetate and methyl propionate.

Methines XXV and XXX

Compound XXIV (0.35 g) was added to a solution of potassium (2 g) in tert-butanol (100 ml) and the resulting solution heated under reflux for 24 hours. A stream of nitrogen was passed through the reaction flask during the reflux period and the effluent gases were passed through a saturated solution of picric acid in ether. The picrate (0.028 g) which precipitated melted at 205-210° (trimethylamine picrate m.p. 216°).

The reaction mixture was cooled, a few ml of water added and the solution evaporated nearly to dryness. The residue was partitioned between water and chloroform. The chloroform solution was dried and evaporated to yield a semicrystalline residue which was purified by chromatography on alumina. An oil (0.17 g) eluted with 1:1 benzene-petroleum ether proved to be methine XXV. The mass spectrum showed a molecular ion peak at m/e 430 and the NMR spectrum showed five ethylenic

protons between 4.5 and 6.0 δ. An analytical sample was obtained by careful chromatography on alumina with petroleum ether as eluant followed by thorough removal of solvent under vacuum, whereupon the compound crystallized and melted at 96-98°.

Calcd. for $C_{30}H_{42}N_2$: C, 83.7; H, 9.8; N, 6.5%.

Found: C, 83.8; H, 9.5; N, 6.8%.

A second fraction (0.18 g) eluted with 1:1 benzene-petroleum ether was rechromatographed on alumina. An oil (0.1 g) was eluted with petroleum ether and its infrared spectrum proved to be identical with XXV obtained above. A second fraction (0.06 g) eluted with 1:1 benzene-petroleum ether was semi-crystalline and appeared to be mostly XXX. The mass spectrum showed peaks at m/e 430 and 416 in the high mass region and at m/e 98 and 112 but not at m/e 58 in the low mass region.

Dimethiodide of Methine XXV, XXXI

Dimethiodide XXXI was obtained as a crystalline precipitate (0.18 g) upon mixing methine XXV (0.15 g) with methyl iodide at room temperature and allowing the solution to stand for 2 hours. The product melted at 188-192° with decomposition.

Zinc dust distillation of XXV

Compound XXV (0.015 g) was heated with zinc powder (2 g) in a sealed tube at 320° for one hour in the manner described by Biemann (58). The yellow liquid which collected in the cool part of the tube was recovered and subjected to vapour phase chromatography on a 4 ft. column of 2.5% SE-30 silicone gum rubber on celite at 210°. Two peaks of retention times 11 (Fraction A) and 17 (Fraction B) minutes were collected.

Fraction A gave a broad maximum at 250 mμ in the ultraviolet. It also showed peaks at m/e 230 and 244 in the high mass region of its mass spectrum. Fraction B showed peaks at m/e 243 and 272 in the high mass region of its mass spectrum.

Methine XXXII

Dimethiodide XXI (0.18 g) was added to tert-butanol (100 ml) containing potassium (2 g). The resulting solution was heated under reflux for 60 hours. A few ml of water were added to the cooled reaction mixture and most of the tert-butanol removed under vacuum. The residue was partitioned between water and chloroform. The chloroform layer was dried over sodium sulfate and the solvent removed under vacuum. The oily residue was chromatographed on alumina. Compound XXXII (0.08 g) was eluted with 1:1 benzene-petroleum ether. The product crystallized on standing and melted at 98-100°. The NMR spectrum had a single peak at 2.2 δ (3 protons) and series of broad multiplets between 4.5 and 6 δ (7 protons). Compound XXXII showed a broad absorption at 235 mμ in the ultraviolet and bands at 910, 985 and 1640 cm^{-1} in the infrared. The mass spectrum had a molecular ion peak at m/e 385 and an intense peak at m/e 160.

Methine XXXIII

N-methyldihydrolobinaline dimethiodide, XXI, (0.7 g) was added to tert-butanol (500 ml) containing potassium (7 g). The resulting solution was heated under reflux for 48 hours. The reaction mixture was cooled, a few ml of water added and most of the tert-butanol removed under vacuum. The residue was partitioned between

water and chloroform, the chloroform layer dried and the solvent evaporated. The semicrystalline residue (0.5 g) was chromatographed on alumina. Elution with 1:20 benzene-petroleum ether yielded crystalline material (0.15 g) which melted at 96-98°. The infrared spectrum of this compound was superimposable on that of compound XXV.

A second fraction was eluted with benzene. This compound, XXXIII, melted at 118-121°. Recrystallization from ether raised the melting point to 125°. Compound XXXIII absorbed at 1680 cm^{-1} in the infrared. The NMR spectrum contained singlets at 2 δ (6 protons) and 2.3 δ (3 protons) and a multiplet centred at 4.6 δ (2 protons). The mass spectrum had a molecular ion peak at m/e 416 and a very intense peak at m/e 58.

Compound XXXIII was converted to the dipicrate with picric acid in methanol. The crystals which separated on cooling the reaction mixture were collected and recrystallized from absolute ethanol. The dried dipicrate melted at 248-250°.

Calcd. for $C_{41}H_{46}N_8O_{14}$: C, 55.3; H, 5.2; N, 12.6%.

Found: C, 55.5; H, 5.4; N, 12.4%.

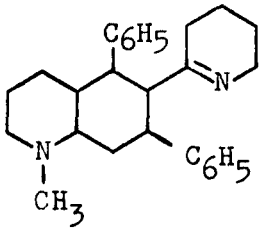
Dimethiodide of Methine XXXIII, XXXIV

Compound XXXIII (0.5 g) was treated with excess methyl iodide (1 ml) in dry acetone (20 ml) at room temperature. After one hour the solvent was evaporated to yield XXXIV which was immediately treated with potassium (2 g) in tert-butanol (100 ml) for 24 hours under reflux. The reaction mixture was cooled, several ml of water added and most of the tert-butanol removed under vacuum. The residue was partitioned between chloroform and water, the chloroform solution

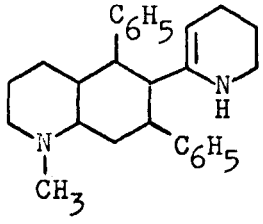
dried and the solvent evaporated. Chromatography of the semicrystalline residue yielded XXXII, melting at 96-98 and giving an infrared spectrum identical with that of XXXII prepared by Hofmann elimination on XXV.

SUMMARY

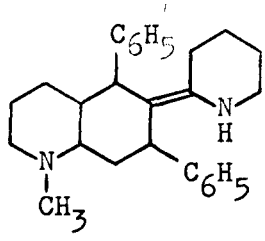
Lobinaline has been degraded through a Hofmann exhaustive methylation sequence to a methine containing only one nitrogen atom. Spectral studies, particularly mass spectrometry and nuclear magnetic resonance spectrometry, on the alkaloid, its derivatives and its Hofmann degradation products have established the presence in the alkaloid of a 2-substituted piperideine ring and a decahydroquinoline ring system which is substituted in three adjacent positions of the carbocyclic ring. The results obtained allow assignment of structure IX to the alkaloid and indicate that the compound also contains



IX



XIVa



XIVb

variable amounts of tautomers XIVa and XIVb.

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