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MASS SPECTROMETRY IN STRUCTURAL AND STEREOCHEMICAL PROBLEMS—XIX*

AKUAMMICINE AND RELATED ALKALOIDS

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Abstract—The mass spectral fragmentation patterns of akuammicine, dihydroakuammicine, tetrahydroakuammicine and many of their relatives are summarized in terms of the most characteristic mass spectral peaks. Plausible mechanisms can be postulated in most instances for these ions and attention is called to the use of this information in the structure elucidation of alkaloids with a 2-methyleneindoline (I) chromophore.

RECENTLY, our two laboratories have been engaged in an extensive program on the isolation and structure elucidation of alkaloids possessing the 2-methyleneindoline chromophore (I),[1] physical methods such as N.M.R. and mass spectrometry playing a crucial role in this work. For mass spectrometry to be effective in the area of such complicated organic molecules,[2,3] it is necessary to study first the mass spectra of a number of structurally closely related substances of known constitution in order to permit a plausible interpretation of the principal fragmentation processes and a reasonable assignment of the most characteristic ion peaks. Only in this manner can the full potentialities of this physical tool be employed in structure studies of unknown members of a particular class of (natural) products.

One of the most important alkaloids possessing the chromophoric system I is akuammicine (IIA), the structure of which has been established by classical chemical means.[4] Mass spectral measurements of akuammicine (IIA) and its derivatives as well as of members of the geissoschizoline[5] series (e.g. IVF) provided the necessary background for the employment of mass spectrometry in the structure elucidation of the related alkaloids mossambine (IIB),[6] echitamidine (IIIC),[7] lochneridine (IIIB)[8]

* For paper XVIII see E. Lund, H. Budzikiewicz, J. M. Wilson and C. Djerassi, *J. Amer. Chem. Soc.* **85**, 941 (1963).

[1] For the most recent paper see M. Plat, J. LeMen, M.-M. Janot, J. M. Wilson, H. Budzikiewicz, L. J. Durham and C. Djerassi, *Bull. Soc. Chim. Fr.* 2237 (1962).

[2] See K. Biemann, *Mass Spectrometry* especially chap 8. McGraw-Hill, New York (1962).

[3] For more recent work not covered in ref. 2 see C. Djerassi, *Pure Appl. Chem.* **6**, No. 4 (1963).

[4a] K. Aghoramurthy and R. Robinson, *Tetrahedron* **1**, 172 (1958); [b] P. N. Edwards and G. F. Smith, *J. Chem. Soc.* 152 (1961); [c] J. Lévy, J. LeMen and M.-M. Janot, *Bull. Soc. Chim. Fr.* 979 (1960); [d] K. Bernauer, W. Arnold, C. Weissmann, H. Schmid and P. Karrer, *Helv. Chim. Acta* **43**, 717 (1960).

[5] See M.-M. Janot, *Tetrahedron* **14**, 113 (1961).

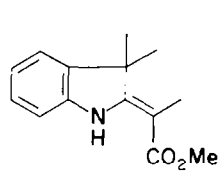
[6] X. Monseur, R. Goutarel, J. LeMen, J. M. Wilson, H. Budzikiewicz and C. Djerassi, *Bull. Soc. Chim. Fr.* 1088 (1962).

[7] C. Djerassi, Y. Nakagawa, H. Budzikiewicz, J. M. Wilson, J. LeMen, J. Poisson and M.-M. Janot, *Tetrahedron Letters* 653 (1962).

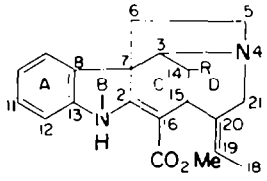
[8] Y. Nakagawa, J. M. Wilson, H. Budzikiewicz and C. Djerassi, *Chem. & Ind.*, 1986 (1962).

and of constituents of *Aspidosperma* species still under investigation. The purpose of the present article is to summarize and systematize the mass spectral fragmentation patterns of alkaloids of this group, information from the related alkaloids aspidospermatine (XE)[9] and condylocarpine (XVIII)[10] being included where pertinent.

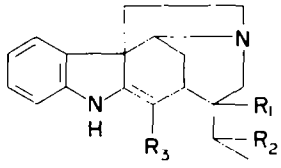
The most straightforward fragmentation pattern is exhibited by derivatives of tetrahydroakuammicine (IVB), where the first step can be assumed[11] to be the opening of ring C in a six-membered transition state (arrows in IV), thus releasing the strain inherent in the fused polycyclic system with concomitant aromatization of the dihydroindole moiety. Further cleavage then occurs at the labilized positions marked α, β and γ in the intermediate V to lead to the observed ions *a*, *b*, *c* and *d*:



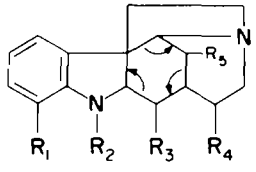
I



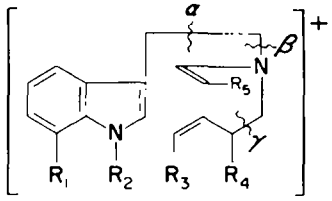
II A R=H
II B R=OH



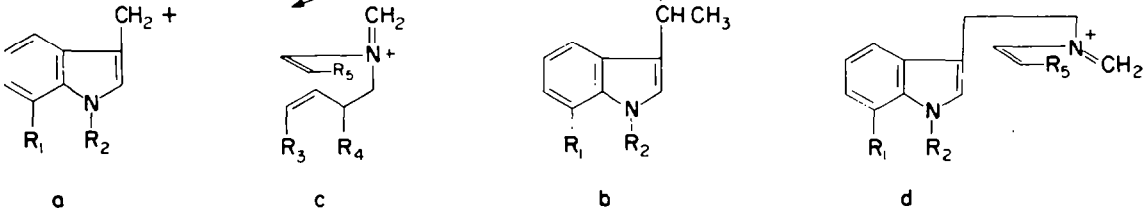
III		R_1	R_2	R_3
	A	H	H	CO_2Me
	B	OH	H	CO_2Me
	C	H	OH	CO_2Me
	D	H	H	CHO



IV



V



a c b d

[9] K. Biemann, M. Friedmann-Spiteller and G. Spiteller, *Tetrahedron Letters* 485 (1961).

[10a] A. Sandoval, F. Walls, J. N. Shoolery, J. M. Wilson, H. Budzikiewicz and C. Djerassi, *Tetrahedron Letters* 409 (1962); [b] K. Biemann, A. L. Burlingame and D. Stauffacher, *Ibid.* 527 (1962).

[11] This was first proposed by Biemann and collaborators (ref. 9) in decarbomethoxytetrahydro (IVC)-and 2,16-dihydro(VIC)-akuammicine, which represent the first members of the akuammicine group where mass spectral information had been recorded.

The basis of these assignments—species *a* and *b* containing only the indole fragment with one or two attached carbon atoms, species *c* only the non-aromatic portion of the molecule, and *d* the indole moiety together with atoms 3, 4, 5, 6, 14 and 21—is outlined in Table 1, which summarizes the shifts (or absence of shifts) of peaks *a* — *d* in the mass spectrum (Fig. 1) of tetrahydroakuammicine (IVB), five positions in the molecule (R_1 — R_5 in IV) having been "labelled" through different substituents. The six important peaks collected in Table 1 offer the following important information:

Molecular ion peaks (M^+ in Table 1) could be observed in the spectra of all alkaloids, thus differentiating immediately between alternate empirical formulae, which were otherwise not distinguishable on the basis of elementary analyses. Cases in point are echitamidine (IIIC)[7] and lochneridine (IIIB)[8] and especially vincadifformine (XIV)[12] and tabersonine.[13] The mass spectrally determined[12,13] molecular weights of the latter two alkaloids demonstrated immediately that they could not belong to the akuammicine class and that there must exist a second group of naturally occurring alkaloids containing the chromophore I.

Other peaks in the high mass range arise from the fragmentation of specific substituents (e.g. loss of CH_3O from CO_2CH_3 enumerated in Table 1) and may, therefore provide some indication of the existence of such groupings.

Peaks a *and* b (Table 1) have been encountered previously[2,14] in indole and dihydroindole alkaloids and have been attributed to an intact indole fragment containing one and two additional carbon atoms. These assignments are corroborated by the observation that appropriate mass unit shifts occur when the aromatic ring (IVJ, IVN, IVO) or N_a (IVE, IVM) are substituted. It should be noted, however, that N-acetate groups are lost during the formation of these ions (e.g. IVD, IVF, IVJ, IVN) with hydrogen transfer (loss of ketene) to give an ion of the same mass as would be expected from the corresponding deacylated compound.

Peak d (Table 1) must contain the indole moiety on the basis of the appropriate shifts upon introduction of substituents in the aromatic ring and at N_a. Furthermore, variations in substituents (R_3 and R_4 in IV) attached to C-16 and C-20 do not affect this peak (e.g. IVA *vs.* IVB *vs.* IVC *vs.* IVI) and consequently cannot contain these carbon atoms, while C-14 (R_5 in IV) is retained (e.g. IVG). Formulation[11] of this ion as *d* seems, therefore, quite reasonable and it should be noted that N-acyl groups are not lost (e.g. IVD, IVF) in the formation of *d*, in contrast to the situation obtaining in *a* and *b*.

Peak c (Table 1) is clearly defined by the observation (Table 1) that variations in the indole portion do not affect it, while the other three labels (R_3, R_4 and R_5 in IV) attached to positions 14, 16 and 20 cause appropriate mass unit shifts.

Through overlap of the above described fragments, mass spectrometry thus permits narrowing down of the point of attachment of a given substituent to one of the following areas—indole nucleus together with C-5 and C-6 *or* together with C-3, C-14 and C-21 *or* together with C-15, C-16 and C-20—precisely the approach which

[13] C. Djerassi, H. Budzikiewicz, J. M. Wilson, J. Gosset, J. LeMen and M.-M. Janot, *Tetrahedron Letters*, 235 (1962).

[13] M. Plat, J. LeMen, M. -M. Janot, J. M. Wilson, H. Budzikiewicz, L. J. Durham, Y. Nakagawa and C. Djerassi, *Tetrahedron Letters*, 271 (1962).

[14] B. Gilbert, J. A. Brissolese, N. Finch, W. I. Taylor, H. Budzikiewicz, J. M. Wilson and C. Djerassi, *J. Amer. Chem. Soc.* **85**, 1523 (1963).

proved so helpful in establishing the structures of mossambine (IIB)[6], echitamidine (IIIC)[7] and aspidospermatine (XE).[9]

Introduction of an ethylidene function as in 2,16-dihydroakuammicine (VIA) alters the mass spectral fragmentation pattern (Fig. 2) in several important respects, which again can be used to advantage in structural diagnosis. The first step can again be assumed[11] to be the opening of ring C through the six-membered transition state indicated by the arrows in VI. In the resulting ion VII, fission of the 20–21 bond is now inhibited by the presence of the extra double bond and no fragment corresponding to *d* in the tetrahydroakuammicine (IVB) spectrum (Fig. 1) is, therefore, found in

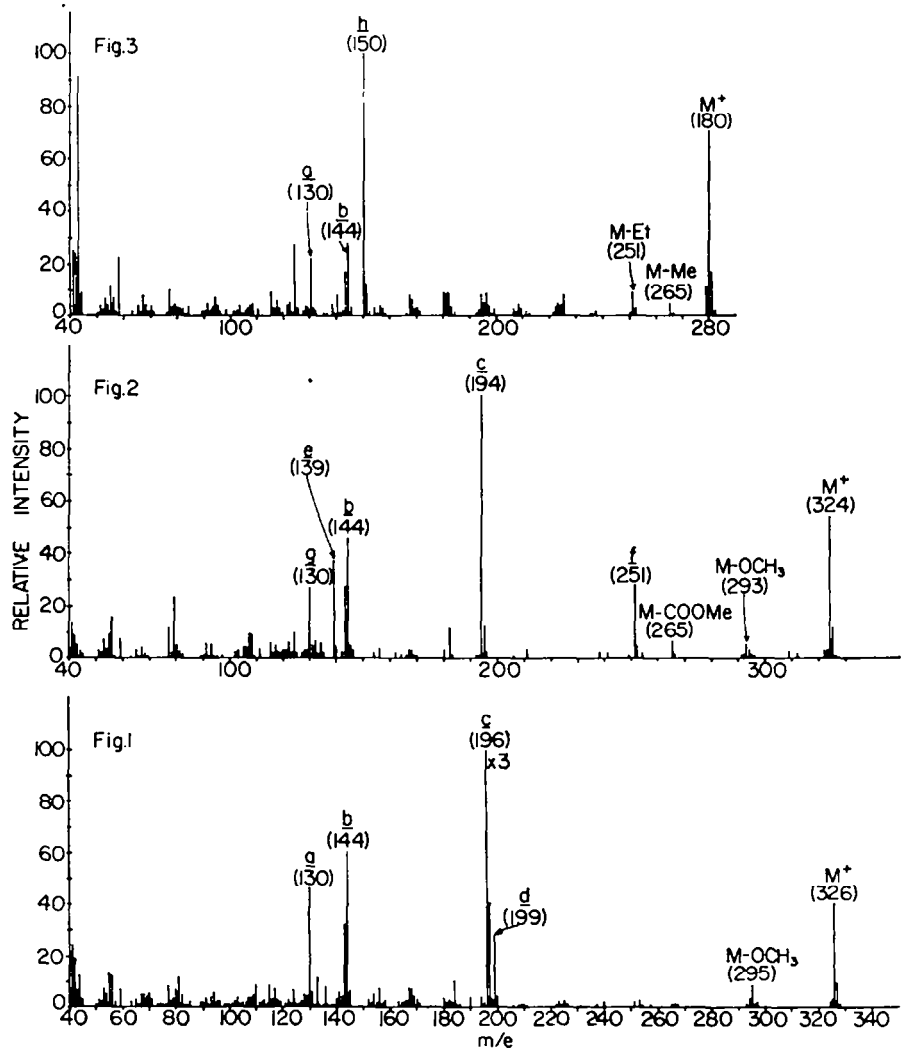


FIG. 1. Mass spectrum of tetrahydroakuammicine (IVB).
FIG. 2. Mass spectrum of 2,16-dihydroakuammicine (VIA).
FIG. 3. Mass spectrum of lithium aluminum hydride reduction product XIIA of 19,20-dihydroakuammicine (IIIA).

Fig. 2. On the other hand, rupture of the N_b–C-21 linkage, being both allylic and adjacent to nitrogen, is favored with the generation of an important peak *e*, which is not present in Fig. 1. It should be noted that in the case of fission of the carbon–carbon bond next to a nitrogen atom (marked α in VII) the driving force is formation

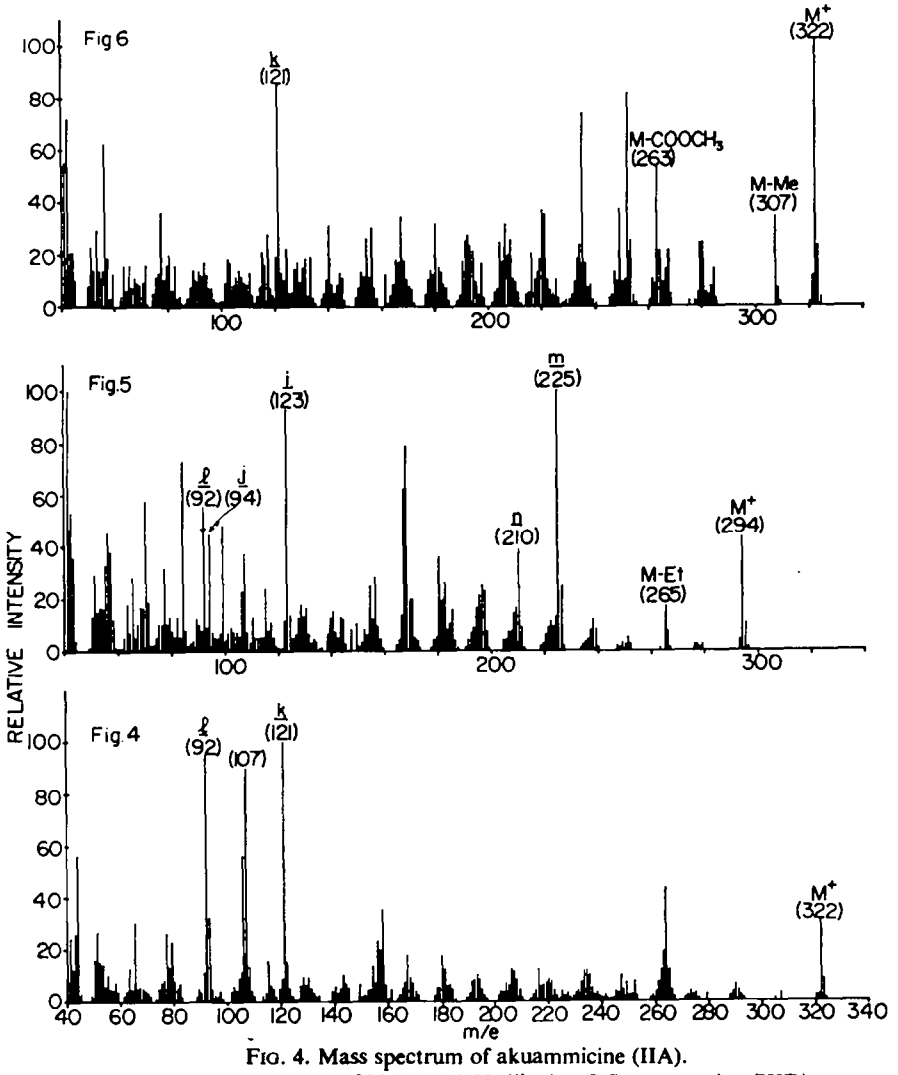


FIG. 4. Mass spectrum of akuammicine (IIA).
FIG. 5. Mass spectrum of N_b-nor-19,20-dihydro-C-fluorocurarine (IIID).
FIG. 6. Mass spectrum of condylocarpine (XVIII).

of an ion stabilized by participation of the non-bonding electrons of the nitrogen atom; therefore the ionized fragment (*c*, *d*) will contain this nitrogen atom. When fission of the nitrogen–carbon linkage occurs (marked β in VII), this type of stabilization is not possible. The carbonium ion *e* appears to be more stable than an ion involving divalent nitrogen, which would be produced by ionization of the other fragment of this cleavage (marked γ in VII).

TABLE 1

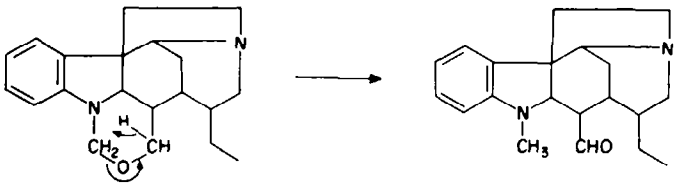
Principal Mass Spectral Peaks of Tetrahydroakuammicine (IVB) and Related Alkaloids

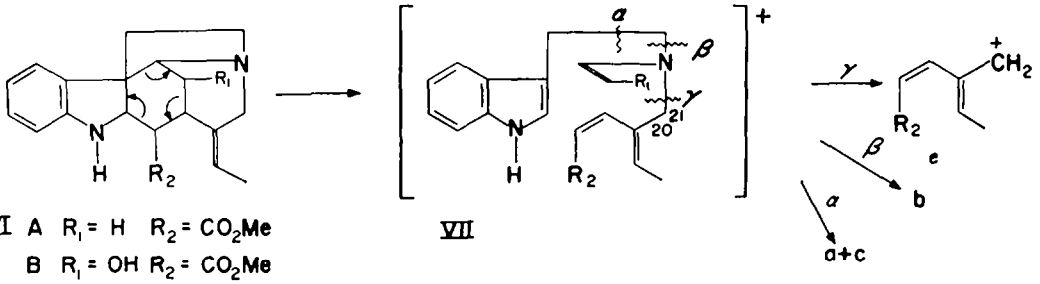
Substance	*Substituents in IV*					*Mass Spectral Peaks* (*m/e*)					
	R_1	R_2	R_3	R_4	R_5	M^+	*a*	*b*	*c*	*d*	other
IVA*	H	H	Me	Et	H	282	130	144	152	199	—
IVB	H	H	CO_2Me	Et	H	326	130	144	196	199	M—OMe
IVC[2]	H	H	H	Et	H	268	130	144	138	199	—
IVD	H	Ac	CO_2Me	Et	H	368	130	144	196	241	M—Ac
IVE†	H	Et	CH_2OH	Et	H	326	158	172	168	227	M—H_2O
IVF	H	Ac	CH_2OAc	Et	H	382	130	144	210	241	M—Ac
IVG	H	H	Me	Et	OH	298	130	144	168	215	—
IVH	H	H	CO_2Me	Et	OH	342	130	144	212	215	M—OCH_3
IVI	H	H	CO_2Me	CHOHMe	H	342	130	144	212	199	M—OCH_3
IVJ[9]	OMe	Ac	H	H	Et	340	160	174	138	?	?
IVK[2]	H	H	H	H	Et	268	130	144	138	227	—
IVL[10b]	H	H	CO_2Me	H	Et	326	130	144	196	227	M—OCH_3
IVM‡	H	CH_2—O—CH_2		Et	H	310	144	158	166	213	M—CH_2O
IVN[2]	OMe(C-11)	Ac	CH_2—O—$CHCH_3$		H	368	160	174	166	271	M—Ac
IVO[2]	OMe(C-11)	H	CH_2—O—$CHCH_3$		H	326	160	174	166	229	—

* The mass spectrum of its C-2 epimer is virtually identical.

† Sample kindly supplied by Prof. A. Bertho (see A. Bertho and M. Koll, *Ber.*, **94**, 2737 (1961)).

‡ The following rearrangement accompanies the initial (arrows in IV) fragmentation:





VI A $R_1 = H$ $R_2 = CO_2Me$
B $R_1 = OH$ $R_2 = CO_2Me$
C $R_1 = R_2 = H$

VII

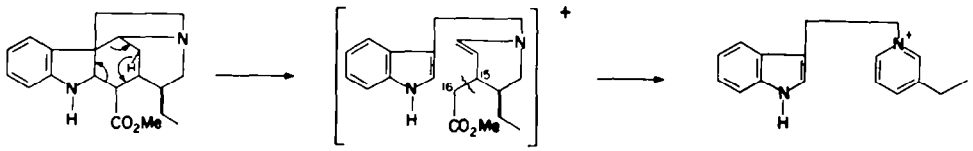
In addition to cleavages α, β and γ in VII giving rise to ions *a*, *b*, *c* and *e* (Fig. 2 and Table 2), there occurs a further fragmentation, involving the expulsion of carbon atom 16 together with its attached substituent and one additional hydrogen. This process is not observed in the tetrahydroakuammicine (IVB) series (Fig. 1 and Table 1)

TABLE 2. PRINCIPAL MASS SPECTRAL PEAKS OF 2,16-DIHYDROAKUAMMICINE (VIA) AND RELATED ALKALOIDS

Substance	Mass Spectral Peaks (*m/e*)						
	M^+	*a*	*b*	*c*	*e*	*f*	others
VIA	324	130	144	194	139	251	$M—OCH_3$; $M—CO_2CH_3$
VIB	340	130	144	210	139	267	$M—H_2O$; $M—OCH_3$; $M—(H_2O + OCH_3)$; $M—CO_2Me$
VIC[8]	266	130	144	136	?	251*	—

* Note that an M-15 peak is absent in the spectra of VIA and VIB.

and thus must be associated with the presence of the 19–20 double bond. We suggest a mechanism involving rupture of ring C with transfer of the C-14 hydrogen atom to C-16 (see arrows in VIII), followed by fission of the doubly allylically activated 15–16 bond (wavy line in IX) and rearrangement of the double bonds, the driving force being the resonance energy gained in the fully aromatic ion *f*. As noted in Table 2, no substituent at C-16 is required for the genesis of this fragment.



VIII IX f

The above mass spectral information combined with that derived from the tetrahydroakuammicine (IVB) spectrum (Fig. 1) permits a further refinement in locating a substituent in an unknown member of this class: ion *f* defines C-16, while a combination of fragments *d* and *e* settles the degree of substitution of C-21. This technique has been used to locate both the side chain and the hydroxyl group in mossambine (IIB)[6] and in echitamidine (IIIC),[7] N.M.R. spectrometry being used to arrive in favor of the finally adduced constitutional formulae. The reverse sequence resulted in the structure elucidation of condylocarpine (XVIII), N.M.R. evidence[10a] reducing the

structural possibilities to two, the final decision being made by analysis of the mass spectrum[10b] of its tetrahydro derivative.

If the ethylidene group is moved from the C-20 position, typical of akuammicine (IIA) and its congeners, to C-14, entry is made into the aspidospermatine group (X), the mass spectra of several representatives having been measured by Biemann and collaborators.[2,9] The principal mass spectral peaks of this group are collected in Table 3, which illustrates the marked differences in fragmentation incident to movement of the ethylidene function from C-20 to C-14. Except for the ubiquitous indole peaks *a* and *b*, the most characteristic fragment of this series is one at *m/e* 136 (*g*), while cleavage giving rise to fragments such as *d* is absent. This seems reasonable—the usual type of fragmentation (arrows in IV) being inhibited since this would

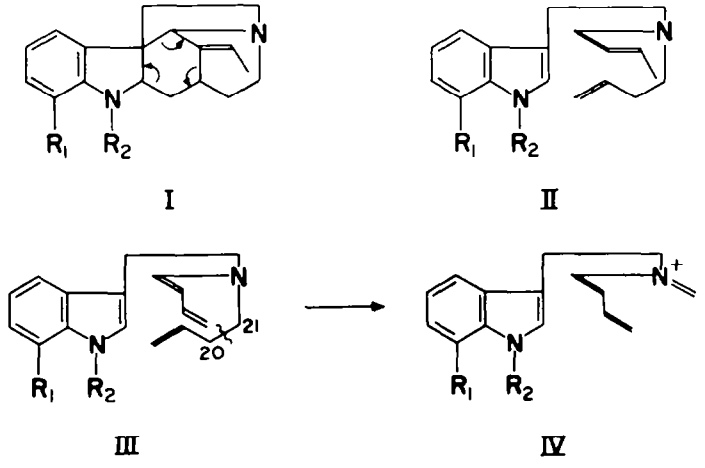
TABLE 3. PRINCIPAL MASS SPECTRAL PEAKS OF ASPIDOSPERMATINE (XE) AND RELATED ALKALOIDS

Substance			Mass Spectral Peaks (*m/e*)*				
	R_1	R_2	M^+	*a*	*b*	*g*	other
XA	H	Me	280	144	158	136	—
XB	H	H	266	130	144	136	—
XC	OMe	H	296	160	174	136	—
XD	H	Ac	308	130	144	136	M—Ac
XE	OMe	Ac	338	160	174	136	M—Ac

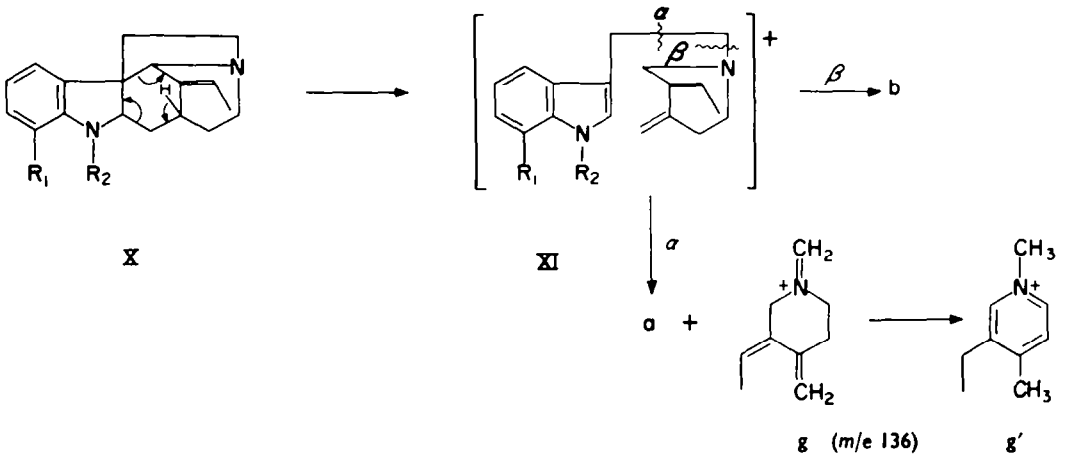
* Spectra reproduced on p. 327 of ref. 2.

produce an allene[15]—and we suggest the following reaction path. Fragmentation of ring C is assumed to proceed (arrows in X) with transfer of the C-15 hydrogen to C-3, followed by the usual cleavages indicated as α or β in the ion XI. Fission of the

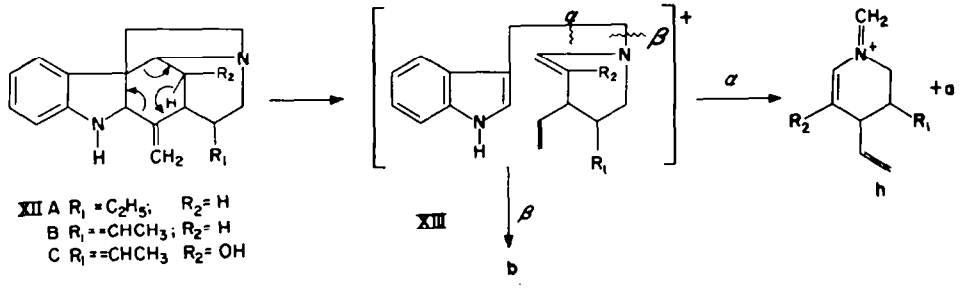
[15] Biemann (ref. 2, 9) assumes operation of the standard tetrahydroakuammicine fragmentation of ring C (arrows in (i)) together with a double bond migration, thus avoiding the intermediacy of allene (ii). The resulting diene (iii) is assumed to undergo rupture of the allylically activated 20–21 bond to give an ion (iv) analogous to *d*. In point of fact, the mass spectra reproduced (ref. 2) by Biemann show the absence of a peak corresponding to *d*, thus making the operation of process (i) very unlikely.



5–6 bond (α in XI) leads to fragment *a* and to a new ion, *g*(*m*/*e* 136 in Table 3), eventual rearrangement of double bonds yielding the aromatic ion *g'*.

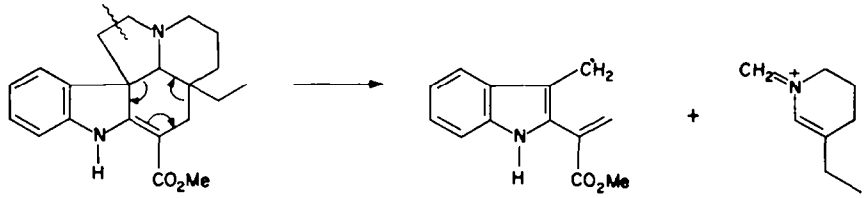


Further evidence that the standard ring C fragmentation (arrows in IV and VI) does not occur when ring C carries an exocyclic double bond, is shown in the mass spectrum (Fig. 3) of the lithium aluminum hydride reduction product[4c] XIIA of 19, 20-dihydroakuammicine. Aside from the usual indole ions *a* and *b*, peaks corresponding *in mass* to fragment *c* are present. These are most readily formulated as *h*(*m*/*e* 150 in XIIA (Fig. 3); *m*/*e* 148 in XIIB; *m*/*e* 164 in XIIC) and are visualized to arise through a hydrogen transfer mechanism (arrows in XII), followed by cleavage of the 5–6 bond in the intermediate ion XIII (wavy line marked α). Fragments, corresponding in mass to *d* are absent.



The parent alkaloid, akuammicine (IIA), possesses a 2–16 double bond, a feature which, as expected, drastically affects the fragmentation pattern as compared to the ring C—saturated derivatives discussed until now. This 2-methyleneindoline moiety (I) is also present in vincadifformine (XIV)[12] and related alkaloids,[1,13] but they undergo a simple retro Diels-Alder fragmentation (arrows in XIV), which is not possible in akuammicine because of the different mode of connection of rings C and D.

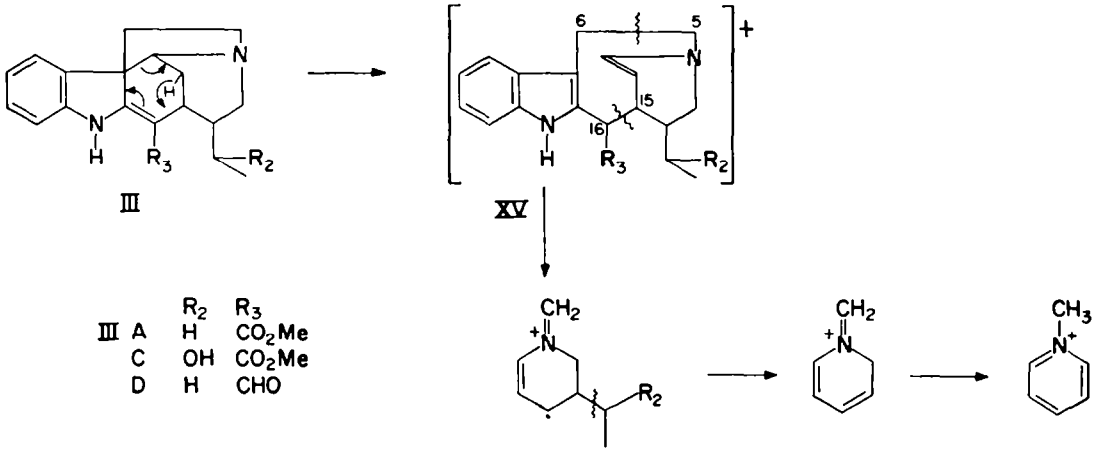
It seems to be generally true of electron impact phenomena, that where no particularly favored transition state can intervene or no bond is especially labile, several fragmentation paths are followed, the masses of the finally obtained peaks being



XIV

determined by the stability of the formed ions and/or neutral species. Another important factor may be the release of strain in highly fused systems. With these points in mind, one may visualize the principal fragments in the mass spectra of 2-methyleneindolines such as akuammicine (IIA) and 19,20-dihydroakuammicine (IIIA); where the substituent labels permit, fragmentation mechanisms will be suggested. A further difficulty in the interpretation of the mass spectra of this group of alkaloids is the fact that most of the naturally occurring ones contain hydroxy groups (e.g. IIB, IIIB, IIIC), which may suffer dehydration prior to the main fragmentation, thus yielding new species that may decompose differently.

The three most intense peaks in the mass spectrum (Fig. 4) of akuammicine (IIA) occur at *m/e* 92, 107 and 121. Exchange of the N_a hydrogen atom with deuterium increases the molecular ion peak by one mass unit, but does not alter the position of the *m/e* 92, 107 and 121 peaks, thus demonstrating that they cannot contain the indole moiety. In 19,20-dihydroakuammicine (IIIA) and the geissoschizoline derivative IIID (N_b-nor-19,20-dihydro-C-fluorocurarine),[16] this group of peaks is found at *m/e* 92, 94, 107 and 123, while in echitamidine (IIIC) or its 0-acetate, these peaks are found at *m/e* 92, 94, 107 and 121. The *m/e* 121 and 123 ions therefore must represent ring D with its C-20 side chain and their genesis may be visualized as follows:



III

	R_2	R_3
III A	H	CO_2Me
C	OH	CO_2Me
D	H	CHO

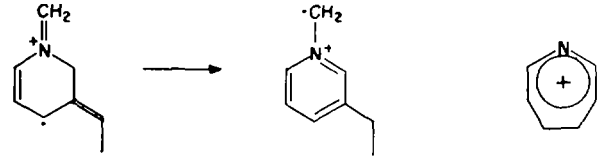
XV

j

Opening of ring C is assumed to be promoted by the transfer of hydrogen from C-14 to C-16 (arrows in III), while cleavage of the 5–6 and 15–16 bonds in the resulting

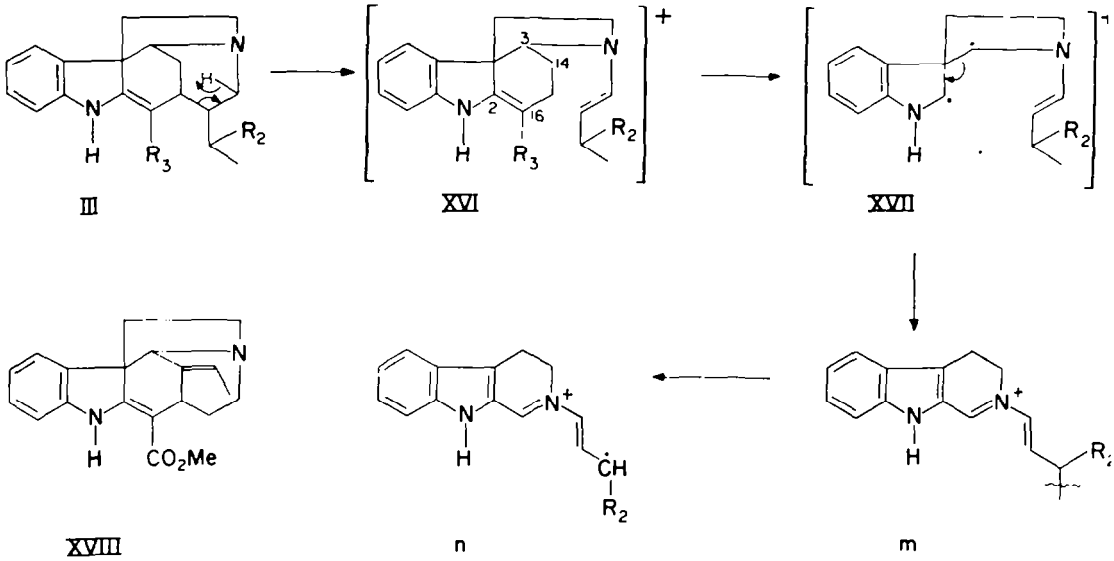
[16] F. Puisieux, Thèse Doc. Sc., Paris (1960).

species XV is to be expected, since these represent obviously the most favored sites of fission. The resulting ion *i* (*m/e* 123 in the spectra of IIIA and IIID (see Fig. 5)) upon further bond fission (wavy line in *i*) would provide, after double bond rearrangement, the stable pyridinium ion *i* (*m/e* 94). In echitamidine (IIIC) if species *i* or one of its precursors suffers dehydration, ion *k* (*m/e* 121) would be formed, which is produced directly in akuammicine (IIA) with its intact ethylidene grouping. This explains, of course, why the *m/e* 94 peak (*j*) is absent in the akuammicine spectrum (Fig. 4). Ion *k* may rearrange to the aromatic structure *k'* and a possible representation for the *m/e* 92 fragment in all of these alkaloids (II and III) may be the azatropylium ion *l*, the *m/e* 107 peak being due to the N-methylazacycloheptatriene cation.



K K' l

The mass spectra of 19,20-dihydroakuammicine (IIIA) or of the geissoschizoline derivative IIID contain a very abundant fragment of *m/e* 225 (Fig. 5), which is shifted to *m/e* 241 in echitamidine (IIIC) and to *m/e* 283 in the latter's 0-acetate. This *m/e* 225 ion must contain, therefore, the C-20 ethyl side chain, but not the C-16 substituent and probably arises from the loss of carbon atoms 14, 15 and 16 with transfer of one hydrogen. No simple path can be given to produce this change, but as a working hypothesis we propose initial cleavage of the 15–20 bond with transfer of the C-21 hydrogen atom (arrows in III)[17] followed by further double bond and hydrogen rearrangement in the ion XVI and eventual fission of the 3–14 and 2–16 linkages. A 1–2 shift of the 3–7 bond (arrow in XVII) would provide the stabilized ion *m* (*m/e*



III XVI XVII

XVIII n m

[17] Hydrogen transfer, rather than simple fission of the 15–20 bond, is suggested in order to generate the 20–21 double bond in *m*, which in turn would rationalize the further loss of methyl to *n*.

225 in Fig. 5), while the *m/e* 210 peak (Fig. 5) would arise from allylically activated loss of the methyl group (wavy line in *m*) to lead to the ion *n* (*m/e* 210). The absence of intense peaks in this mass range in the akuammicine spectrum (Fig. 4) is consistent with the above mechanism, since the presence of the 19–20 double bond would already tend to make the first step a rather unfavorable one.

Condylocarpine (XVIII)[10] exhibits a mass spectrum (Fig. 6), which bears only little resemblance to that (Fig. 4) of akuammicine (IIA); most notably, there are absent the latter's intense *m/e* 92 and 107 peaks. It has already been noted above that the presence of an ethylidene function may affect the fragmentation of ring C and the absence of "labelled" condylocarpine analogs makes it difficult at this stage to propose plausible fragmentation paths. The mass spectrum of the alkaloid, however, does serve as a valuable fingerprint in distinguishing it from its isomer akuammicine (IIA).

In conclusion, it can be stated that the presently accumulated information on the mass spectral fragmentation behavior of such 2-methyleneindoline (chromophore I) alkaloids can be of considerable help in structure work, especially if the corresponding 2,16-dihydro (VI) or 2,16; 19,20-tetrahydro (IV) analogs are also available. As indicated above, the mass spectra of such derivatives can often lead to the exact location of certain substituents and when combined with N.M.R. analysis and the more conventional U.V. and I.R. spectral measurements, these physical tools can lead with a minimum of chemical manipulation to precise structural definitions.

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