APPLICATION OF MASS SPECTROMETRY IN THE STUDY OF SOME ISOQUINOLINE ALKALOIDS

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By

CHI-KUEN YU, B.Sc.

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AUTHOR: Chi-Kuen Yu, B.Sc. (Taiwan Normal University)

SUPERVISOR: Professor D. B. MacLean

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SCOPE AND CONTENTS:

The structures of three new tetrahydroprotoberberine alkaloids, caseanadine, cavidine and apocavidine have been determined on the basis of mass spectral and p.m.r. data.

The mass spectra of spirobenzylisoquinoline alkaloids with different oxygenated substituents in the five-membered ring have been recorded. Fragmentation mechanisms are proposed to account for the major peaks in all the spectra. High resolution mass measurements have been used to aid in the interpretation of the spectra. The structure of a new spirobenzylisoquinoline alkaloid, F-38, has been elucidated by spectroscopic methods.

ACKNOWLEDGEMENTS

It is a great pleasure to express my sincere gratitude to Professor D. B. MacLean for his guidance and encouragement during the course of this work.

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GENERAL INTRODUCTION

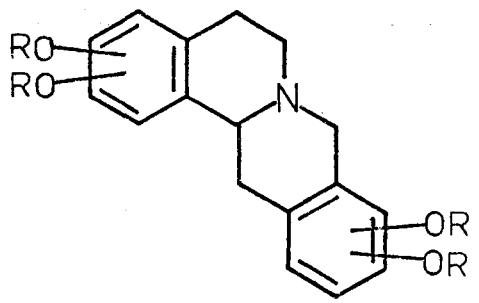
In the last decade, mass spectrometry has been used extensively in the structural studies of natural products, including alkaloids. In this thesis mass spectrometry has been applied in the elucidation of the structures of several new alkaloids of the isoquinoline group. Accordingly, in the introduction to this thesis a survey of the literature on the application of mass spectrometry to isoquinoline alkaloids has been included.

The mass spectra of tetrahydroprotoberberine (1) alkaloids have been studied thoroughly and new alkaloids of this type are readily recognized by their fragmentation pattern. Three new alkaloids, caseanadine, cavidine and apocavidine, were isolated recently and were recognized by their mass spectra as alkaloids of the tetrahydroprotoberberine type. The substitution patterns of the various rings, however, could not be determined from the mass spectroscopic data, and proton magnetic resonance (p.m.r.) data had to be used to obtain this information. By a combination of these methods, the structures of these alkaloids have been determined unambiguously.

The structure of ochotensimine, the first member of the spirobenzylisoquinoline (2) group of alkaloids to have its structure resolved, was determined in 1964. A number of alkaloids of similar type have been isolated since and their structures elucidated. The mass spectra of these alkaloids, however, have not been studied systematically.

Part of the work reported in this thesis is devoted to a study of the fragmentation, upon electron impact, of alkaloids of this group in which different oxygenated substituents are present in the five-membered ring. High resolution mass measurements were used extensively to determine the composition of each ion. In addition, several model compounds of the spiroisoquinoline type were prepared and used to aid in the interpretation of the fragmentation. It has been found that the fragmentation pattern is affected greatly by the substitution in the five-membered ring.

The characteristic fragment of spirobenzylisoquinoline compounds were found in the spectrum of the new alkaloid, F-38. The final structure of F-38 was resolved by a combination of mass spectrometry and other spectroscopic methods.



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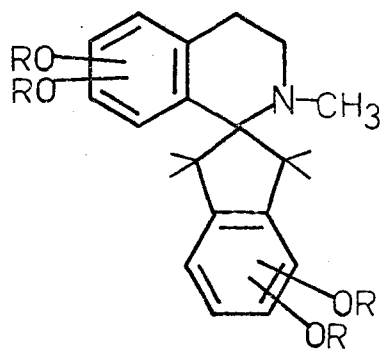

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HISTORICAL INTRODUCTION

Instrumentation

The historical development of mass spectrometry goes back many years. As early as 1886, Goldstein discovered positive rays in a low pressure electrical discharge tube. Later, W. Wien, in 1898 (1), showed that the rays of Goldstein were deflected in electric and magnetic fields and then established that these rays carried a positive electrical charge. In 1912, J.J. Thomson (2) discovered that neon consisted of a mixture of two different isotopes (mass 20 and 22) rather than only a single isotope by using a parabola mass spectrograph. This observation of the existence of stable isotopes is perhaps the greatest achievement that can be claimed by mass spectrometry.

The prototypes of modern mass spectrometers were built by Dempster (3) and Aston (4). The two designs are suited for different purposes, the former instrument is better suited for measuring the relative abundances of ionic species, while the latter is particularly useful for accurate mass measurement. Since then, both types of instrument have been modified and refined. During the past two decades, the development of sophisticated electronic devices has made reliable mass spectrometers available commercially. An excellent account of the history and instrumentation of mass spectrometers is available in books by Kiser (5) and Roboz (6).

Two common types of mass spectrometers used in organic chemistry laboratories will be discussed briefly.

A. Single-focusing mass spectrometer

Single-focusing mass spectrometers are often of the sector type. The principle of the sector instrument is the same as that of Dempster's original design. It was first introduced by Nier in 1940 (7). The sector magnetic analyzer is shown schematically in Figure 1. Positive ions produced in the ion source are accelerated by a potential difference of a few thousand volts between two plates. It can be seen that deflection takes place in a wedge-shaped magnetic field H and that direction focusing is present. The ion beam enters and leaves the field at right angles to the boundary, so the deflection angle is equal to the wedge angle θ. The ions passing through the slit S_2 impinge on a detector. The geometry is symmetrical in that the source and the detector are equidistant from the magnet. The relationship between the radius r of the ion path in the field, the accelerating voltage V, the strength of the magnetic field H, and the mass to charge ratio (m/e) can be derived in the following way.

The potential energy eV of the particle is equal to its kinetic energy.

$$eV = \frac{1}{2} mv^2 \qquad (1)$$

In the magnetic field, the force evH experienced by the ion will be balanced by a centrifugal force mv^2/r.

$$Hev = \frac{mv^2}{r}$$

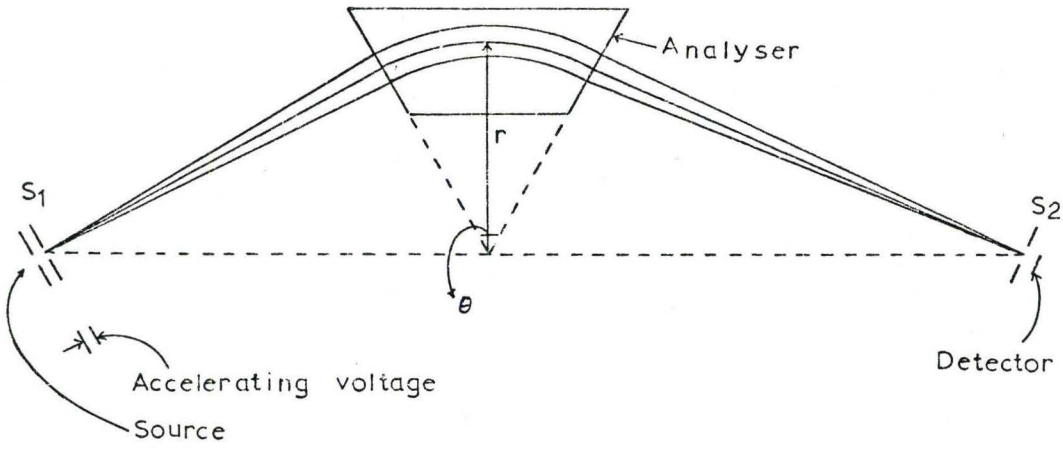


Figure 1. A sector mass spectrometer

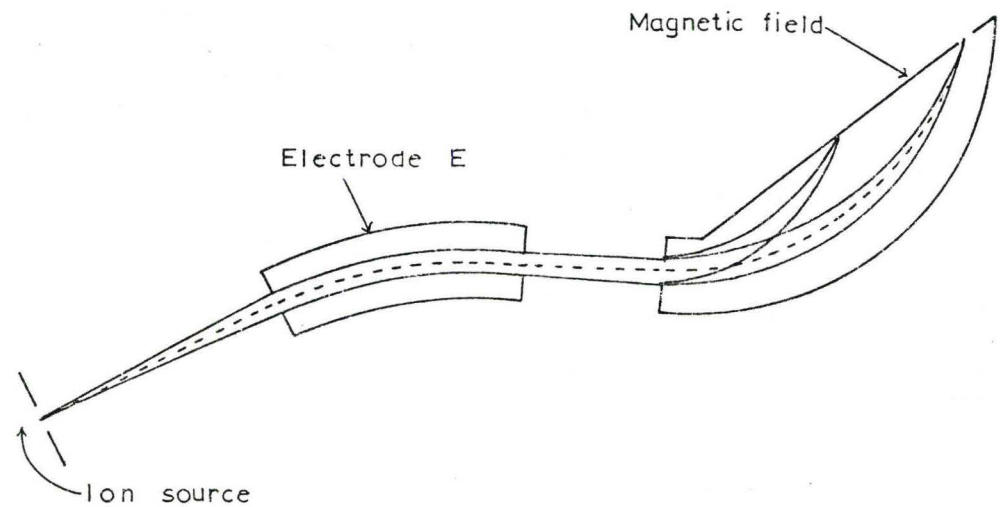


Figure 2. Double focusing design of Mattauch and Herzog

$$r = \frac{mv}{He} \tag{2}$$

Elimination of v from equation (1) and (2) gives

$$\frac{m}{e} = \frac{H^2r^2}{2V} \tag{3}$$

At constant H and V, ions with given m/e will be deflected and reach the detector. By changing V or H different m/e groups arrive at the detector successively. In the case of electric scanning, H is kept constant, and a continuous decrease of V in a reproducible manner leads to the recording of the ions. The same is achieved if V is kept constant and the magnetic field is increased slowly. Both practices have advantages as well as disadvantages. With the magnetic scan, a larger mass range can be covered in a single sweep. With electric scanning the resolution and intensity fall off at lower accelerating potential because of the increased relative contribution of initial thermal and kinetic energy of the particles. On the other hand, electric scanning is easier to achieve, but the mass range is limited.

The choice of sector angle depends on each designer's consideration. Currently, instruments with sector angles, θ, of 60° and 90° are all available.

B. Double-focusing mass spectrometer

The geometry of Mattauch and Herzog for attaining double focusing for all masses is shown in Figure 2. The main reason for introducing a radial electric field between the source and magnetic field is for velocity focusing.

If an ion of charge, e, is projected into a radial electric field, E, at right angle to the boundary, it experiences a force eE normal to the direction of motion, which is balanced by a centrifugal force mv^2/r. The result is a circular orbit of which the radius r is simply calculated.

$$eE = \frac{mv^2}{r}$$

$$r = \frac{mv^2}{eE} \qquad (4)$$

At constant E, ions with given e deflect according to their kinetic energy. A slit is placed between the electrostatic analyzer and the magnetic field to let ions of certain energy pass through. The energy spread, which is a serious problem in single focusing mass spectrometers, is eliminated in this manner. After subjecting to both velocity and direction focusing, ions with different mass can be separated cleanly. Thus, high resolution is achieved.

The term "resolution" is used to describe the extent of peak separation from the neighbouring one. The height of the "valley" between two adjacent peaks of equal intensity is, therefore, an indication of the extent of peak separation. The quantitative definition of "resolution" is rather arbitrary. A currently accepted definition (8) states that the resolution of a mass spectrometer is equal to $M/\Delta M$ with the specification that two ion beams, M and $M + \Delta M$, of equal intensity be recorded as two peaks with the valley between them no greater than ten per cent of the intensity of the peak M, i.e. $\Delta H/H = 10\%$, as shown in Figure 3.

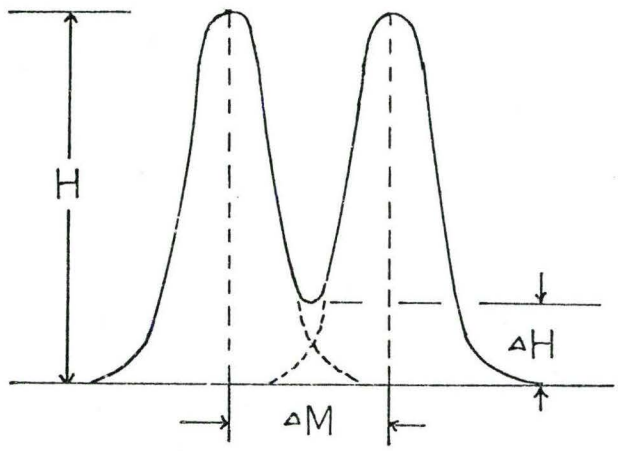


Figure 3. "per cent valley" definition of resolution

The resolving power of single-focusing magnetic deflection mass spectrometers is usually between 300 and 600. With such resolving power, only nominal masses will be recorded from the low mass end up to 600. As the resolving power increases, ions with small mass differences can be separated. Two compositions, CO and N_2, cannot be distinguished by low resolution mass spectrometry, because both compounds will be recorded at m/e 28. At high resolution, the ions can be detected as separated peaks, since the exact masses for CO and N_2 are 27.99491 and 28.00614 mass units, respectively. The need for this high resolution in organic mass spectrometry becomes obvious when one considers that for any nominal mass number many possible combinations of carbon, hydrogen, oxygen, and nitrogen exist. Moreover, the number of possible combinations increases rapidly with mass.

High resolution mass spectrometry was introduced to organic chemists by Beynon (9), who demonstrated the potential power of the method in solving organic chemical problems. If the mass can be determined within a certain limit, one can assign elemental compositions to the ions. The loss of two molecules of carbon monoxide successively from the molecular ion of anthraquinone (9) was confirmed by measuring masses of the fragment ions accurately. Until about 1964, the technique of high resolution mass spectrometry had normally been applied only to establish the elemental compositions of the most abundant, or potentially most diagnostic, fragment ions. Such an approach is wasteful, for it is clear that in ignoring the elemental compositions of many ions, much useful information may be discarded.

Currently, the spectra of both a sample and a reference compound are recorded on a photographic plate. Since the distances of the various focal points are directly related to the square root of the corresponding masses, see Equation (3), the accurate mass of any species can be determined by measuring the exact distance of this line relative to two other lines produced by ions of known composition, usually perfluorokerosene. The distance and the intensity of each line can be read on to magnetic tape through the use of an automatic comparator-densitometer. The tape can then be processed by the computer to give ion compositions.

The problem of time-consuming calculation of the mass of each ion, used earlier, was solved by the introduction of computer techniques. A large amount of information can be presented in a concise and clear

form by the use of "element maps", introduced by Biemann (10). Other methods of presentation have been discussed in the book of Budzikiewicz, Djerassi and Williams (11). Computers have also been used to recognize the molecular ion and for simple interpretation of the mass spectrum (12, 13).

A high resolution mass spectrum requires only a small sample. Set-ups have been described in which gas chromatographs are linked directly to the mass spectrometer. Several methods have been used to extract the sample from carrier gas. These topics have been reviewed (14).

Theory and Interpretation

When neutral molecules collide with electrons of given kinetic energy, the collision may be elastic or inelastic. In the former case, the internal energies of the two particles are preserved, and no structural change occurs in the molecule. In the latter case, many possible changes may occur (6). The two most important processes in mass spectrometry are ionization and dissociative ionization, in which the positive ions produced can be accelerated, deflected and detected in the mass spectrometer. The ionization process produces the molecular ion and dissociative ionization gives rise to various fragment ions, the intensity of which depends on the bond strength and the configuration of the molecule.

The so-called quasi-equilibrium theory of mass spectrometry, originally developed by Eyring and his co-workers (15), is based on the following assumptions.

(1) The prime process in ion formation is the transfer of energy from the bombarding electron to the neutral molecule, resulting in the formation of a molecular ion, which is in an electronically excited state.

(2) The transition from excited state to many low-lying electronic states can transfer electronic energy into vibrational energy. The excess energy is rapidly distributed over all internal degrees of freedom. Fragmentation of the parent ion occurs when sufficient vibrational energy is concentrated in a particular bond.

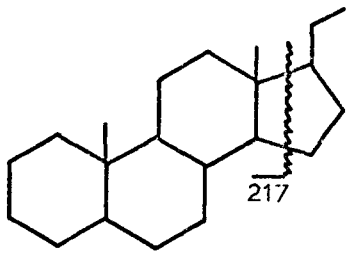
(3) The fragment ions that are observed in the mass spectrum are formed in a series of competing and consecutive unimolecular reactions which are similar to the rate processes of conventional chemical kinetics.

(4) The fragment ions thus formed may again have a sufficient amount of excitation energy to undergo further decomposition.

The theory has been applied to several molecules of rather small dimension (16), and the results are only in semi-quantitative agreement with experiments. The poor agreement between practice and theory is due to the poor mathematical approximations and the many parameters involved in the calculation. For most organic compounds, the interpretation has to be made through a mechanistic approach. This approach is concerned with rationalization of how and why certain fragmentations occur. By making a few simple assumptions which are based largely on analogy to ground state organic chemistry, a fairly consistent picture is obtained which applies to the vast majority of cases.

Under relatively high ionization voltage (50 ev - 100 ev), the initial reaction $M + e \longrightarrow M^{\dagger} + 2e$ can take place on any bond in the molecule (16), giving an electronically excited species, which decomposes statistically after dissipating the excess electronic energy through vibrational energy to yield species of lower-lying excited electronic states. It is quite reasonable to apply this theory to saturated hydrocarbons, because the carbon-carbon bond strengths are nearly equal, and similar to those of the carbon-hydrogen bonds. For unsaturated compounds or molecules containing heteroatoms, the situation is quite different. If one considers the molecular ion as a resonance hybrid of the various canonical forms usually drawn in organic chemistry, and if one of them appears to be clearly more stable, then it is both reasonable and useful to represent the molecular ion by that canonical form. Frequently this turns out to be the one with the charge residing on the heteroatom or unsaturated center. This assumption was used extensively in the interpretation of organic spectra by Budzikiewicz, et al. (11) and supported by experimental evidence (17, 18, 19). This concept of charge localization was challenged recently by Mandelbaum and Biemann (20), who prefer the dynamic distribution concept, which distributes the charge throughout the molecule, statistically maximized at the site of the lowest ionization potential. Kinstle and Oliver (21) concluded from their experiments that it is difficult to predict the extent of charge mobility in a particular molecule, but that in some instances a dynamic distribution of charge appears to operate. Although further experimental evidence is needed to prove or disprove this concept,

nevertheless, the assumption of charge localization is a useful tool in predicting which bonds are likely to break in a given molecule. An excellent example was given by Budzikiewicz et al. (11), in which the spectra of the steroids, 5α-pregnane (3) and 20β-dimethylamino-5α-pregnane (4), were compared.



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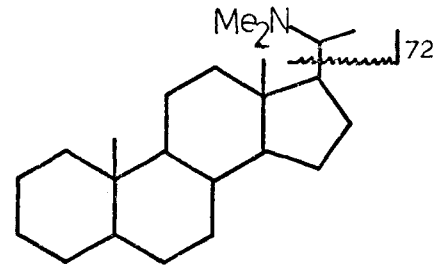


4

The multiplicity of peaks in the mass spectrum of 3 is a reflection of the approximately equal ease with which the various carbon-carbon or carbon-hydrogen bonds are broken, cleavage of tertiary certers (for example, m/e 217) being somewhat preferred. Introduction of a dimethylamino group at C-20, as in 4 leads to a dramatic simplification of the mass spectrum. Charge localization is assumed on the nitrogen atom, and the bond cleavage beta to the nitrogen atom will give a strong fragment ion, $(CH_3)_2\overset{+}{N}=CH-CH_3$, at m/e 72.

Other rules of predicting which peaks will be predominant in a mass spectrum have been summarized by McLafferty (22). Often the reasons behind such rules can be postulated using concepts of modern physical-organic chemistry such as resonance, hyperconjugation, polarizability, inductive effects, steric effects and so on.

The useful generalizations made above are largely a result of experience, because there is no assurance that the fragmentation

process and the structures of molecular ions and fragment ions are actually as predicted. Since the ionic species cannot be isolated and examined in the classical sense, other techniques have to be used to achieve information about ion structures.

(1) Isotope labelling: Isotope labelling is a widely used method not only in mechanistic studies, but also in structural elucidation, in which a stable isotope is introduced specifically by a known reaction. The fragment containing the stable isotope can be detected by the shift of the peak in the spectrum as compared with the unlabelled one. Ion structures and fragmentation mechanisms can sometimes be deduced in this manner. The methods for introducing heavy isotopes into organic compounds have been discussed extensively by Budzikiewicz et al. (23).

An isotope effect has been found in many cases with discrimination against deuterium (24). Thus, caution must be taken in the interpretation of mechanisms involving heavy isotopes.

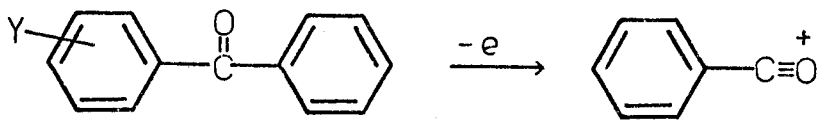
(2) Metastable peaks: The rate of decomposition of ions in a mass spectrometer may be such that some of these ions will reach the collector without decomposition, others will decompose prior to acceleration, and yet others actually decompose after acceleration but before entry into the analyzer. Ions with a half-life of the order of 10^{-6} sec. are sufficiently long-lived to be accelerated out of the ionization chamber, but decompose in transit and are recorded neither as m_1 nor m_2, but as a small diffuse peak. These peaks are called metastable peaks and the process giving rise to these peaks, $m_1^+ \longrightarrow m_2^+ + m_3$, is termed a

metastable transition. The peak m^* observed in the spectrum can be correlated with the decomposing ion m_1 and daughter ion m_2 by the following expression.

$$m^* = \frac{m_2^2}{m_1} \qquad (5)$$

Until very recently the presence of metastable peaks for the transition $m_1^+ \longrightarrow m_2^+ + m_3$ has been regarded as evidence that the neutral atoms of mass $(m_1 - m_2)$ are ejected in a one step process as a single entity. Usually this is correct, but some instances have been found in which the appropriate metastable peaks are likely to correspond to a two-step process (25). Extra caution has to be taken in citing the metastable evidence to support the proposed mechanism, in which the fragment ion is favored in one step process. The shape of the metastable peak has been found to be related to accelerating voltage and the kinetic energy released during the transition (26, 27). It is apparent that the shape of a metastable peak (27) may be a sensitive function of the structures of the parent and daughter ions. Hence ions identical in structure and energy should in principle be identified by the appearance of identical metastable peaks for their further decomposition. Shannon and McLafferty (28) have classified $C_2H_5O^+$ ions into a few distinct structural types on this basis.

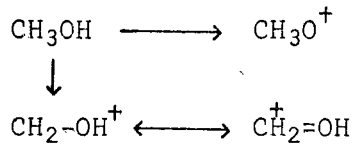
(3) Kinetic approach: The kinetic approach to mass spectra has been developed extensively by Bursey and McLafferty (29, 30). This method was used for deducing the structure of ions. In the decomposition of substituted benzophenones (5), benzoyl ion (6) is formed. If the



5 6

ion 6 is formed with the same energy distribution, its rate of further decomposition will be independent of the substituent Y and the same in all cases. The ratio of peak intensities $C_6H_5CO^+/M^+$ will be a measure of the rate of formation of 6 from the parent ion 5 . In the plot of ratio of ion intensities against Hammet σ values for a series of substituted benzophenones, a straight line was obtained. Thus, the intact structure of the substituted ring must be retained up to the transition state. In contrast, a common molecular ion species was proposed in order to account for the fact that the rates of formation and rates of subsequent decomposition of $M-C_2H_4$ ions derived from substituted phenetoles are independent of the position of the second substituent (31).

(4) Energy Cycles: It is possible to define in some cases the mode of formation and the structure of fragment ions by using data on ionization and appearance potentials, heats of formation and bond dissociation energies. An explicit example is provided in the decomposition of methanol (32). There are two structural possibilities for the M - 1 peak as shown below. From appearance potential measurement, the ion, CH_3O^+, is excluded because the appearance potential calculated



for its formation is greater than that observed. The appearance potential for the alternative structure would be expected to be lower because of resonance stabilization.

Mass Spectrometry of Isoquinoline Alkaloids

The full potential of mass spectrometry to solve structural problems in complex organic molecules was first shown by Biemann, who settled the carbon skeleton of sarpagine by the mass spectral method (33) instead of by other conventional methods. This success triggered the rapid development and remarkable improvement of this instrumental method.

The applications of mass spectrometry to the chemistry of natural products have been discussed in detail in books by Budzikiewicz et al. (23, 34) and reviewed by Biemann (35). Hence the discussion in this section will be limited to those cases which relate to the present research, namely isoquinoline alkaloids.

The remarkable success of mass spectrometry, when applied to the structural elucidation of indole alkaloids, is undoubtedly due to those structural features, which make the indole alkaloids exceptionally suitable for mass spectral studies. Firstly, the well recognizable molecular ion peak which is observed in all indole alkaloids, represents a system of stability in the molecule, namely, the nitrogen-containing

TABLE I

Mass Spectra of Isoquinoline Alkaloids

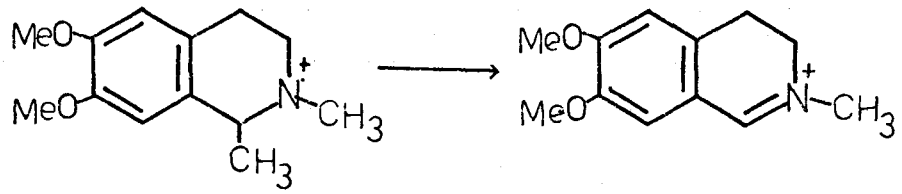
Simple isoquinoline alkaloids	36
1-Benzylisoquinoline alkaloids	37, 38, 39, 40, 41
Pavine and Isopavine alkaloids	42, 43
2-Benzylisoquinoline alkaloids	44, 45
Bisbenzylisoquinoline alkaloids	46, 47, 48, 49, 50
Cularine and related alkaloids	37, 51
Proaporphine alkaloids	52, 53, 54
Aporphine alkaloids	37, 55, 56
Protoberberine alkaloids	37, 39, 57, 58, 59, 60, 61
Protopine alkaloids	62, 63, 64, 65
Phthalide alkaloids	37, 35
Spirobenzylisoquinoline alkalcids	66, 109
Rhoeadine and related alkaloids	67, 68, 69, 70, 71, 72
Morphine and related alkaloids	73, 74, 75, 76, 77, 78
Hasubanonine and related alkaloids	79
Emetine and related alkaloids	80, 81, 82
Erythrina alkaloids	83, 84
Amaryllidaceae alkaloids	65, 85, 86, 87, 88, 89, 90
Benzophenanthridine alkaloids	91, 92
Dibenzopyrrocoline alkaloids	93
Phenethylisoquinoline and Colchicine alkaloids	65, 94

heteroaromatic nucleus. Secondly, the alicyclic nitrogen-containing system results in the breaking of bonds in a specific way with the ensuing pattern being characteristic of a particular ring system. Similar features are present in isoquinoline alkaloids. Molecular ion peaks of most alkaloids, with the exception of simple 1-substituted isoquinoline systems (vide infra), are observable and in some cases these even become the base peaks, e.g. proaporphine alkaloids.

The two most plausible sites to accommodate the charge density in the molecular ion of an isoquinoline alkaloid are the aromatic ring and the nitrogen atom. The strong molecular ion peak and the prevalence of doubly-charged ions in aromatic compounds lend support to the contention that the aromatic nucleus constitutes a center of stabilization for the positive charge (11). The concept of charge localization which facilitates greatly the interpretation of the mass spectra of amines, also applies to isoquinoline alkaloids. A list of references dealing with the mass spectra of isoquinoline alkaloids is shown in Table I.

1-substituted Isoquinoline Alkaloids

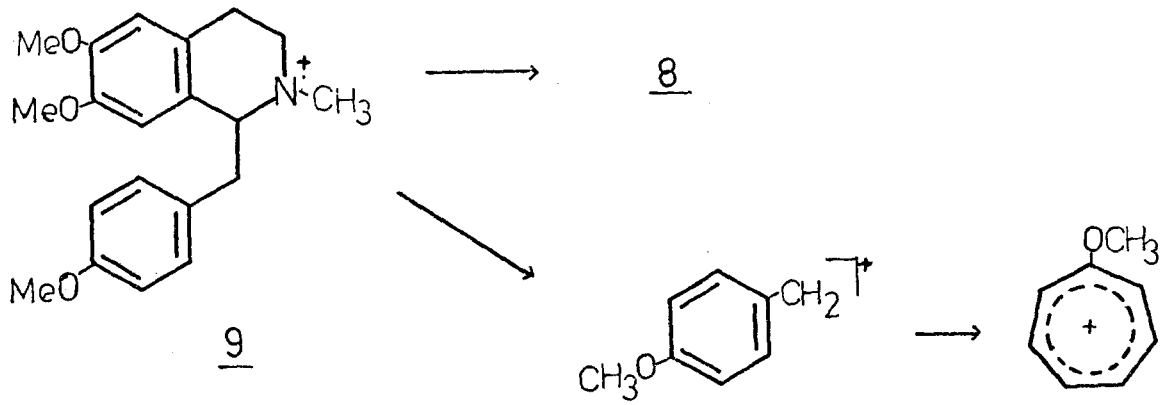
The most readily broken bond in the 1-substituted 1,2,3,4-tetrahydroisoquinolines molecules is the one which is benzylic and β to the nitrogen atom. As a result, the molecular ion peak is usually very small and the strongest peak comes from the cleavage of the weakest bond. As shown in the spectrum of the simple isoquinoline alkaloids, carnegine (7) (36), the only significant peak is the base peak 8 . The immonium ion (8) formed by simple cleavage is so stable

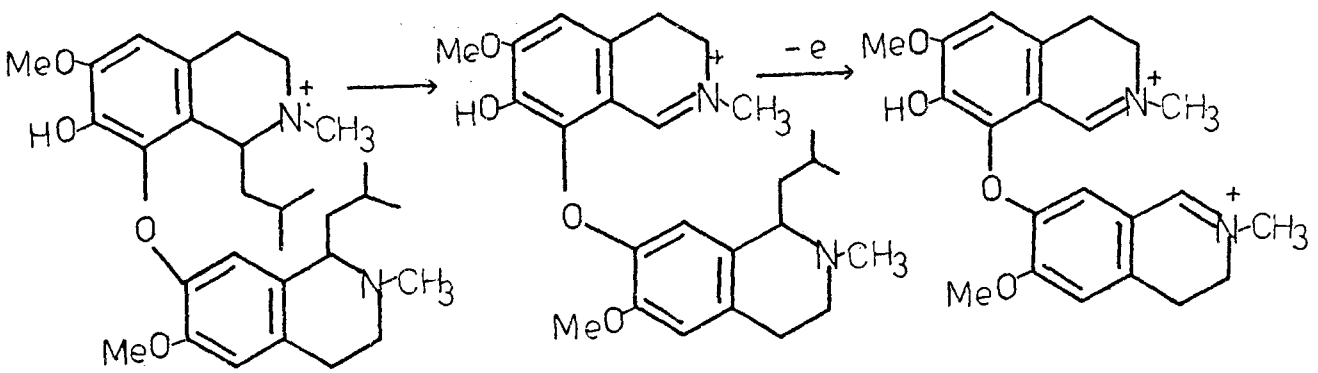


7 8

that hardly any further decomposition is discernible. Similar behavior is observed in the mass spectra of 1-benzylisoquinoline (37, 38) and phthalide isoquinoline alkaloids (37). In both cases, the base peak is formed by fission of a bond which is doubly benzylic and β to the nitrogen atom. The molecular ion peak has an intensity of less than 1% of the base peak, m/e 206, in the spectrum of N.O.O.-trimethylcoclaurine (9). The benzylic cleavage of the parent ion can give either ion 8 or ion 10. Since there is extensive conjugation in ion 8, and the nitrogen atom can well accommodate the positive charge, it is not surprising that ion 8 is the base peak ion in this spectrum. Ion 10, which can transfer into the stable tropylium ion, is found to have an intensity of twenty per cent of the base peak. Both ions 8 and 10 can lose neutral fragments such as CH_3, H, or CO to give small daughter ions. The fragmentation mechanism was confirmed by deuterium labelling and by the shift technique.

It has been reported that the parent ion peak is not observed in the spectrum of the phthalide isoquinoline alkaloid, hydrastine (37).


MeO
MeO
N
CH3
MeO
8
9
CH2
CH3O
OCH3
10


MeO
HO
N
CH3
O
N
CH3
MeO
MeO
HO
N
CH3
O
N
CH3
MeO
-e
MeO
HO
N
CH3
O
N
CH3
MeO
11
12
13

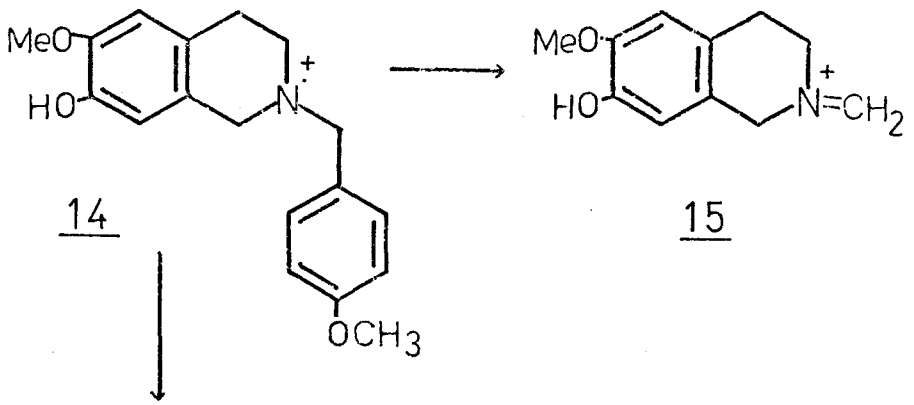
Apparently the presence of the additional oxygen function makes the doubly benzylic bond weaker than in a 1-benzylisoquinoline.

This behavior is also observed in the dimeric cleavage product of isopilocereine (11) (23, 95), in which the most important fragments arise from successive β-cleavages to give the singly charged ion 12, m/e 439, and the doubly charged ion 13, m/e 191. As expected, the molecular ion peak in this case is very small. This characteristic pattern can be recognized very easily and has been used to identify new alkaloids, such as thalifendlerine (39).

Primary skeleton information about a new alkaloid is obtained much more easily by the mass spectral method than by classical chemical degradations. Another advantage of the mass spectral method is that only a small amount of material isolated from the plant is needed to get a spectrum while chemical degradation usually requires many times that amount.

2-Benzylisoquinoline

As the position of the benzyl group attached to the isoquinoline ring changes, dramatic changes in the mass spectra occur. When the benzyl group is at the 2-position, e.g. sendaverine (14) (45), the base peak is no longer the immonium ion obtained by simple cleavage as in 1-substituted isoquinolines, but instead ion 10. If fission of the bond β to the nitrogen atom occurs, the ion 15 will be formed. The small intensity of ion 15 seems to imply that this is an unfavorable process. The parent peak is moderately strong, since there is not a bond of comparable weakness to that in 1-substituted isoquinoline alkaloids.



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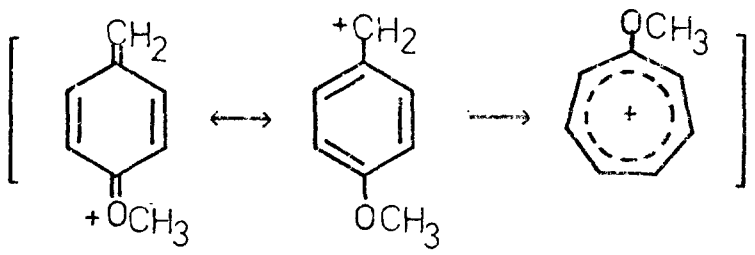

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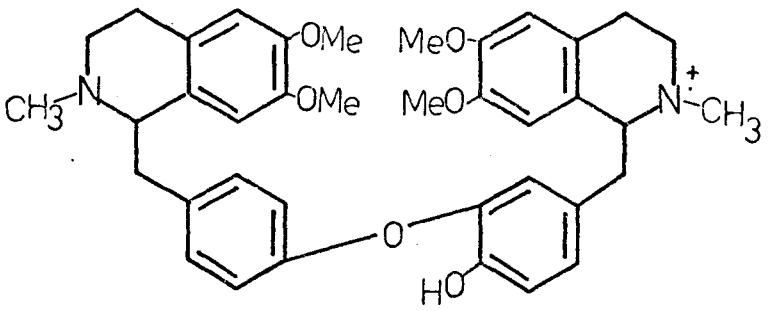
Bisbenzylisoquinoline alkaloids

Several publications dealing with the mass spectral fragmentation of bisbenzylisoquinoline alkaloids have appeared (46, 47, 48, 49, 50). The alkaloids can be divided into three main types.

Type 1, One ether linkage: Dauricine (16) is a dimer of 1-benzylisoquinoline joined together through a single ether bond, and its mass spectrum is thus similar to that of the monomers (47). The spectrum exhibits a very small molecular ion peak at m/e 624, and consists of almost a single peak at m/e 206 which is due to the cleavage of the doubly benzylic bond with charge retention on the dihydroisoquinolinium ion (8). Since both tetrahydroisoquinoline moieties are identical, only one peak of this type is observed. Charge retention



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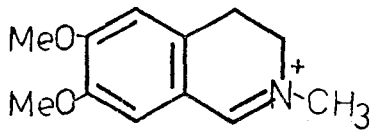

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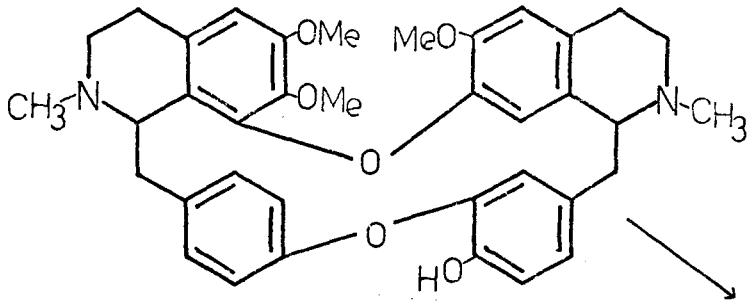

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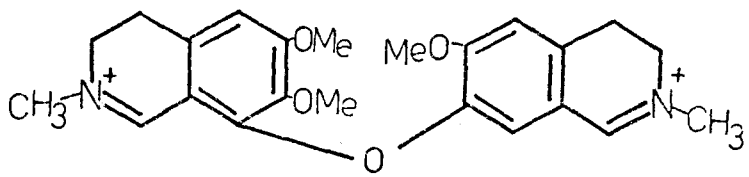

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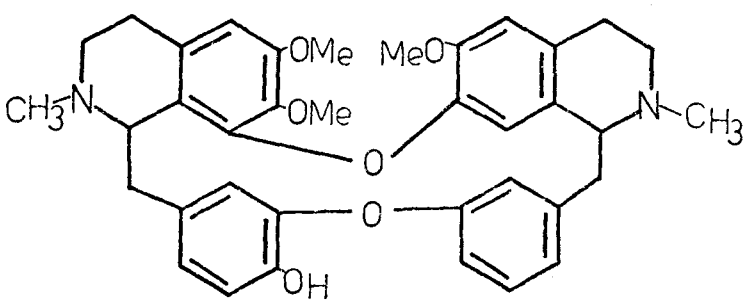


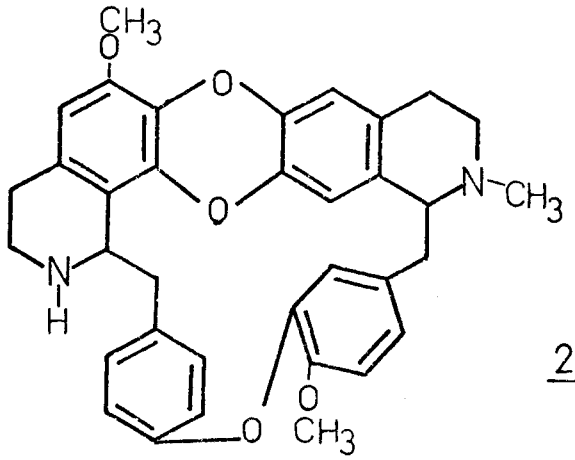
19

on the benzyl group is not favored compared to the dihydroisoquinolinium ion. Thus, the benzyl ion peak at m/e 418 is small but still perceptible.

Type II, Two ether linkages, head to head: The characteristic peak in this type of alkaloid, e.g. oxyacanthine (17), is an intense doubly charged peak, as evidenced by the isotope peak appearing at half a mass unit higher. The base peak, ion 18, arises from the cleavage of both isoquinoline benzyl bonds in a doubly charged molecule. Despite the fact that two positive charges on a single molecule are highly unstable, the two positive charges in ion 18 are well separated and well stabilized in two separated dihydroisoquinoline systems. The greatly increased intensity relative to Type I of the molecular ion peak results because at least two bonds must be broken before any fragment ion can be formed. A singly charged ion corresponding in composition to 18 minus one hydrogen is observed as a moderate peak at m/e 395. The substituents on the two isoquinoline can be deduced from this ion.

It is obvious that the change of position of the ether linkage between the two isoquinoline parts or two benzyl groups does not affect the fragmentation process, as demonstrated in the spectra of oxyacanthine (17) (47) and berbamine (19) (35), where the two spectra are similar, even though they were recorded on different machines.

Also included in this group is the alkaloid triboline (20) (48). Although there are three ether linkages in this molecule, the main fragmentation process should be the same as it is for 17 or 19. Examination of the spectrum shows a definite similarity.



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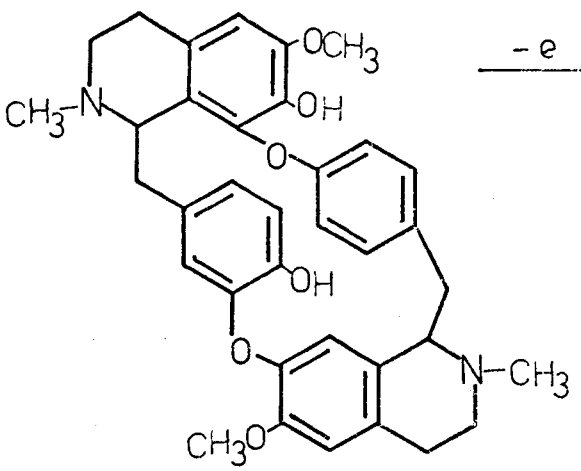

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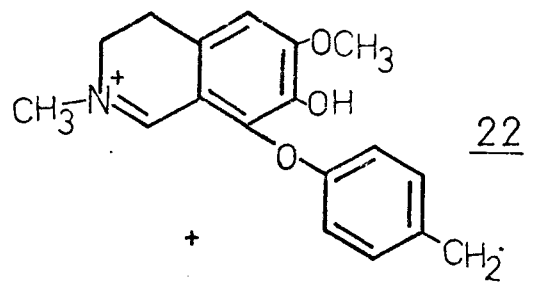

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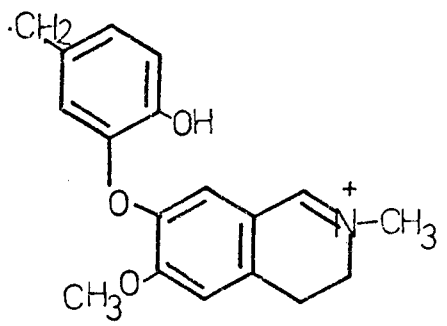


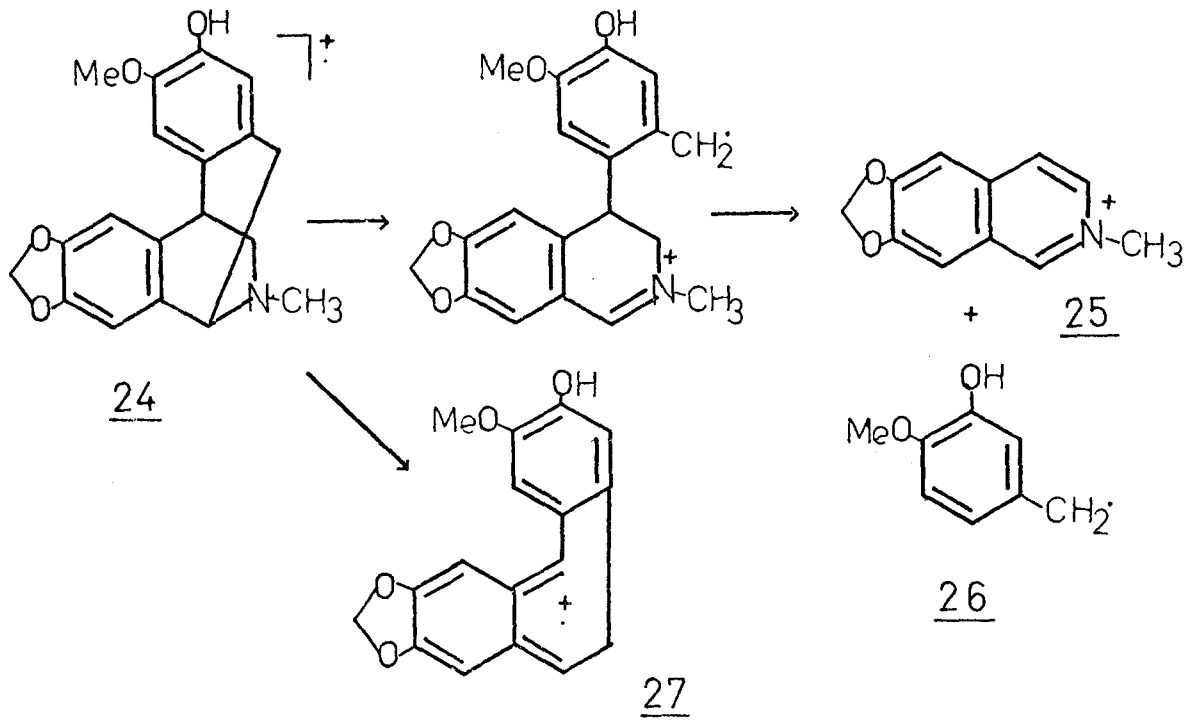
23

Type III, Two ether linkages, head to tail: When the ether linkage connects different rings, extensive differences in the spectrum are produced. Unlike oxyacanthine or berbamine, there is no doubly charged ion in the spectrum of chondodendrine (21) (47); instead, two singly charged species, 22 and 23, are formed, each coming from different parts of the molecule. In this case, ions 22 and 23 happen to appear at the same mass, m/e 297. It was found in O-ethyl-O-methyl-isochondodendrine (48) that both ions were observed in the spectrum.

Thus, mass spectrometry is a convenient tool to distinguish the three types of bisbenzyl-tetrahydroisoquinoline alkaloids, as they have distinct fragmentation patterns. Several new alkaloids have been identified with the aid of mass spectrometry, such as repanduline (96) and penduline (97). The determination of the substitution pattern, however, is still dependent on other methods.

Pavine and Isopavine Alkaloids

Only a few mass spectra of alkaloids of this type have been reported in the literature (42, 43). However, the characteristic peaks displayed in the spectra can be recognized easily and useful conclusions can be drawn about their fragmentation. The strong molecular ion peak in the spectrum of amurensine (24), an isopavine alkaloid, is compatible with the structure, in which there is no simple bond cleavage which can give rise to stable daughter ions. The base peak ion, 25, arises from a series of bond fissions, namely, benzylic cleavage followed by aromatization of the nitrogen-containing ring with expulsion of 26 as a neutral fragment. It is not surprising that the fully aromatic ion



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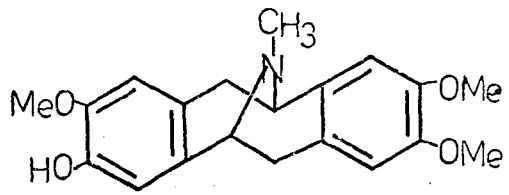
26

27



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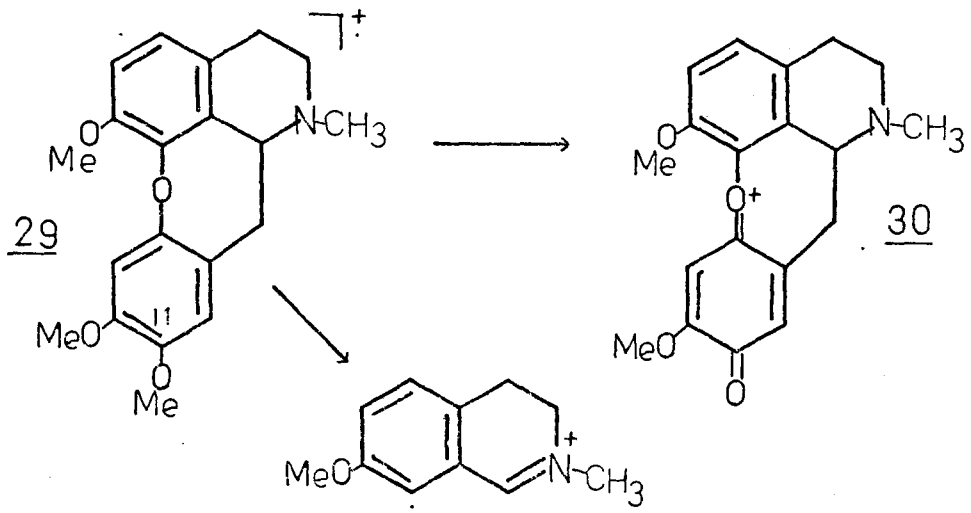

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31

25 is the most stable ion.

The difference between pavine and isopavine alkaloids lies in the fact that the central carbon skeleton between the two aromatic rings in the pavine alkaloids is symmetrical, e.g. norargemonine (28). Both bonds β to the nitrogen atom can be broken upon electron impact to form an intermediate ion which decomposes further to give the isoquinolinium ion. Two strong peaks are observed in the spectrum of the pavine alkaloid, norargemonine. In cases where the substituents on both rings are identical, only one strong peak corresponding to the isoquinolinium ion exists in the spectrum. In order to differentiate pavine alkaloids of this type from their isopavine counterparts one can use the M-43 peak which is observed in the isopavine but not in the pavine series. The M^+-43 peak, ion 27, formed through the retro-Diels-Alder process, has a moderate intensity in the isopavine series. This process is often found in six membered rings with one double bond, and has been discussed by Biemann (98). A similar difference would be expected for secondary bases of the pavine and isopavine alkaloids.

Cularine and related alkaloids

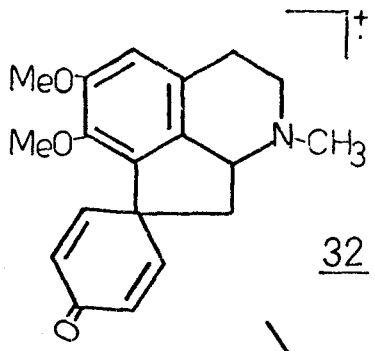
Cularine alkaloids exhibit rather clean spectra (37, 51) which owe their stability to the existence of a tetracyclic ring system. The only two strong peaks in the spectrum of cularine (29) are the parent peak and the quinonoid ion 30, which arises from the expulsion of a methyl radical from the C-11 OMe. One small but recognizable peak appears in the middle of the spectrum with an odd mass number, which apparently forms from the cleavage of the benzylic and the ether linkage to form ion 31.

Proaporphine and Aporphine alkaloids

Biogenetically, proaporphine alkaloids were proposed by Barton and Cohen (99) to be the precursors of aporphine alkaloids. They also bear some similarities in their mass spectral fragmentation. The tetracyclic ring system in which there are no easy bond cleavages leads to a stable molecular ion, which forms the base peak in proaporphine and in most aporphine alkaloids. The strong M-1 peak is an indication of the stability of the quaternary ammonium ions, 33 and 37, formed by losing a hydrogen atom from the carbon adjacent to the nitrogen atom. Moderately strong peaks appearing in the high mass region at m/e 268, 34, and at m/e 284, 36, are formed through the retro-Diels-Alder decomposition of the molecular ions 32 and 35, respectively, with expulsion of $CH_2{=}NCH_3$. Peaks of low intensity show the loss of small radicals such as CH_3, OH or OCH_3 from the molecular ions 32 and 35 as well as from ions 34 and 36. No significant peaks appear in the spectra further down. The only way to distinguish between these two groups is through the peak, M-CHO, formed by losing CO from ion 33 in the proaporphine alkaloids. In instances where the amine is secondary, the ions M-CH_2=NH and M-CHO can be differentiated by high resolution mass spectrometry.

Protoberberine Alkaloids

A systematic study of the mass spectral fragmentation of this group of alkaloids has been reported (60). The moderately strong molecular ion peak, 38, and the base peak account for most of the ion current. The retro-Diels-Alder opening of ring C gives the ion 41 which



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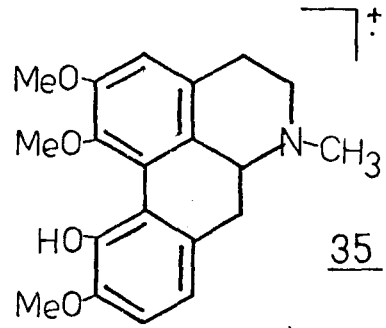

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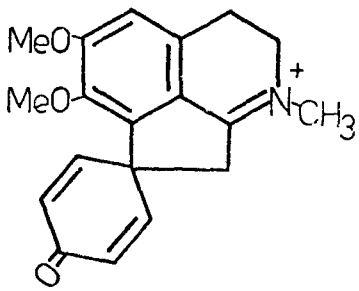

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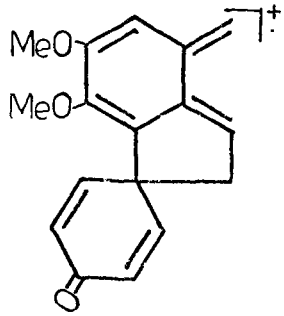

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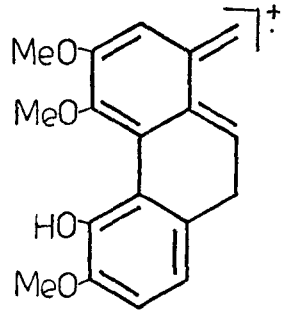

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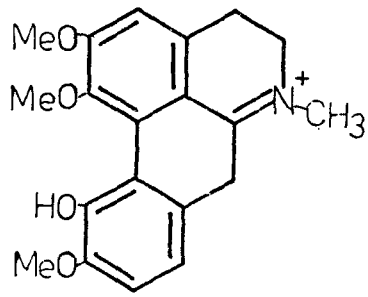

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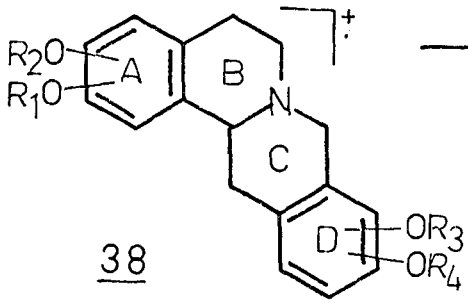

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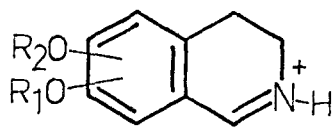

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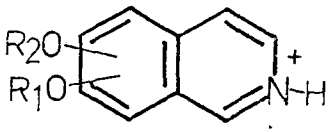

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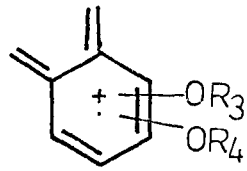

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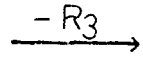


42

is the base peak in most compounds examined. In the instances where there is a hydroxyl group in ring D, the base peak shifts to the ion 39 arising from retro-Diels-Alder decomposition with one hydrogen transfer. Ion 42 is found to be strong when the substituents at C-9 and C-10 are methoxyl groups. These differences are very useful in structural studies on new members of this group of alkaloids. Isoquinolinium ion, 40, is found in all of the spectra, but its intensity is small.

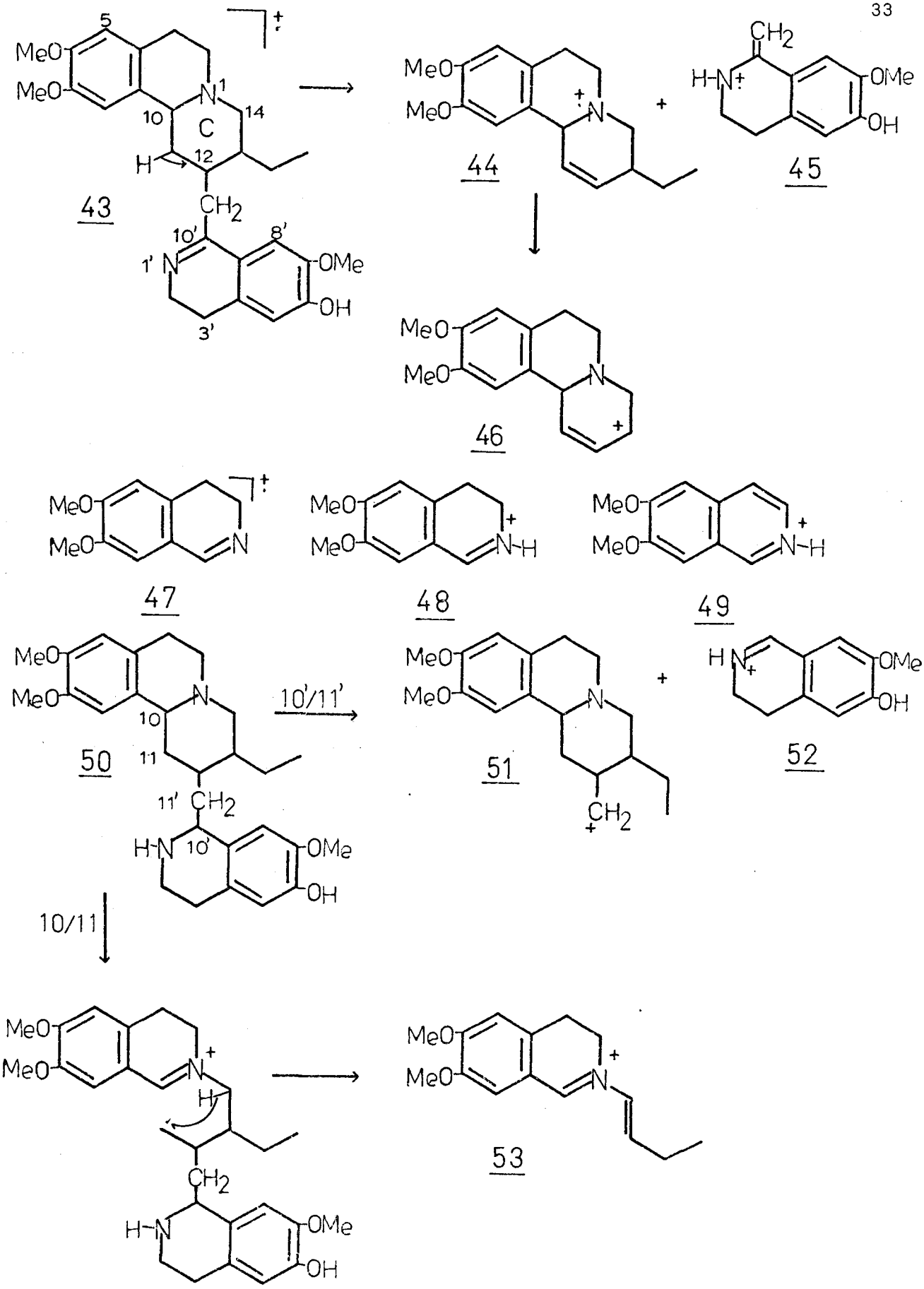
Emetine and Related Alkaloids

The mass spectra of emetine alkaloids have been studied in considerable detail by Budzikiewicz et al. (80). They found that the double bond at 1', 10' determines the general features of the spectrum.

Allylic cleavage of the psychotrine ion 43 followed by hydrogen transfer produces ions 44 and 45, both of which are strong. The odd electron ion, 44, then loses its ethyl side chain to give the base peak, ion 46.

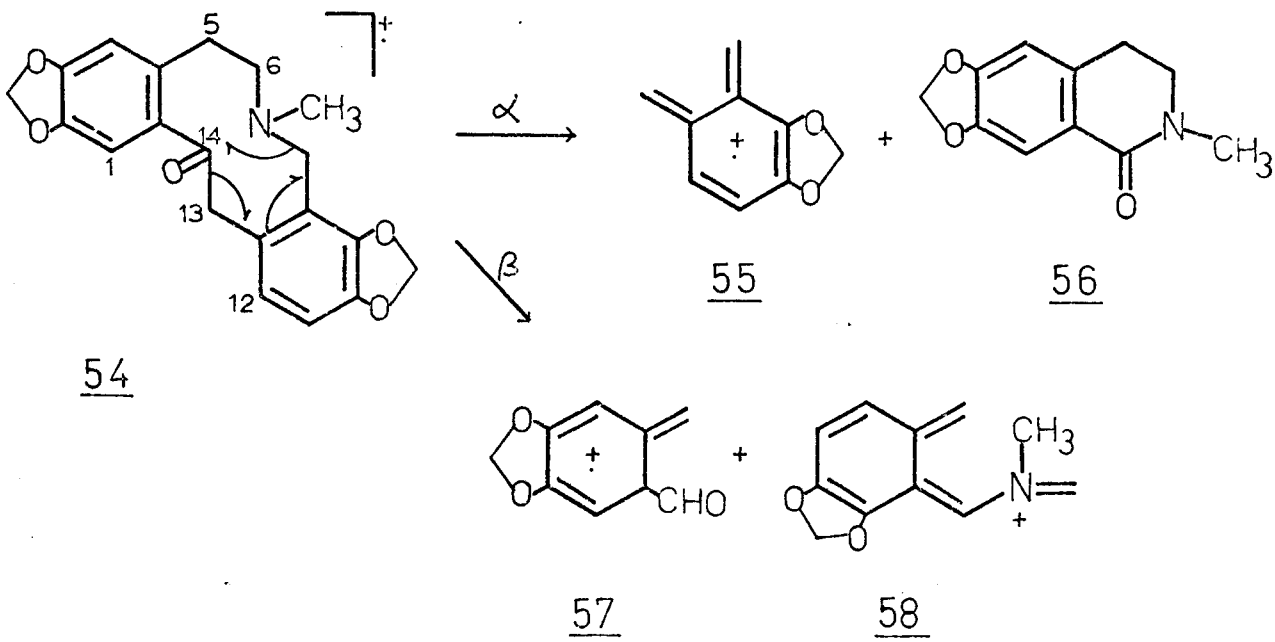
The cyclic cleavage of ring C with hydrogen transfer to or from the isoquinoline ring gives a series of peaks, 47 m/e 191, 48 m/e 192 and 49 m/e 190.

The spectrum of the saturated analogue, cephaeline (50) is dominated by ions formed by benzylic cleavage of bonds 10', 11', and 10, 11. In the former case, two ions 51 and 52 are formed with the latter predominant. In the C-10, C-11 bond cleavage, a series of electron shifts and a hydrogen transfer gives the ion 53.


MeO
MeO
5
1
10
C
14
12
H
CH2
10'
8'
1'
N
OMe
OH
3'
43
44
45
46
47
48
49
10'/11'
50
11
11'
10'
51
52
10/11
53

Protopine Alkaloids

In contrast to the tetracyclic alkaloids mentioned previously, the molecular ion peak of protopine (54) (65) itself and other members of this family is comparatively small. The introduction of a keto group into the molecule accelerates the retro-Diels-Alder type decomposition (α process). As a result, the parent peak is very small. The base peak is usually ion 55, which is derived from the α process and carries most of the ion current. Other characteristic peaks corresponding to the ion structures 57 and 58 arise from the benzylic 5,6 and 13, 14 cleavage with one hydrogen transfer (β-process). If the substituent at C-9 is a hydroxyl group an additional strong peak appears, its composition corresponds to 56 plus one hydrogen. Its genesis is probably derived from the α process with the transfer of phenolic hydrogen to the nitrogen, as in the case of the protoberberine alkaloids.



Rhoeadine and related alkaloids

The alkaloid, rhoeadine, was earlier formulated as a modified benzyl or phthalide isoquinoline (100, 101), but its structure was revised to 59 on the basis of mass spectral and other chemical evidence.

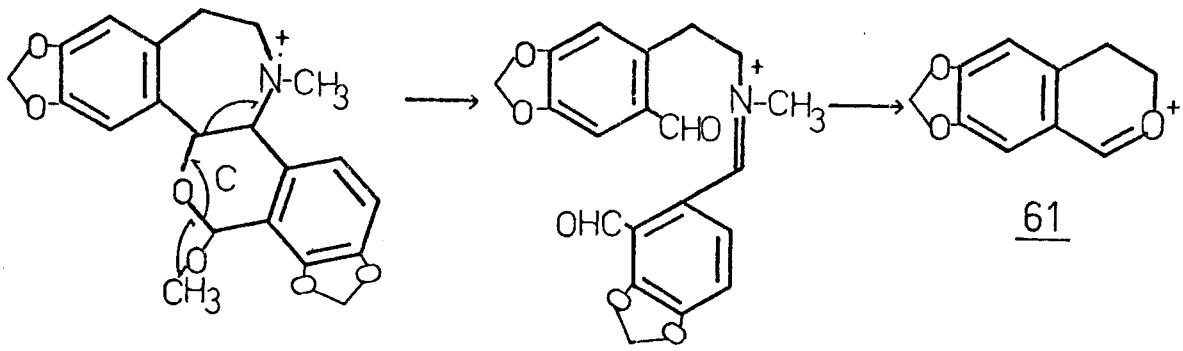
As mentioned earlier, the mass spectra of benzyl and phthalide isoquinolines exhibit little or no molecular ion peak, while rhoeadine (59) shows a parent peak about half the intensity of the base peak.

The rhoeadine alkaloids may be divided into two groups, acetal and hemiacetal, mutually convertible by hydrolysis and etherification, respectively. The mass spectra of each series of compounds also exhibit a distinct pattern. The intense $M-CH_3$ peak in the acetal group, but not in the hemiacetal group, shows clearly that the methyl group must be lost from the methoxyl group on ring C. The process is shown in 59. The ion 60 may decompose further to give the base peak, ion 61 at m/e 177, a process which is supported by a metastable peak.

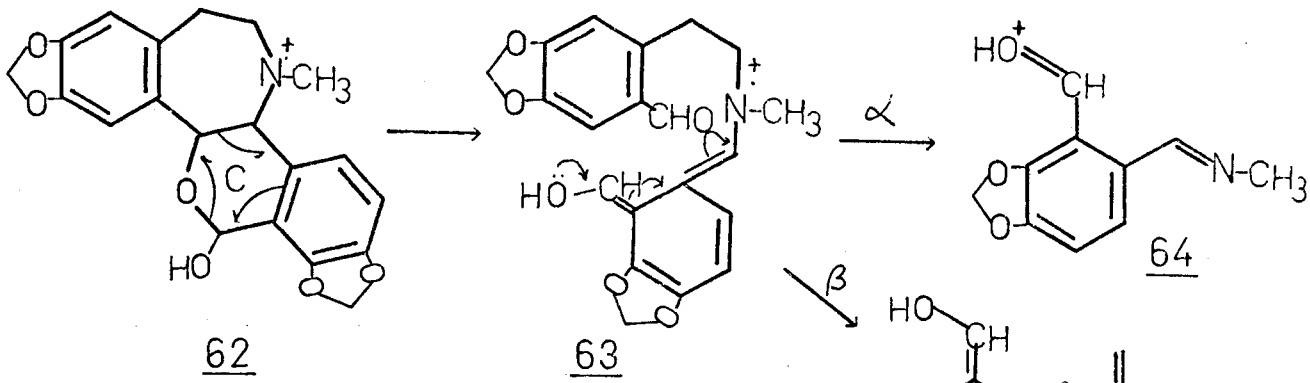
On the other hand, the hemiacetal rhoeagenine ion (62) prefers to fragment by the retro-Diels-Alder mechanism to form ion 63, which can then undergo either α or β cleavage to give ions 64 and 65, respectively. As a general rule, β cleavage will be favorable, because the bond is benzylic and β to the nitrogen. The base peak is indeed the ion 65. This process also occurs in the rhoeadine ion 59, but to a lesser extent.

Spirobenzylisoquinoline Alkaloids

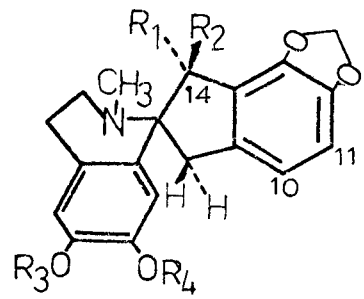
Mass spectral methods have been used in the structural investigation of spirobenzylisoquinoline alkaloids. The three minor alkaloids



59 60 61



62 63 64 65



66	$R_1+R_2=O$, $R_3+R_4=CH_2$
67	$R_1=H$, $R_2=OH$, $R_3=R_4=CH_3$
68	$R_1=R_4=H$, $R_2=OH$, $R_3=CH_3$
69	$R_1=H$, $R_2=OH$, $R_3+R_4=CH_2$

fumariline (66), fumaricine (67) and fumaritine (68) have been shown to have the same carbon skeleton by examination of the mass spectra of 67, 68 and the reduction product of 66, 69 (109). The fragmentation patterns of these compounds were similar and the substituents in the ring could be deduced from the peak shift. This information could not be obtained easily by other methods.

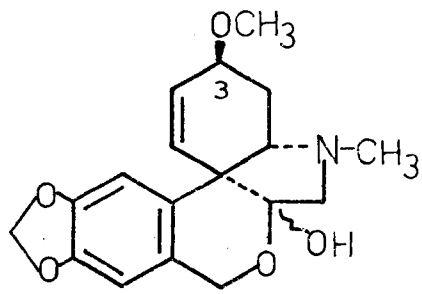
High resolution mass measurements confirmed the molecular formulas and also gave the elemental composition of the fragments. The base peak ions of 67, 68 and 69 have been confirmed to be the dihydro-isoquinolinium ion (109).

The study of the detailed fragmentation of these alkaloids forms part of this thesis and will be discussed in next Chapter.

Morphine, Amaryllidaceae and Related Alkaloids

A number of publications dealing with the mass spectra of morphine and Amaryllidaceae alkaloids have appeared in the literature. However, the mass spectra of the alkaloids lack the characteristic intense peaks in the medium mass region and, therefore, are difficult to interpret. The peaks in the high mass region, which merely reflect the loss of small substituents or a few carbon atoms of a ring system, offer little useful information for structural studies. This difficulty is due to the polycyclic nature of the ring systems and the absence of characteristic cleavages. Two salient features are obvious. Firstly, the molecular ion usually gives the base peak. A cursory survey of these spectra will suffice to prove this point. Secondly, minor structural variations are frequently sufficient to cause drastic

changes in the mass spectra. For example, the case of the Amaryllidaceae alkaloids, tazettine (70) and criwelline (71) (87), can be cited. The only difference between these two isomers is the configuration at C-3, but the base peak for 70 is at m/e 247, while a peak of only small intensity is observed in this region for 71. A large difference is also observed between the spectra of the morphine alkaloids codeine and neopine (76), where the only difference between them is the position of the double bond. As a result, no generalizations will be made on these alkaloids. Further work is needed for a complete understanding of the factors affecting their fragmentations.



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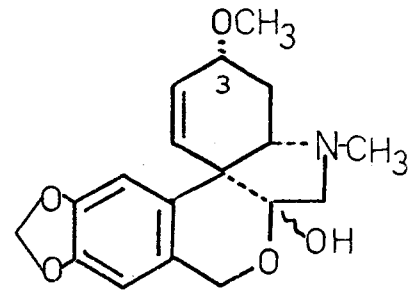

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The above discussion is by no means comprehensive and only general features of the spectra of most alkaloids are outlined. It is apparent that some inherent difficulties are involved in the application of electron impact mass spectrometry to structural determinations in this series, such as the difficulty in identifying molecular ions in phthalide isoquinoline alkaloids and the complexity of the spectra of the Amaryllidaceae alkaloids. Some of these difficulties could be overcome by using low electron energies or chemical ionization. As demonstrated recently by Fales (65), the mass spectra of Amaryllidaceae

alkaloids are simplified dramatically by using chemical ionization. Chemical ionization mass spectra usually provide intense molecular ions which are sometimes absent in electron impact spectra. Nevertheless, electron impact mass spectrometry is still a useful and informative physical method for structural studies.

The Structure of Spirobenzylisoquinoline Alkaloids

The spirobenzylisoquinoline alkaloids, ochotensine and ochotensimine, were first isolated from Corydalis ochotensis Turcz (Fumariaceae) by Manske (102) in 1940. Their structures were fully elucidated by McLean and coworkers in 1964 (103, 104). The assigned structures were supported by an x-ray analysis of ochotensimine methiodide (105).

Very recently, the structures of six additional alkaloids, fumaricine, fumariline, fumaritine (109), sibiricine (66), ochrobirine (107), and fumarophycine (128), with a spirobenzylisoquinoline system have been reported. All of these alkaloids were isolated in small amounts and it was necessary in the elucidation of their structures to rely heavily on physical methods.

The most extensively used method was p.m.r. spectroscopy. The structural determination of ochotensine and ochotensimine was also based mainly on the p.m.r. spectra of the alkaloids and of two Hofmann degradation products (104). As p.m.r. techniques have become more sophisticated, more structural information and stereochemical conformation can be obtained. The first successful application of the nuclear Overhauser effect to the isoquinoline alkaloids was the confirmation of the structure of fumariline (66) and fumaricine (67) (108).

In their first publication (106), the assignment of structure to 66 and 67 was based on the similarity of 66 to a known synthetic compound, and the stereochemistry of 67 at C-14 was left unassigned. In a later paper (108), the NOE technique was used to confirm the assigned structure, and also to define the orientation of the hydroxyl at C-14. The NOE technique was also used in the elucidation of the structure of sibiricine (66) and ochrobirine (107).

So far biosynthetic experiments have not been carried out on these alkaloids. A biogenetic-type synthesis, however, of spirobenzylisoquinoline alkaloids was reported to be successful (110). The spirobenzylisoquinoline skeleton was derived from a diphenolic dihydroprotoberberine salt through a base catalyzed rearrangement. Whether the plant follows the same route or another pathway remains to be resolved.

DISCUSSION OF RESULTS

Caseanadine

An examination of Corydalis caseana A. Gray was reported in 1938 by Manske and Miller (111) who isolated ten alkaloids. Of the ten alkaloids six are tetrahydroprotoberberines, three are of the protopine type and one is a phthalide isoquinoline.

A new alkaloid, $C_{20}H_{23}NO_4$, which was assigned the trivial name, caseanadine, was recently isolated from this source. The ultraviolet spectrum has λ max (MeOH) 282 mμ and the infrared spectrum ν max ($CHCl_3$) 3540 $cm.^{-1}$ (OH absorption) and bands in the region 2700-2800 $cm.^{-1}$ (Bohlmann bands). The spectral properties are not inconsistent with a tetrahydroprotoberberine.

The mass spectrum of caseanadine shows a molecular ion at m/e 341 (100) as expected and fragment ions at m/e 326 (7), 310 (20), 178 (8), 176 (6), 164 (82) and 149 (38).* This pattern is characteristic of tetrahydroprotoberberines (60) substituted with two methoxyl groups in ring D and with one hydroxyl and one methoxyl group in ring A. According to Chen and MacLean (60), the intense molecular ion peak as well as peaks at m/e 149 indicate that the substituents in ring D are at C-9 and C-10. It has been postulated that ions m/e 164 and 149 have the structure 72 and 73, respectively.

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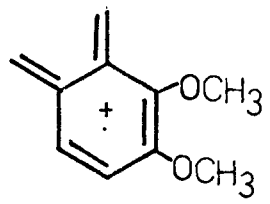
* Ion intensities are shown in brackets



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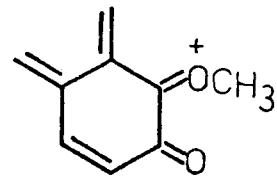

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The ions at m/e 178 and 176 are the dihydroisoquinolinium ion and isoquinolinium ion, respectively. The composition of all the ions discussed above have been confirmed by high resolution mass measurements as shown in Table II. Unfortunately, the mass spectrum does not define the sites of substitution in ring A.

TABLE II

Compositions of Major Ions in the Spectrum of Caseanadine

m/e	Composition	m/e	Composition
341	$C_{20}H_{23}NO_4$	178	$C_{10}H_{12}NO_2$
340	$C_{20}H_{22}NO_4$	176	$C_{10}H_{10}NO_2$
326	$C_{19}H_{20}NO_4$	164	$C_{10}H_{12}O_2$
310	$C_{19}H_{20}NO_3$	149	$C_9H_9O_2$

The 100 MHz p.m.r. spectrum of caseanadine in $CDCl_3$ is shown in Figure 4. The spectrum confirms the presence of four aromatic protons and three methoxyl groups. The presence of the doublet centered

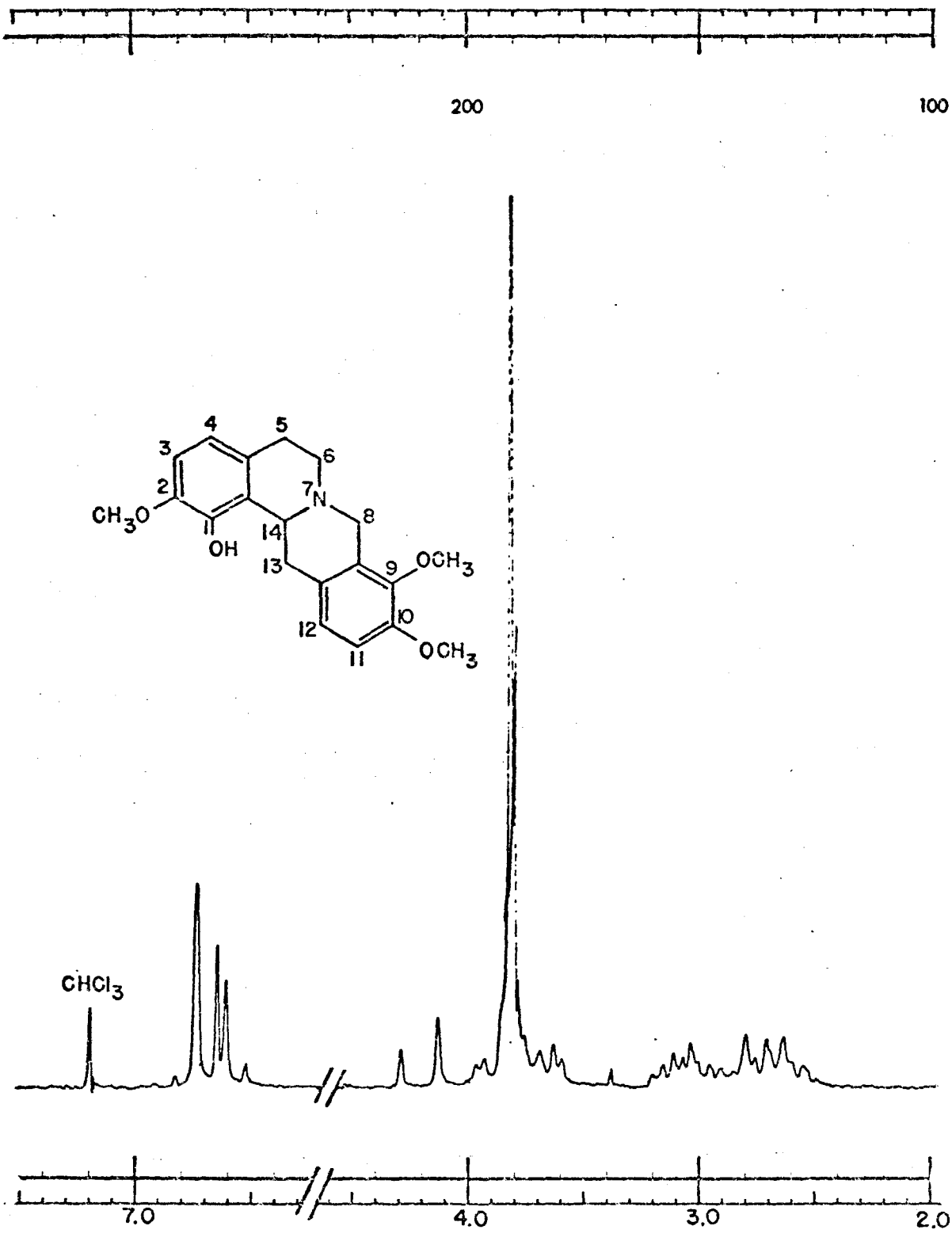
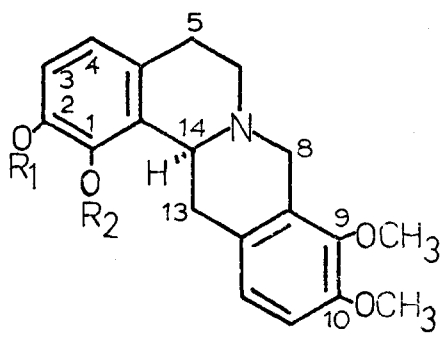


Figure 4. P.m.r. spectrum of caseanadine in chloroform-d

near 4.26 is characteristic of the protoberberines in which ring D carries an oxygen substituent at C-9 (60). The signal constitutes one-half of an AB quartet associated with the protons at C-8. The other half of this signal is obscured in this spectrum by the methoxyl signals. The aromatic region definitely shows one AB system with indications that another may also be present.

The spectrum of caseanadine Figure 5 in DMSO-d_6 was more informative, particularly in the aromatic region. The presence of two AB systems is clearly discernible there and thus the substituents in ring A must be at the 1,2 or 3,4 positions. The previous demonstration that caseamine (59) and caseadine (112) have substituents at the 1- and 2-position suggests that caseanadine may be similarly substituted in ring A. The presence of two AB quartets in the aromatic region along with the p.m.r. evidence that C-9 carries an oxygen substituent defines the substitution in ring D. Thus caseanadine may be represented as in 74 where $R_1 + R_2 = H + CH_3$. The assignment of the OH group to C-1



74 $R_1=CH_3$, $R_2=H$

75 $R_1=CH_3$, $R_2=CH_3-CO$

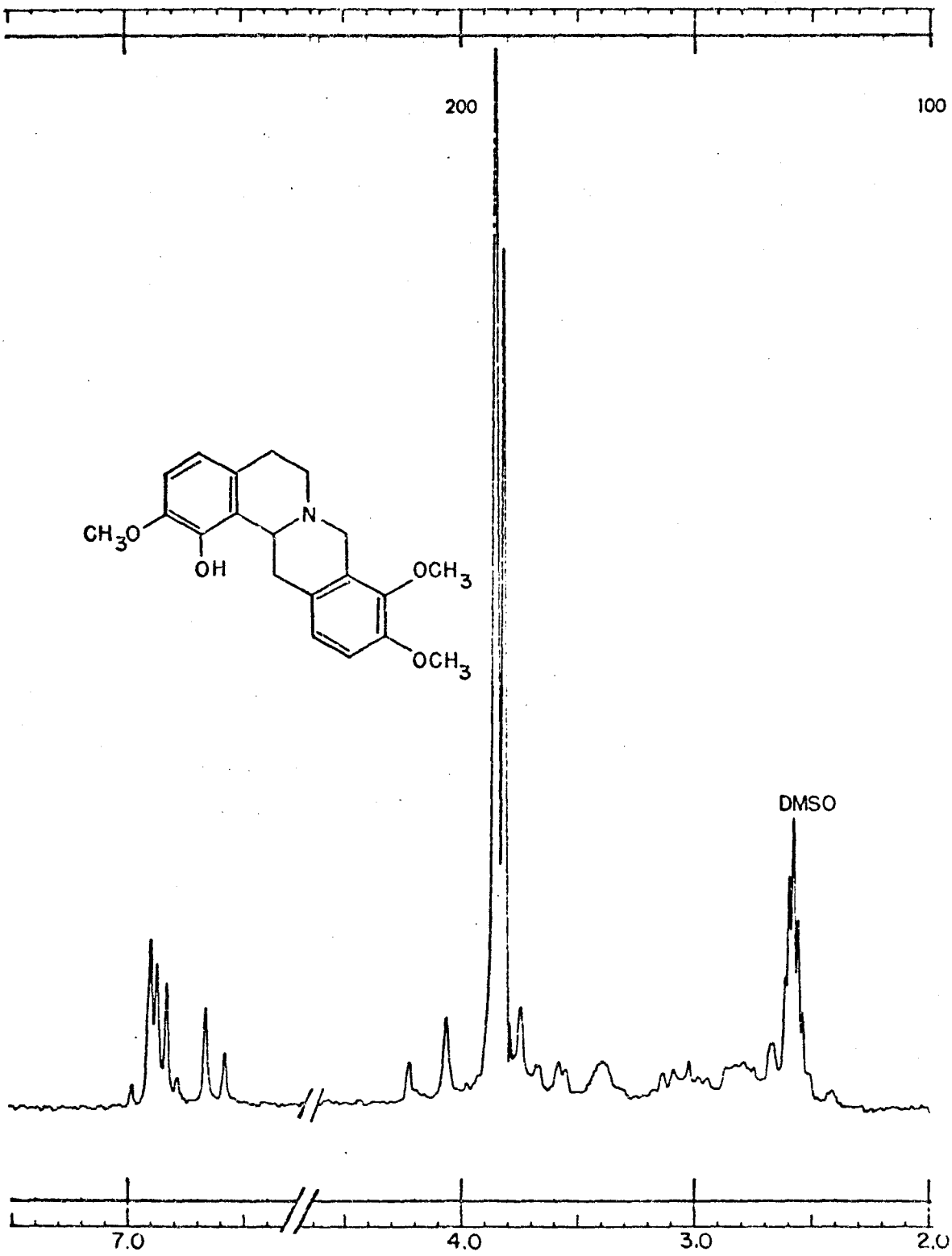


Figure 5. P.m.r. spectrum of caseanadine in dimethyl sulfoxide-d_6

rests on decoupling evidence on caseanadine and on an examination of the p.m.r. spectrum of its O-acetyl derivative 75 shown in Figure 6.

The p.m.r. spectrum of 75 shows that two aromatic protons have shifted downfield in 75 and, therefore, they must be in the ring carrying the O-acetyl group. The chemical shift of the remaining (ring D) protons has not changed appreciably. Moreover, it is obvious from the height of the signals that the lowest field proton of 75 is the highest field proton of 74. The magnitude of the shift (ca. 0.4 ppm) is expected for a proton para to a phenolic hydroxyl group which has undergone acetylation (113). The high field signal of 74 and the low field signal of 75 are both broader (less sharp) than the proton to which they are coupled. This suggests that these protons are broadened by ortho-benzylic coupling whereas the protons to which they are coupled are less strongly long-range coupled to meta and/or para protons (114). Double irradiation experiments lend support to the assignment of structure 74. Irradiation of 75 in the region 280-324 Hz from TMS caused the low field proton to sharpen relative to that proton to which it is directly coupled. The effect is most pronounced in the region 294-310 Hz from TMS. The effect is shown in Figure 7a. A similar effect is observed in 74 when the solution in DMSO-d_6 is subjected to irradiation in the region 265-284 Hz as shown in Figure 7b. The signals which are under irradiation in both systems must be one or other of the signals associated with the protons at C-5 or C-13. With the orientation of substituents of structure 74 irradiation at C-13 would not affect the protons of ring A. The only other benzylic proton

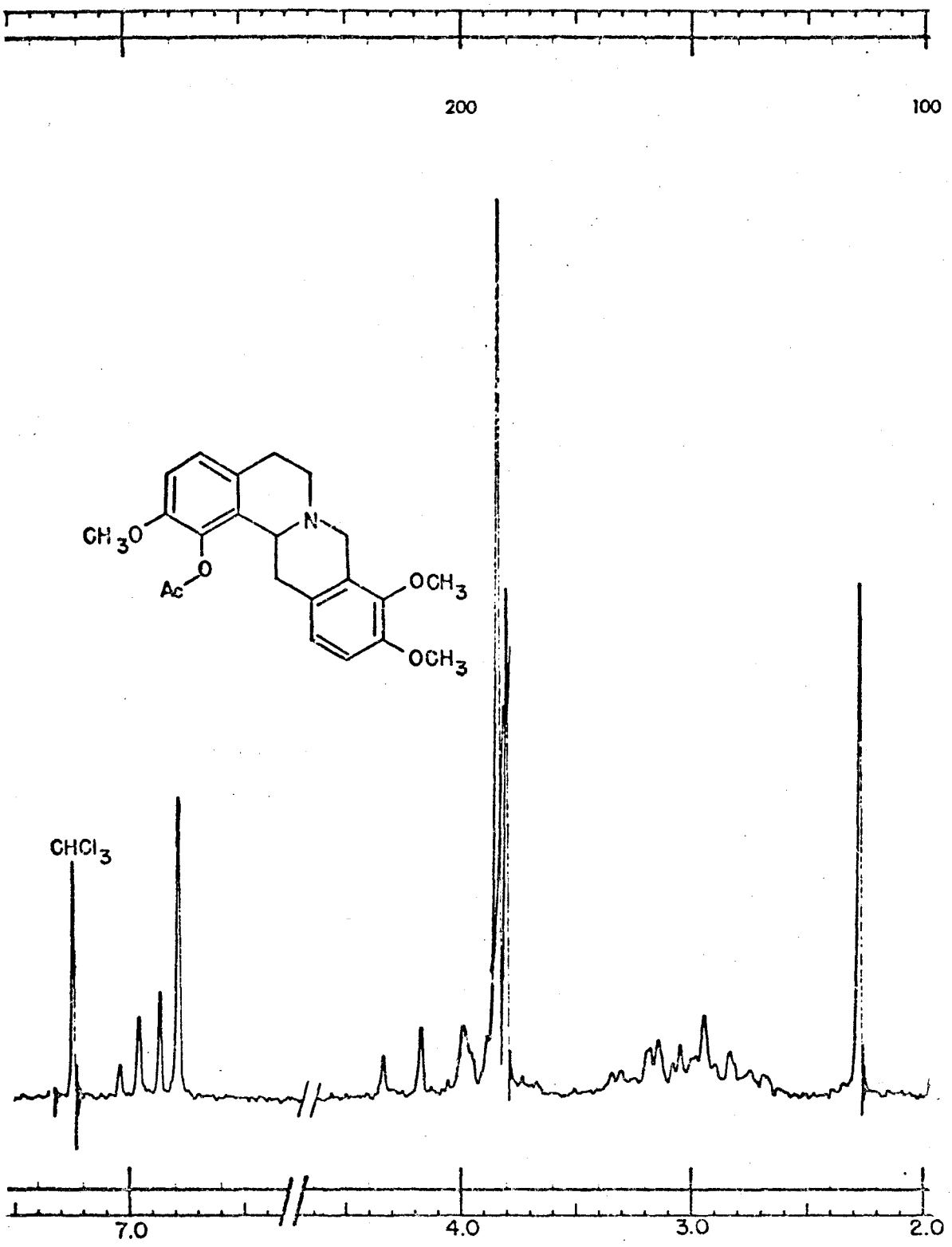


Figure 6. P.m.r. spectrum of O-acetyl caseanadine in chloroform-d

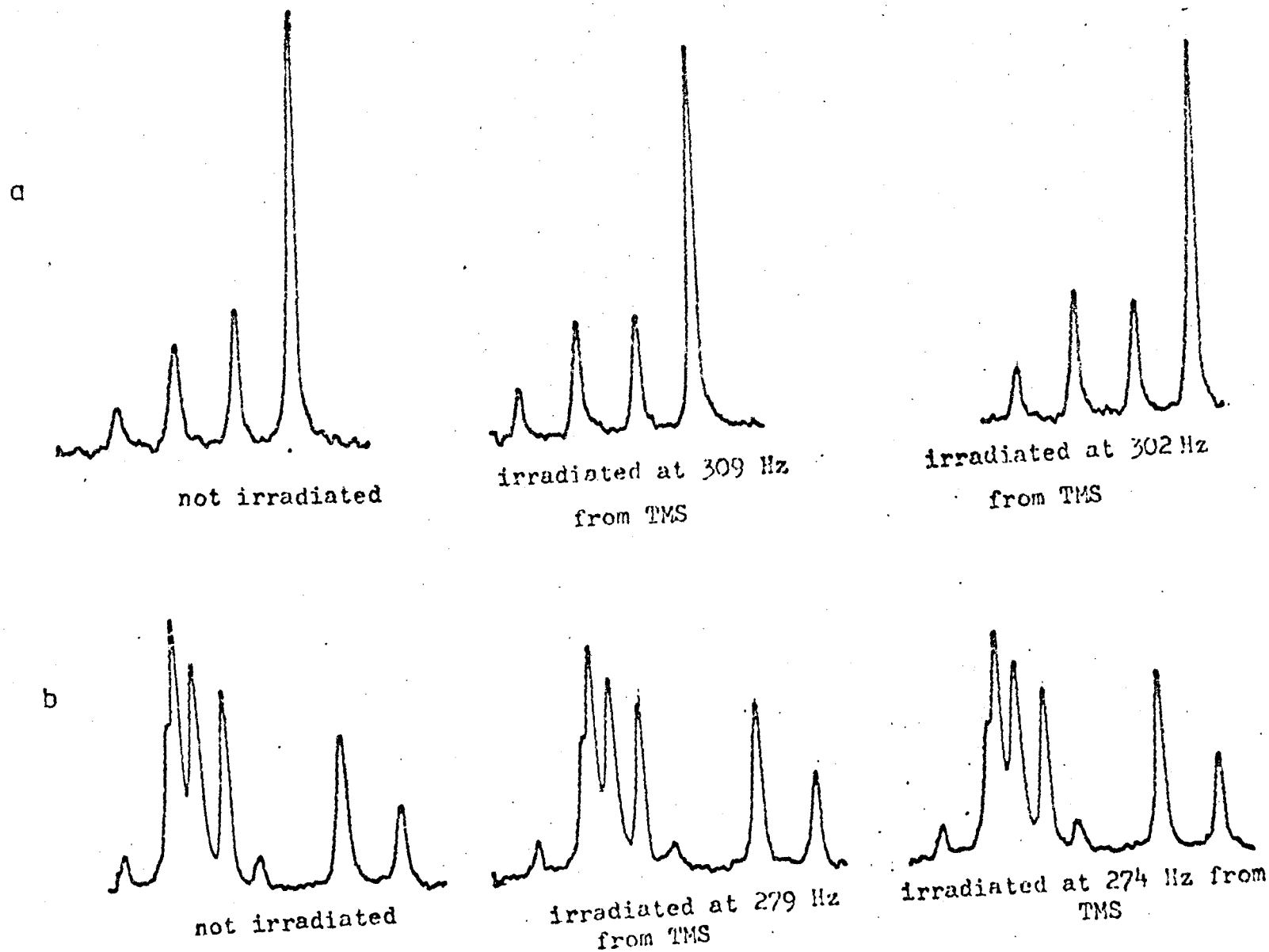
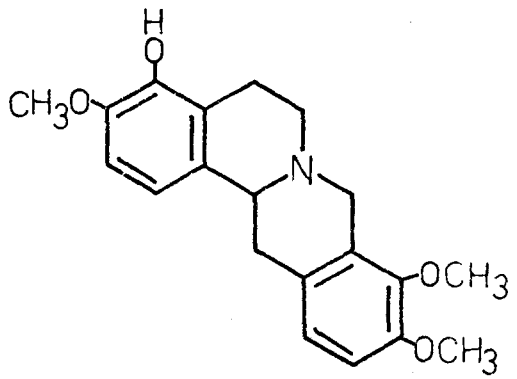


Figure 7. P.m.r. irradiation experiments of a) O-acetyl caseanadine, b) caseanadine

which can be long range coupled to the aromatic protons of ring A is situated at C-14. This proton has not been assigned in this spectrum but since it is benzylic and alpha to nitrogen one would expect to find it at lower field than 3 ppm from TMS. In the 13-methylprotoberberines this proton is clearly defined and appears in the region 3.7 ±0.1.

The alternative structure 76, although unlikely on biogenetic



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grounds, must also be considered. If 76 were the correct structure, the low field proton of the AB quartet of 75 in ring A would be at C-1, and the high field proton of the AB quartet of 74 in ring A would be that at C-1. Irradiation at C-5 would affect the proton at C-1 less than that at C-2 since meta-benzylic coupling is weaker than para (114), a circumstance which is contrary to the observed result. In any event, in structure 76 the proton at C-1 would be expected to be broader, even after irradiating C-5, than that at C-2 because it would still be ortho-benzylic coupled to C-14. Such is not the case. Nevertheless, the irradiating field may be affecting the protons at C-13. These protons

will not be longe-range coupled to C-1 but irradiation at C-13 might increase the area of the proton at C-1 through a nuclear Overhauser effect (115) and give the misleading impression that a decoupling had occurred. An examination of the peak width at half height definitely shows a decoupling of the low field aromatic proton of 75 and the high field aromatic proton of 74. For 74 the width at half height of the high field aromatic proton is 1.9 Hz in the uncoupled spectrum, and in the decoupled spectrum, 1.0 Hz. For the other half of the AB quartet the figures were 1.4 and 1.0. Only structure 74 is compatible with these observations.

Thus, the decoupling experiments establish the orientation of the substituents in ring A. A trans-quinolizidine conformation of rings A and B in caseanadine is indicated by the presence of Bohlmann bands in its i.r. spectrum (116). The large negative optical rotation of caseanadine in $CHCl_3$ suggests the absolute configuration shown in the diagram in which the C-14 hydrogen has an α-orientation (117).

13-Methyltetrahydroprotoberberines

From Corydalis thalictrifolia Franch. (118) and from Corydalis tuberosa DC. (C. cava) (119) a number of tetrahydroprotoberberine and several 13-methyltetrahydroprotoberberine alkaloids have been isolated along with alkaloids of other isoquinoline systems. Two new alkaloids, one from each of these species have been isolated.

The first of these two new alkaloids, $C_{21}H_{23}NO_4$, from C. thalictrifolia, was assigned the trivial name, cavidine 77. The base was optically inactive, melted at 193°C and had λ max (MeOH) 285 mμ in

its ultraviolet spectrum (u.v.). The infrared spectrum (i.r.) showed the absence of carbonyl or hydroxyl absorption but had absorption in the 2700-2800 $cm.^{-1}$, Bohlmann bands (116, 120). The mass spectrum of cavidine is recorded in Figure 8f. When the spectrum of cavidine is compared with that of thalictrifoline 78, Figure 8e, the two spectra are nearly identical. This suggests that cavidine is likely an isomer of thalictrifoline. Since the mass spectrum does not define the substitution pattern, it was necessary to use p.m.r. spectrometry in order to assign the positions of substituents.

The 100 MHz p.m.r. spectrum of 77 is shown in Figure 9. The presence of a $CH-CH_3$ group is apparent from the signal centred at 0.98, J=7 Hz, and the presence of two methoxyl groups and of one methylenedioxy group by the signals at 3.88 (area 6) and 5.93 (area 2), respectively. The presence of four protons in the aromatic region is established by integration but the spectrum in the aromatic region in $CDCl_3$ is disappointing in that it is impossible to draw any conclusions about the substitution pattern. In DMSO-d_6, however, two singlets and an AB quartet were clearly discernible (see inset Figure 9). The presence of an AB quartet centred at 4.07 and 3.52δ, J_{AB}=14 Hz, ascribed to the protons at C-8, indicates that there is an oxygen substituent at C-9 (60). The signal centred at 3.74 is attributed to the proton at H-14 which is coupled (J=3 Hz) to the adjacent cis proton at C-13 (vide infra). The remaining unassigned signals in the aliphatic region arise from protons at C-13, C-5, and C-6.

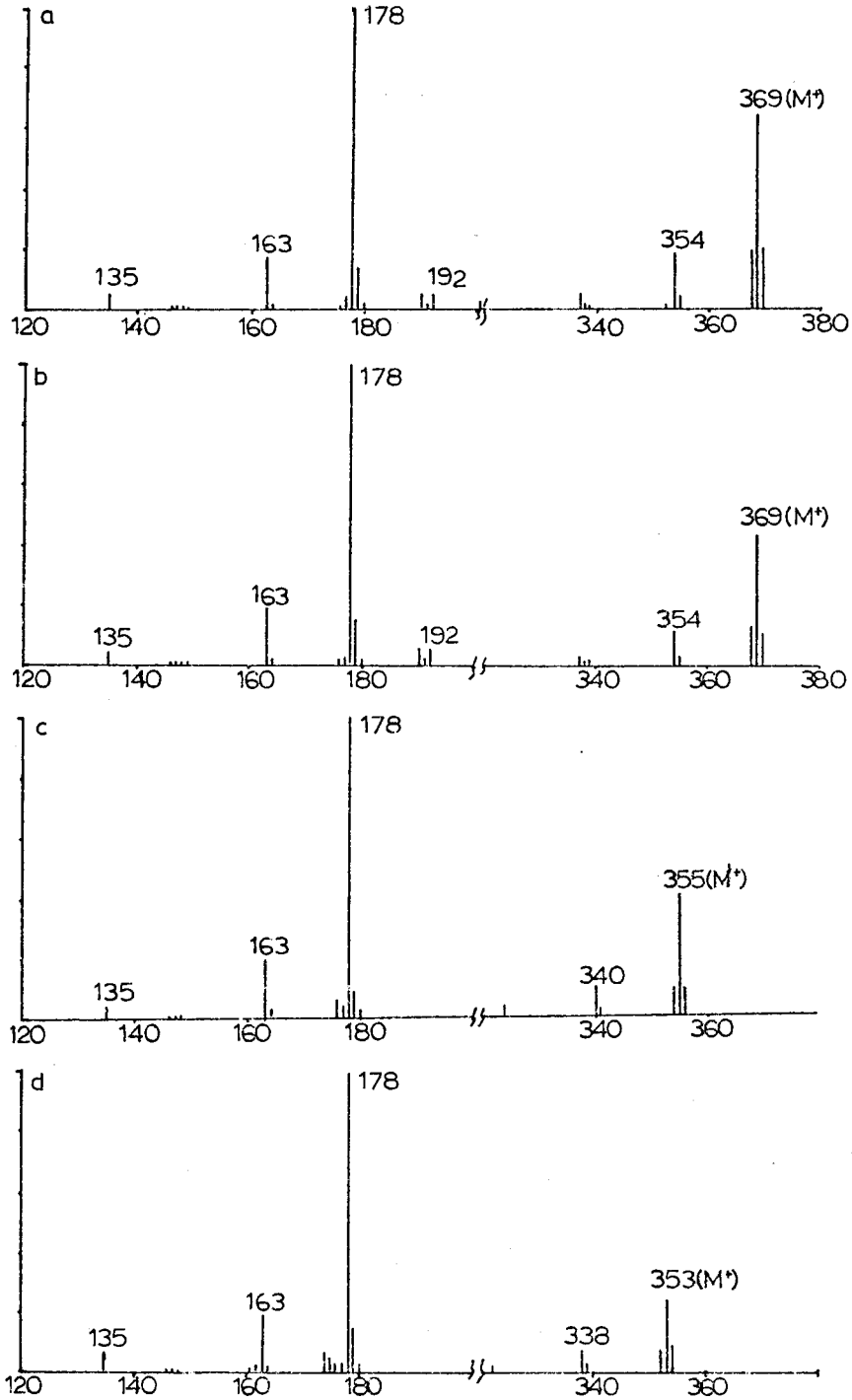


Figure 8. Mass spectra of a) (+)-corydaline, b) meso-corydaline, c) (+)-corybulbine, d) (+)-thalictricavine

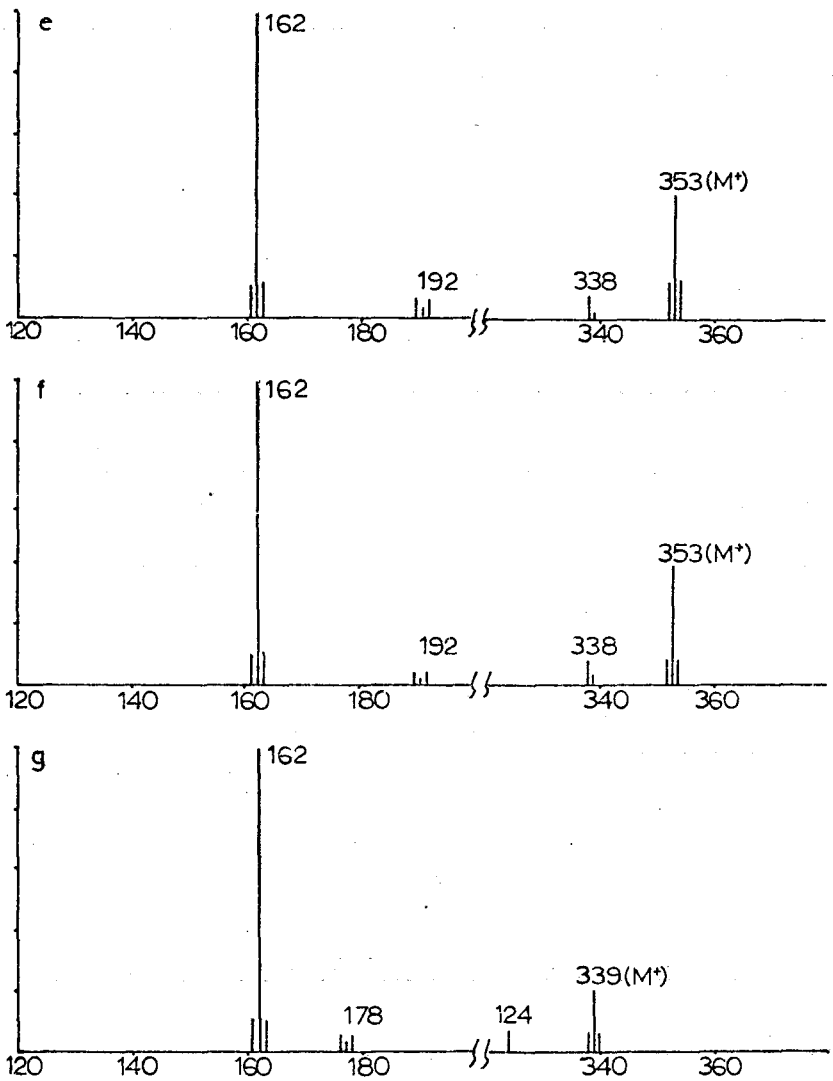


Figure 8. Mass spectra of e) (+)-thalictrifoline, f) (±)-cavidine, g) (±)-apocavidine

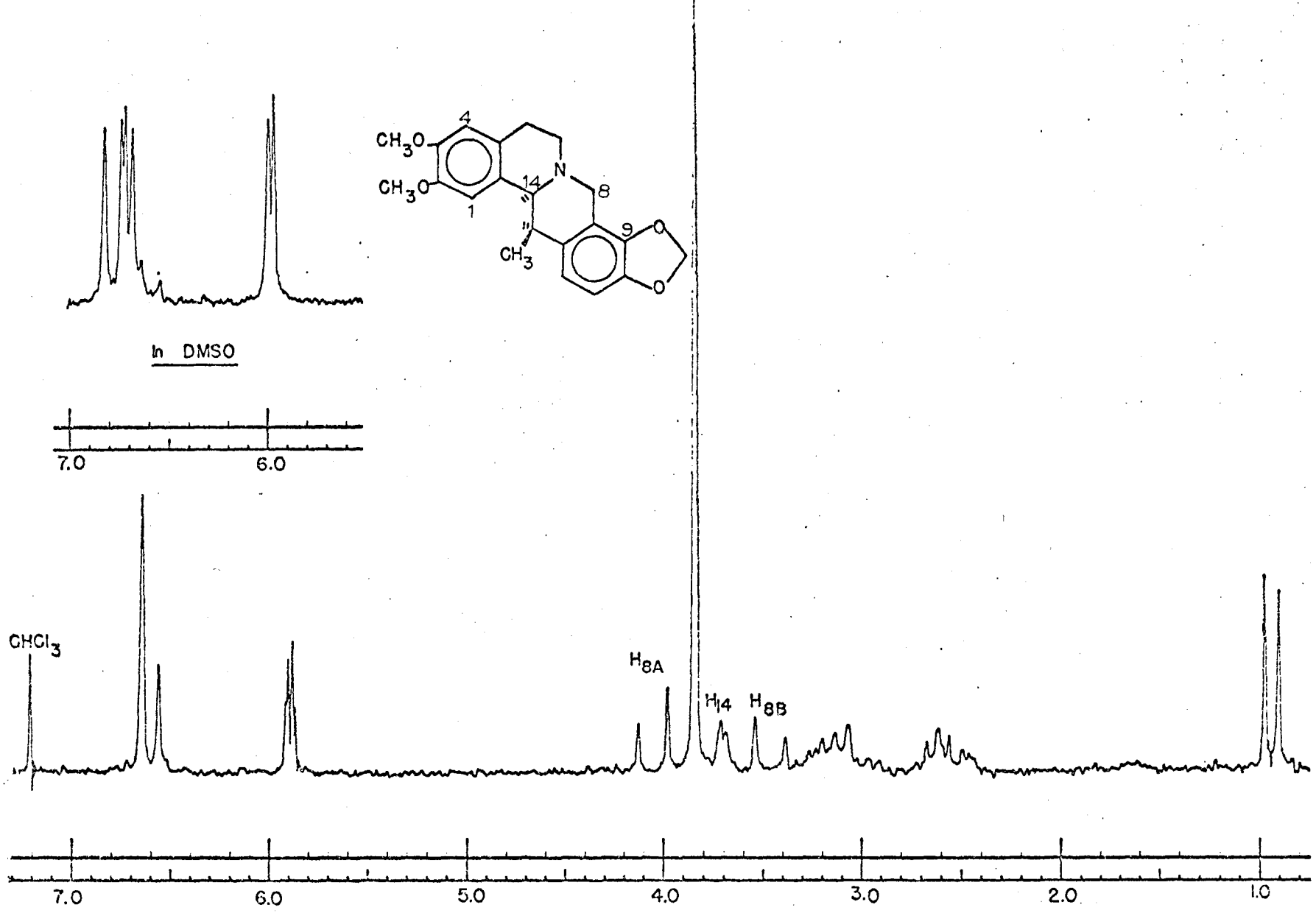


Figure 9. P.m.r. spectrum of cavidine

The spectroscopic examination indicated that cavidine is a 13-methyltetrahydroprotoberberine with a methylenedioxy group at C-9 and C-10 in ring D and with two methoxyl groups in ring A. The substitution pattern is similar to that in thalictrifoline and in an alkaloid described by Taguchi and Imaseki (121) which they designated "Base II". They showed that "Base II" was a stereoisomer of thalictrifoline by converting both alkaloids to a common dehydro base. Upon reduction the dehydro base gave a mixture of thalictrifoline and ± "Base II" (m.p. 191-92). Jeffs (122, 123) has inferred that the C-13 and C-14 hydrogens in Base II are cis while in thalictrifoline they are trans to one another. Base II has a melting point close to that of cavidine. Moreover, the i.r. and p.m.r. spectra of cavidine are similar to those published by Taguchi and Imaseki (121) for ± "Base II". It seems likely that cavidine and ± "Base II" are identical, but it was not possible to obtain an authentic sample of "Base II" for comparison.

The second base, also optically inactive, $C_{20}H_{21}NO_4$, now named apocavidine 79, is a demethylated derivative of 77. This was apparent from an examination of its mass spectrum and its p.m.r. spectrum. The mass spectrum showed a similar fragmentation pattern to that of cavidine. The p.m.r. spectrum, Figure 10, in $CDCl_3$, shows a signal corresponding to a single $CH\text{-}CH_3$ (0.98δ, J=7 Hz), a single methoxyl at 3.87δ and a methylenedioxy group centred at 5.93δ. The aromatic region integrates for four aromatic protons and there is present an AB quartet corresponding to the protons at C-8 and a doublet corresponding to the proton at C-14. The phenolic hydroxyl shows up as a broad signal centred near

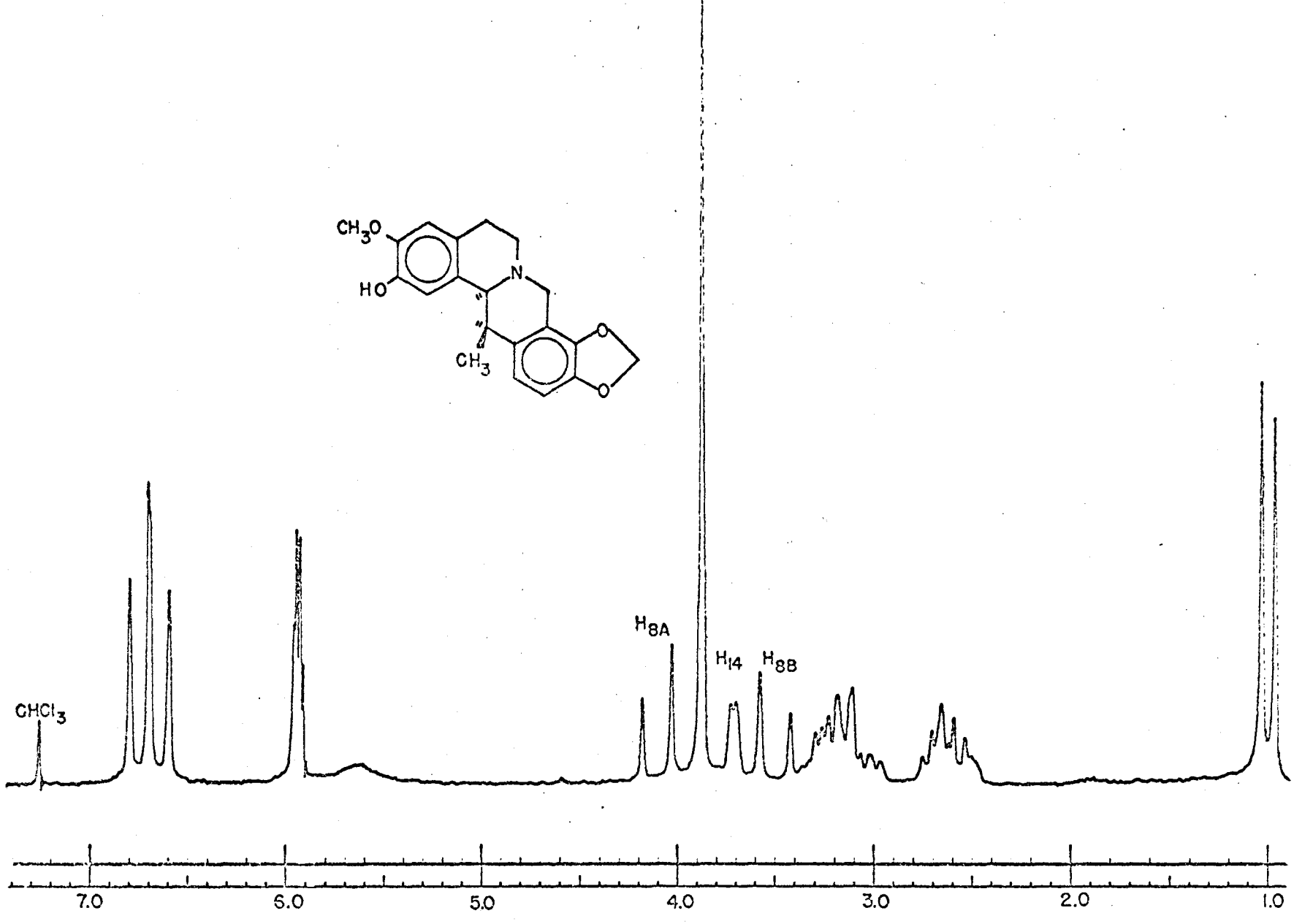
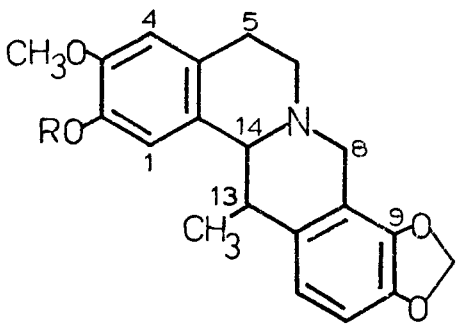


Figure 10. P.m.r. spectrum of apocavidine

5.68. The relationship of 77 and 79 was confirmed by conversion of 79 to 77 by treatment with diazomethane.

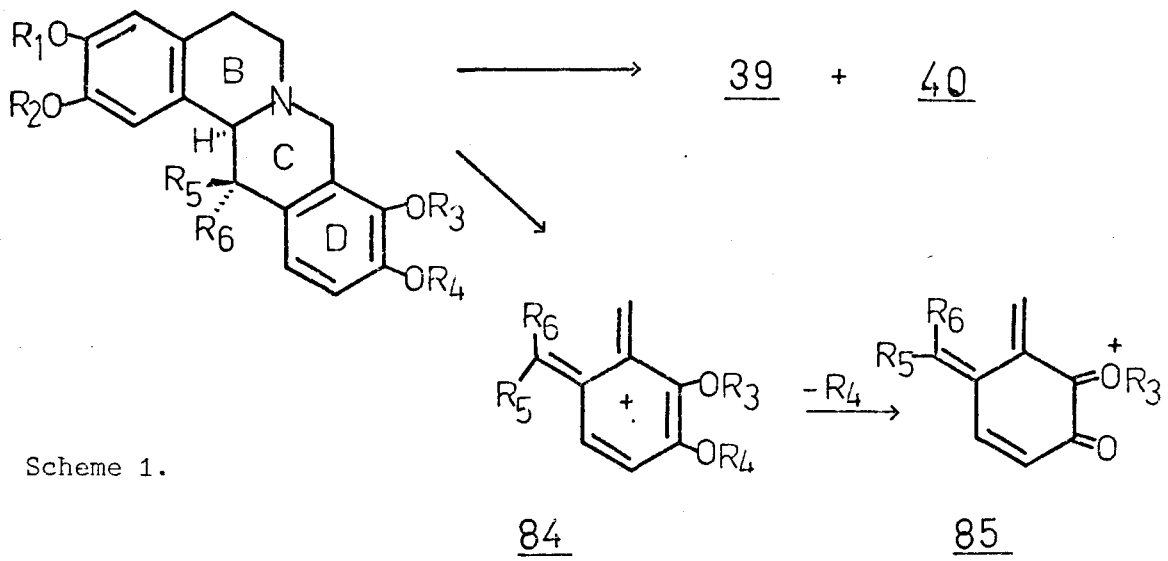


77 R=CH_3

79 R=H

From double irradiation experiments and through nuclear Overhauser effect studies (115, 108) it was possible to assign the position of the OH group in apocavidine. First, it was established that the proton to which the C-methyl group is coupled is also coupled to the signal centred at 3.71δ which must therefore be attributed to C-14. Irradiation at 3.71 causes the low field aromatic proton at 6.78δ to sharpen relative to the aromatic proton at 6.58. Thus the signal at 6.78δ is assigned to H-1. Irradiation at 2.6δ sharpens the signal at 6.58 relative to that at 6.78. Thus the signal at 6.58δ must be attributed to C-4. The irradiated proton must be one of those at C-5. Upon irradiation of the methoxyl signal the area of the signal at 6.58δ increased by 23% but the area of the signal at 6.78 was unaffected. Thus the methoxyl group was assigned to C-3 and the hydroxyl group to C-2.

The mass spectra of seven 13-methyltetrahydroprotoberberine alkaloids are recorded in Figure 8. The mass spectral fragmentation pattern of the 13-methyl derivatives are rather simple and straightforward and similar to tetrahydroprotoberberine (60). In the high mass region, the spectra show a molecular ion peak, usually about 40% of the base peak, and small peaks corresponding to the ions, $M-CH_3$ and $M-OCH_3$. The base peak ion 84 arises from the retro-Diels-Alder opening of ring C, as shown in Scheme 1.



Scheme 1.

84 85

If the charge remains on the other moiety of the molecule, ions 39 and 40 will be formed. Both ions are small but discernible. The formation of ions of type 39 requires the transfer of a hydrogen from either ring C or D to ring B. The origin of the hydrogen in this transfer has not been investigated.

When the substituents in ring C are methyl groups, as in corydaline, ion 84 can decompose further to form ion 85 by losing a

methyl group. The origin of the methyl group in this decomposition has not been investigated. However, in analogy to the spectra of tetrahydroprotoberberine (60), R4 is, presumably, lost preferentially to form the more stable ion 85, which can expel a molecule of carbon monoxide to give the ion at m/e 135. When the substituents in ring D are methylenedioxy, no such process is possible.

The composition of fragment ions discussed above have been confirmed by high resolution mass measurements, as shown in Table III.

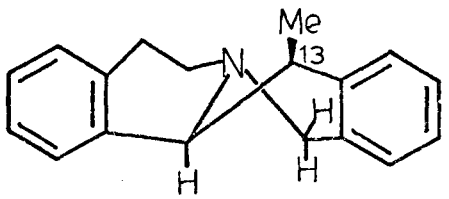
TABLE III

Compositions of Major Ions in the Spectra of Cavidine and Apocavidine

Cavidine		Apocavidine	
m/e	Composition	m/e	Composition
353	$C_{21}H_{23}NO_4$	339	$C_{20}H_{21}NO_4$
338	$C_{20}H_{20}NO_4$	324	$C_{19}H_{18}NO_4$
192	$C_{11}H_{14}NO_2$	178	$C_{10}H_{12}NO_2$
190	$C_{11}H_{12}NO_2$	176	$C_{10}H_{10}NO_2$
162	$C_{10}H_{10}O_2$	162	$C_{10}H_{10}O_2$

The stereochemistry at C-13, as expected, has no effect on the fragmentation pattern. The spectra of Hcorydaline and mesocorydaline, in which the C-13 and C-14 hydrogen are cis and trans to one another, respectively, are almost identical with only small variations in intensity. A similar phenomenon is observed in the spectra of thalictrifoline and cavidine.

The conformation of the 13-methyltetrahydroprotoberberines has been discussed by Jeffs (122, 123). He has concluded that in those compounds in which the C-13 and C-14 hydrogens are cis to one another, the quinolizidine system assumes a trans conformation as shown in 86. The C-13 methyl will adopt an axial conformation and all compounds of this stereochemistry exhibit Bohlmann bands in their i.r. spectra.



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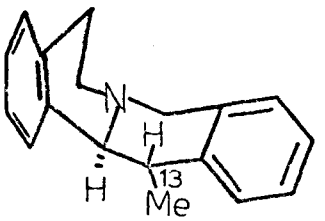

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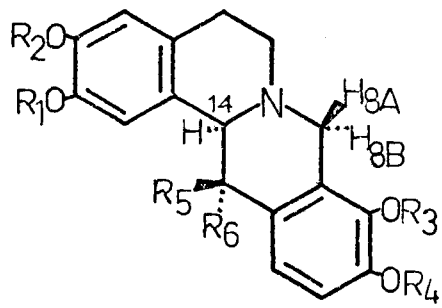
The two new bases described above fall into this category since both have Bohlmann bands in their i.r. spectra. The diastereomeric compounds in which the hydrogen at C-13 and C-14 are trans to one another are considered by Jeffs to adopt a cis-quinolizidine conformation, as shown in 87, for to do otherwise would result in strong non-bonded interactions between the C-CH_3 and the hydrogens at C-1 and C-12. As expected, compounds of this configuration, e.g. meso-corydaline and thalictrifoline, do not exhibit Bohlmann bands.

The p.m.r. data of a number of these compounds are listed in Table IV. Only two alkaloids with the hydrogen at C-13, C-14 trans to one another were available, namely, thalictrifoline and meso-corydaline.

TABLE IV

Selected Signals in the P.m.r. Spectra of Some 13-methyltetrahydroprotoberberines

		Chemical shift in p.p.m. (δ)				Coupling Constant Hz
		H-8A	H-8B	13-CH_3	H-14	$J_{H_{13}-H_{14}}$
(+)-corydaline (80)	$R_1=R_2=R_3=R_4=R_5=CH_3$ $R_6=H$	4.19	3.49	0.97	3.68	3.0
meso-corydaline (81)	$R_1=R_2=R_3=R_4=R_6=CH_3$ $R_5=H$	4.13	3.97	1.48	3.62	7.5
(+)-corybulbine (82)	$R_1=R_3=R_4=R_5=CH_3$ $R_2=R_6=H$	4.20	3.55	0.99	3.70	3.0
(+)-thalictricavine (83)	$R_1+R_2=CH_2$; $R_6=H$ $R_3=R_4=R_5=CH_3$	4.21	3.52	0.99	3.69	3.0
(+)-thalictrifoline (78)	$R_1=R_2=R_6=CH_3$ $R_3+R_4=CH_2$; $R_5=H$	4.02	3.89	1.48	3.68	7.5
(±)-cavidine (77)	$R_1=R_2=R_5=CH_3$ $R_3+R_4=CH_2$; $R_6=H$	4.07	3.52	0.98	3.74	3.0
(±)-apocavidine (79)	$R_2=R_5=CH_3$ $R_3+R_4=CH_2$ $R_1=R_6=H$	4.08	3.50	0.98	3.71	3.0



The spectrum of thalictrifoline is shown in Figure 11. It is obvious that there are three noticeable differences between the two groups of diastereomers. The first is the chemical shift of the C-Me group, the second is the coupling constant between the protons at C-13 and C-14, and the third is the chemical shift of the two protons at C-8. The chemical shift of the C-Me in those compounds with cis hydrogens is near 1.0δ while it is near 1.5δ in the system with trans hydrogens at these centres, an observation also made by Shamma et al. (124) for two synthetic 13-methyltetrahydroprotoberberines. In the systems in which the hydrogens are trans, the C-Me group lies nearly in the plane of ring D and is, therefore, deshielded. The coupling constants between H-13 and H-14 are ca. 3.0 Hz in the systems with cis hydrogens and ca. 7.5 Hz in the system with trans hydrogens. The coupling constants are expected for the systems, in which the dihedral angle approaches 90° in one case and 180° in the other. The H-14 signal is clearly visible in all spectra.

In all the spectra of the cis compounds the protons at C-8 appear as an AB quartet (J=16 Hz) with a large chemical shift (0.6-0.7)

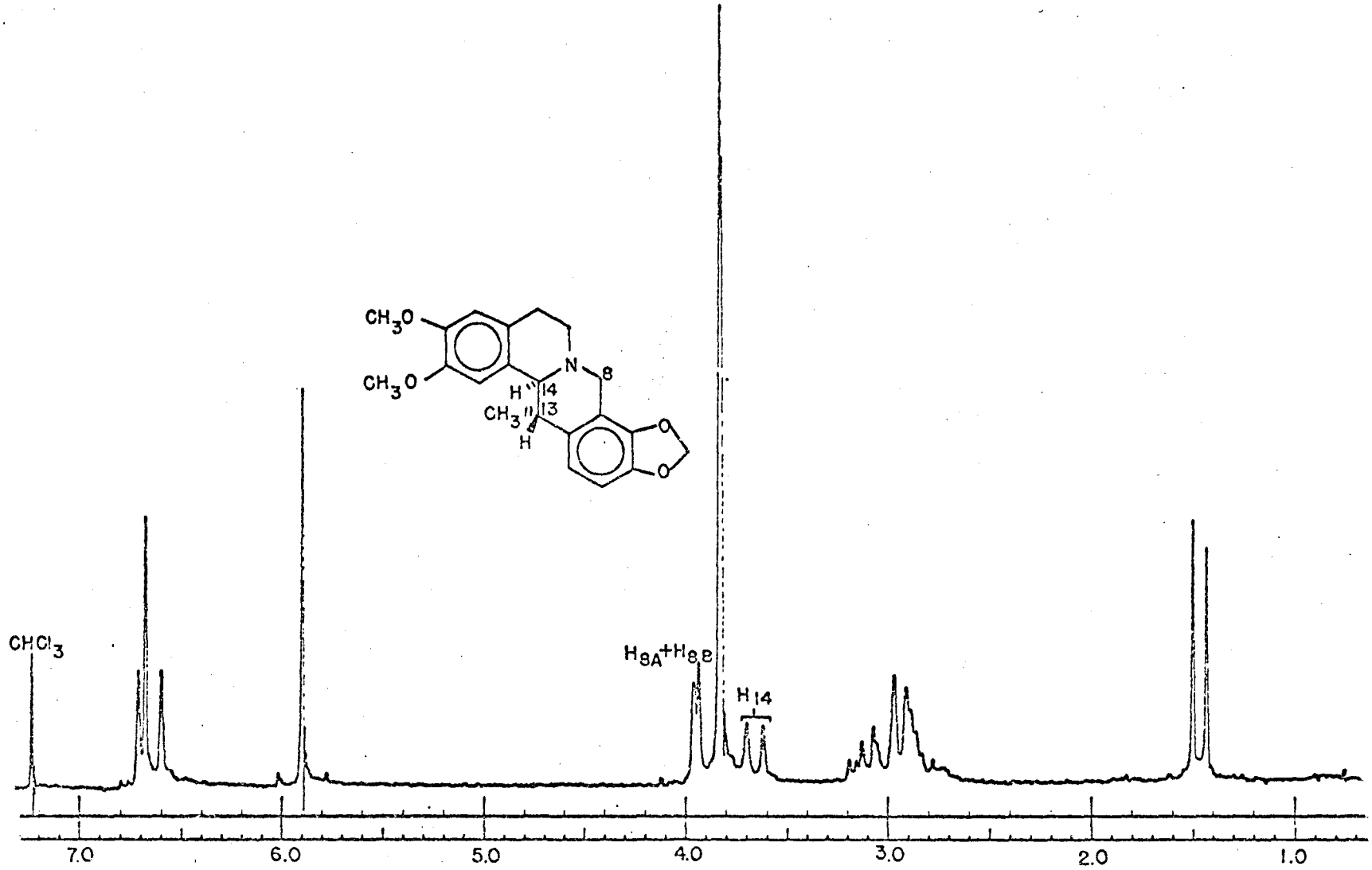


Figure 11. P.m.r. spectrum of thalictrifoline

between them. The lower field proton is probably equatorial and deshielded by the aromatic ring D, the lone pair on the adjacent nitrogen, and the oxygen at C-9. In the two compounds with trans hydrogens at C-13 and C-14 the chemical shifts of the two protons at C-8 are very much smaller (0.1-0.2 ppm) with the high field proton moving downfield. Thus the p.m.r. spectra are diagnostic of the configuration of these systems. The results of the p.m.r. study are in complete accord with and lend support to the stereochemical assignment made on the basis of i.r. and conformational arguments. Structures 86 and 87 are typical representations of trans and cis-fused tetrahydroprotoberberines.

P.m.r. spectroscopy has been used previously to assign conformation in saturated cyclic systems in which nitrogen occupies a bridgehead position (125 and the references cited therein). It has been shown that, in trans-fused system, the two protons on the methylene adjacent to nitrogen have a much larger difference in chemical shift than the analogous protons in a cis-fused system and that the proton at lower field in the trans-fused system is the equatorial proton. The difference in chemical shift has been attributed to the deshielding effect of the lone pair on the nitrogen. In the trans-fused system only one proton, the equatorial one, is deshielded by the lone pair. In the cis-fused system both protons are equally affected since the lone pair bisects the angle between the geminal protons.

Mass Spectra of Spirobenzylisoquinoline Compounds

Extensive work has been done on the mass spectra of many isoquinoline alkaloids as discussed in the previous Chapter. No systematic study, however, of the mass spectra of spirobenzylisoquinoline alkaloids has been carried out. The first reported spectra of these alkaloids (104), i.e. ochotensine and ochotensimine, gave no interpretation about their fragmentation. The reported data showed no characteristic fragmentations which could be used for diagnostic purposes. On the other hand, the spirobenzylisoquinoline compounds with oxygenated substituents in the five-membered ring show distinct fragmentation patterns. The present work will deal with the fragmentation pattern of several known alkaloids of the spiro series with the aid of high resolution mass spectrometry and some synthetic model compounds.

Mass Spectra of Fumaricine (67), Fumaritine (68) and Dihydrofumariline (69)

The mass spectra of 67, 68, and 69 are recorded in Figure 12. The base peak, ion 90, in the spectra of these compounds has been postulated (109) to arise from the process illustrated in Scheme 2. This mechanism has been supported by a deuterium labelling experiment, in which the hydroxyl hydrogen was replaced by deuterium. The base peak then shifts one mass unit higher. The small peak two mass units lower than the base peak is probably due to the isoquinolinium ion 91, formed from 88 by C-C bond cleavage followed by the loss of one hydrogen from the isoquinoline moiety and rearrangement of another or by loss of two hydrogens from 90. Both ions, 90 and 91, are characteristic of

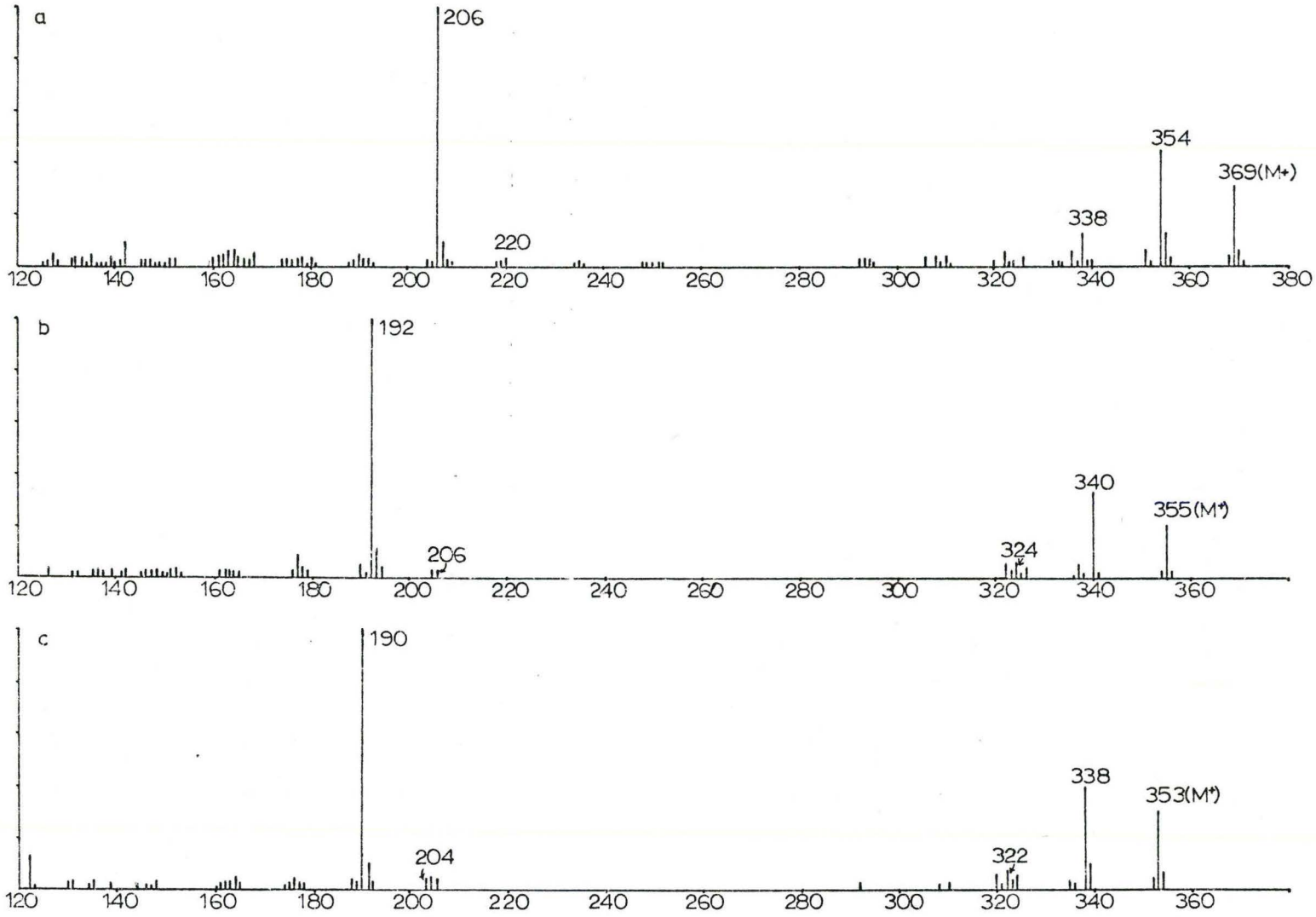
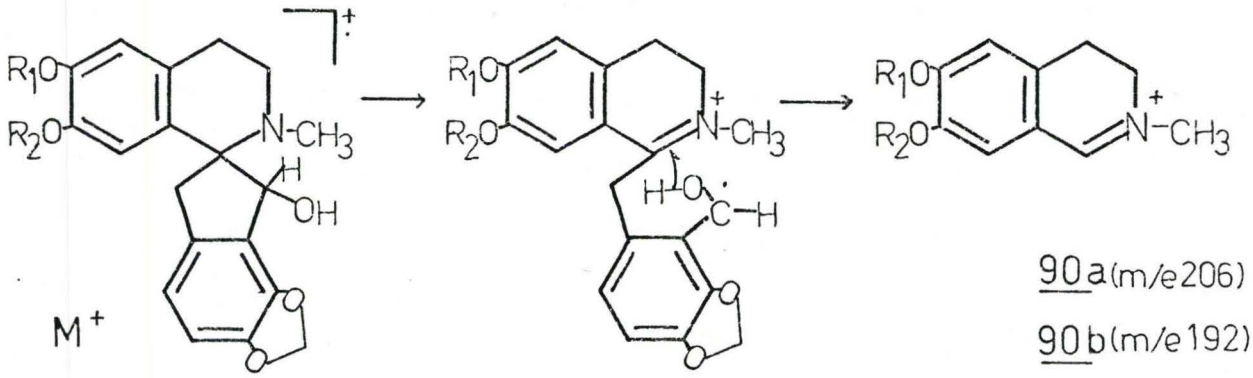
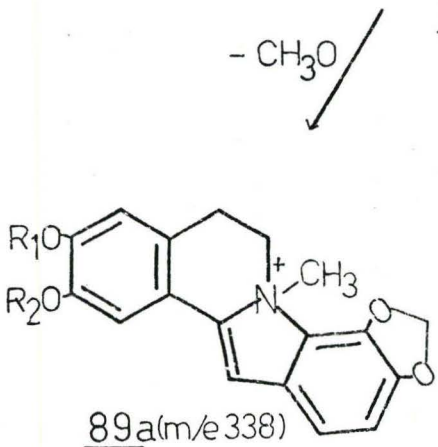
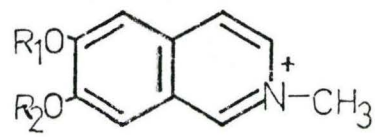
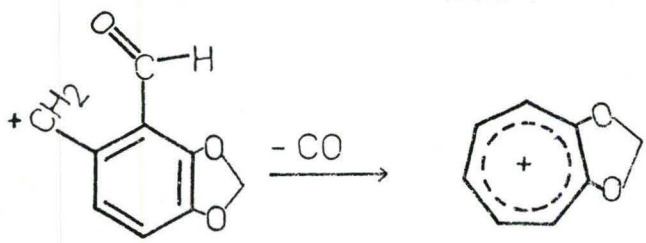


Figure 12. Mass spectra of a) fumaricine, b) fumaritine, c) dihydrofumariline









a $R_1=R_2=CH_3$

b $R_1=CH_3$, $R_2=H$

c $R_1+R_2=CH_2$

Scheme 2

many isoquinoline alkaloids. The same bond rupture with charge retention by the other portion of the molecule leads to ion 92, m/e 163, which upon losing CO yields ion 93, m/e 135. These ions are too small to be of any diagnostic value.

On the high mass end, the M-CH_3 ion shows a constant intensity relative to the molecular ion ((intensity of M-CH_3)/(intensity of M) is about 1.4-1.7), which indicates that the source of the methyl radical must be from a common structural unit, presumably the N-CH_3 group. In dihydrofumariline (69), the only source of M-CH_3 ion is the methyl group attached to the nitrogen atom. Thus, it is assumed that the methyl group at nitrogen is lost quite readily upon electron impact. The reason for this is not clear.

The M-31 peak is observed in all three cases and its composition has been determined as M-OCH_3. In 67 and 68, this ion may be attributed to the loss of OCH_3 from the substituent in ring A. In 69, however, the ion, M-31, can only come from another source. A reasonable speculation about its formation is that ion 88 expels a neutral fragment CH_2O and a radical H· to form ion 89. As will be seen later, ion 89 occurs in most, if not all, of the spirobenzylisoquinoline compounds with oxygenated substituents in the five-membered ring. It may be that ions 89a and 89b also contribute significantly to the M-31 peak in 67 and 68, respectively.

A small ion, fourteen mass units higher than the base peak, has been found to contain one nitrogen and three oxygen atoms. The structure of this ion will be discussed in a later section.

TABLE V

Compositions of the Major Ions in the Mass Spectra of Fumaricine (67), Fumaritine (68) and Dihydrofumariline (69)

Fumaricine		Fumaritine		Dihydrofumariline	
m/e	Composition	m/e	Composition	m/e	Composition
369	$C_{21}H_{23}NO_5$	355	$C_{20}H_{21}NO_5$	353	$C_{20}H_{19}NO_5$
368	$C_{21}H_{22}NO_5$	354	$C_{20}H_{20}NO_5$	339	$C_{19}H_{17}NO_5$
354	$C_{20}H_{20}NO_5$	340	$C_{19}H_{18}NO_5$	338	$C_{19}H_{16}NO_5$
338	$C_{20}H_{20}NO_4$	326	$C_{19}H_{20}NO_4$	322	$C_{19}H_{16}NO_4$
326	$C_{19}H_{20}NO_4$	324	$C_{19}H_{18}NO_4$	204	$C_{11}H_{10}NO_3$
220	$C_{12}H_{14}NO_3$	322	$C_{19}H_{16}NO_4$	190	$C_{11}H_{12}NO_2$
206	$C_{12}H_{16}NO_2$	206	$C_{11}H_{12}NO_3$	188	$C_{11}H_{10}NO_2$
204	$C_{12}H_{14}NO_2$	192	$C_{11}H_{14}NO_2$		
190	$C_{11}H_{12}NO_2$	190	$C_{11}H_{12}NO_2$		
		177	$C_{10}H_{11}NO_2$		

The compositions of the fragment ions of 67, 68 and 69 are listed in Table V.

Mass Spectrum of Fumariline (66)

The spectrum of fumariline (66), Figure 13, is distinctly different from that of 67, 68, and 69. It does not show an intense peak corresponding to 90, since there is not a readily available hydrogen to facilitate the process for its formation. The isoquinolinium ion, m/e 188, however, is still discernible in the spectrum, since a process, in which hydrogen is lost and undergoes rearrangement in the isoquinoline moiety, is still feasible. The base peak now appears at m/e 322. High resolution mass spectrometry has shown that this peak is a singlet and that it corresponds in composition to $C_{19}H_{16}NO_4$. It is apparently formed by loss of CO followed or preceded by loss of H. In the absence of a labelling experiment, the hydrogen is assumed to come from H-9, and the process proposed for the formation of the base peak, ion 89c, is shown in Scheme 3.

Ion 89c is expected to be an extremely stable species, since it is an even electron ion and the double bonds are fully conjugated. It is not surprising to find that this species is also found in dihydrofumariline, sibiricine, ochrobirine, and other compounds of this type (vide infra).

Upon loss of a molecule of formaldehyde from the methylenedioxy group, a process which is not very favorable but feasible (126), the ion 89c yields an ion at m/e 292, which decomposes further to give an ion at m/e 264. Since these processes are not favorable energetically,

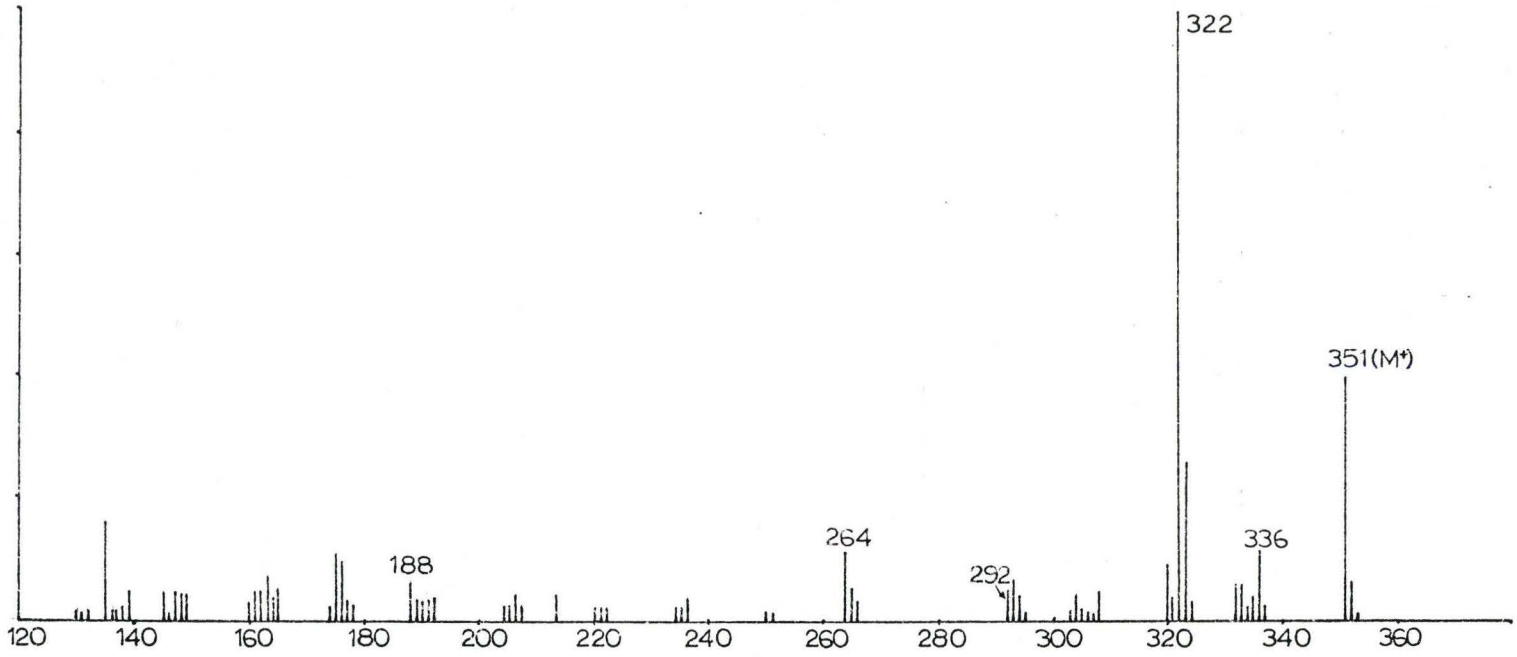
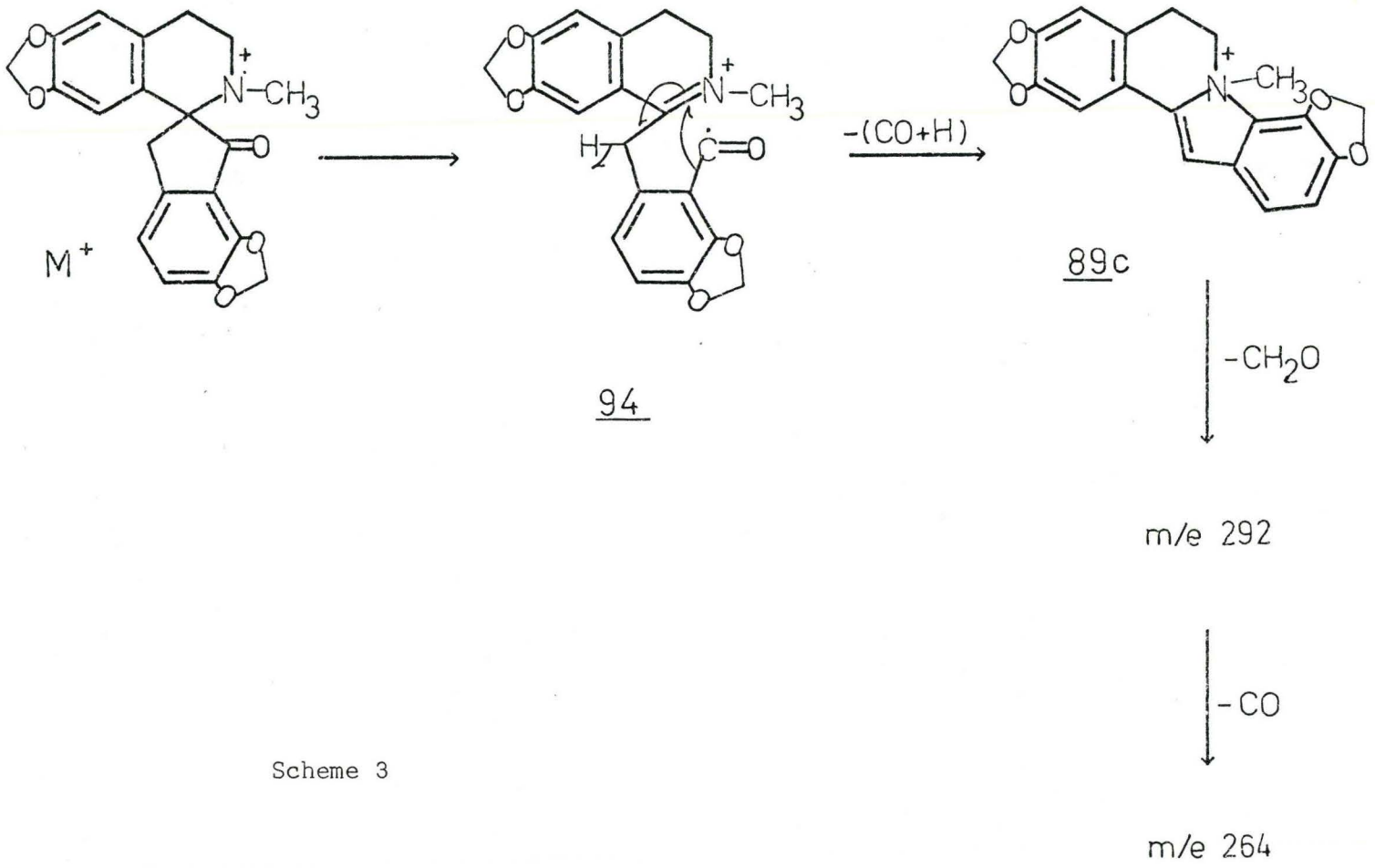


Figure 13. Mass spectrum of fumariline



Scheme 3

the peak intensities are small. A peak corresponding to M-CH_3 at m/e 336 is small but observable in the spectrum. The source of this methyl radical is probably the methyl group attached to the nitrogen atom.

The compositions of the fragment ions of 66 are shown in Table VI.

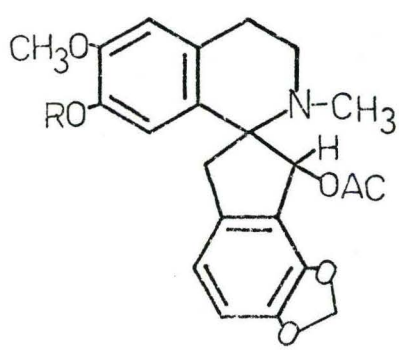
TABLE VI

Compositions of the Major Ions in the Mass Spectrum of Fumariline (66)

m/e	Composition	m/e	Composition
351	$C_{20}H_{17}NO_5$	292	$C_{18}H_{14}NO_3$
336	$C_{19}H_{14}NO_5$	264	$C_{17}H_{14}NO_2$
322	$C_{19}H_{16}NO_4$	188	$C_{11}H_{10}NO_2$

Mass Spectra of Fumarophycine and Diacetylfumaritine

Fumarophycine, an alkaloid isolated from Fumaria officinalis of Bulgarian origin by Mollov et al. (127), has been shown to have the structure 95 (128). When fumaritine or fumarophycine is treated with acetic anhydride and pyridine the diacetyl derivative 96 is obtained. The mass spectra of 95 and 96 are recorded in Figure 14.



95 R=H

96 R=CH_3-CO

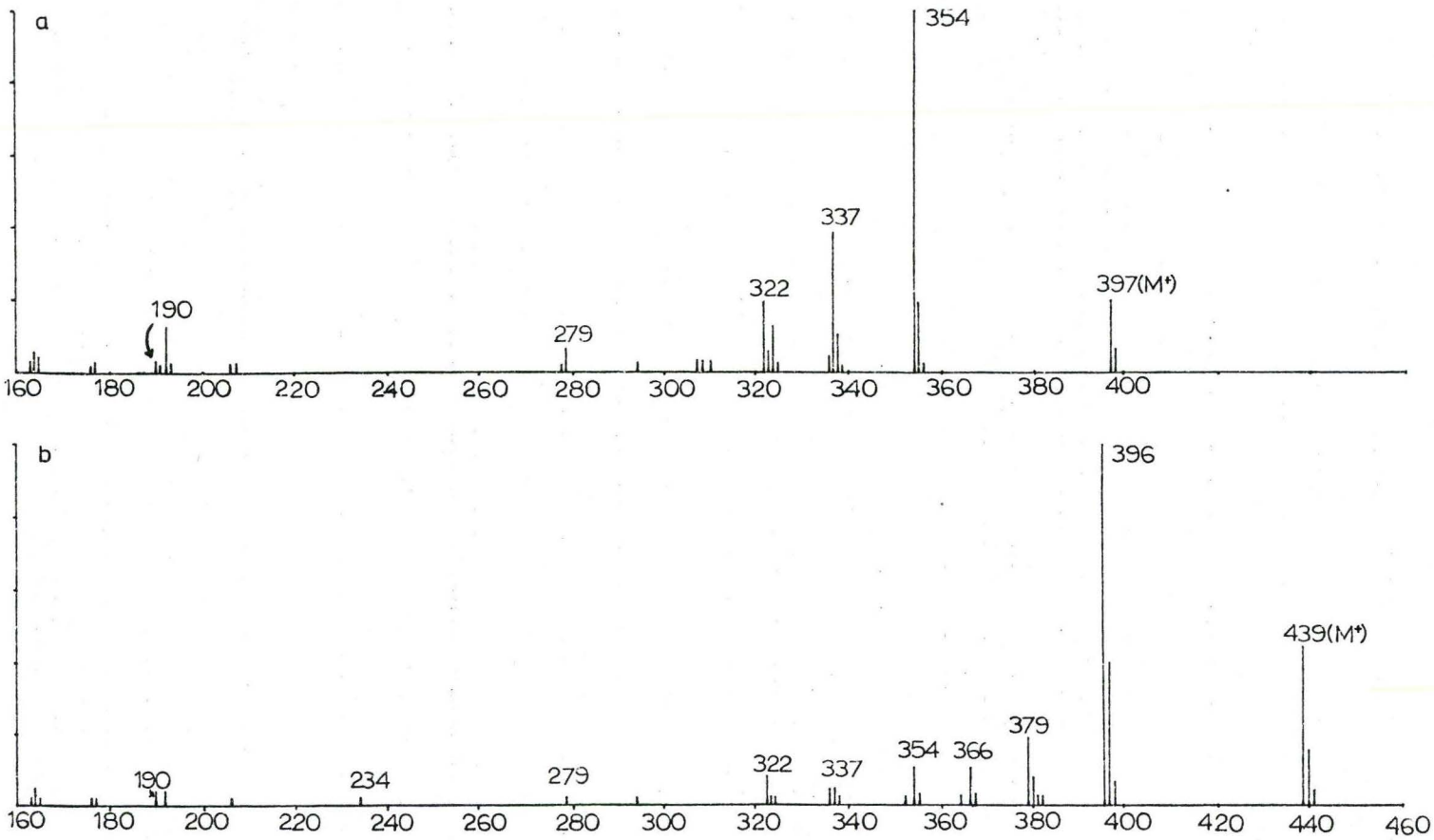


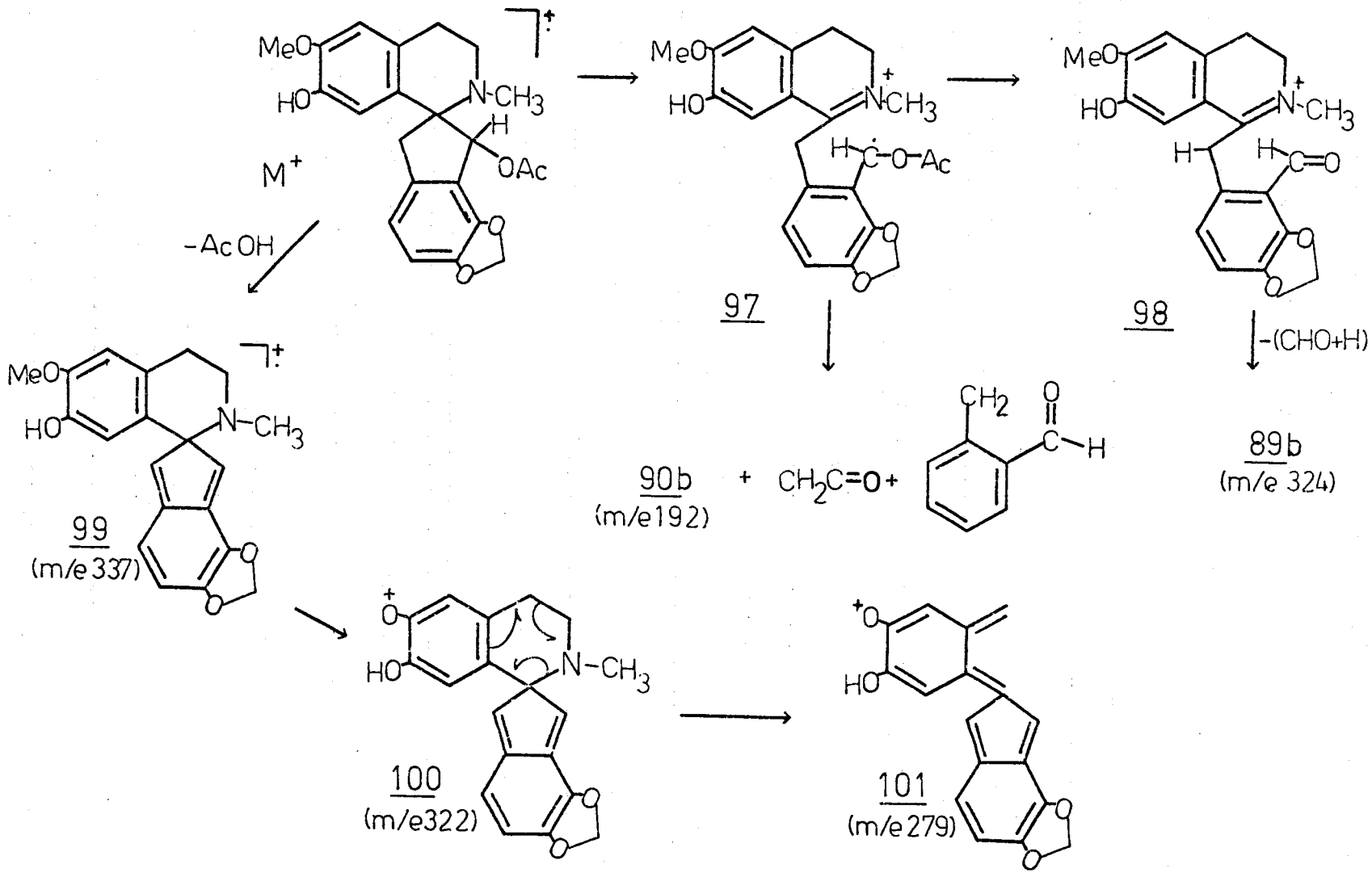
Figure 14. Mass spectra of a) fumarophycine, b) diacetylfumaritine

Introduction of an acetyl group to fumaritine alters the fragmentation pattern greatly. The base peak, ion 98, in this spectrum now arises from the expulsion of the acetyl radical as shown in Scheme 4. Ion 98 can again lose (CHO+H) to form the stable ion 89b.

The characteristic dihydroisoquinolinium ion of fumaritine can still be observed, but in much reduced intensity. Hydrogen could be transferred from the acetyl hydrogen with concomitant expulsion of a molecule of ketene and another neutral fragment to form the dihydroisoquinolinium ion 90b, as illustrated in Scheme 4. Since the process involved a hydrogen transfer from methyl rather than from the hydroxyl group, this ion is not as intense as in the case of fumaritine. It is also possible to form fumaritine first by loss of ketene and then transfer hydrogen from the hydroxyl group.

When a molecule of acetic acid is expelled from the molecular ion, ion 99 is presumably formed. Charge localization on the aromatic ring may facilitate the expulsion of the methyl group from the ion 99 to form ion 100, which may then decompose by a retro-Diels-Alder process to form ion 101.

An alternative speculation about the genesis of these ions comes from an examination of the spectrum recorded at higher temperature, about 250°C. Under these conditions, the only observable peaks in the high mass range are m/e 337, 322, and 279. Presumably, thermal elimination of the acetic acid occurs at high temperature and subsequent electron impact produces ions 99, 100, and 101. It is not known whether this process also occurs at 150°C, which was the temperature used to record the spectrum of fumarophycine.


MeO
HO
N-CH3
H
OAc
M+
-AcOH
97
98
-(CHO+H)
89b
(m/e 324)
90b
(m/e192)
+ CH2C=O+
99
(m/e337)
100
(m/e322)
101
(m/e279)

Scheme 4

TABLE VII

Compositions of the Major Ions in the Mass Spectra of Fumarophycine (95) and Diacetylfumaritine (96)

Fumarophycine		Diacetylfumaritine	
m/e	Composition	m/e	Composition
397	$C_{22}H_{23}NO_6$	439	$C_{24}H_{25}NO_7$
354	$C_{20}H_{20}NO_5$	397	$C_{22}H_{23}NO_6$
337	$C_{20}H_{19}NO_4$	396	$C_{22}H_{22}NO_6$
324	$C_{19}H_{18}NO_4$	366	$C_{21}H_{20}NO_5$
322	$C_{19}H_{16}NO_4$	354	$C_{20}H_{20}NO_5$
279	$C_{17}H_{11}O_4$	337	$C_{20}H_{19}NO_4$
192	$C_{11}H_{14}NO_2$	336	$C_{20}H_{18}NO_4$
190	$C_{11}H_{12}NO_2$	322	$C_{19}H_{16}NO_4$
164	$C_9H_8O_3$	324	$C_{13}H_{16}NO_3$
		206	$C_{11}H_{10}O_4$
		192	$C_{11}H_{14}NO_2$
		190	$C_{11}H_{12}NO_2$
		164	$C_9H_8O_3$

When an acetyl group is attached to the molecule of fumarophycine, peak shifts are observed. Since ketene is one of the stable and favored neutral departing species in acetate esters, ions with acetyl groups can expel ketene. Thus, ions 98, 99, 89b, and 90b, and their acetyl analogues at m/e 396, 379, 366, and 234 are all observed in the spectrum of 96. Since the carbon skeleton of 95 and 96 are the same, their fragmentation patterns will be similar. That is indeed the case.

The compositions of the major fragment ions of fumarophycine and diacetylfumaritine are shown in Table VII.

Synthetic Model Compounds

During the study of alkaloid F-38 (vide infra), model compounds were prepared in order to gain further insight into the fragmentation of compounds with the spirobenzylisoquinoline skeleton. A description of their synthesis follows.

In 1968, Kametani et al. (129) reported the condensation of 3-hydroxy-4-methoxyphenylethyl amine with ninhydrin to form a compound which he named 3-hydroxy-2-methoxyochotensinan-8,13-dione. When, however, in this laboratory the amine hydrochloride was used in the condensation with ninhydrin, a different product was obtained. The pink semicrystalline solid could not be purified easily through recrystallization as reported. Accordingly, without further purification, the solid was methylated with diazomethane and recrystallized from a small amount of methanol. Yellow prismatic crystals were obtained. The infrared spectrum showed no hydroxyl absorption, but two absorptions in the carbonyl region at 1700 and 1745 cm^{-1}. The 60 MHz p.m.r.

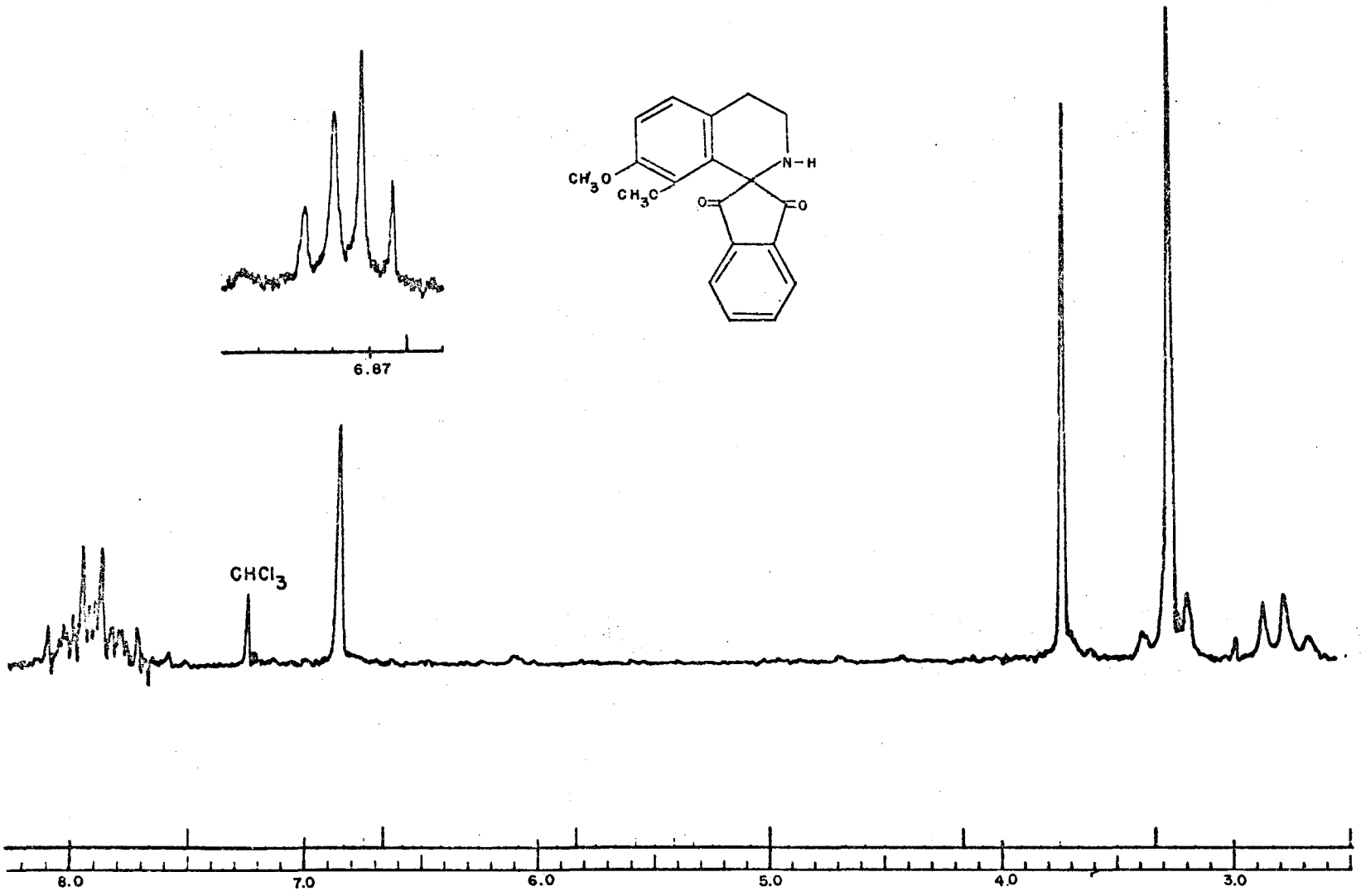
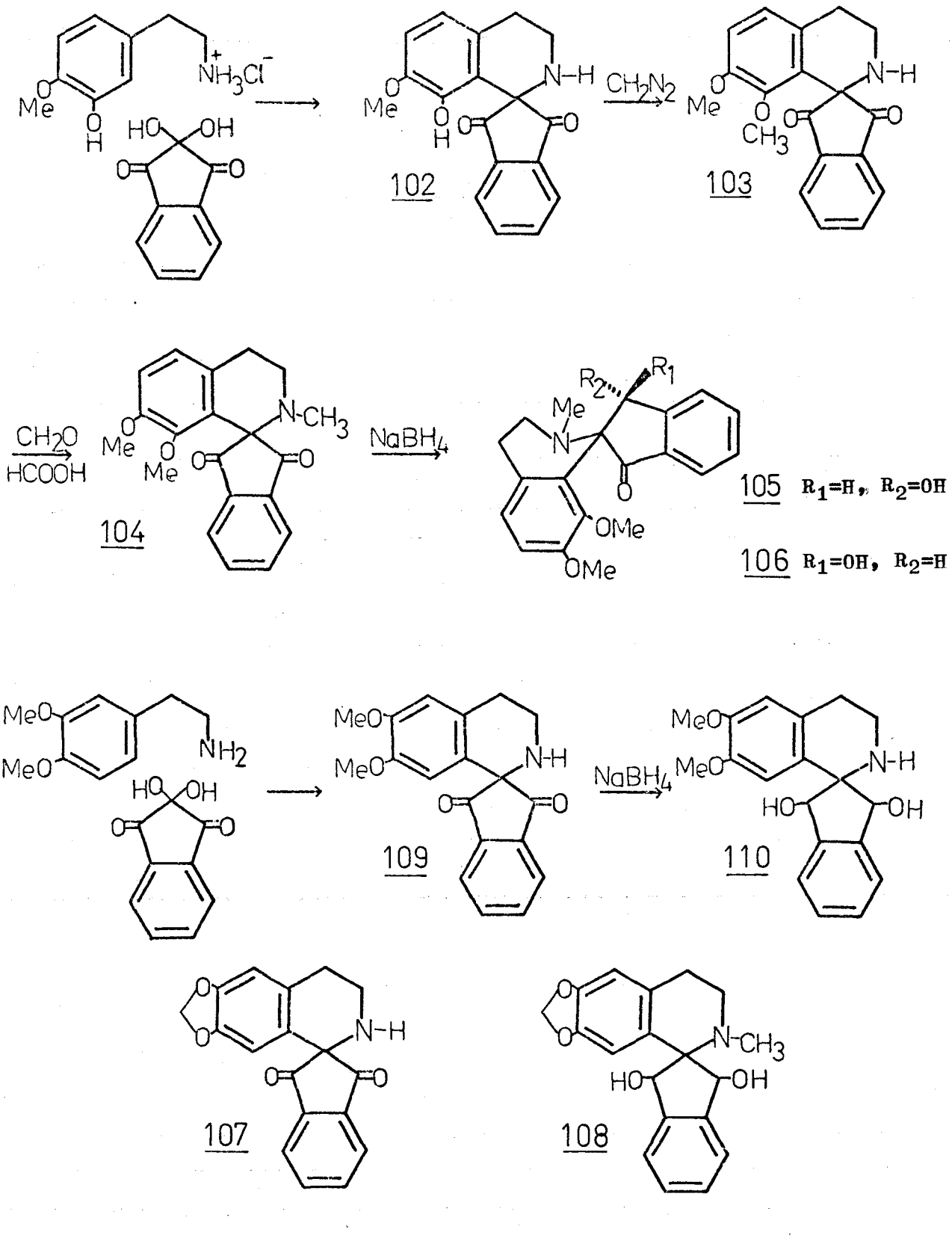


Figure 15. P.m.r. spectrum of 103

spectrum, Figure 15, shows 2 methoxyl signals at 3.28δ and 3.73δ, one singlet of area 2 at 6.86δ corresponding to two aromatic protons in ring A, a multiplet of area 4 in the region 7.7-8.1δ arising from the protons in ring D and signals from the remaining protons in the range 2.5-3.5δ. When the spectrum was run on the 220 MHz instrument, the aromatic signal at 6.86δ was split into a well defined AB quartet with a coupling constant of 8 Hz (see inset Figure 15). It is clearly demonstrated that during the condensation of ninhydrin and 3-hydroxy-4-methoxyphenylethylamine under our conditions cyclization took place at the carbon ortho rather than para (129) to the hydroxyl group. The structure of the product must be 102, Scheme 5.

In the interim, Manske and Ahmed (130) reported the synthesis of an analogue of ochrobirine in high yield. They simply treated 3,4-methylenedioxyphenethylamine with ninhydrin in absolute ethanol at low temperature, and were able to isolate pure para cyclized product 107, which was later transferred into 108, the analogue of ochrobirine, by successive methylation and sodium borohydride reduction.

In order to simulate this reaction, 3,4-dimethoxyphenethylamine was condensed with ninhydrin at ice-water temperature. The product 109, bright yellow crystals, melted at a different temperature from 103. The p.m.r. spectrum of 109 showed a pattern similar to 103 with the exception that two singlets at 5.91 and 6.65δ appeared in the aromatic region replacing the AB quartet of 103. Apparently, the cyclization occurs at the para position in this instance as shown in Scheme 5. The signal at 6.65δ is broader than the other singlet, presumably because


Me
O
H
NH3Cl
HO
OH
O
O
CH2N2
N-H
CH3
102
103
CH2O
HCOOH
N-CH3
NaBH4
R2
R1
Me
N
OMe
104
105 R1=H, R2=OH
106 R1=OH, R2=H
MeO
NH2
109
110
107
108

Scheme 5

of benzylic coupling to H-5. The same situation has also been observed in the spectrum of sibiricine (66) in which two singlets, one at 6.54, the broader signal, and one at 6.04δ, were assigned to H-4 and H-1, respectively.

When compound 103 was methylated with formic acid and formaldehyde and reduced with sodium borohydride, two major products were detected on thin layer chromatography (T.L.C.). The two major compounds were separated by thick layer chromatography and subjected to spectroscopic investigation. Both products, 105 and 106, were dihydro compounds as shown by their mass spectra. The i.r. spectra of both compounds show both hydroxyl and carbonyl absorption. Apparently, only one carbonyl group was reduced in this reaction, possibly because of steric hindrance. When one of the carbonyl groups is reduced, the interaction between R_1 and OCH_3 at C-1 (see 105 or 106) may be such that this OCH_3 group is forced to sit behind the remaining carbonyl group. The interaction between R_2 and $N\text{-}CH_3$ may force the N-methyl group to stay in front of the carbonyl group. As a result, the carbonyl group is not readily accessible to hydride attack under mild reaction conditions and only dihydro compounds, 105 and 106, are obtained.

The assignment of stereochemistry to 105 and 106 is based on two facts. Firstly, on T.L.C. plates compound 105 has a higher Rf value than that of 106, indicating that the latter is adsorbed more firmly on the surface of the silica gel. Since hydrogen bonding in 105 may reduce the interaction between adsorbent and compound (131), it moves faster on T.L.C. Secondly, when the p.m.r. spectra of both compounds

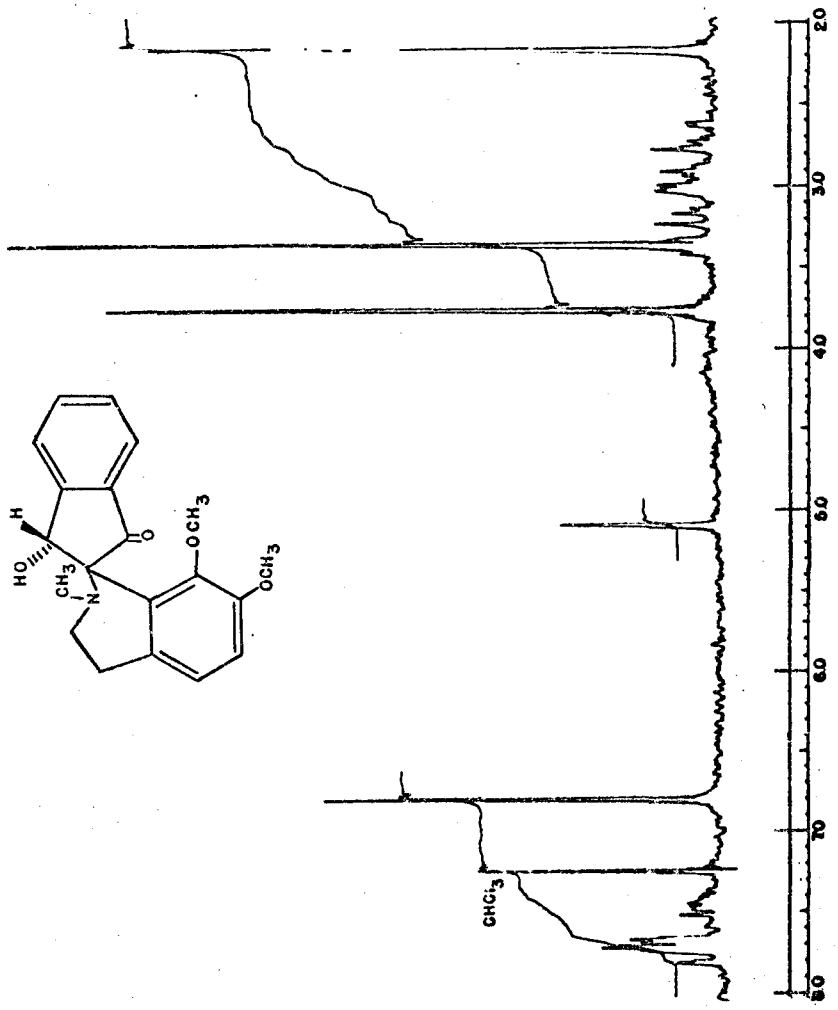


Figure 16. P.m.r. spectrum of 105

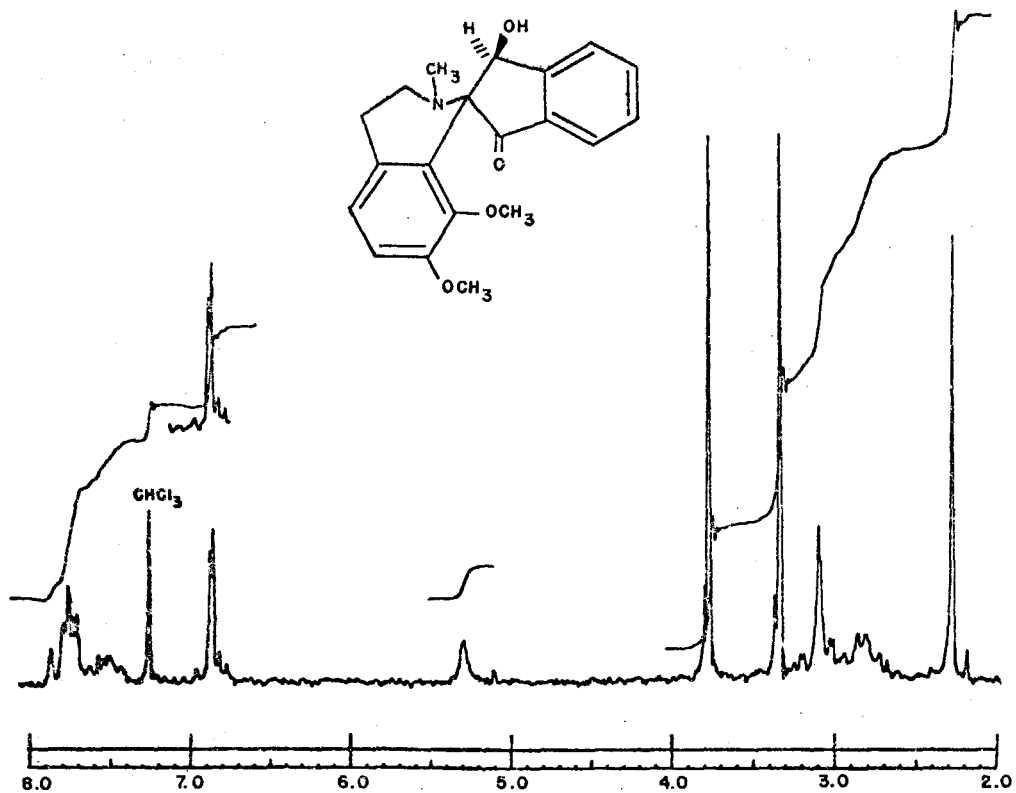


Figure 17. P.m.r. spectrum of 106

are compared, as shown in Figures 16 and 17, the only significant difference is the signal at 5.08δ in 105 and the very broad signal centred at 5.26δ in 106. These signals are assigned to the C-9 protons in each case. Analogous signals have been observed in ochrobirine (107), in which the broad signal at 5.42δ was assigned to the proton at H-14 on the same side of the plane as the $N-CH_3$ group while the sharp singlet at 4.88δ was assigned to H-9 which is on the opposite side of the ring. Thus, the stereochemistry of 105 and 106 is confirmed.

When 109 was reduced with sodium borohydride one major compound 110 was detected on T.L.C. The molecular ion peak in the mass spectrum appears four mass units higher than in 109, indicating that both carbonyl groups are reduced in this instance. Since there is less steric hindrance present in this molecule, both carbonyl groups are accessible to hydride attack.

Mass Spectra of 105 and 106

The spectra of 105 and 106 are nearly identical but vary slightly in peak intensity. Although the substitution pattern in ring A is different from that of the natural alkaloid, sibiricine, the basic carbon skeleton is still the same and, therefore, can be used as a model to gain a better understanding of the fragmentation mechanism of this series. The spectrum of 105 is recorded in Figure 18.

The base peak is the molecular ion peak, reflecting the stability of the molecular ion. Most fragments result from the cleavage of the two benzylic bonds which are β to the nitrogen. When the bond α to the hydroxyl group is broken, ion 111 is formed. The decomposition

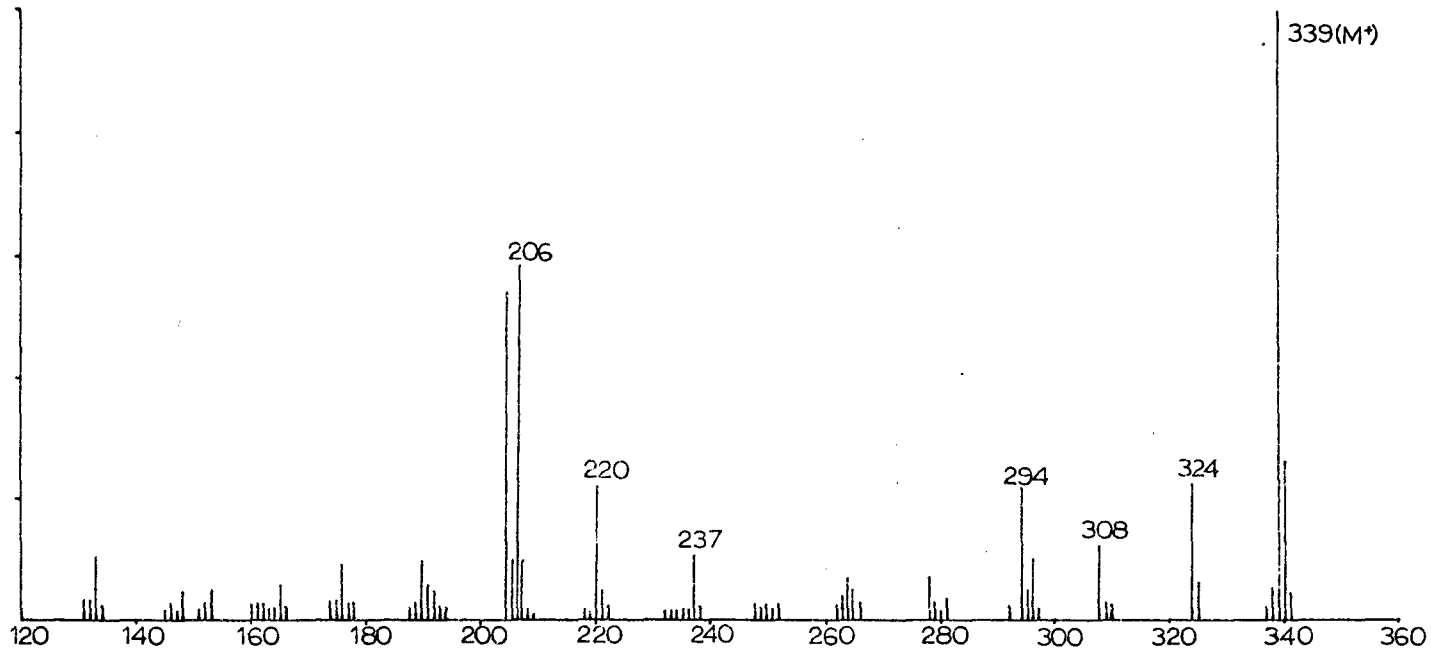
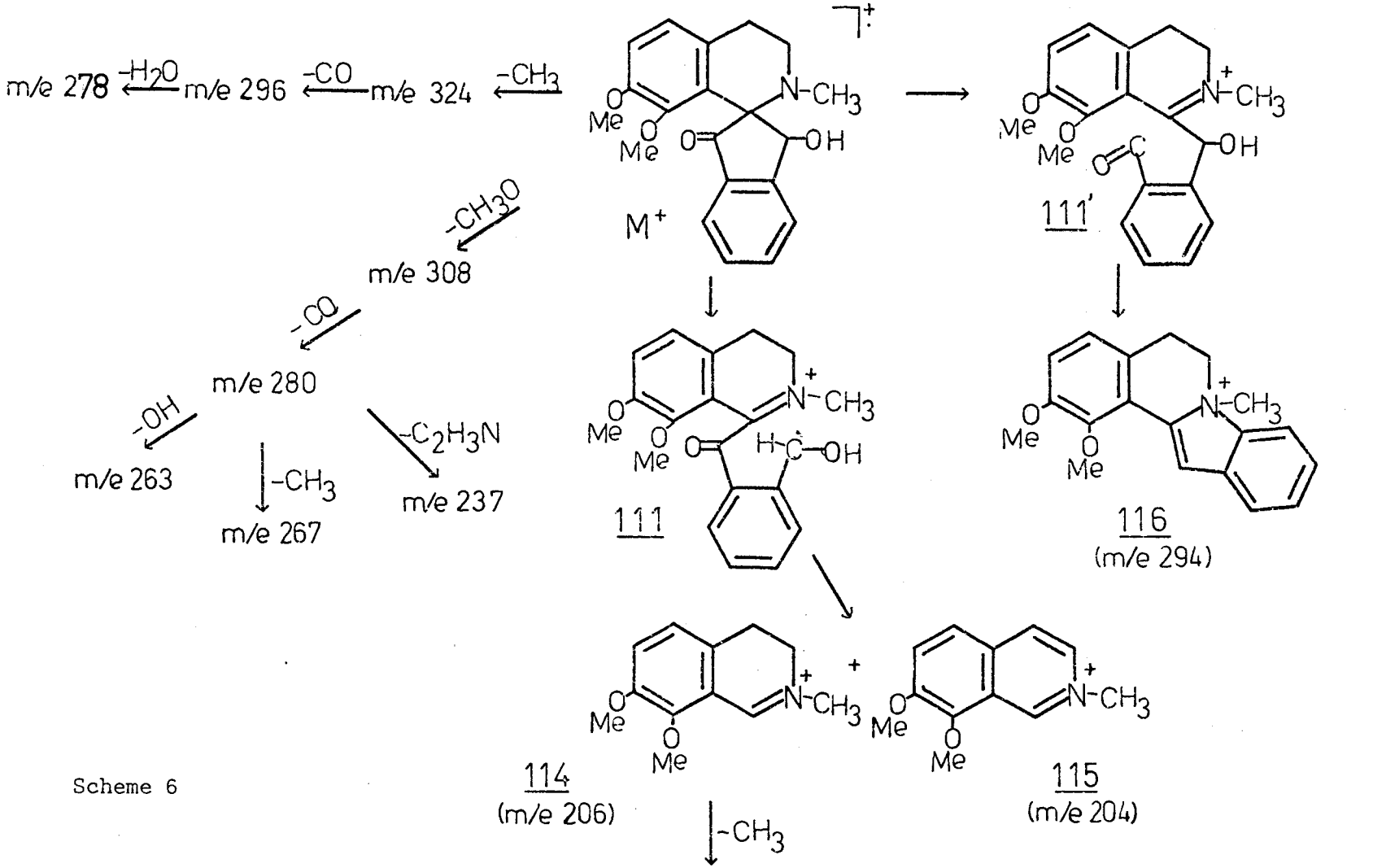


Figure 18. Mass spectrum of 105


m/e 278
-H2O
m/e 296
-CO
m/e 324
-CH3
M+
-CH3O
m/e 308
-CO
m/e 280
-OH
m/e 263
-CH3
m/e 267
-C2H3N
m/e 237
111
111'
116
(m/e 294)
114
(m/e 206)
115
(m/e 204)
-CH3
m/e 191
-CH3
m/e 176
-CO
m/e 148

Scheme 6

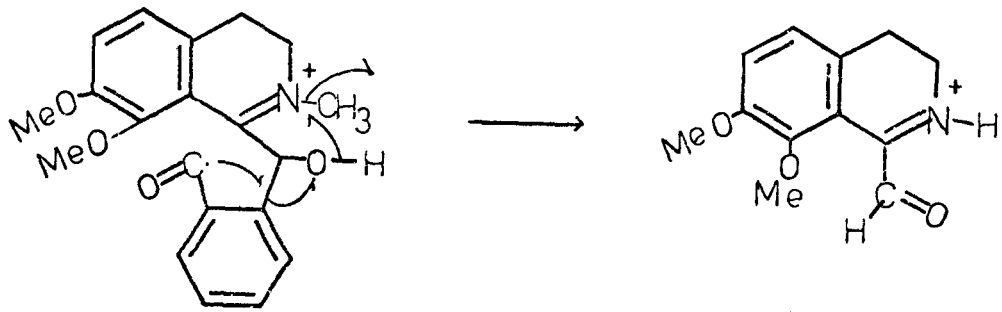
of 111 will follow a process analogous to that in the fumaricine series (109) to give the ions, 114 and 115. Ion 114 can lose two CH_3 radicals and CO successively to give ions m/e 191, 176, and 148, respectively, as shown in Scheme 6. The intensity of ions 114 and 115 are nearly alike whereas in the corresponding ions of fumaricine, the ion of higher mass is much more intense.

If the benzylic bond α to the carbonyl is broken, the product ion can be represented as 111'. The expulsion of a molecule of CO and an OH radical from 111' yields the stable ion 116, a moderately intense peak. From the information available, it is hard to determine whether this is a concerted or a stepwise process. The process, however, is most likely similar to that described for the genesis of the base peak in the spectrum of fumariline in which hydrogen instead of a hydroxyl radical is expelled. The ion structure should be similar, as represented in 116.

In the high mass range, there are a series of small peaks corresponding to the loss of small fragments, such as CH_3, $CH_3O\cdot$, $OH\cdot$, and CO from the molecular ion. Several sequences of decomposition are likely to occur in the manner shown in Scheme 6. It has been shown that ion m/e 237 contains no nitrogen. This ion may possibly come from the retro-Diels-Alder decomposition of ring B of the ion of m/e 280.

A moderately intense peak at m/e 220 has been shown to have the composition $C_{12}H_{14}NO_3$. It, therefore, comes from the isoquinoline moiety. Two possible mechanisms are proposed as shown in Scheme 7.

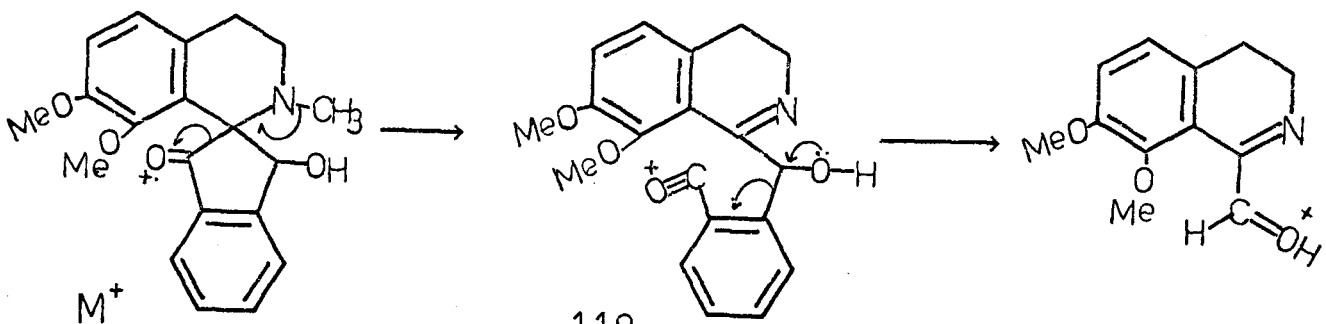
a.



111'

117

b.



M+

118

117'

Scheme 7

In the first mechanism "a", ion 111' can give rise to ion 117 through electron transfer and hydrogen rearrangement with concomitant expulsion of the methyl radical from the nitrogen. The alternative route "b" involves the expulsion of methyl radical from the nitrogen atom to form an intermediate ion 118, which then shifts the positive charge to oxygen to form the daughter ion 117'. Both processes involve the loss of methyl radical from the nitrogen atom and there is evidence to support this speculation. In the spectrum of dihydrofumariline, the $M-CH_3$ ion has an intensity of 38% of the base peak which indicates that the loss of the methyl group occurs readily in the spirobenzylisoquinoline system. In the absence of any further evidence, both processes are possible.

Ions of type 117 or 117' occur widely in the spectra of the spirobenzylisoquinoline compounds with hydroxyl groups in the five-membered ring, and can be used for diagnostic purposes. The intensity of this ion, however, is very small in the spectra of fumaritine, fumaricine and dihydrofumariline, and care should be taken to identify this ion.

The compositions of the major ions of 105 are shown in Table VIII.

Mass Spectrum of Sibiricine (119)

The spectrum of sibiricine is recorded in Figure 19.

The benzylic cleavage of the molecular ion may give either 120 or 120', both of which may decompose further to give a series of peaks similar to those from 105. Ion 120' can decompose by loss of CO and OH

TABLE VIII

Compositions of the Major Ions in the Mass Spectrum of 105

m/e	Composition	m/e	Composition
339	$C_{20}H_{21}NO_4$	264	$C_{17}H_{14}NO_2$
324	$C_{19}H_{18}NO_4$	263	$C_{18}H_{17}NO$
308	$C_{19}H_{18}NO_3$	237	$C_{16}H_{13}O_2$
296	$C_{18}H_{18}NO_3$	220	$C_{12}H_{14}NO_3$
294	$C_{19}H_{20}NO_2$	206	$C_{12}H_{16}NO_2$
280	$C_{18}H_{18}NO_2$	204	$C_{12}H_{14}NO_2$
278	$C_{18}H_{16}NO_2$	190	$C_{11}H_{12}NO_2$
265	$C_{17}H_{15}NO_2$	176	$C_{10}H_{10}NO_2$

to give 122, which loses CH_2O and CO successively to yield ions m/e 292 and 264, respectively. Ion 120 can give rise to the dihydroisoquinolinium ion m/e 190 and the small isoquinolinium ion m/e 188.

Ion M-CH_3 has an intensity about 30% of the base peak and presumably, the methyl group attached to the nitrogen is lost.

Ion M-CHO may be derived from the loss of the CHO radical from the methylenedioxy group. Further decomposition of this ion can give rise to ion m/e 295 by expulsion of the neutral fragment, CH_2=N-CH_3, from ring B.

An intense peak, fourteen mass units higher than the dihydroisoquinolinium ion, has been shown again to have one nitrogen and three oxygen atoms. The genesis of this ion, 121 or 121', must be the same

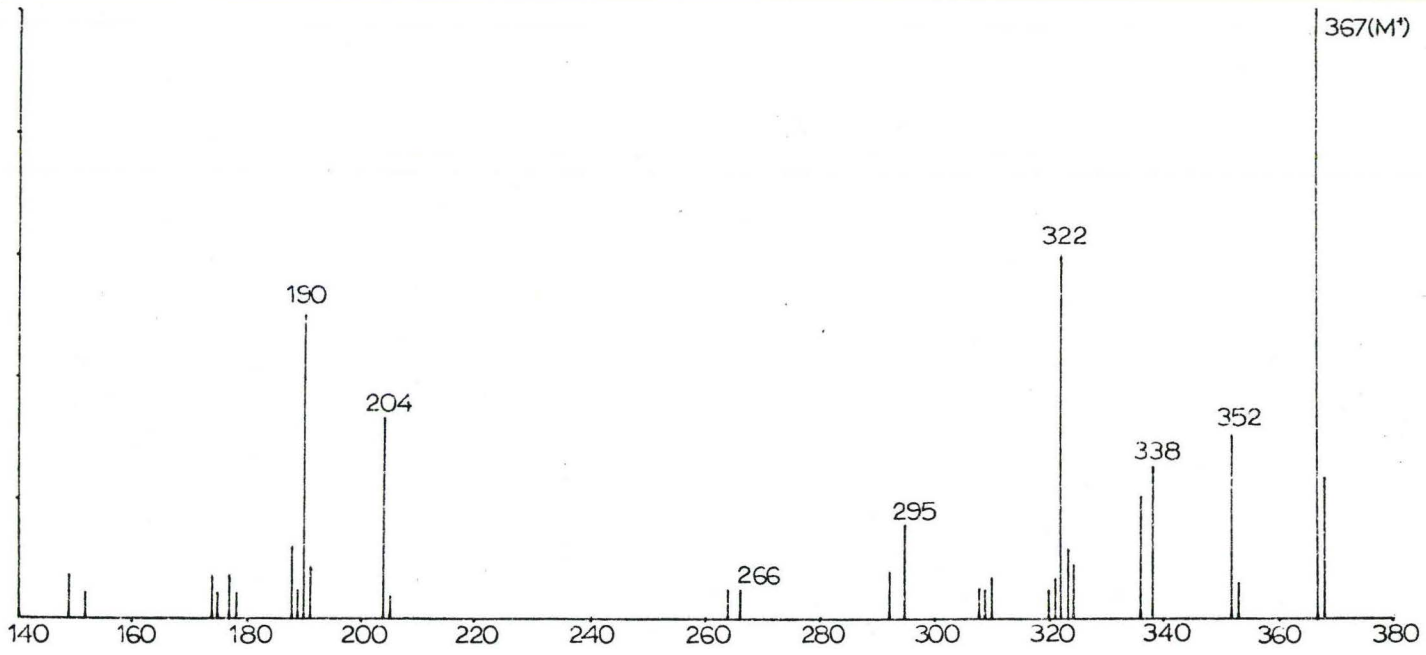
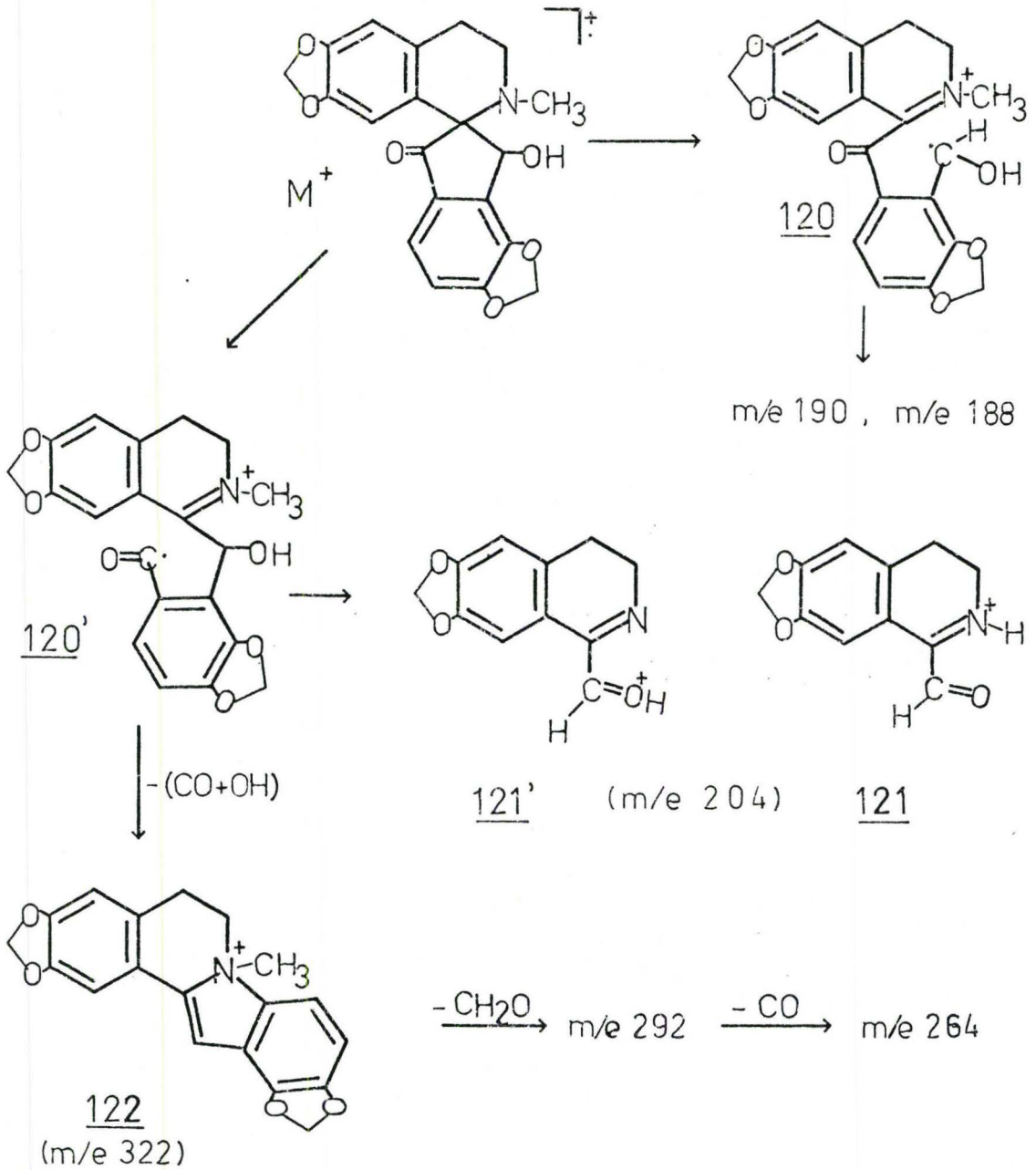


Figure 19. Mass spectrum of sibiricine (119)


M+
120
m/e 190 , m/e 188
120'
-(CO+OH)
121'
(m/e 204)
121
122
(m/e 322)
-CH2O
m/e 292
-CO
m/e 264

Scheme 8

as that of 117 or 117'. The fragmentation pattern is shown in Scheme 8 and the composition of major ions is shown in Table IX.

TABLE IX

Compositions of the Major Ions in the Mass Spectrum of Sibiricine (119)

m/e	Composition	m/e	Composition
367	$C_{20}H_{17}NO_6$	264	$C_{17}H_{14}NO_2$
352	$C_{19}H_{14}NO_6$	204	$C_{11}H_{10}NO_3$
338	$C_{19}H_{16}NO_5$	190	$C_{11}H_{12}NO_2$
336	$C_{19}H_{14}NO_5$	188	$C_{11}H_{10}NO_2$
322	$C_{19}H_{16}NO_4$	177	$C_9H_5O_4$
310	$C_{18}H_{16}NO_4$	174	$C_{10}H_8NO_2$
295	$C_{17}H_{11}O_5$	149	$C_9H_9O_2$
292	$C_{18}H_{14}NO_3$	149	$C_8H_5O_3$
266	$C_{16}H_{10}O_4$		

In conclusion, the spectra of 105 and 119 bear many similarities. Both have the molecular ion peak as the base peak, an intense M-(CO+OH) ion, a dihydroisoquinolinium ion peak, and an ion of type 121. All are recognized readily and are quite diagnostic. Thus an examination of a model compound can be of great help in interpreting the spectrum of a more complicated natural alkaloid.

Mass Spectra of 108, 110, and Ochrobirine (123)

Mass spectra of 108, 110, and ochrobirine are recorded in Figure 20.

When there are two hydroxyl groups in the five-membered ring, the spectra show a pattern distinct from that of sibiricine. The parent peak is only 10 per cent of the base peak or even less, while in sibiricine it is the base peak. The dihydroisoquinolinium ion is still present but in reduced intensity. The M-H_2O and the base peak (M-CH_3O_2) may be formed in the processes shown in Scheme 9.

The loss of H_2O from 124 leads to the more stable radical 125, in which the radical centre is benzylic and also conjugates to the immonium ion. The M-H_2O peak may also come from the molecular ion directly to form ion 125'. The base peak, ion 126, can be derived either from 125 by losing a CHO radical or from 124 by concerted elimination of the neutral fragment, CH_2O and the OH radical.

The latter process is analogous to the elimination of (CO+OH) from sibiricine, in which no M-OH ion peak can be detected. The course of further decomposition of 126a, 126b, and 126c is different, each depending on the substituents either in ring A or in ring D. Ion 126a loses a CHO radical followed by expulsion of CO to give small peaks at m/e 249 and 221, while ion 126c can lose either CHO or CH_2O, presumably from different rings, to yield ions at m/e 293 and 292, each of which is capable of losing a molecule of CO to produce the respective ions at m/e 265 and 264. The situation is quite different in 126b, where two methoxyl groups are in ring A, the ejection of the stable neutral

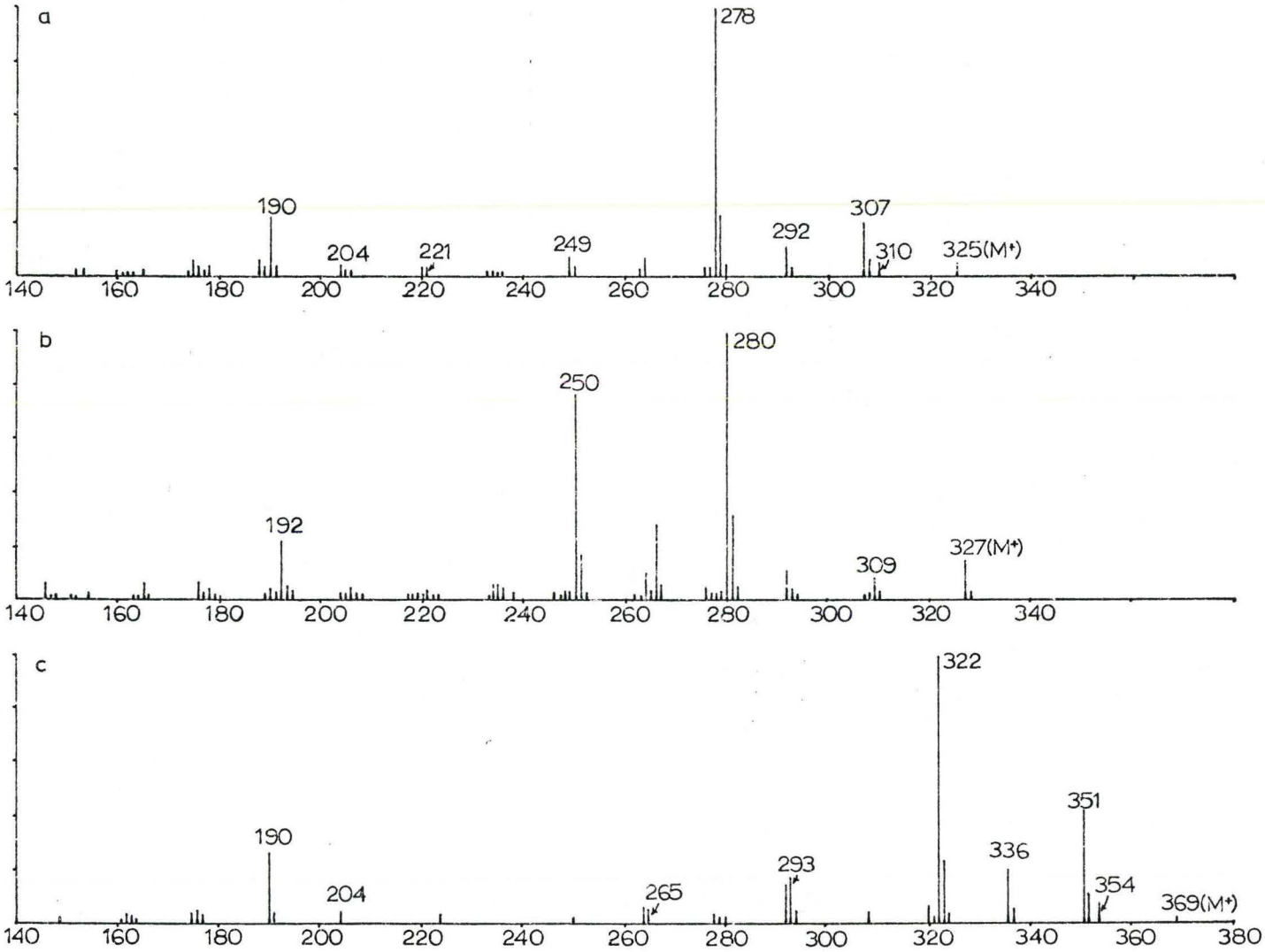
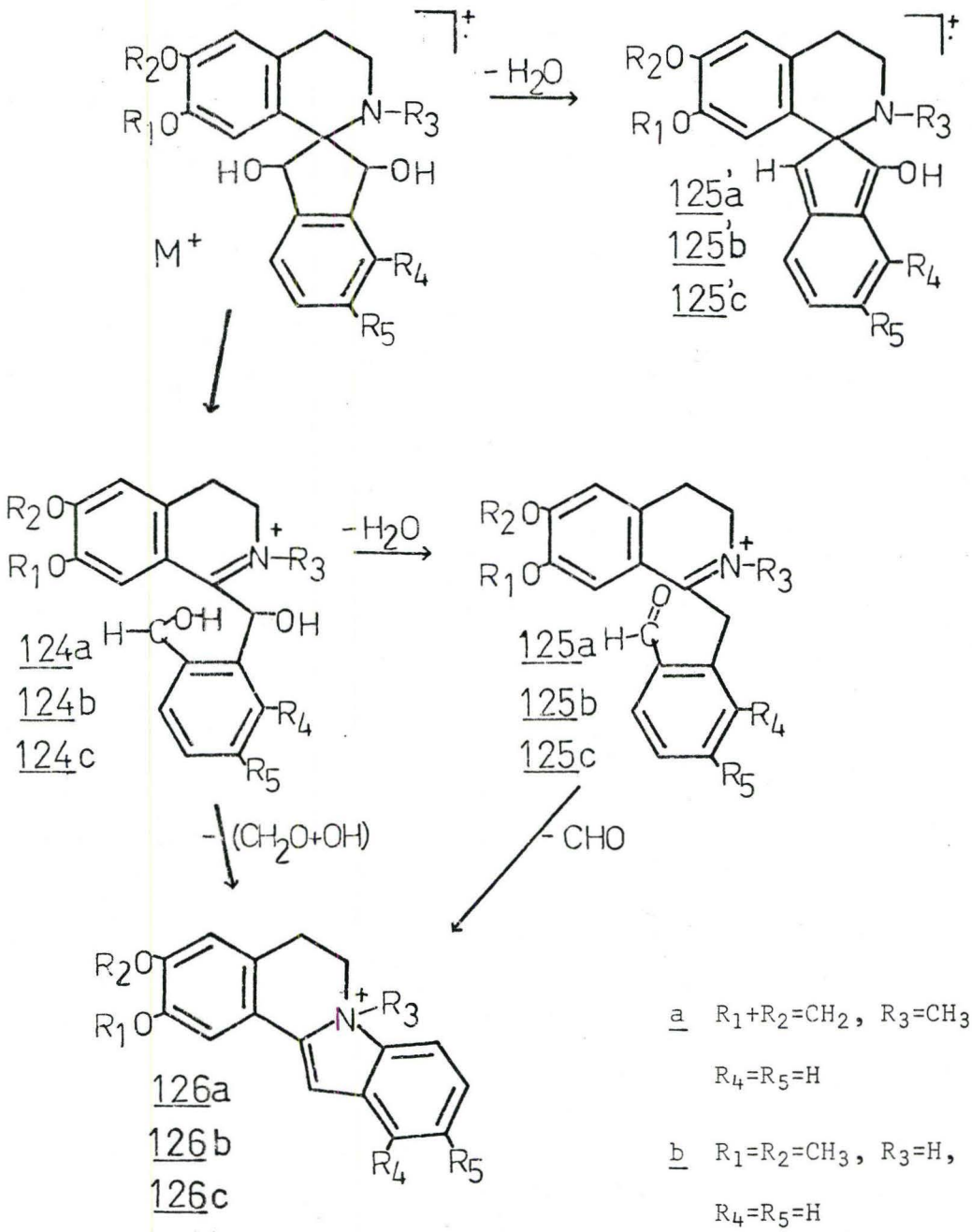


Figure 20. Mass spectra of a) 108, b) 110, c) ochrobirine (123)



a $R_1+R_2=CH_2$, $R_3=CH_3$
$R_4=R_5=H$

b $R_1=R_2=CH_3$, $R_3=H$,
$R_4=R_5=H$

c $R_1+R_2=CH_2$, $R_3=CH_3$
$R_4+R_5=O-CH_2-O$

Scheme 9

TABLE X

Compositions of the Major Ions in the Mass Spectra of 108, 110 and 123

108		110		123	
m/e	Composition	m/e	Composition	m/e	Composition
325	$C_{19}H_{19}NO_4$	327	$C_{19}H_{21}NO_4$	369	$C_{20}H_{19}NO_6$
310	$C_{18}H_{16}NO_4$	309	$C_{19}H_{19}NO_3$	354	$C_{19}H_{16}NO_6$
308	$C_{19}H_{18}NO_3$	292	$C_{19}H_{18}NO_2$	352	$C_{20}H_{18}NO_5$
307	$C_{19}H_{17}NO_3$	281	$C_{18}H_{19}NO_2$	351	$C_{17}H_{20}NO_5$
292	$C_{18}H_{14}NO_3$	280	$C_{18}H_{18}NO_2$	322	$C_{19}H_{16}NO_4$
278	$C_{18}H_{16}NO_2$	266	$C_{17}H_{16}NO_2$	321	$C_{19}H_{15}NO_4$
264	$C_{17}H_{14}NO_2$	265	$C_{17}H_{15}NO_2$	308	$C_{18}H_{14}NO_4$
249	$C_{17}H_{15}NO$	264	$C_{17}H_{14}NO_2$	307	$C_{18}H_{13}NO_4$
204	$C_{11}H_{10}NO_3$	251	$C_{17}H_{17}NO$	294	$C_{18}H_{16}NO_3$
190	$C_{11}H_{12}NO_2$	250	$C_{17}H_{16}NO$	293	$C_{18}H_{15}NO_3$
188	$C_{11}H_{10}NO_2$	192	$C_{11}H_{14}NO_2$	292	$C_{18}H_{14}NO_3$
175	$C_{11}H_{11}O_2$	190	$C_{11}H_{12}NO_2$	265	$C_{17}H_{15}NO_2$
175	$C_{10}H_9NO_2$			264	$C_{17}H_{14}NO_2$
174	$C_{10}H_8NO_2$			250	$C_{16}H_{12}NO_2$
				204	$C_{11}H_{10}NO_3$
				190	$C_{11}H_{12}NO_2$
				188	$C_{11}H_{10}NO_2$

fragment, CH_2O, to give an even electron ion seems to be a favorable process. The daughter ion peak, m/e 250, is quite intense. In the high mass range, when $R_3=CH_3$, there is a small peak corresponding to M-CH_3. There is also a peak corresponding to M-(CH_3 + H_2O), which can be derived either from ion M-CH_3 by losing OH_2 or from ion M-OH_2 by losing CH_3 radical.

In the spectra of 108 and 123, there is a small peak fourteen mass units higher than the dihydroisoquinolinium ion and its composition has been determined to be $C_{11}H_{10}NO_3$. The formation of this ion will be similar to that in sibiricine and therefore has the structure 121 or 121'.

The composition of major ions of 108, 110, and 123 are shown in Table X.

In summary, the spectra of these three compounds are quite characterstic and can be recognized quite readily. The dihydroisoquinolinium ion will reveal that the alkaloid in question is an isoquinoline one, and the base peak, M-(CHO + H_2O) will show that it is of the ochrobirine type. Thus, mass spectrometry can be used to identify the carbon skeleton of these compounds.

F-38

In 1938 Manske (132) isolated and identified several isoquinoline alkaloids from the plant, Fumaria officinalis L. Among these were protopine, cryptopine, ± stylopine, + and ± scoulerine, and sinactine. Besides these alkaloids of established structure, he reported the presence of two new alkaloids, F-37 ($C_{21}H_{23}NO_5$) and F-38 ($C_{20}H_{19}NO_6$).

Two minor alkaloids were found later (133) in the same plant corresponding in composition to $C_{20}H_{21}NO_5$ and to $C_{20}H_{17}NO_5$, which were named fumaritine and fumariline, respectively. These alkaloids and F-37, fumaricine, have been shown to have a spirobenzylisoquinoline system (109). In this section the structure of F-38 will be reported.

F-38 has absorption ascribed to a hydroxyl (3450 cm^{-1}) and a carbonyl (1700 cm^{-1}) group in its i.r. spectrum. An examination of its 100 MHz p.m.r. spectrum in DMSO-d_6, Figure 21, shows one singlet of area one at 8.65δ, which is assigned to a phenolic hydroxyl proton, since the chemical shift of this signal is lower than normal for aromatic protons. A total area of seven protons is observed in the region 6-7.5δ. One AB quartet centred at 7.18 and 7.36δ (J_{AB}=8 Hz) is assigned to ortho aromatic protons and a signal at 6.26δ with an area of two to methylenedioxy protons. The three remaining singlets, two of which are probably due to para aromatic protons and one to an alcoholic OH, are at 7.24, 6.68 and 6.29δ. A methoxyl signal at 3.80δ is apparent in the spectrum. A signal at 4.33δ (area 1) is probably due to a benzylic hydrogen geminal to a hydroxyl group. One of the disadvantages of the p.m.r. spectrum in DMSO-d_6 is the intense signal, arising from undeuterated DMSO, around 2.5δ which interferes with the signals derived from the compound. Unfortunately, F-38 is only slightly soluble in $CHCl_3$ and therefore a spectrum in $CDCl_3$ could not be obtained.

When F-38 was treated with diazomethane, F-38 methyl ether 129 was obtained. The p.m.r. spectral data of F-38 methyl ether in DMSO-d_6 and $CDCl_3$ are given in Table XI. In DMSO-d_6, the appearance of one

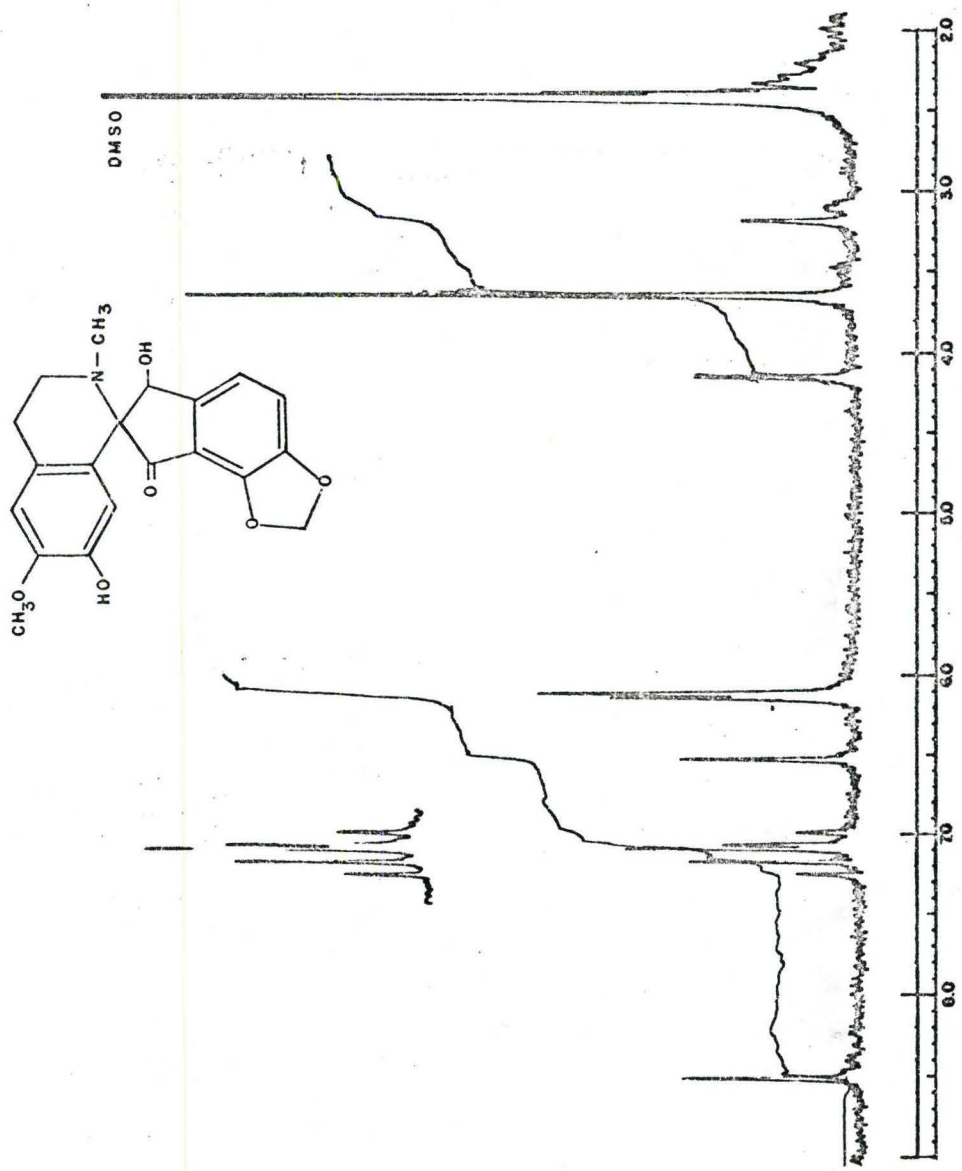
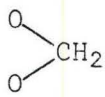
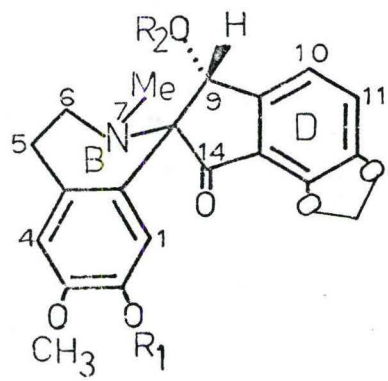


Figure 21. P.m.r. spectrum of F-38

TABLE XI

The P.m.r. Signals of 128, 129, and 130 in P.p.m. (δ)

	128 (in DMSO-d_6)	129 (in DMSO-d_6)	129 (in $CDCl_3$)	130 (in $CDCl_3$)
H-1	7.24	7.41	7.29	7.30
2-OR_1	8.65	3.87	3.93	3.92
3-OCH_3	3.80	3.81	3.87	3.84
H-4	6.68	6.75	6.61	6.53
7-N-CH_3	--	--	2.53	2.63
12, 13 	6.26	6.28	6.18	6.12
H-10	7.18	7.21	7.13	7.09
H-11	7.36	7.38	7.13	7.09
H-9	4.33	4.33	4.45	4.98
9-OR_2	6.29	6.44	6.18	2.22



128 $R_1=R_2=H$

129 $R_1=CH_3$, $R_2=H$

130 $R_1=CH_3$, $R_2=CH_3-CO$

additional methoxyl signal at 3.87δ and the disappearance of the signal at 8.65δ confirms that one phenolic hydroxyl group is present in F-38. In the region 6-7.5δ, one AB quartet, one methylenedioxy, and three singlets are still observable. A signal corresponding to the benzylic hydrogen geminal to a hydroxyl group stays at 4.33δ. When deuterium oxide was added to the solution of F-38 methyl ether in DMSO-d_6, the signal at 6.44δ disappeared. Thus, this observation provides convincing evidence for the presence of an active hydrogen in the molecule, presumably, a hydroxyl hydrogen.

When the p.m.r. spectrum of F-38 methyl ether was run in $CDCl_3$, Figure 22, a signal appeared at 2.53δ (area 3), which was obscured in the spectrum in DMSO-d_6, and is assigned to an N-methyl group.

In order to confirm the presence of one hydroxyl group in the molecule, F-38 methyl ether was acetylated with acetic anhydride and pyridine. The p.m.r. spectrum of acetylated F-38 methyl ether 130 in $CDCl_3$ shows an acetyl group signal at 2.22δ. The signal assigned to the benzylic hydrogen geminal to the hydroxyl group shifts from 4.45 in F-38 methyl ether down to 4.98δ in the acetylated compound. In the region 6-7.5δ, a signal at 7.09 (area 2) is assigned to two ortho protons, a signal at 6.12 (area 2) to the methylenedioxy, and signals at 7.30 (area 1) and 6.53 (area 1) to two para aromatic protons. These data suggest clearly that a unit, $-\overset{|}{\underset{|}{C}}-CH(OH)-\overset{|}{\underset{|}{C}}-$ is present in the molecule of F-38.

Although i.r. and p.m.r. data reveal much information about the functional groups and the structural units, the skeleton is still unknown.

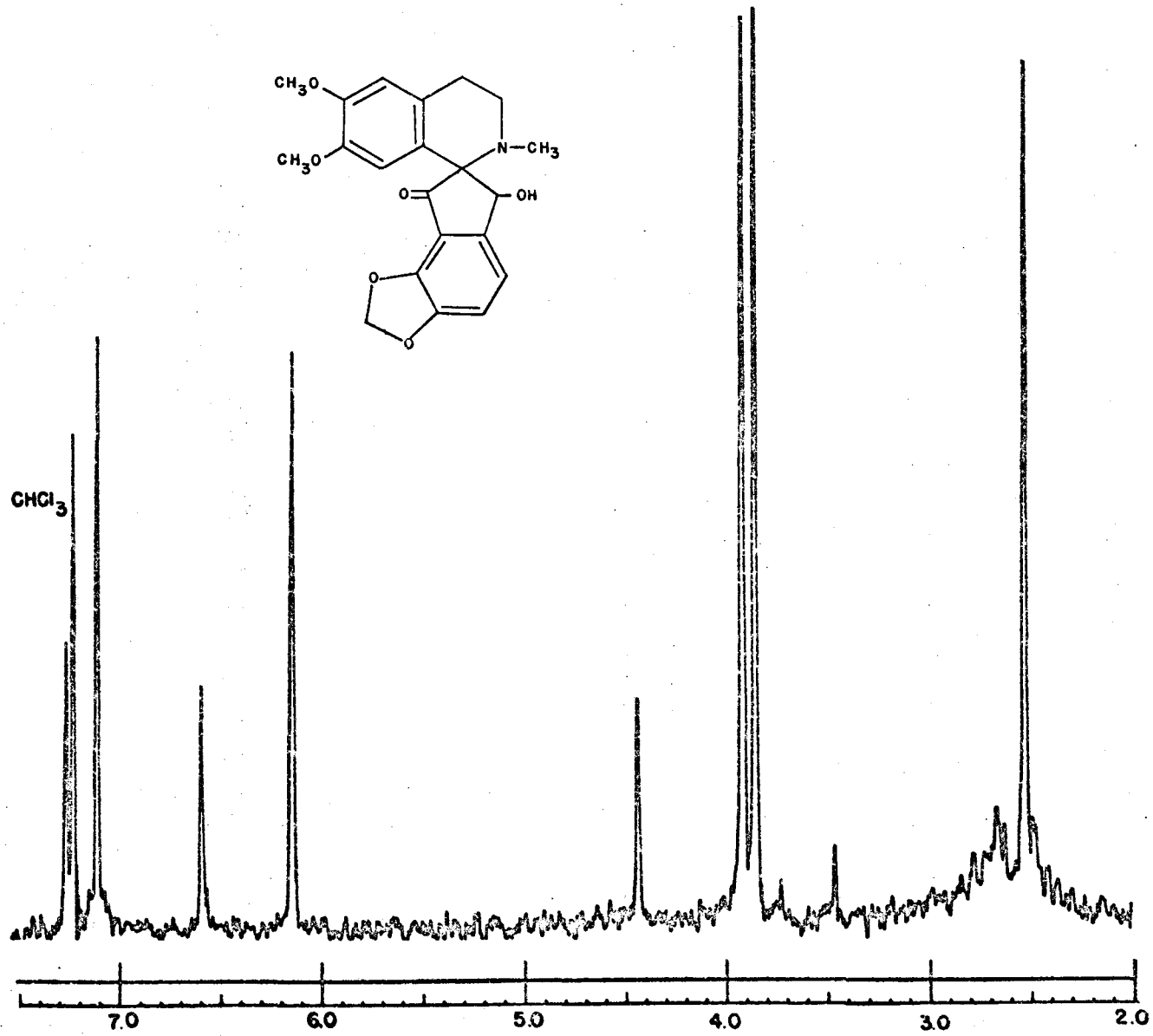
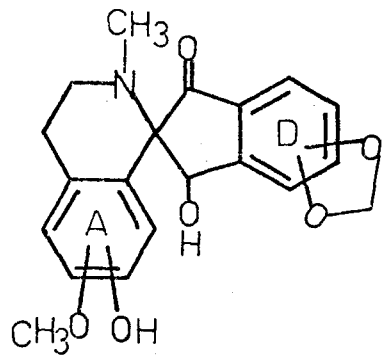


Figure 22. P.m.r. spectrum of F-38 methyl ether

Since F-38 occurs in the same plant as fumaricine, fumariline and fumaritine, it may be structurally related to these alkaloids. Its properties preclude it belonging to the protoberberine, phthalide isoquinoline or the protopine groups. When the mass spectrum of F-38 is compared with those of the spiroisoquinoline group found in the same plant, definite conclusions cannot be drawn about its structure. When, however, the spectra of sibiricine and F-38 were compared, there appeared a remarkable similarity. Both compounds show the molecular ion peak as the base peak and most of the intense ions occur in the high mass end and in the region between m/e 140-200. Moreover, many peaks characteristic of a spiroisoquinoline with a ketone and a hydroxyl group in the five-membered ring are present in F-38. It is, therefore, reasonable to assume that both compounds have the same carbon skeleton. The substitution pattern can be deduced from the presence of the dihydroisoquinolinium ion m/e 192, which shows that one hydroxyl and one methoxyl group, must be present in ring A. Therefore, the methylenedioxy group must be in ring D.

The spectral data suggest the structure 127, but for the assignment of stereochemistry and the substitution pattern further p.m.r. data is required.

In alkaloid F-38, the chemical shift difference of the aromatic AB quartet (δ_{HA}=7.18, δ_{HB}=7.36) indicates that the carbonyl is ortho to the methylenedioxy group. In those compounds, in which the carbonyl group is ortho to the aromatic protons, the chemical shift difference between two adjacent protons is much larger. For example, a difference



127

of 0.4 ppm is observed in sibiricine because of the strong deshielding effect of the carbonyl function on the ortho proton. The substitution in ring D has been confirmed by an NOE experiment, in which an NOE of 9% is observed at H-10 when H-9 is saturated (see 128).

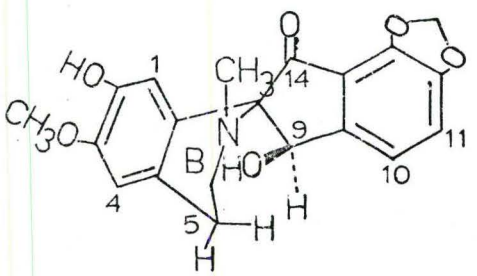
The establishment of the substitution pattern in ring A is based on the following NOE experiment. Saturation of H-9 causes a 4% increase in area of the absorption at 7.24δ which must be assigned to H-1, while saturation of the O-CH_3 results in an increase in area of 25% at 6.68δ, presumably H-4. These data suggest that the hydroxyl and methoxyl groups in ring A are at C-2 and C-3, respectively. The structure of F-38 is shown in 128. Further evidence for the stereochemistry at C-9, as shown in 128, comes from the zero increase in area of H-9, when the N-CH_3 is irradiated. If H-9 and N-CH_3 were on the same side of the plane of the five-membered ring, a large NOE would be expected. Thus, NOE data establish the stereochemistry at C-9. All the NOE data are given in Table XII.

TABLE XII

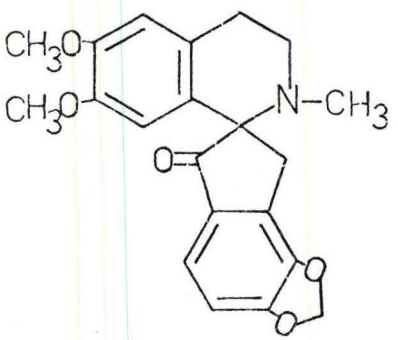
Nuclear Overhauser Effects in 128

Irradiated protons	Observed protons	% Area increase	Internuclear distance (Å)*
H-9	H-1	4	3.35
H-9	H-10	9	2.88
H-5	H-9	16	2.65
N-CH_3	H-9	0	
O-CH_3	H-4	5	

* As measured from a Dreiding Molecular Model.



131



132

133

An unusually large NOE is observed between H-9 and H-5. Also the chemical shift of H-1 is further downfield than in related alkaloids, e.g. sibiricine and fumariline. All the above facts can be explained by the conformation as shown in 131, in which ring B is in a twist conformation and the OH is hydrogen bonded to the nitrogen. The hydrogen bonding to the nitrogen causes the five-membered ring to be twisted such that the carbonyl group is closer to ring A with H-1 in the deshielding zone. H-9 is also forced to be closer to H-5. No such effect, however, is observed in sibiricine, where the stereochemistry at C-9 is different. Thus, the structure 128 is fully compatible with the mass spectral, i.r. and p.m.r. data.

One other piece of evidence supporting the proposed structure of F-38 comes from the u.v. spectrum. The reported u.v. spectrum of 132 (134), ν max (EtOH); 237 (4.52)*, 292 (4.13), and 310 (4.01), is similar to that of sibiricine, ν max (EtOH); 205 (4.80), 240 (3.94), 291 (3.91) and 313 (3.99), but differs greatly from that of F-38 methyl ether, ν max (EtOH); 208 (4.45), 235 (4.35), 259 (3.96), 286 (3.37) and 350 (3.40). On the other hand, the u.v. spectrum of 133, ν max (EtOH); 234 (4.47), 260 (4.08), 283 (3.60) and 354 (3.52), is similar to that of F-38 methyl ether. The spectra of 132 and 133 are a composite of two chromophoric systems, i.e. the tetrahydroisoquinoline system and the 1-indanone system. The absorptions corresponding to the latter chromophore are different for different substitution patterns. The resemblance

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* Values of log ε max are shown in brackets.

of the u.v. spectrum of F-38 methyl ether to that of 133 indicates that they have the same chromophoric system.

Mass Spectra of F-38 and Its Methyl Ether

The characteristic ions in the spectra of 105 and 119 should also be found in the spectrum of F-38, because all have the same carbon skeleton. It turns out that all these ions, though varying in intensity, are observed in the spectrum (Figure 23). The spectrum is dominated by the molecular ion and the M-CH_3 ion. Ions, 135, the dihydroisoquinolinium ion (m/e 192) and 136 have been confirmed by high resolution mass measurements. The fragmentation pattern is shown in Scheme 10.

When the spectrum is examined more closely, however, the spectrum seems to be more complicated than that of 119. Many additional peaks appear in the high mass range, such as M-CO and its subsequent decomposition product ions. Multiplicity in the middle of the spectrum (m/e 150-220) appears, as shown in Figure 23. One possible explanation for these differences is that the substitution of methoxyl and hydroxyl in ring A may increase the probability of the decomposition of the molecular ion as well as the fragment ions. Thus, when the expulsion of CO from the molecular ion to form ion 137 occurs, the charge may remain in the aromatic ring.

The retro-Diels-Alder decomposition of 137 can produce ion 138, m/e 298. If the fragment C_3H_7N is lost from 137, ion m/e 284 will be formed. The latter process occurs rarely in the mass spectra of isoquinoline alkaloids and its genesis is still in doubt. In the absence of any other information, the proposed process seems feasible, because

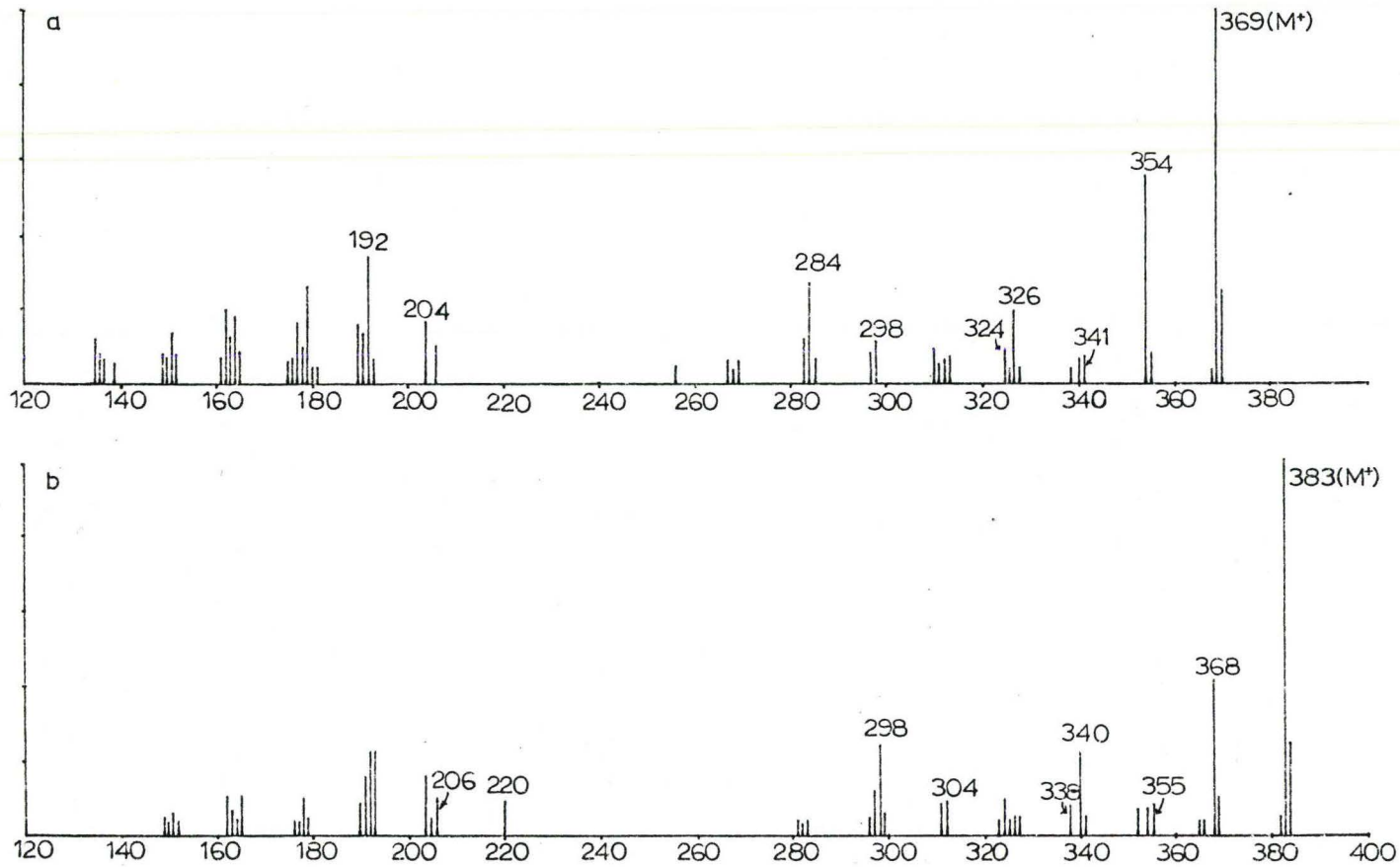
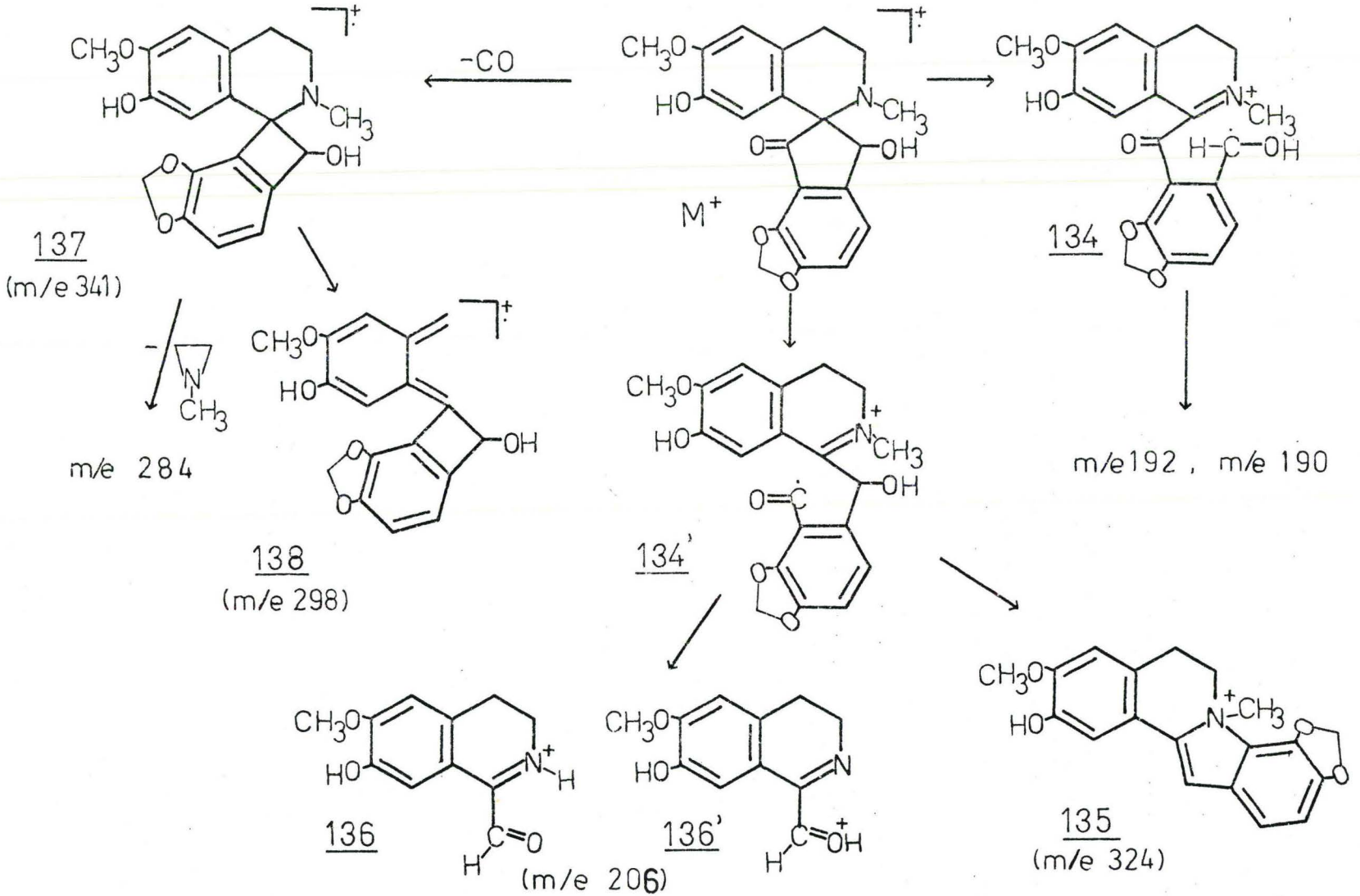


Figure 23. Mass spectra of a) F-38, b) F-38 methyl ether


-CO
137
(m/e 341)
m/e 284
138
(m/e 298)
M+
134
m/e192 , m/e 190
134'
136
(m/e 206)
136'
135
(m/e 324)

Scheme 10

it involves only two simple bond cleavages with the expulsion of a stable neutral fragment, 1-methyl aziridine.

In the high mass range, there is a peak of composition $C_{18}H_{16}NO_5$, corresponding to the loss of CH_3CO. Since there is no acetyl group in the molecule, the source of this radical may be the aromatic ring. It is well documented that aromatic methyl ethers (11) can lose CH_3 and CO successively to give an ion, $M-CH_3CO$.

In the region m/e 150-200, most peaks are doublets and most of them also contain a nitrogen atom as determined by high resolution mass measurements. The source of these ions must be the isoquinoline moiety. The only useful and characteristic ion in this region is the dihydro-isoquinolinium ion, m/e 192, which is the most intense peak in this area.

The spectrum of F-38 methyl ether is also recorded in Figure 23.

In the high mass range (above m/e 250), all the peaks are fourteen mass units higher than the corresponding peaks in F-38, and the peak intensities vary little. In the range between m/e 150 and 200, the multiplicity of the peaks is much reduced in comparison to that of F-38. The peaks at m/e 192 and 206 in F-38 are shifted to m/e 206 and 220 in F-38 methyl ether. These ions correspond to the isoquinolinium ion and the ion containing one nitrogen and three oxygens. The compositions of all these ions has been confirmed by high resolution mass measurements as shown in Table XIII.

In conclusion, the spirobenzylisoquinoline alkaloids with one hydroxyl and one ketone group in the five-membered ring give very characteristic fragment ions. Many small peaks, however, especially in

TABLE XIII

Compositions of Major Ions in the Spectra of F-38 and Its Methyl Ether

F-38		F-38 methyl ether	
m/e	Composition	m/e	Composition
369	$C_{20}H_{19}NO_6$	383	$C_{21}H_{21}NO_6$
354	$C_{19}H_{16}NO_6$	368	$C_{20}H_{18}NO_6$
341	$C_{19}H_{19}NO_5$	355	$C_{20}H_{21}NO_5$
340	$C_{19}H_{18}NO_5$	354	$C_{20}H_{20}NO_5$
326	$C_{18}H_{16}NO_5$	340	$C_{19}H_{18}NO_5$
324	$C_{19}H_{18}NO_4$	338	$C_{20}H_{20}NO_4$
310	$C_{18}H_{16}NO_4$	324	$C_{19}H_{18}NO_4$
298	$C_{17}H_{14}O_5$	312	$C_{18}H_{16}O_5$
297	$C_{17}H_{13}O_5$	311	$C_{18}H_{15}O_5$
284	$C_{16}H_{12}O_5$	298	$C_{17}H_{14}O_5$
283	$C_{16}H_{11}O_5$	297	$C_{18}H_{17}O_4$
206	$C_{11}H_{12}NO_3$	297	$C_{17}H_{13}O_5$
204	$C_{11}H_{10}NO_3$	283	$C_{17}H_{15}O_4$
192	$C_{11}H_{14}NO_2$	282	$C_{17}H_{14}O_4$
192	$C_{10}H_{10}NO_3$	281	$C_{17}H_{13}O_4$
190	$C_{11}H_{12}NO_2$	220	$C_{12}H_{14}NO_3$
190	$C_{10}H_8NO_3$	206	$C_{12}H_{16}NO_2$
179	$C_{10}H_{13}NO_2$	204	$C_{12}H_{14}NO_2$
177	$C_{10}H_9O_3$	204	$C_{11}H_{10}NO_3$
177	$C_{10}H_{11}NO_2$	193	$C_{10}H_{11}NO_3$
164	$C_9H_{10}NO_2$	192	$C_{11}H_{14}NO_2$
164	$C_9H_8O_3$	192	$C_{11}H_{12}O_3$
162	$C_9H_8NO_2$	178	$C_{10}H_{10}O_3$
162	$C_9H_6O_3$	178	$C_{10}H_{12}NO_2$
		165	$C_{10}H_{13}O_2$
		162	$C_9H_6O_3$
		162	$C_9H_8NO_2$

the medium mass range (m/e 150-200), seem very sensitive to the type and position of the substituents. Since they are small and variable, no diagnostic value can be attached to them.

EXPERIMENTAL

Apparatus, Methods and Materials

Mass spectra were determined on a C.E.C. 21-110B double-focusing mass spectrometer. Samples were introduced through a direct inlet system. Spectra are plotted in terms of relative abundance, with the most intense peak (base peak) taken as 100%. Only those peaks with an intensity equal to or greater than 3% of the most intense peak have been recorded.

The high resolution mass spectra were recorded on Ilford Q-2 photographic plates, which were developed in the usual manner (135). The spectra were then recorded on magnetic tape, using a Gaertner comparator-densitometer linked to a Datex system. They were processed on a CDC-6400 computer using a modified version of the HIRES program of Tinnicliff and Wadsworth (136).

The p.m.r. spectra were routinely run on a Varian A-60 or Varian T-60 instrument in $CDCl_3$, using tetramethylsilane as internal standard. The 100 MHz spectra were recorded using the frequency sweep mode of a varian HA-100 spectrometer. Samples were dissolved in $CDCl_3$ or DMSO-d_6 using added TMS as the internal locking signal. Chemical shifts were measured relative to TMS using a V4315 frequency counter incorporated in the instrument. Double irradiation was achieved by employing a Hewlett-Packard 201C audiogenerator at the desired frequency.

A Perkin-Elmer 337 Grating Infrared spectrometer was used to record infrared (i.r.) spectra and a Cary 14 spectrometer was used for the u.v. spectrum.

Melting points were determined on a Kofler hot stage and are uncorrected.

The alkaloids, caseanadine, cavidine, apocavidine, fumariline, fumaricine, fumaritine, sibiricine, ochrobirine and F-38, and compounds, 107 and 108, were obtained from Dr. R.H.F. Manske of the University of Waterloo. Fumarophycine was obtained from Dr. N.M. Mollov of the Bulgarian Academy of Science, Sofia, Bulgaria.

O-acetylcaseanadine

Caseanadine (10 mg) was dissolved in acetic anhydride (2 ml) and pyridine (0.5 ml). The mixture was allowed to stand at room temperature for 12 h. It was then evaporated to dryness under reduced pressure. The residue, after neutralization, was dissolved in ether, washed several times with aqueous sodium carbonate, and the solution dried over anhydrous potassium carbonate. The residue obtained upon evaporation of the solvent failed to crystallize. Thin layer chromatography showed only one spot which had a different Rf value from the starting material. The p.m.r. spectrum, Figure 6, demonstrates the presence of the O-acetyl group and clearly indicates the absence of unacetylated material. The mass spectrum has a molecular ion at m/e 385 (55) with major fragment ions at m/e 164 (100) and 149 (53). Insufficient material was available for a combustion analysis. Accurate mass measurement of the molecular ion gave the following result:

Calcd. for $C_{22}H_{25}NO_5$, 383.173; found, 383.169.

7-Methoxy-8-hydroxy-1,2,3,4-tetrahydroisoquinoline-1-spiro-2'-(1',3'-indandione) (102)

3-Hydroxy-4-methoxyphenylethylamine hydrochloride (940 mg), prepared according to the method of Ramirez (137), was added to a solution of ninhydrin (950 mg) in absolute alcohol (20 ml). The mixture was left at room temperature for 5 days. The solvent was evaporated, the residue dissolved in hot water, basified with saturated sodium bicarbonate solution and extracted with chloroform. The chloroform extract was dried over sodium sulfate and evaporated to dryness. The red semicrystalline solid obtained from chloroform-hexane melted at 92-95°C. The mass spectrum showed a molecular ion at m/e 309.

7,8-Dimethoxy-1,2,3,4-tetrahydroisoquinoline-1-spiro-2'-(1',3'-indandione) (103)

Compound 102 (70 mg) was dissolved in methanol (10 ml) and excess diazomethane in ether was added to the solution which was then allowed to stand for 3 hours. The solvents were allowed to evaporate and the residue recrystallized from methanol. The yellow crystalline prisms melted at 158°C.

Infrared spectrum: $\nu_{C=O}$ ($CHCl_3$) 1700, 1745 cm^{-1}.

Anal. Calcd. for $C_{19}H_{17}NO_4$: C, 70.6; H, 5.3

Found: C, 70.6; H, 5.4

7,8-Dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquincline-1-spiro-2'-(1',3'-indandione) (104)

Compound 103 (50 mg) was mixed with formic acid (2 ml) and formaldehyde (2 ml), and heated on a steam bath for 12 h. Water was added to the mixture which was basified with saturated sodium bicarbonate solution and extracted with chloroform. The chloroform extract was washed with water, dried over sodium sulfate and evaporated to dryness. The yellow residue was crystallized from methanol-ether, m.p. 158-9°C.

Infrared spectrum: $\nu_{c=o}$ ($CHCl_3$) 1700, 1745 cm^{-1}

Anal. Calcd. for $C_{20}H_{19}NO_4$: C, 71.2; H, 5.6; N, 4.2.

Found: C, 71.2; H, 5.8; N, 4.3

6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline-1-spiro-2'-(1',3'-indandione) (109)

The procedure for preparing 109 was similar to that for the preparation of 107 (130), but 3,4-dimethoxyphenylethylamine was used instead of 3,4-methylenedioxyphenylethylamine. The product 109 was recrystallized from chloroform-hexane, m.p. 175-9°C.

Infrared spectrum: $\nu_{c=o}$ ($CHCl_3$) 1710, 1750 cm^{-1}

Anal. Calcd. for $C_{19}H_{17}NO_4$: C, 70.6; H, 5.3; N, 4.6

Found: C, 70.5; H, 5.3; N, 4.6

6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline-1-spiro-2'-(1',3'-indandiol) (110)

Compound 109 (100 mg) was dissolved in ethanol and sodium borohydride (100 mg) was added. The mixture was allowed to stand at

room temperature for 2 hours. The solvent was evaporated, water was added, and the mixture was extracted with ether. The ether extract was dried over potassium carbonate and evaporated to dryness. Compound 109 was purified on basic silica gel by thick layer chromatography and, after elution, recrystallized from ethanol, m.p. 176-180°C.

Infrared spectrum: ν_{OH} ($CHCl_3$) 3560 cm^{-1}

Anal. Calcd. for $C_{19}H_{21}NO_4$: C, 69.7; H, 6.4; N, 4.2

Found: C, 69.5; H, 6.3; N, 4.3

Preparation of 105 and 106

Sodium borohydride (200 mg) was added slowly to a solution of 104 (220 mg) in methanol (10 ml) and the mixture stirred at room temperature for 10 h. The solvent was evaporated, water (5 ml) was added and the aqueous suspension was extracted with ether. The ether extract was dried over potassium carbonate and evaporated to dryness. The mixture was separated by thick layer chromatography on basic silica gel (developed in methanol-chloroform 1:20). After the silica gel was scraped off and eluted with $CHCl_3$-MeOH, the fast moving fraction was crystallized from ethanol to give 105 (65 mg), m.p. 171-3°C.

Anal. Calcd. for $C_{20}H_{21}NO_4$: C, 70.8; H, 6.2; N, 4.1

Found: C, 70.8; H, 6.2; N, 4.3

Infrared spectrum: $\nu_{C=O}$ ($CHCl_3$) 1705 cm^{-1}

The slow moving fraction was crystallized from ethanol to give 106 (67 mg), m.p. 183-6°C.

Anal. Calcd. for $C_{20}H_{21}NO_4$: C,70.8; H, 6.2; N, 4.1

Found: C, 70.7; H, 6.2; N, 4.2

Infrared spectrum: $\nu_{c=o}$ ($CHCl_3$) 1710 cm^{-1}

Methylation of F-38

F-38 (25 mg) was dissolved in methanol (5 ml) and excess diazomethane in ether added to the solution which was then allowed to stand for 3 hours. The solvents were allowed to evaporate and the residue recrystallized from methanol-ether, m.p. 245-6°C.

Ultraviolet spectrum: λ_{max} (EtOH) 208, 235, 259, 286, and 350 mμ; log ε 4.45, 3.96, 3.37, and 3.40.

Infrared spectrum: ν_{OH} (KBr) 3450 cm^{-1}; $\nu_{c=o}$ (KBr) 1700 cm^{-1}

Acetylation of F-38 Methyl Ether

F-38 methyl ether (17 mg) was dissolved in acetic anhydride (4 ml) and pyridine (2 ml). The mixture was stirred at room temperature under a nitrogen atmosphere for 60 h. The excess acetic anhydride and pyridine were evaporated under reduced pressure, the residue triturated in aqueous sodium carbonate, and the suspension extracted several times with chloroform. The chloroform extract was dried over sodium sulfate, and evaporated to a residue which could not be induced to crystallise but on an analytical thin layer plate it showed only a single spot. The mass spectrum showed a molecular ion at m/e 425. Insufficient material was available for a combustion analysis.

SUMMARY

The structure of three new tetrahydroprotoberberine alkaloids, caseanadine, cavidine, and apocavidine have been determined. The characteristic fragmentations of their mass spectra reveal the basic carbon skeleton of these alkaloids. The p.m.r. data have been used to determine their substitution pattern in the rings.

The mass spectra of several spirobenzylisoquinoline alkaloids have also been examined. It has been shown that the fragmentation pattern is affected mainly by the substitution pattern in the five-membered ring. When there is only one hydroxyl group in the five-membered ring, e.g. fumaricine, the base peak ion is the dihydroisoquinolinium ion which is derived from benzylic bond cleavage followed by hydrogen rearrangement and another benzylic bond cleavage. If a carbonyl group is present in the five-membered ring, e.g. fumariline, the molecular ion will expel a molecule of carbon monoxide and a hydrogen radical to form a tetracyclic ion, 89c, as a base peak ion. In the case of ochrobirine-type compounds, in which there are two hydroxyl groups in the five-membered ring, the molecular ion is usually very small. The base peak ion of these compounds is derived from the molecular ion by expulsion of a molecule of formaldehyde and a hydroxyl radical. In still another type, e.g. sibiricine, in which one hydroxyl and one carbonyl group are attached to the five-membered ring, the base peak is the parent peak. These differences in the mass spectra provide a

convenient way to differentiate the different types of alkaloids.

A new alkaloid, F-38, has been found to have the same characteristic fragments in the mass spectrum as that of sibiricine. The final structure of F-38 (128) has been resolved by a combination of spectroscopic methods.

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