MASS SPECTROMETRIC INVESTIGATIONS IN THE STEROID, TERPENOID AND ALKALOID FIELDS*

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INTRODUCTION

The present lecture is to introduce the colloquium on "Modern Physical Methods in the Chemistry of Natural Products". The crucial rôle which the many physico-chemical methods play in modern natural products research has been well summarized by Thompson[1] at the first I.U.P.A.C. International Symposium on the Chemistry of Natural Products in Melbourne, and another general survey covering this area has been published recently by Pinder[2]. It seemed more appropriate, therefore, that this lecture deal with a specific physical method rather than with a general summary of all physical methods currently available. As the subject of optical rotatory dispersion[3]—especially as it pertains to developments in the natural products field—has already been covered at the first I.U.P.A.C. Symposium on the Chemistry of Natural Products[4], I have decided to discuss in some detail recent applications of mass spectrometry.

While this physical method has been employed extensively in organic chemistry for well over twenty years[5], its use has been limited largely to volatile substances of relatively low molecular weight and the most spectacular applications occurred in the petroleum field. The rather recent developments in improved sample inlet systems have permitted the extension of mass spectrometry to relatively non-volatile organic substances and, during the past four years, this tool has gained increased importance in the field of natural products, notable contributions having been made by Reed[6], Bergström, Ryhage and Stenhagen[7], and by Biemann[8]. The Swedish workers[7] concentrated especially on fatty acids and steroids, while the American group[8] emphasized amino-acids, peptides and alkaloids. No attempt will be made to summarize these important studies, which have already been reviewed by the authors themselves[6–8]. Rather, the present article will concern itself almost exclusively with some of the mass spectrometric investigations conducted in our laboratory at Stanford University during the past sixteen months in the fields of steroids, terpenoids and alkaloids—precisely the area of natural products chemistry to which this Second International Symposium is limited.

DETERMINATION OF MOLECULAR WEIGHT

The determination of molecular weights is perhaps the best known application of mass spectrometry in general organic chemistry. Considerably

* Paper XXV in the series "Mass Spectrometry in Structural and Stereochemical Problems". For preceding article see ref. 17.

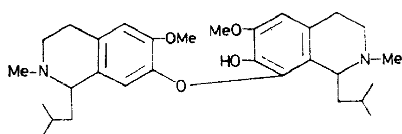
less than 1 mg of material is required and the accuracy of the method (with present commercially available instruments) in the mass range below 800 is such that a secure distinction between two alternative molecular weights, such as 756 and 757 (see discussion below), is possible. As the use of the more complicated and expensive double-focusing instruments[5] becomes prevalent, elementary analyses will be largely circumvented as empirical formulae can then also be established, the method being capable of resolution between alternatives, as, for example, $C_{12}H_{17}O_3$ (209·185) and $C_{12}H_{19}NO_2$ (209·208).

The use of mass spectrometry for molecular weight determination and, hence, for the establishment of the correct empirical formula becomes particularly important in those polycyclic natural products where $\pm CH_2$ has only a small effect upon the calculated carbon and hydrogen values. Frequently, it is not possible to differentiate between two or more possible empirical formulae simply on the basis of microanalyses, and in *Table 1* are listed nine indole alkaloids which have recently been investigated in our laboratories and where mass spectrometric molecular weight determination had to be employed to correct or to differentiate among earlier published empirical formulae based solely on elementary analyses.

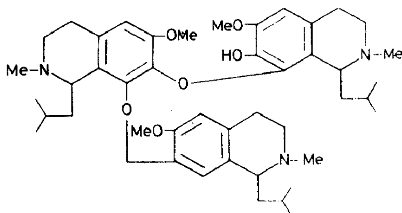
Table 1

Alkaloid	*Anal. (Lit.)*	*Mass Spec.*	*Reference*
Pyrifoline	$C_{23}H_{30}N_2O_3$	$C_{22}H_{30}N_2O_3$	33
Refractine	$C_{22}H_{26}N_2O_4$ $C_{23}H_{28}N_2O_4$	$C_{23}H_{28}N_2O_4$	34a
Spegazzinine	$C_{21}H_{28}N_2O_3$ $C_{22}H_{30}N_2O_3$	$C_{21}H_{28}N_2O_3$	36
Stemmadenine	$C_{21}H_{26-28}N_2O_3$	$C_{21}H_{26}N_2O_3$	52
Vindolinine	$C_{21}H_{24-26}N_2O_2$ $C_{22}H_{26}N_2O_2$	$C_{21}H_{24}N_2O_2$	48
Tabersonine	$C_{20}H_{24-26}N_2O_2$	$C_{21}H_{24}N_2O_2$	49
Echitamidine	$C_{20}H_{22}N_2O_3$ $C_{20}H_{26}N_2O_3$	$C_{20}H_{24}N_2O_3$	51
Lochneridine	$C_{19}H_{24}N_2O_3$	$C_{20}H_{24}N_2O_3$	53
Aspidofiline	$C_{20}H_{22}N_2O_2$	$C_{21}H_{26}N_2O_2$	36

An even more striking illustration is the cactus alkaloid pilocereine, for which structure (I) was proposed[9a] on the basis of extensive degradation experiments interpreted in terms of the empirical formula $C_{30}H_{44}N_2O_4$ (496), a Rast molecular weight determination[10] having yielded a value of 532. Recently[9b], we had occasion to examine the mass spectrum of pilocereine methyl ether and found instead of the expected molecular weight of 510 ($C_{31}H_{46}N_2O_4$) a molecular ion peak at *m/e* 757 corresponding to the empirical formula $C_{46}H_{67}N_3O_6$ (757). The analytical figures, aside from the molecular weight, are, of course, equally compatible with the higher empirical formula and so are all of the earlier degradation experiments[9a], which are best interpreted in terms of the very unusual " trimeric " structure (II), rather than the " dimeric " (I). Subsequent chemical work[9b] in our laboratory settled exactly the point of attachment of the third tetrahydroisoquinoline nucleus in pilocereine, the " trimeric " nature of which has been confirmed further by N.M.R. measurements.



(I)



(II)

Another recent illustration of the utility of mass spectrometric molecular weight determinations can be cited from the sterol field, where elementary analyses are essentially useless to differentiate among closely related empirical formulae. Pearl and Harrocks[11] reported the isolation from aspenwood of a sterol (m.p. 137°) and an alcohol (m.p. 165°); on the basis of elementary analyses these substances were assigned the respective empirical formulae $C_{32}H_{56}O$ and $C_{32}H_{54}O$, which would be unique in the plant sterol field. Through the kind co-operation of Dr I. A. Pearl, samples of the two substances became available to us and mass spectrometric measurements showed molecular ion peaks corresponding to m/e 414 and 426, thus showing that the correct formulae were in fact $C_{29}H_{50}O$ and $C_{30}H_{50}O$. The impossibility of utilizing standard elementary analyses for differentiating between such possible formulae is illustrated in *Table 2*.

Table 2

	Aspenwood sterol (m.p. 137°)		*Aspenwood alcohol* (m.p. 165°)	
Empirical formula	$C_{32}H_{56}O$	$C_{29}H_{50}O$	$C_{32}H_{54}O$	$C_{30}H_{50}O$
Molecular weight	456	414 (mass spec.)	454	426 (mass spec.)
Carbon content	84·14%	83·99%	84·51%	84·44%
Hydrogen content	12·36%	12·15%	11·97%	11·81%

A similar situation was encountered recently[12] in the fluoroboric acid-catalysed ring expansion of steroidal Δ^4-3-ketones, where elementary analysis could not distinguish between the seven- and eight-membered homologues, while a decisive answer could be provided by mass spectrometric molecular weight determination.

STRUCTURAL AND STEREOCHEMICAL APPLICATIONS IN THE STEROID FIELD

While occasional mass spectrometric measurements have been performed in the steroid series[13], the only systematic study on a group of closely related substances has been performed by the Swedish investigators[7] notably in the bile acid series. In order to utilize mass spectral fragmentation patterns for more sophisticated purposes—notably to elucidate structural and possibly also stereochemical features—it is absolutely necessary to conduct measurements on a large number of closely related substances in order to examine the

effect of minor structural or stereochemical variations upon the mass spectrum. Once specific mass spectral features have been noted by such an empirical approach, then it is possible to suggest plausible fragmentation mechanisms to explain the formation of such ion peaks, using accepted generalizations from free radical or carbonium ion chemistry (preferred stability of tertiary over secondary ions, of allylic ions over non-conjugated ones, *etc.*) as guide posts. Finally, while not absolutely necessary for empirical use in structure studies, it is highly desirable to confirm as many of the peak assignments as possible by the use of suitably labelled substrates, deuterium marking being especially convenient. This type of confirmatory work is particularly important at this stage of development, when the analysis of mass spectral fragmentation patterns is only just being introduced as an important tool in structure work dealing with complicated organic substances, notably in the natural products field.

This has been precisely the approach used by us in our earlier development[3] of optical rotatory dispersion as a general tool in organic chemistry, where closely related steroids were used initially to arrive at empirical generalizations. Consequently, many of the same substances were employed in our first mass spectrometric measurements and, just as in the case of optical rotatory dispersion, principal attention was given to carbonyl compounds, chiefly because of their greater thermal stability as compared to alcohols or acetates*.

In our first paper[14] dealing with the mass spectrometric fragmentation patterns of steroids with ketone groups in all eleven possible nuclear positions, it was shown that attachment of a carbonyl function at a given nuclear carbon atom often gave rise to some characteristic mass spectral peaks. An illustration is offered in *Figure 1*, in which it is shown that saturated 11-oxo steroids undergo principally fission of ring B by cleavage (1) of the 6–7 and 9–10 bonds, accompanied by subsidiary peaks involving rupture of the 5–6 and 9–10 (marked (2) in *Figure 1*) or 7–8 and 9–10 linkages (marked (3) in *Figure 1*). Aside from the details of hydrogen transfer, the general peak assignments can be made by comparing the mass spectrum of 5β-androstan-11-one (III) with that of 5β-pregnan-11-one (IV), the latter's additional ethyl substituent at C–17 serving as a marker. Since the charge remains predominantly with the oxygen-containing fragment, peaks (1), (2) and (3) in the 5β-androstan-11-one (III) spectrum are shifted by 28 mass units in the 5β-pregnan-11-one (IV) spectrum.

A similar type of cleavage of the ring adjacent to the carbonyl group is observed (*Figure 1*) among 1-oxo steroids (V, VI), the characteristic ring B fission peaks (corresponding to wavy lines (1), (2) and (3) in *Figure 1*) occurring at lower mass range, as compared to 11-ketones, since rings C, D and part of ring B are lost, the charge remaining with the oxygen-containing ring A. Again, these assignments can be made in a straightforward manner, by simply taking into account the molecular weight differences of the C–17 " labels ", the carbomethoxy group in methyl-1-oxo-5β-etianate (V) or the iso-octyl side chain of cholestan-1-one (VI) serving for this purpose. Aside

* Such functional groups often lead to species resulting from the loss of water (M–18) or of acetic acid (M–60) and this feature can often be used to advantage for diagnostic purposes (see, for instance, ref. 52 for detection of hydroxyl group in stemmadenine).

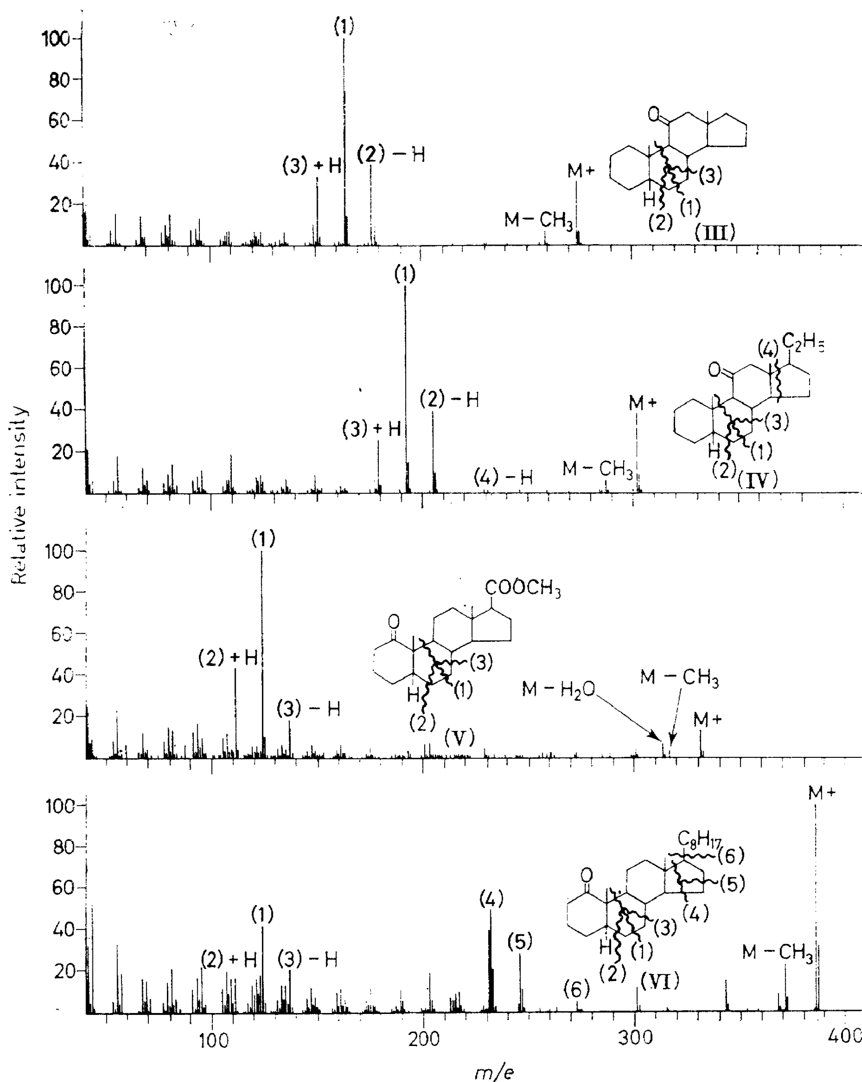


Figure 1

from these typical features, it should be noted that in the mass spectra of 11-oxo steroids (III, IV), the C–17, but not a C–3, substituent will affect the position of the characteristic (1), (2) and (3) peaks, while the reverse will be true of 1-oxo steroids (V, VI), since the C–17 side chain is not retained in the peaks corresponding to fissions (1), (2) and (3).

In *Figure 2* are reproduced characteristic fragmentations for 4-oxo (IX, X) and 6-oxo (VII, VIII) steroids and, by using two pairs of isomers, it is possible to demonstrate that stereochemical conclusions can also be derived from an inspection of mass spectra, although it is usually desirable or even

indispensable that the spectra of both isomers be available[15]. In decalones, the peak corresponding to fission at the ring juncture is usually much more pronounced in the *cis* than in the *trans* isomer[16], and the same phenomenon is also observed among steroids. Thus it will be noted in *Figure 2*, that fission

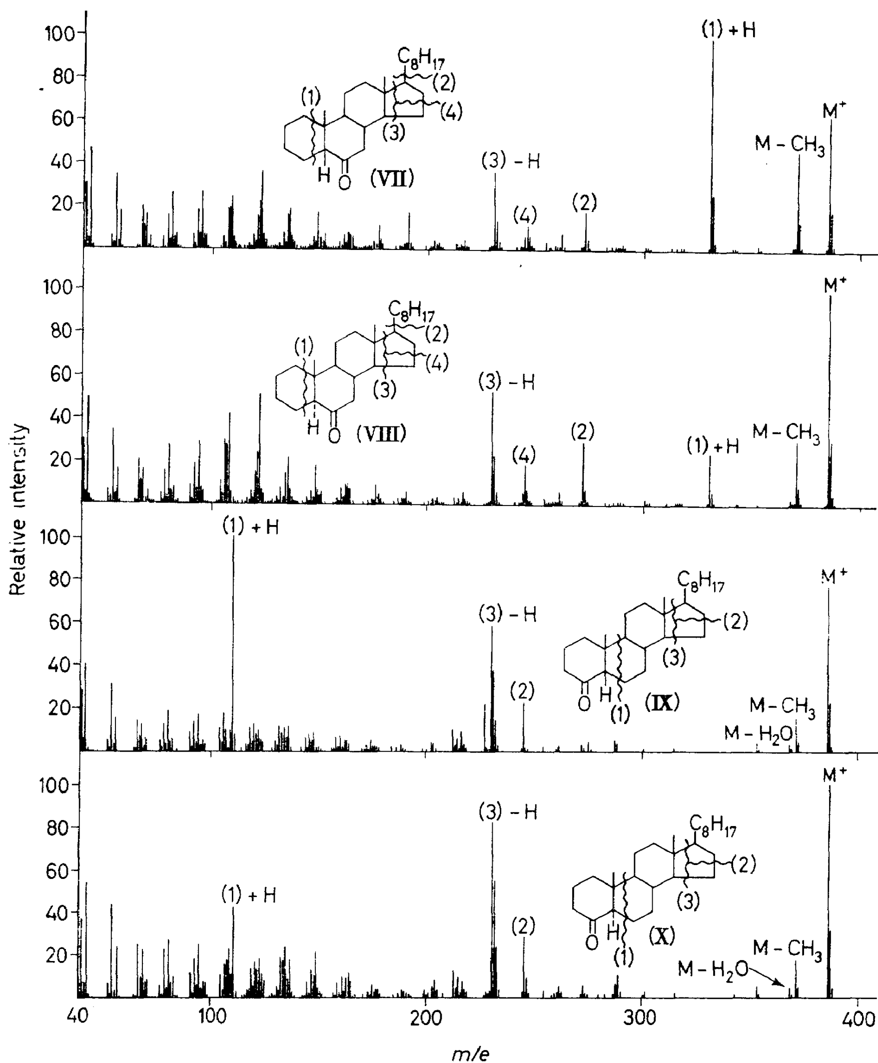


Figure 2

(1) is much more pronounced in coprostan-6-one (VII) and coprostan-4-one (IX) than in cholestan-6-one (VIII) and cholestan-4-one (X).

As indicated above, the elucidation of the mechanism of these fragmentation processes and especially of the hydrogen transfer reactions requires the use of deuterated analogues, the preparation of which is often very laborious. In *Figure 3* are outlined a few of the mono- and poly-deuterated analogues of

5α-androstan-11-one which have been synthesized in this laboratory[17] and which serve to identify unambiguously the assignments of peaks (1)–(5), simply by noting the shifts that occur upon introduction of deuterium into various positions. The mechanistic implications of this work will be discussed elsewhere[17], but it is pertinent to note at this time that the availability

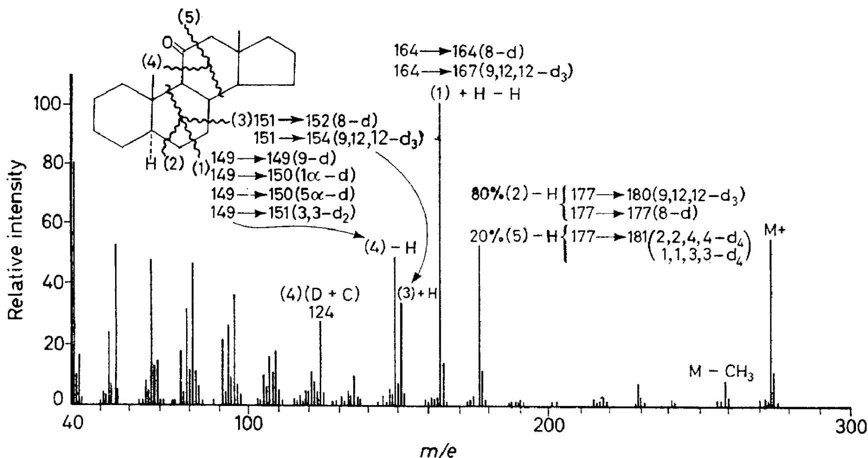
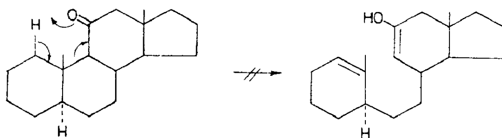


Figure 3

of 1 α-d-1,1,3,3-d_4- and 3,3-d_2-labelled 5-α-androstan-11-one[17] eliminated the most obvious[14] mechanism involving rupture of the 9–10 linkage, namely initial transfer of a hydrogen atom from C–1 to the 11-oxygen atom:



A particularly striking illustration of the information which can be deduced from suitably deuterated analogues is provided in *Figure 4*, in which is shown a portion (*m/e* 210 → molecular ion) of the mass spectra* of 17β-ethyl-5α-androstan-16-one (XI), 17β-isopropyl-5α-androstan-16-one (XII) and cholestan-16-one (XIII) as well as of several of their deuterated analogues which have recently been synthesized in our laboratory[18]. The peak at *m/e* 259 in the spectra of all three 16-ketones (XI–XIII) is due to loss of the C–17 substituent and the angular C–18 methyl group (fission (1) in *Figure 4*) and this process is initiated by transfer of the C–22 hydrogen atom (cholestane numbering) as illustrated in *Figure 4* with (XII). Particularly striking is the observation that this transfer involves only the C–22 (and not the C–21) hydrogen atoms as demonstrated by the fact that no movement of the peak

* The mass spectra of the remaining members of this homologous series of 16-oxo steroids, 5α-androstan-16-one and 17β-methyl-5α-androstan-16-one have already been reproduced in ref. 14 and ref. 19, respectively.

is observed with 21,21,21-d_3-cholestan-16-one (XIII), while a one-unit shift is encountered in the 22,22-d_2-cholestan-16-one spectrum. The most likely explanation for this phenomenon is the completely preferential loss of a $CH_3CH{=}CHR$ rather than $CH_2{=}CHCH_2R$ radical in the hydrogen transfer step outlined in *Figure 4* for (XII).

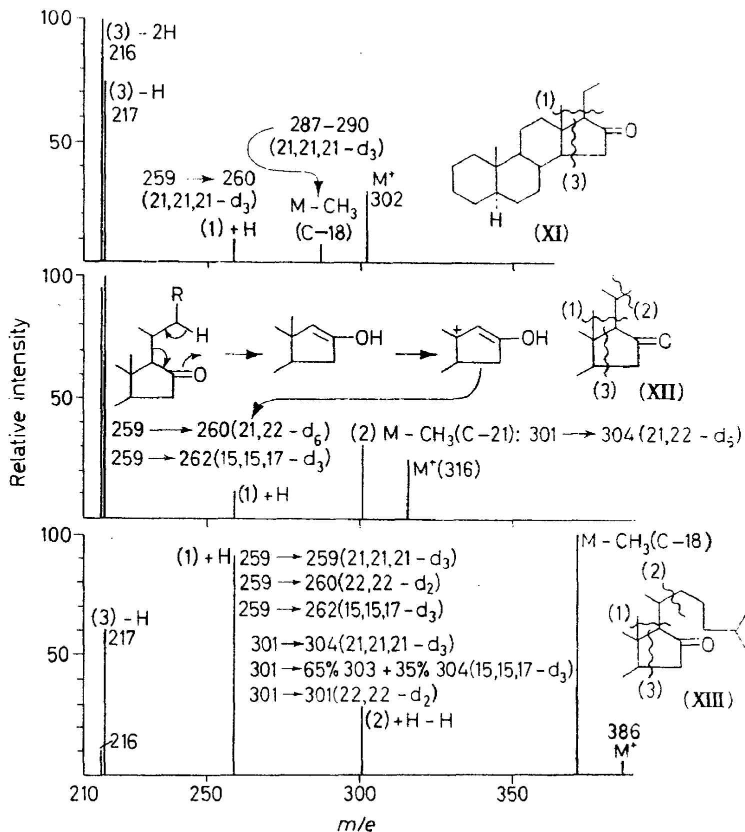


Figure 4

Of further interest is the conclusion that generation of the *m/e* 301 ion (fission (2) in *Figure 4*) involves chiefly rupture of the 20–22 linkage, rather than of the 20–21 bond, as shown by the observation (*Figure 4*) that the $M{-}CH_3$ peak in the 17β-isopropyl-5α-androstan-16-one (XII) spectrum is due to loss of the C–22 methyl group, while in cholestan-16-one (XIII) the expulsion of the angular C–18 methyl group is responsible for this peak*.

Two other groups of steroids which have been examined mass spectrometrically in some detail in our laboratory are the steroidal sapogenins[20], in which the most characteristic fragmentations involve the spiroketal side chain, and oestrogens[21]. The latter represent an instructive group, since plausible mechanisms for many of their principal peaks could be proposed.

* The alternate possibility—namely expulsion of the C–26 or C–27 methyl groups—has been excluded by deuterium labelling in those positions.

As an example, there is reproduced in *Figure 5* the mass spectrum of oestrone methyl ether (XIV) with representations for the principal peaks at *m/e* 160, 173, 186 and 199.

The *m/e* 160 peak clearly encompasses rings A and B—as demonstrated[21] by the mass spectra of C–1 and C–6 labelled derivatives—and its mode of

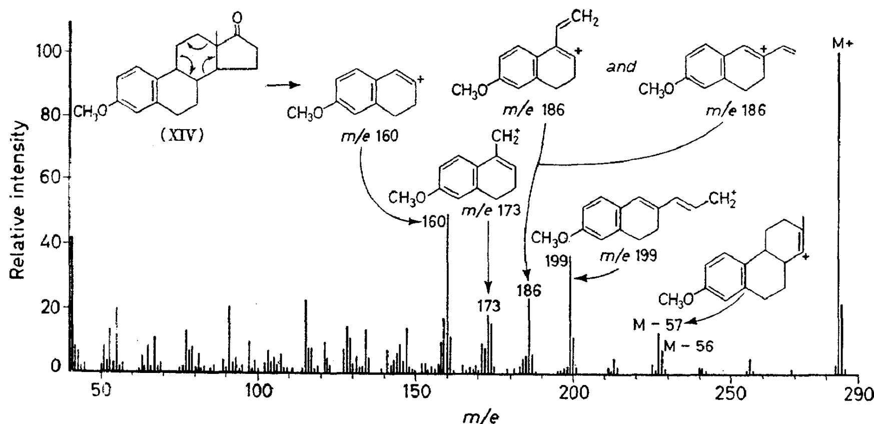


Figure 5

formation is indicated in *Figure 5*. The *m/e* 186 ion is made up of approximately equal amounts of two species as shown by the observation that it is shifted in part to *m/e* 187 upon introduction[21] of deuterium in the 15α-position. Consequently, part of *m/e* 186 does not include C–15 and hence must involve rings A and B (see *m/e* 160) as well as carbon atoms 9 and 11. A plausible mechanism*, involving transfer of one hydrogen atom each from C–9 and C–11, is postulated in *Figure 6 via* intermediates (XIV) and (XV), while fission of the 11–12 bond in (XV) would lead to the *m/e* 173 ion indicated in *Figure 5*. The other species contributing to the *m/e* 186 peak must encompass C–15 (and hence C–14) because of its shift in 15-d-estrone methyl ether and a possible mode of formation is indicated in *Figure 6* through intermediates (XVI) and (XVII). Confirmation of these mechanisms, especially as far as the suggested hydrogen transfers are concerned, would involve preparation of the rather inaccessible 8, 9 and 11-deuterated analogues, but there is little doubt that the structural assignments of these peaks are correct.

The nature of the *m/e* 199 peak in the mass spectrum (*Figure 5*) of oestrone methyl ether (XIV) is especially interesting and various labels (*e.g.* 16,16-d_2, 16-methyl, 16,16-difluoro, 13-nor-18-propyl, *etc.*) demonstrate that it must include rings A, B and C–16, but not the angular methyl group nor C–17. Therefore, carbon atoms 14 and 15 must also be included and the mechanism* outlined in *Figure 7*, involving the conventional ketone fission adjacent to the carbonyl group (stabilization of charge in (XVIII) through —C≡O+),

* For the sake of simplicity only, all bond fissions are written as homolytic fissions.

followed by transfer of a C–15 hydrogen (XIX), and finally the C–8 hydrogen atom (XX), appears most likely. Indeed the first hydrogen transfer (XIX) indicated in *Figure 7* could be established by observing[21] the appropriate one mass unit shift in the mass spectrum of 15α-d-estrone methyl ether (XXI).

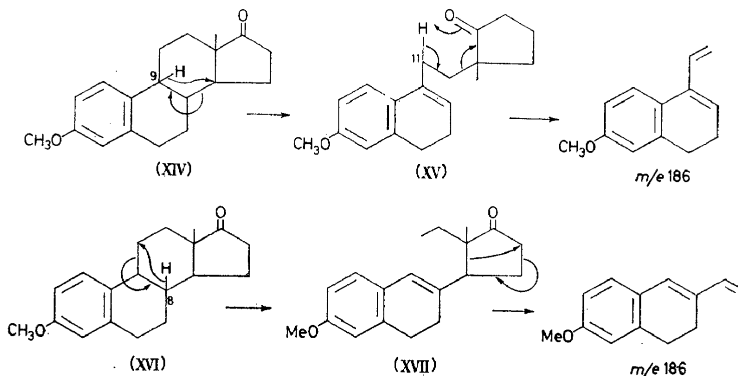


Figure 6. Proposed genesis of *m/e* 186 peak; in 15-d-oestrone methyl ether, *m/e* 186 → *m/e* 186 + *m/e* 187 (all species carry positive charge)

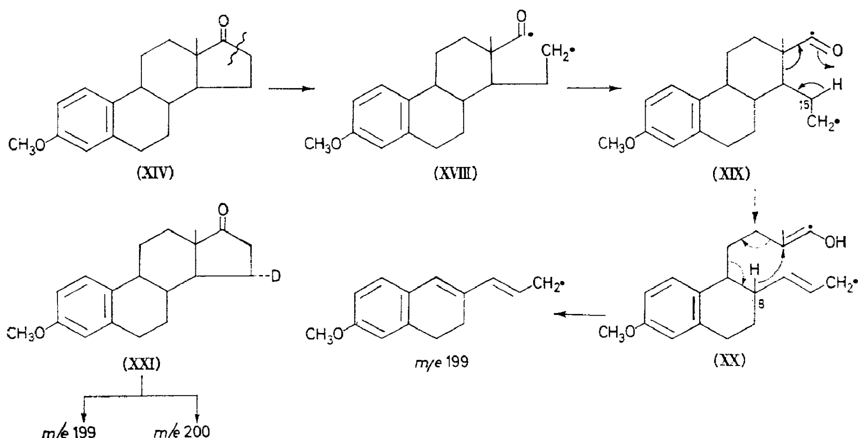


Figure 7. Proposed genesis of *m/e* 199 peak (all species carry positive charge)

In virtually all of the mass spectra of the many oestrogens examined in our laboratory[21], the molecular ion peak is also the most intense one in the spectrum (see *Figure 5*) and it would seem that mass spectrometry of oestrogens—possibly combined with gas phase chromatography—may prove to be a very efficient analytical procedure in certain biochemical experiments with such steroids.

STRUCTURAL APPLICATIONS AMONG TRITERPENES

Utilizing the same type of approach outlined above with steroids, mass spectra have been measured in our laboratory of over fifty different triterpenoids. As already indicated in a preliminary communication[22], the

principal fragmentations are associated with nuclear unsaturation and, in spite of the rather high melting points (range 200–330°), it was possible to observe molecular ion peaks in virtually all substances.

Members of the α- and β-amyrin class are in general not readily distinguished by mass spectrometry, since they exhibit the identical principal fragmentation process—a retro-Diels-Alder reaction involving the Δ^{12}-double bond. This is illustrated in *Figure 8* with the mass spectrum of methyl oleanolate 3-acetate (XXII), the most important high mass peak (*m/e* 262) representing rings D and E together with a portion of ring C. Further loss of one of the extranuclear substituents—especially the carbomethoxy function—from this species is also very characteristic and is noticed in *Figure 8* in a very strong *m/e* 203 peak. As outlined earlier[22], a wide variety of substituents can be introduced without affecting this characteristic mode of fragmentation. Indeed, even additional double bonds, such as a Δ^{15}-bond, do not affect this process, and the important peaks (*Figure 8*) of methyl 15-dehydro-oleanolate 3-acetate (XXIII) occur in the same region as the oleanolate analogue (XXII) except for the necessary two mass unit shift.

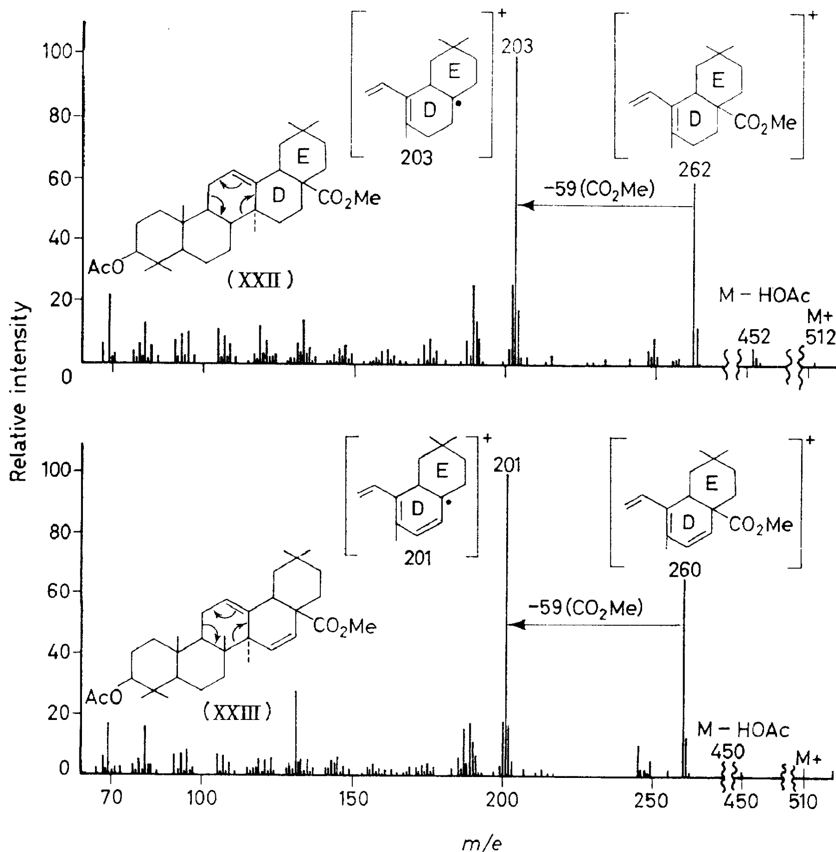


Figure 8

The crucial rôle of the Δ^{12}-double bond in this fragmentation process is illustrated in *Figure 9* with the mass spectra of two isomeric oleanenes. The Δ^{12}-oleanene (XXIV) spectrum represents the parent spectrum of the α- and β-amyrin class and is directly comparable with that (*Figure 8*) of methyl oleanolate 3-acetate (XXII) except that loss of the methyl group in (XXIV) from the initial retro-Diels-Alder fragment (*m/e* 218 in *Figure 9*) is not as pronounced as the corresponding loss of the carbomethoxy group (*m/e* 262 → *m/e* 203 in *Figure 8*) in (XXII). The other spectrum reproduced in *Figure 9* is that of Δ^{18}-oleanene (XXV), the fundamental hydrocarbon of the germanicol group of triterpenes, and its main fragmentation process differs in many details from that of its isomer (XXIV). Three of the peak assignments are marked directly on the spectrum (for the sake of clarity only, they are represented as radical ions to illustrate the points of bond fission), their correctness being established through comparisons[22] with the spectra of several substituted analogues.

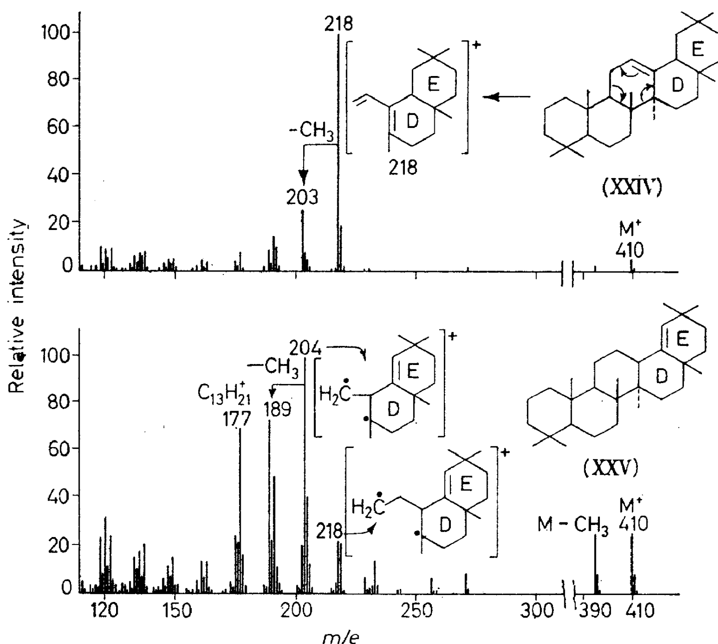


Figure 9

In contrast to the above triterpenes (*Figures 8* and *9*), where the important high mass range peaks represent rings D and E, the reverse situation is encountered in members of the taraxerol group, as demonstrated with the mass spectrum (*Figure 10*) of taraxerone (XXVI). In this series, the reverse Diels-Alder fragmentation leads to an ion (*m/e* 300) in which rings A, B and C are retained and it would appear a simple matter to decide on the basis of a mass spectrum whether an unknown triterpene belonged to this class or to the amyrins.

The information contained in the mass spectra in *Figures 8–10* will not only help in assigning membership of an unknown triterpene to one of the major sub-classes, but the exact position of the important peaks at the same time will yield information about whether a given substituent is in rings A and B or D and E. Thus a comparison of the mass spectra of the methyl

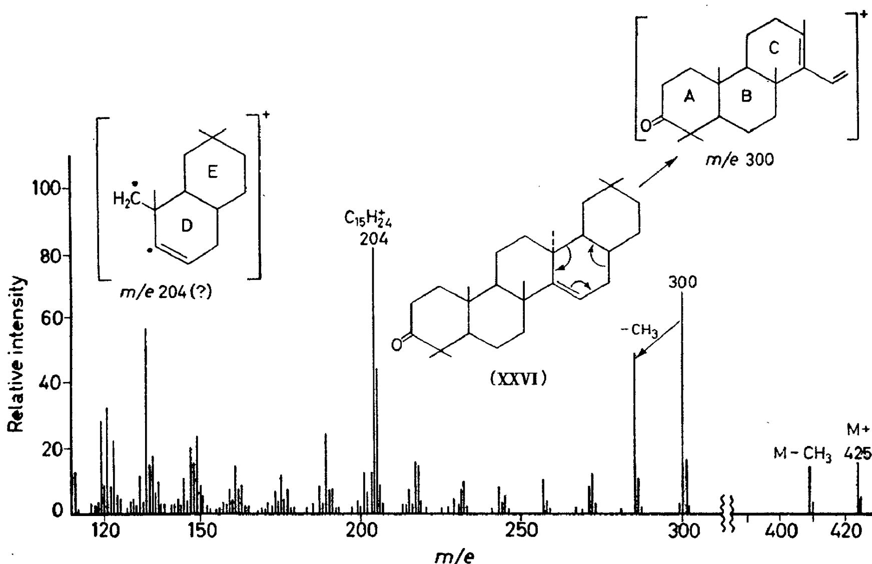


Figure 10

ester (XXII) and the hydrocarbon (XXIV) shows that the carbomethoxy group must be attached to rings D and E (or carbon atoms 11, 12 and 27); if the carbomethoxy group had been located at C–4—a rather common occurrence among triterpenes—then the *m/e* 262 peak in (XXII) would actually have moved to *m/e* 218, accompanied by a relatively small *m/e* 203 peak (see *Figure 9*).

At times, even more precise information about the actual locus of a substituent, notably a carbonyl group, can be derived from the occurrence of hydrogen transfers accompanying the typical fragmentations outlined above. Thus, the principal fragmentation of 15-ketones of the amyrin series, such as 15-oxoerythrodiol diacetate (XXVII), does not yield the expected ion *m/e* 290 derived from the usual retro-Diels-Alder process involving the Δ^{12}-double bond, but rather an ion of mass *m/e* 291 (*Figure 11*). This almost certainly involves a transfer of a hydrogen atom from C–7 (XXVII) or the angular C–26 methyl group, followed by cleavage of the doubly allylically activated 9–11 bond. The occurrence of such a hydrogen transfer in the mass spectrum of an unknown triterpenoid coupled with the negative rotatory dispersion Cotton effect of such 15-oxo-triterpenes[23]—two measurements which could be conducted on a total of about 1 mg of material—would provide very strong presumptive evidence that one is dealing with a 15-oxo derivative of the amyrin series.

Hydrogen transfers have also been observed among 11-oxo-triterpenoids, notably in 11-keto-Δ^{12}-oleanenes. As indicated in *Figure 11*, methyl-18-dehydroglycyrrhetate acetate (XXVIII) shows its important fragmentation peak at *m/e* 315 rather than *m/e* 314, the most likely candidates for the transfer being the hydrogen atoms attached to C–1 (XXVIIIa) or the angular methyl group (XXVIIIb).

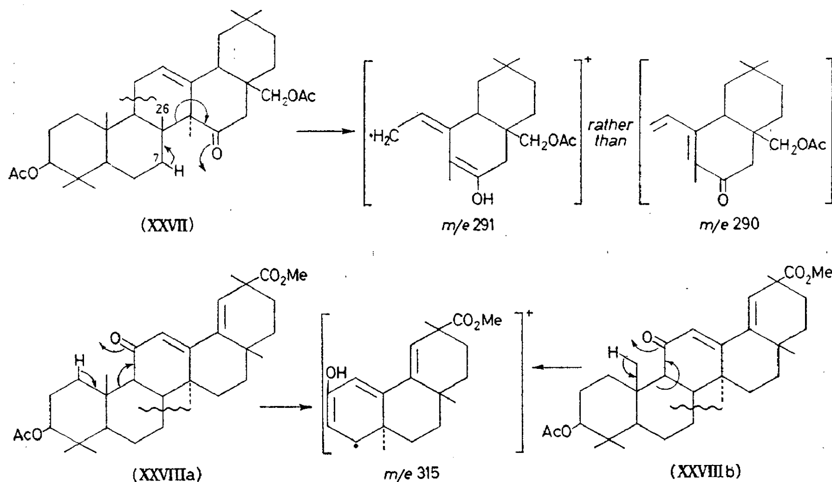


Figure 11. Examples of hydrogen transfer reactions

STRUCTURAL AND STEREOCHEMICAL APPLICATIONS IN THE FIELD OF INDOLE ALKALOIDS

Biemann and collaborators[8] were the first to employ mass spectral fragmentation patterns for structural conclusions in the indole and dihydroindole alkaloid series. Aside from indole alkaloids belonging to the sarpagine[24] and ibogamine[25] groups, they concentrated especially on aspidospermine (XXIX) and its congeners. Thus they were able to demonstrate[26] that the mass spectrum of aspidospermine (XXIX) exhibited a characteristic M–28 peak (XXX), evidently due to the expulsion of ethylene (see arrows in XXIX) with concomitant relief in strain of the highly fused polycyclic system and aromatization of the dihydroindole moiety, as well as a base peak at *m/e* 124. The latter was assigned[26] the plausible representation (XXXI) resulting from fission in (XXX) of the activated 10–11 double bond with formation of the benzylic type radical (XXXII) and the stable ion (XXXI) (*m/e* 124).

During the past three years there has been under way in our laboratory an extensive programme on the isolation and structure proof of polycyclic indole and dihydroindole alkaloids, notably from Brazilian *Aspidosperma* species[27], this latter work being performed in collaboration with Dr B. Gilbert of the Instituto de Quimica Agricola in Rio de Janeiro. As mass spectrometry was employed extensively in these investigations, several pertinent examples from our laboratory of its use in the structure elucidation of such alkaloids will now be cited.

The observation by Biemann *et al.*[26] that the appearance of M–28 (XXX) and *m/e* 124 (XXXI) peaks may be considered diagnostic of an aspidospermine (XXIX) skeleton proved very helpful in the structure elucidation[28] of pyrifolidine (XXXIII), the mass spectrum of which also exhibited its base peak at *m/e* 124 (XXXI) as well as a substantial M–28 peak. Usually, though not always, substitution at carbon atoms 3 and 4 does not affect the genesis of the *m/e* 124 base peak and only reflects itself in shifts in the peak corresponding to (XXX), due to the molecular weight increment associated with the C–3 or C–4 substituent. Thus, spegazzinine (XXXIV) and spegazzinidine (XXXV)[29] show their most intense peak at *m/e* 124 (XXXI), but instead of an M–28 peak, their mass spectra are now characterized by one at M–44 corresponding to the expulsion (see arrows in XXXV) of the elements of vinyl alcohol. Similarly, in dihydrovincadifformine[30] (*Figure 12*)

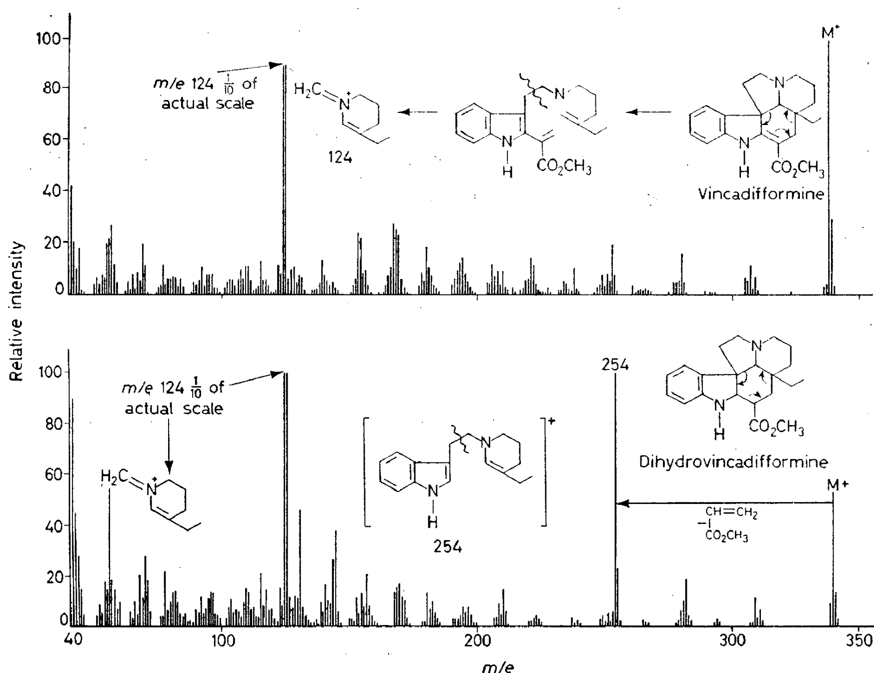
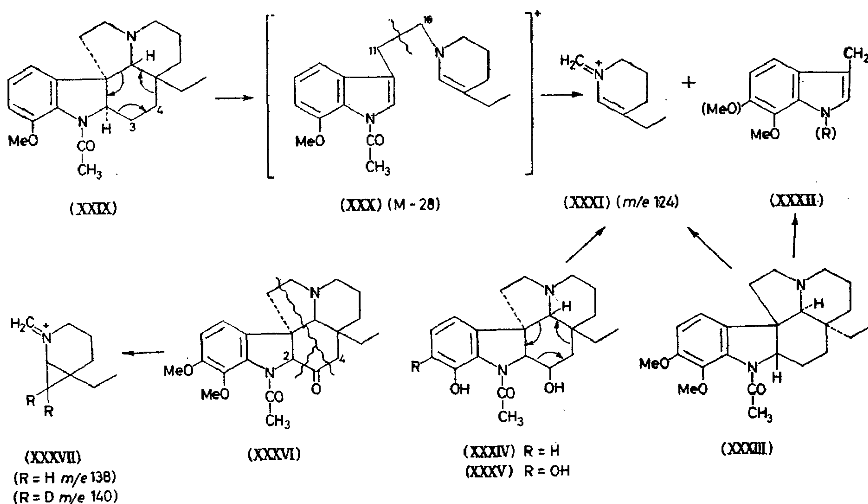


Figure 12

the presence of the carbomethoxy substituent at C–3 is now demonstrated quite clearly in a strong M–86 peak (*m/e* 254) due to expulsion of the C–3 and C–4 bridge as methyl acrylate. The base peak remained at *m/e* 124, which was also true of the mass spectrum (*Figure 12*) of vincadifformine[30] itself, the presence of the 2,3-double bond, however, preventing the elimination of carbon atoms 3 and 4.

That some caution has to be exercised in the interpretation of such mass spectra is illustrated in the ketone (XXXVI) derived[29] from spegazzinidine (XXXV). In contrast to the original alkaloid, which underwent[29] the

standard aspidospermine-type[26] fragmentation (XXIX → XXX → XXXI) with formation of the base peak at *m/e* 124 (XXXI), the spectrum of the ketone (XXXVI) reflected a different kind of rupture with expulsion of carbon monoxide, the most intense peak now appearing at *m/e* 138. Its representation as (XXXVII, R=H) was verified by the observation that this peak shifted to *m/e* 140 (XXXVII, R=D) in the 2,4,4-d_3 analogue of (XXXVI), thus demonstrating that C–4, but not C–2, was retained in



that ion. It is interesting to note that movement of the keto group from C–3 (XXXVI) to C–4[31] again restores the " normal " aspidospermine fragmentation process.

Substitution in ring D can also be recognized mass spectrometrically, as has been demonstrated recently in our laboratory with the *Aspidosperma* alkaloids aspidoalbine (XXXVIII)[32] and pyrifoline (XLIII)[33]. The former has been shown by N.M.R. spectroscopy to possess a tri-oxygenated aromatic substitution pattern, which so far has been unique in the dihydroindole alkaloid series. The additional tetrahydrofuran ring in aspidoalbine (XXXVIII) does not affect the aspidospermine-like fragmentation process discussed above and its mass spectrum exhibits an M–28 peak (XXXIX) as well as a base peak at *m/e* 138 (XL). The attachment of this ether bridge to ring D, and adjacent to N(b), is confirmed by the observation (see *Figure 13*) that lithium aluminium hydride reduction of *N*-depropionyl-*O*-methyl-aspidoalbine leads to *N*-depropionyl-*O*-methylaspidoalbinol (XLI, R=H), the principal mass spectral peak of which now occurs at *m/e* 140 (XLII, R=H) and is shifted to *m/e* 141 (XLII, R=D) when the reduction is performed with lithium aluminum deuteride (XLI, R=D).

Pyrifoline (XLIII)[33] represents the first alkaloid, of what is now recognized to be a fairly large group[34], based on a hexacyclic skeleton in which, formally speaking, the angular ethyl group of aspidospermine (XXIX) is involved in an additional carbocyclic ring terminating at C–2. The structure

elucidation[33] of pyrifoline (XLIII) and its ring A demethoxylated relative, refractidine, rested heavily on an analysis of the mass spectral fragmentation patterns of various derivatives coupled with extensive N.M.R. measurements. No classical degradations of any kind were performed and, in the strictest sense, none of the carbon atoms (with the exception of the *N*-acetyl function) of this alkaloid have been " captured " in the form of degradation products. As shown in *Figure 14*, virtually all of the important peaks in the mass

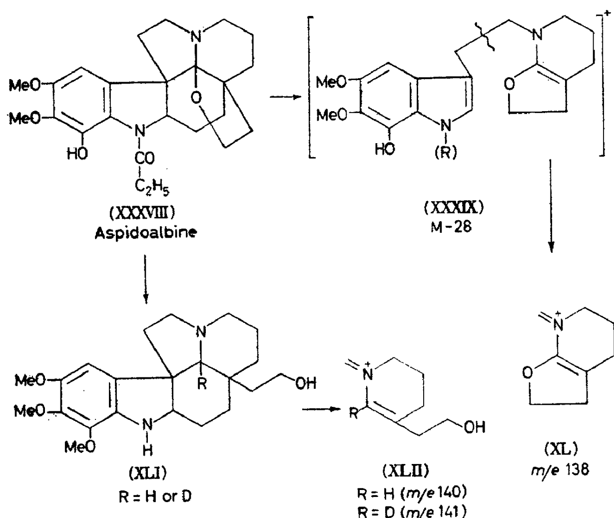


Figure 13

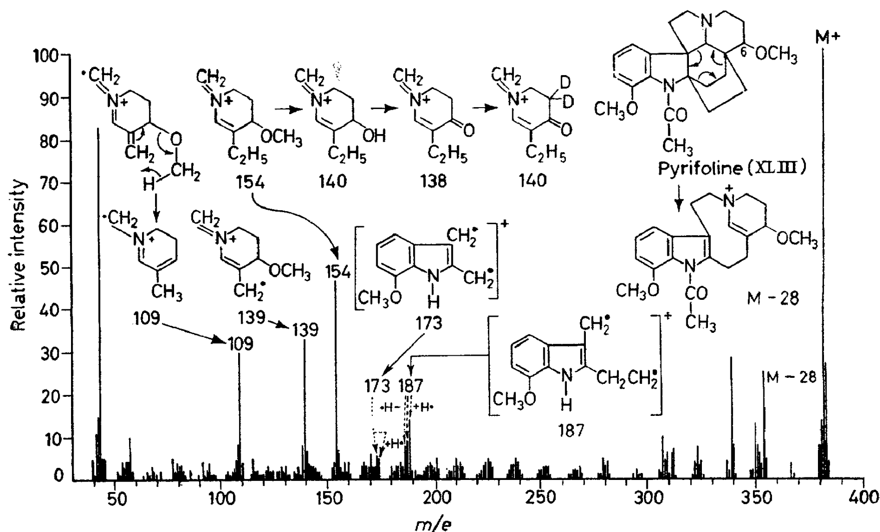


Figure 14

spectrum of pyrifoline (XLIII) have been assigned plausible structures on the basis of a number of " labels ", the assignment of the very characteristic *m/e* 154 peak being used as an example. It could not represent the indole portion of the molecule, because its position was not affected by substituents on N(a) (*e.g.* CH_3, CD_2H). On the other hand, it had to encompass the aliphatic methoxyl function known to be present from N.M.R. measurements and Zeisel determination. This feature, as well as the immediate environment of the methoxyl group, could be demonstrated mass spectrometrically by observing that conversion of the methyl ether function to an alcohol group shifted the *m/e* 154 peak to *m/e* 140, while oxidation to a ketone resulted in a further movement to *m/e* 138 (see *Figure 14*). That the keto group (shown to be part of a six-membered ring by infra-red spectroscopy), and hence the methoxyl substituent, contained only two adjacent hydrogen atoms was verified by deuterium exchange, two deuterium atoms having entered as noted by a two mass unit shift in the molecular ion peak and in the original *m/e* 138 peak of the ketone, which now appeared at *m/e* 140.

The information gained in the interpretation of the pyrifoline (XLIII) mass spectrum (*Figure 14*) could be employed to good effectiveness in establishing the structure of aspidofiline, an alkaloid isolated by Antonaccio[35] from the leaves of *Aspidosperma pyrifolium**, and assigned the empirical formula $C_{20}H_{22}N_2O_2$ on the basis of elementary analysis. Infra-red, ultra-violet and N.M.R. measurements indicated the presence of a 7-hydroxy-*N*-acetyldihydroindole system and the mass spectrum (*Figure 15*) of aspidofiline immediately suggested the expression (XLIV)[36]. Thus the molecular ion

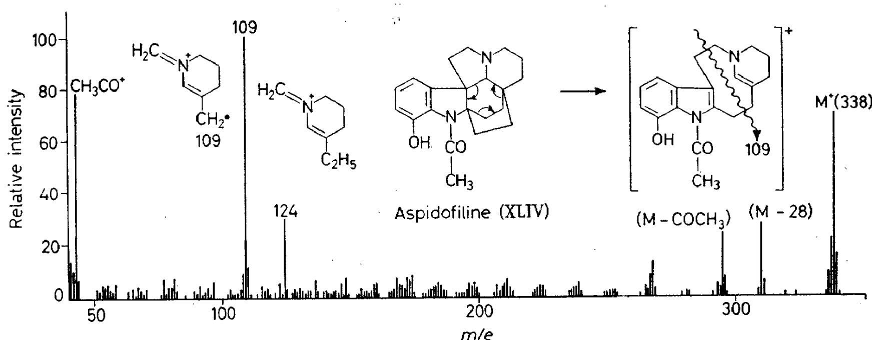
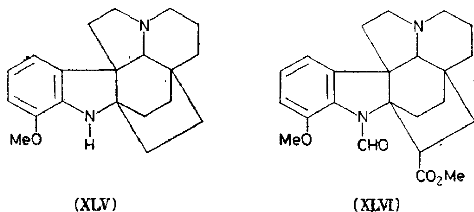


Figure 15

peak at *m/e* 338 required modification of the earlier assumed[35] empirical formula $C_{20}H_{22}N_2O_2$ to $C_{21}H_{26}N_2O_2$, while the M–28 peak was indicative of the presence of an aspidospermine-like bridge (XXIX) involving C–3 and C–4. Most importantly, the intense peaks at *m/e* 109 and 124 were analogous to the *m/e* 139 and 154 peaks in the pyrifoline spectrum (*Figure 14*), the thirty mass unit increase being due to the latter's aliphatic methoxyl substituent, and they have also been observed earlier in the mass spectra[34c] of

* This is the same plant from which pyrifoline was isolated.

refractine (XLVI) and its relatives. The correctness of structure (XLIV) for aspidofiline was established by direct chemical conversion[36] of pyrifoline (XLIII) to *N*-deacetyl-*O*-methylaspidofiline (XLV).



The above examples were all drawn from the dihydroindole series. In connection with our investigation[37] of the alkaloid constituents of *Aspidosperma polyneuron*, there have been examined the mass spectra of a number of indole alkaloids related to yohimbine (XLVII). Here again, several characteristic fragmentations have been noted, which serve very readily to permit assignment of an alkaloid to this group. The mass spectrum of yohimbine (XLVII) itself is reproduced in *Figure 16* and it will be noted that its most characteristic features are represented by an enormous M–1 peak, shown by deuterium marking to be due in part to loss of the C–3 hydrogen atom, and peaks at *m/e* 184, 170, 169 and 156. The assignments made in *Figure 16* for these peaks are quite rigorous, since they could be substantiated[37] by deuterium labelling in positions 3, 5 and 6 as well as by studying the effect of functional substituents in various other positions of the molecule. Plausible paths to these fragments are indicated in *Figure 16*.

The mass spectra of many of the stereoisomers of yohimbine (XLVII) have also been examined[37] but they resemble that (*Figure 16*) of yohimbine

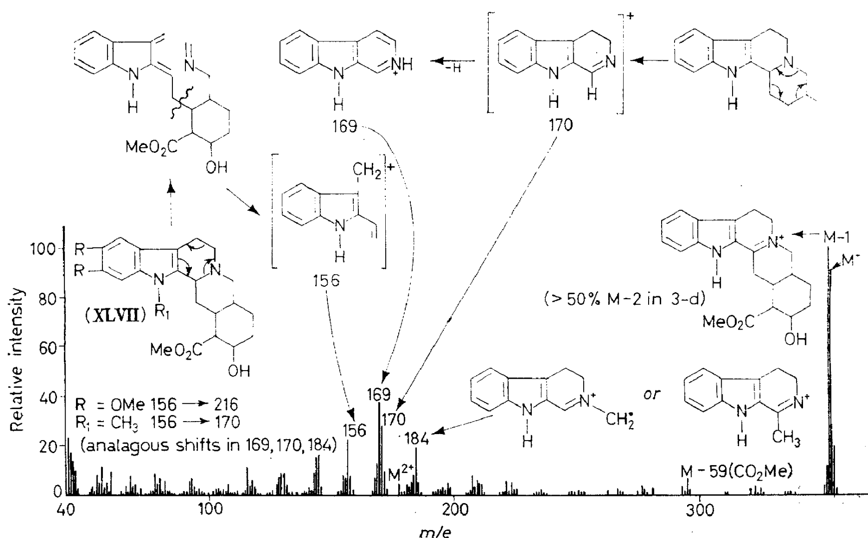


Figure 16

sufficiently closely so that no stereochemical assignments are possible by mass spectrometry.

The fragmentation patterns of the ring-E heterocyclic indole alkaloids, such as ajmalicine and tetrahydroalstonine, follow the same general paths as observed in *Figure 16* for yohimbine (XLVII). This is illustrated in *Figure 17*, the principal difference being the appearance of a peak at *m/e* 225 associated with a retro-Diels-Alder fragmentation of ring E as outlined in *Figure 17*. The various assignments were again confirmed[37, 44] by deuterium labelling at positions 3, 5, 6 and 14 as well as by methoxyl substitution in the aromatic ring. Noteworthy is the observation (*Figure 17*) that the relative intensities of the *m/e* 156, 169, 170 and 184 peaks now differ substantially, depending upon the D/E stereochemistry, and that the mass spectra can be

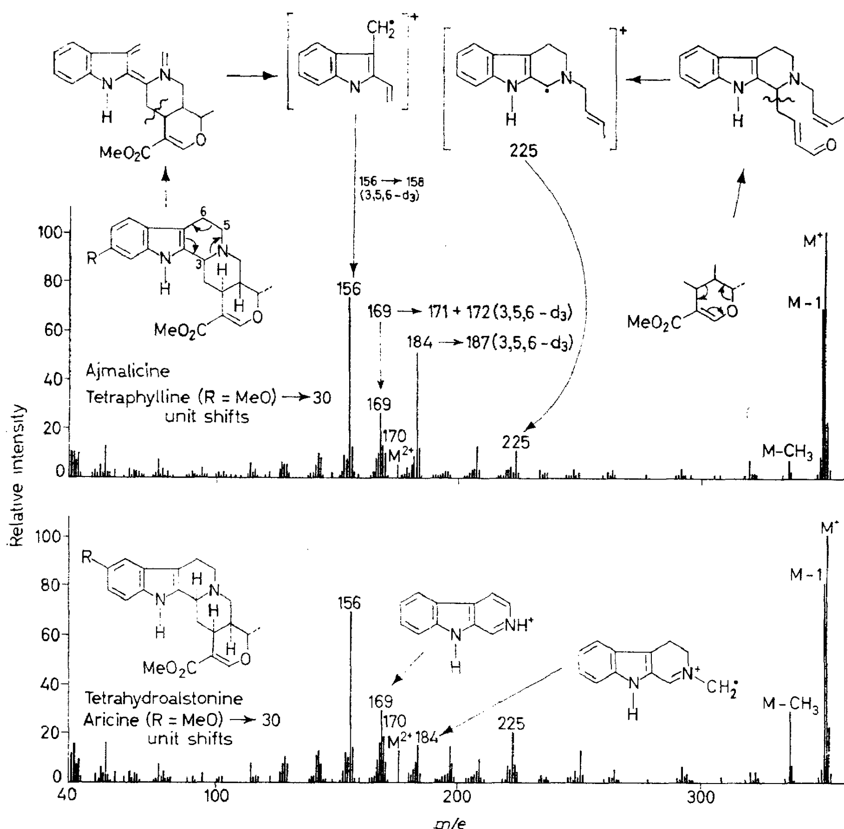


Figure 17

used in this series for stereochemical assignment purposes. Thus it was found (as indicated in *Figure 17*) that tetraphylline exhibited a mass spectrum of the ajmalicine type (except for the thirty mass unit increment due to the extra aromatic methoxyl function), while that of aricine resembled closely the intensity relationships of the tetrahydroalstonine spectrum. These

conclusions[37] are in complete accord with recent stereochemical assignments[38] reached by N.M.R. measurements and rate studies on methiodide formation.

Another group of indole alkaloids, the mass spectra of which have been measured in our laboratory, are the bases eburnamine, eburnamonine and eburnamenine isolated and characterized by Bartlett and Taylor[39]. These alkaloids are derived from another novel pentacyclic system and, through the courtesy of Dr W. I. Taylor[39], specimens were made available for mass spectrometry. As shown in *Figure 18*, the mass spectra are relatively simple and are characterized by several extremely typical peaks due to generation of almost completely conjugated, stable ions. The mass spectra of eburnamine and eburnamenine proved to be completely identical, as no molecular ion peak could be observed with eburnamine, the first peak being the dehydration peak (M–18) corresponding to the molecular ion of eburnamenine. The two principal peaks in that series are the one at *m/e* 249, associated with the loss of the allylically activated angular ethyl group, and the *m/e* 208 peak, which is quite clearly due to the aromatic ion resulting from fragmentation of ring C and cleavage of one of the allylic bonds (see *Figure 18*). Such a type of mass spectrum can be considered to be very indicative of an

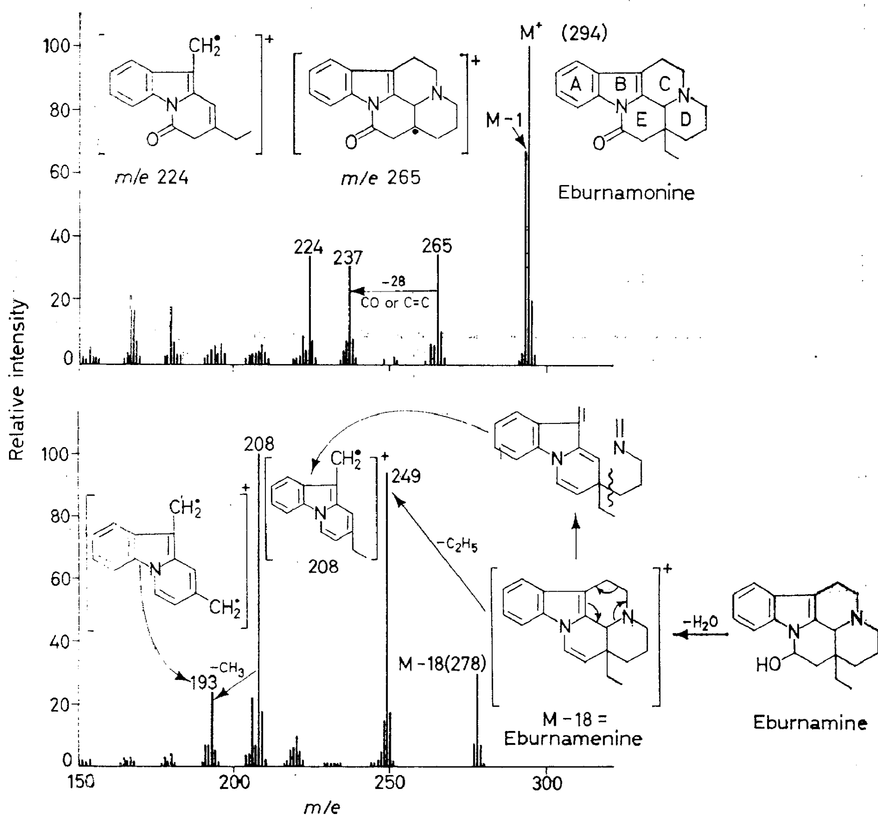


Figure 18

eburnamine skeleton and this criterion has proved to be very helpful in confirming[40] structure (XLVIII), proposed[41] recently for vincamine, as well as in establishing structure (XLIX)[40] for a methoxylated analogue of vincamine.

Of the three important peaks (*m/e* 265, 237, 224) in the high mass range of the eburnamonine spectrum (*Figure 18*), those at *m/e* 265 and 224 are completely analogous to the *m/e* 249 and 208 peaks of the eburnamenine spectrum, the sixteen mass unit difference being due to the additional amide oxygen. The third important peak (*m/e* 237) in the eburnamonine spectrum (*Figure 18*) represents the loss of 28 mass units from the *m/e* 265 peak, but we have been unable to establish whether this involves* the loss of carbon monoxide or of ethylene (from ring D), although, mechanistically, the former is more likely.

Recently, there have been developed procedures[42, 43] for converting indole alkaloids of the yohimbine (XLVII) and ajmalicine class to the corresponding oxindoles, thus making available a variety of naturally occurring, as well as hitherto unknown, oxindoles. A group of representative oxindole alkaloids has been examined mass spectrometrically in our laboratory and the mass spectrum of one typical representative, mitraphylline (L), is reproduced in *Figure 19*. It will be noted that, aside from the strong molecular ion peak, the spectrum is characterized by a single, intense peak

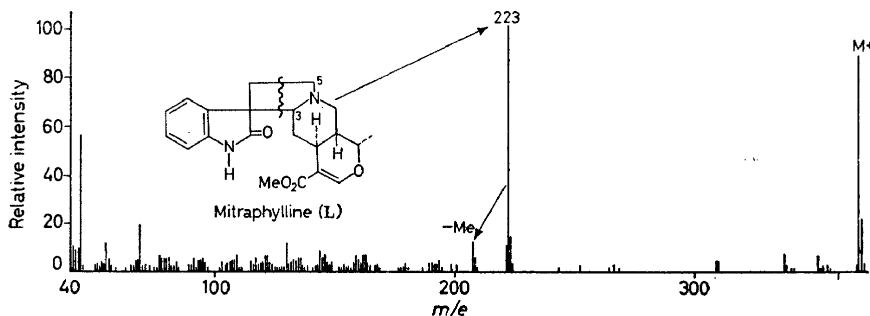


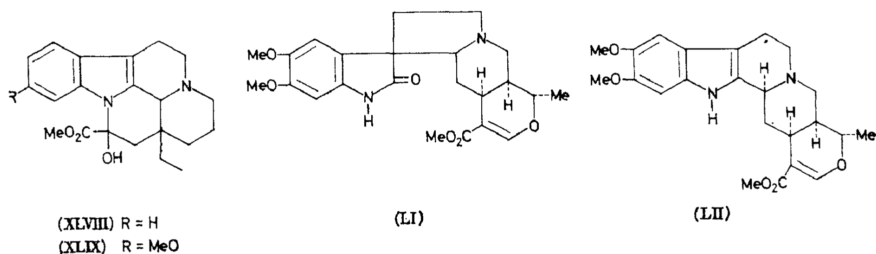
Figure 19

at *m/e* 223, representing the hydroaromatic portion of the molecule arising from fission (see wavy line in L) at C–3 and C–5. The appearance of such a peak thus represents a very convenient means of detecting membership of an unknown base among this class of alkaloids and an illustration of its utility can be presented by the recent structure elucidation of the alkaloid carapanaubine (LI), isolated[44] from *Aspidosperma carapanauba*.

The ultra-violet, infra-red and N.M.R. spectra of carapanaubine (LI) resembled in many respects the corresponding spectra of the well-known *Rauwolfia* alkaloid, isoreserpiline (LII)[38, 45], which has recently been encountered[46] in *Aspidosperma discolor*, but the elementary analysis and mass spectrum showed the presence of one additional oxygen atom. In fact, a comparison of the mass spectrum (*Figure 19*) of mitraphylline (L) with that

* This question could be settled by measuring the mass spectrum in a double focusing instrument (see ref. 5) or by labelling with ^{18}O.

(not reproduced) of carapanaubine (LI) showed that the latter clearly must be of the oxindole type, in view of the appearance of a very strong *m/e* 223 peak, while peaks associated with the indole moiety in the mitraphylline (L) spectrum (*Figure 19*)—such as *m/e* 130, 144, 159—were shifted by sixty mass units corresponding to the two extra aromatic methoxyl groups. This



conclusion could be verified completely by direct comparison with a subsequently synthesized specimen[47] of isoreserpiline oxindole.

The above brief survey of some of the recent mass spectral information which has been accumulated with indole and dihydroindole alkaloids should suffice to illustrate the power of this tool in structural alkaloid chemistry. In collaboration with Profs. M.-M. Janot and J. Le Men of the Faculté de Pharmacie in Paris, the structures of the novel aspidospermine-like alkaloids, minovincine[54], vindolinine[48] and tabersonine[49], and of the akuammicine-like alkaloids[55], minovincinine[54], mossambine[50] and echitamidine[51], have been elucidated. In each instance, mass spectrometry, together with N.M.R. spectroscopy, has played a crucial rôle and this also applied to our collaborative studies with Prof. A. Sandoval (Instituto de Quimica, Mexico City) on the structure determination[52] of stemmadenine and condylocarpine.

CONCLUSION

The application of mass spectrometry to structural and stereochemical studies in the field of complicated natural products is still in its infancy. What is needed at this time are extensive mass spectral measurements on closely related substances of the type described in this article to permit the recognition of the most important fragmentation paths associated with given structural types. There is no question whatsoever that mass spectrometry now represents a powerful adjunct to the structural chemist's armamentarium of physical methods and that this tool offers information not generally available from other physicochemical measurements. The requirements of microquantities of material, and the combination of sensitivity to the detection of impurities and yet simultaneous non-interaction of such mixture components, make mass spectrometry one of the most important measurements in the initial examination of a new natural product.

In the type of structural applications described in the present paper, the combination of mass spectrometry and optical rotatory dispersion can frequently give a great deal of information in the steroid and triterpenoid fields, while mass spectrometric measurements combined with N.M.R. data have proved especially valuable for the rapid structure elucidation of

complicated alkaloids. In our own laboratory, the structures of well over twenty polycyclic indole and dihydroindole alkaloids have been elucidated in less than two years largely by the judicious use of these two physical methods.

I would like to express my deep appreciation to two of my postdoctoral research fellows, Drs H. Budzikiewicz and J. M. Wilson, who were intimately concerned with the work described in this article.

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