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MASS SPECTROMETRY IN STRUCTURAL AND STEREOCHEMICAL PROBLEMS[1]

UNSATURATED PENTACYCLIC TRITERPENOIDS[2]

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RECENT studies have demonstrated that mass spectrometry may be a powerful adjunct to the structure elucidation of polycyclic natural products such as alkaloids[3,4] and steroids.[5] These developments have been made possible by improved inlet systems, which permitted the vaporization in an undecomposed state of relatively non-volatile substances. We should now like to report some of our results with pentacyclic triterpenes[6] (usual melting point range 200-300°), which show that mass spectrometry (250° heated all-glass inlet system) of such high melting natural products with a variety of substituents is feasible and that the recognition of the principal mass spectral peaks can offer some extremely valuable structural information.

The most common naturally occurring pentacyclic triterpenes are members of the α- (A, $R_6 = CH_3$; $R_7 = H$) or β- (A, $R_6 = H$; $R_7 = CH_3$) amyrin

[1] Part VIII. For paper VII see C. Djerassi, H. Budzikiewicz, J.M. Wilson, J. Gosset, J. Le Men and M.-M. Janot, Tetrahedron Letters 235 (1962).

[2] Supported by grants No. A-4257 and RG-6840 from the National Institutes of Health, U.S. Public Health Service.

[3] See K. Biemann, Angew. Chem. 74, 102 (1962).

[4] See papers II [B. Gilbert, J.M. Ferreira, R.J. Owellen, C.E. Swanholm, H. Budzikiewicz, L.J. Durham and C. Djerassi, Tetrahedron Letters 59 (1962)] to VII (ref. 1) from this laboratory.

[5] See a H. Budzikiewicz and C. Djerassi, J. Amer. Chem. Soc. 84, April (1962); b R. Ryhage and E. Stenhagen, J. Lipid Res. 1, 361 (1960).

[6] The mass spectrum of only one such substance, the hydrocarbon α-amyrene, seems to have been measured [P. de Mayo and R.I. Reed, Chem. & Ind. 1481 (1956); R.I. Reed, J. Chem. Soc. 3432 (1958)].

series, all of them being characterized by the presence of a 12-13 double bond. This feature has proved to be readily recognizable by mass spectrometry, since the molecular ion undergoes a type of reverse Diels-Alder fragmentation (see arrows in A)[7] to furnish a very characteristic peak due to an ion of type B. As illustrated in Fig. 1 for methyl ursolate (VIII), this peak (m/e 262) is one of the most intense ones in the spectrum and is followed by a second important peak (m/e 203 in Fig. 1) corresponding to the loss of the C-17 angular substituent. That ion B^+ is actually the precursor of this second peak could be established definitely by the appearance of a metastable peak at m/e 158.5. In Table 1 are collected a few selected triterpenes of the α- (VIII-XI) and β- (I-VII) amyrin series, which illustrate the range of different substituents which have been covered. The mass spectra of all of these substrates showed a strong peak corresponding in mass (see Table 1) to fragment B as well as a second one in which the C-17 substituent has been eliminated. This second peak (B-R_5 in Table 1) is not very intense (ca. 20% of B^+ peak) when R_5 is methyl, but becomes very strong (see Fig. 1) when the angular R_5 substituent is methoxycarbonyl (III, IV, VI, VIII) or CH_2OAc. An additional feature, peculiar to certain substituents, is the rather small loss of acetic acid (M-60) from the molecular ion[8] in triterpenoid 3β-acetates (in contrast to the situation obtaining among steroidal 3β-acetates), while a very substantial M-60 peak (as well as B^+-60) is associated with the loss of acetic acid from a 28-hydroxy acetate (e.g. II). The diagnostic utility of this observation is strengthened by the absence of such a strong peak in the isomeric

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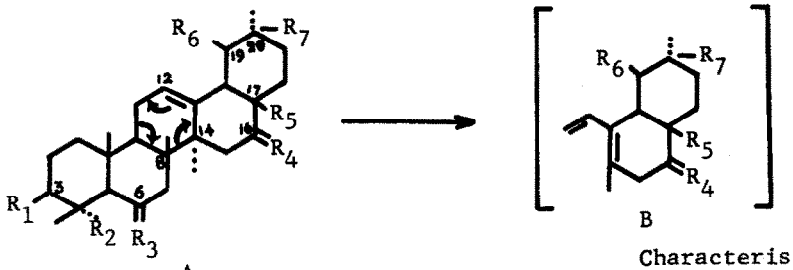
[7] Biemann (ref. 3) has already called attention to this type of bond cleavage in the monocyclic olefin α-ionone.

[8] Molecular ion peaks could be observed for all of the triterpenes mentioned in this paper except for methyl siaresinolate 3-acetate (VI) where dehydration of the C-19 hydroxyl group was noted (highest peak corresponding to M-18).

TABLE 1



A B

Characteristic mass spec. peaks (m/e)

	R_1	R_2	R_3	R_4	R_5	R_6	R_7	B^+	$(B-R_5)^+$
I	H	CH_3	H_2	H_2	CH_3	H	CH_3	218	203
II	AcO	CH_3	H_2	H_2	CH_2OAc	H	CH_3	276[a]	203
III	AcO	CH_3	O	H_2	CO_2CH_3	H	CH_3	262	203
IV	O	CH_3	H_2	O	CO_2CH_3	H	CH_3	276	217[b]
V	AcO	CH_3	H_2	O	H	H	CH_3	218	-
VI	AcO	CH_3	H_2	H_2	CO_2CH_3	α-OH	CH_3	278	[c]
VII	AcO	CH_3	H_2	H_2	CH_3	H	CH_2OAc	276	203($B-R_7$)
VIII	HO	CH_3	H_2	H_2	CO_2CH_3	CH_3	H	262	203(Fig.1)
IX	AcO	CH_3	H_2	H_2	CH_3	CH_3	H	218	203
X	AcO	CH_3	H_2	O	CH_3	CH_3	H	232	-
XI	O	CO_2CH_3	H_2	H_2	CH_3	CH_3	H	218	203

[a] Also m/e 216 (276 - HOAc).

[b] An even stronger m/e 216 peak may be associated with further loss of one proton and generation of a phenolic D ring.

[c] The loss of water by dehydration of the 19-hydroxyl group intervenes, thus giving rise to peaks at m/e 260 (278 - H_2O) and 201 (260 - R_5).

diacetate VII.

We feel justified in stating, therefore, that a mass spectrum on less than 1 mg of a triterpenoid can determine whether or not the substance is based on the usual α- or β-amyrin skeleton and which of its substituents are located in rings D and E. In a number of instances, the location of the substituent can be defined more precisely by observing the ease of elimination of the angular group attached to C-17 and/or the operation of hydrogen transfer reactions.[9] Thus, 15-oxoerythrodiol diacetate (XII) does not show the m/e 290 peak, expected on the basis of the fragmentation A → B, but rather exhibits its most intense peak (above mass 50) at m/e 291 (ion $\underline{C}^+$) due to the type of hydrogen transfer indicated by the arrows in XII as had already been noted earlier[5a] with 15-keto steroids. The presence of the C-28 acetate grouping is indicated by the ready loss of acetic acid as revealed in substantial M-60 (m/e 480) and $\underline{C}^+$-60 peaks (m/e 231).

Our earlier[5a] steroid studies indicated that among 11-keto steroids, rupture of the 9-10 bond is favored due to a cyclic transition state involving transfer of the C-1 hydrogen atom. A completely analogous situation has now been observed with 11-keto triterpenoids, the most intense peak in the mass spectra of 18α-11-oxo-β-amyrin acetate (XIV) and methyl 18-dehydroglycyrrhetate acetate (XV) occurring at m/e 273 (ion D, R = CH_3) and m/e 315 (ion D, R = CO_2CH_3 with 18-19 double bond), which is readily rationalized by the hydrogen transfer indicated by the arrows in XIV and rupture at the allylically activated C-8 center.

The presence of additional double bonds in rings D or E of Δ^{12}-unsaturated triterpenoids does not seem to affect the principal fragmentation process (A → B). Thus the mass spectra of 15- and 21-dehydro methyl oleanolate 3-acetate (A, R_1 = OAc; R_2 = R_7 = CH_3; R_3 = R_4 = R_6 = H;

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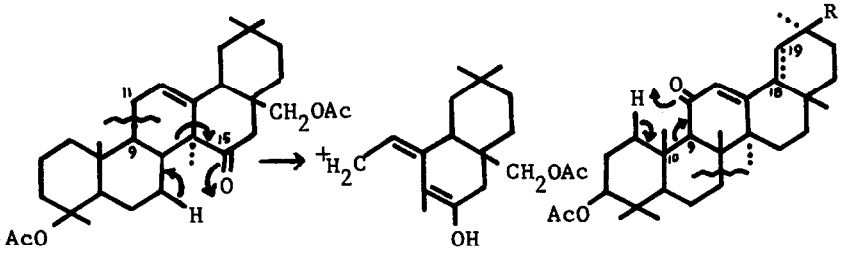
[9] F.W. McLafferty, Analyt. Chem. 31, 82 (1959).

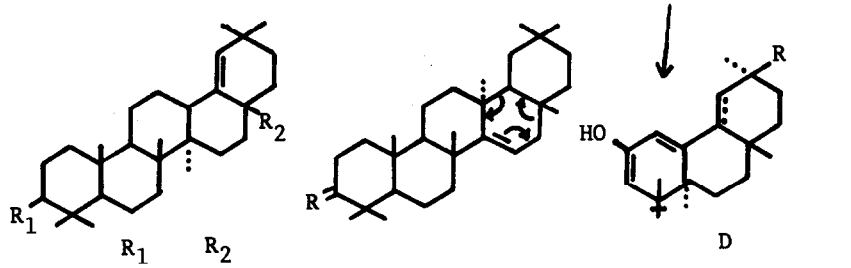
$R_5 = CO_2CH_3$ with double bonds at 15-16 or 21-22) closely resembled that of methyl ursolate (VIII) (Fig. 1) in the region above m/e 190, except that the latter's two most characteristic peaks at m/e 262 ($\underline{B}^+$) and 203 ($\underline{B}^+-R_5$) now occurred at m/e 260 and 201.

If instead of adding a further double bond, the single center of unsaturation is located elsewhere, then the characteristic fragmentation



XII C XIV R = CH_3

XV R = CO_2CH_3 (with 18,19-double bond)



D

	R_1	R_2
XVI	H	CH_3
XVII	AcO	CH_3
XVIII	HO	CO_2CH_3
XIX	O	CO_2CH_3

XX R = OH / H

XXI R = O

pattern of the Δ^{12}-unsaturated triterpenoids is altered drastically, thus making mass spectrometry a very sensitive tool for locating double bonds in such systems. Three illustrations can be offered in support of this statement.

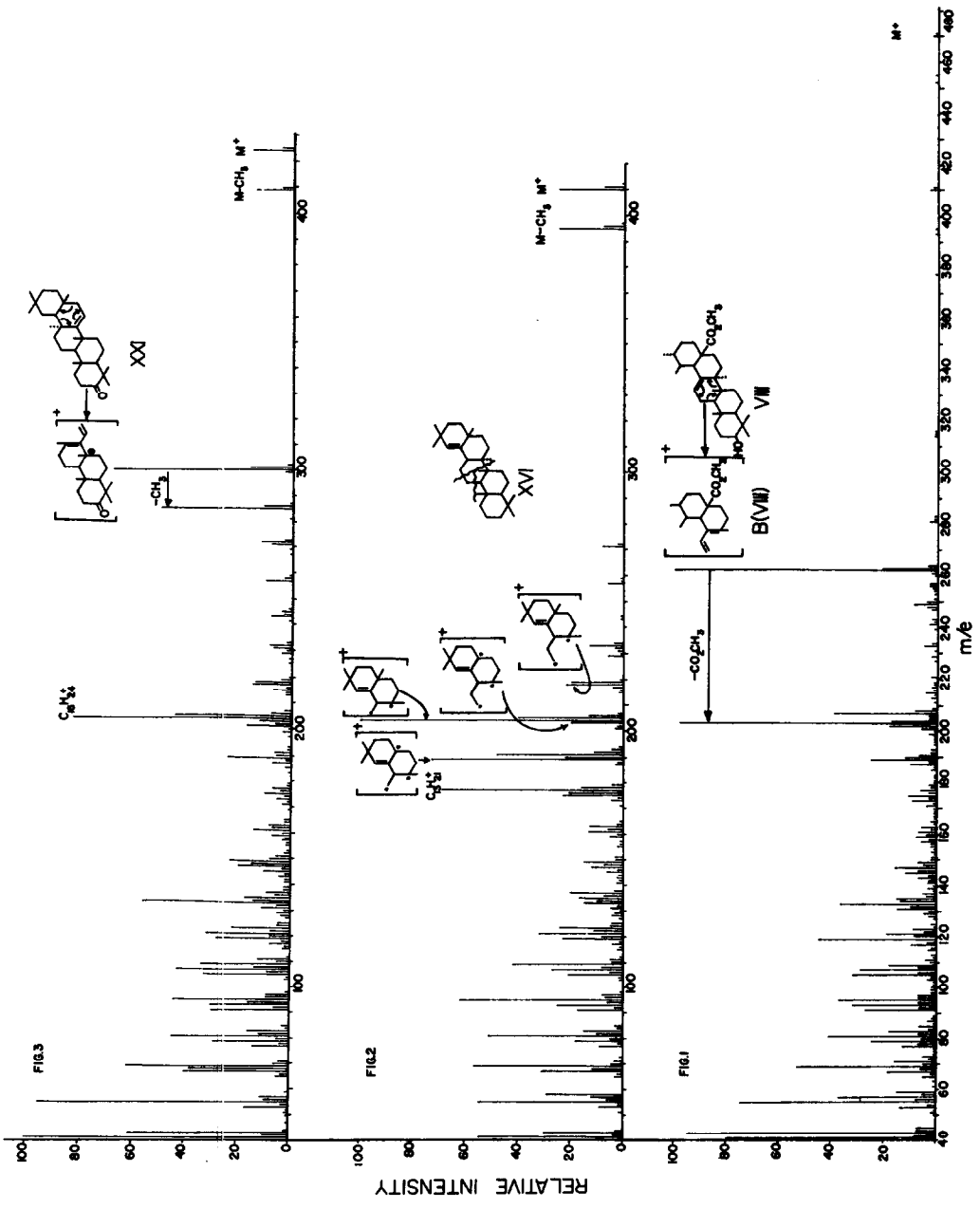


FIG.3
FIG.2
FIG.1
RELATIVE INTENSITY
m/e
M+
M-CH3 M+
-CH3
-CO2CH3
XXI
XVI
VIII
B(VIII)

While Δ^{12}-oleanene (IA) exhibits its strongest mass spectral peak at m/e 218 (IB in Table 1), $\Delta^{13(18)}$-oleanenes (e.g. 3-oxo or 3β,24-diacetoxy) do not show any strong peaks between the molecular ion and m/e 205, which appears to be due to cleavage of the 11-12 and 8-14 bonds coupled with rearrangement of one hydrogen atom.

The second group deals with Δ^{18}-oleanenes; as four representatives were available differing in substitution at C-3 and/or C-17 (XVI-XIX), several assignments could be made by observing the coincidence or shift of a given peak. The mass spectrum of the parent hydrocarbon, Δ^{18}-oleanene (XVI) is reproduced in Fig. 2 and it will be noted immediately that no major cleavage occurs in the unsaturated terminal ring. Since the spectrum of germanicol acetate (XVII) exhibits the same three major peaks at m/e 177, 189 and 204 (as well as the accompanying ones at m/e 203 and 218) with about the same intensity, they cannot contain ring A. The spectra of methyl morolate (XVIII) and methyl moronate (XIX) retain only one of these three principal peaks, namely the one at m/e 189 (accompanied by the m/e 203 peak), thus showing that it cannot include the C-17 substituent. We have been unable to give reasonable formulations to the m/e 177 peak ($C_{13}H_{21}^{+}$), while the remaining assignments are based on the appropriate shift (of 44 mass units) in the spectra of XVIII and XIX. The ready loss of the C-17 substituent - as would be expected from the activating influence of the double bond - represents one of the characteristic features of the mass spectra of this group of substances. For the sake of clarity, radical ions are used in Fig. 2 to indicate the principal bond cleavages, but it is quite likely that the corresponding cyclopropyl and cyclobutyl bonds should actually be completed or that unsaturated conjugated systems are formed by hydrogen shifts.

The last class of mono-unsaturated triterpenoids to be considered are

taraxernes, taraxerol (XX) and taraxerone (XXI) actually having been measured. The mass spectrum of the latter is reproduced in Fig. 3 and of the three most important peaks at m/e 300, 285 and 204, only the latter is also found in the spectrum of taraxerol (XX), thus demonstrating that it cannot incorporate ring A. A possible formulation for the m/e 204 peak ($C_{15}H_{24}^{+}$) would be rings D and E together with C-12 arising by rupture of the 11-12 and 8-14 bonds. A retro-Diels-Alder fragmentation (see arrows in XX and XXI) - similar to the process A — B among the Δ^{12}-oleanenes - immediately accounts for the m/e 300 peak, while the m/e 285 peak involves the further loss of methyl, most likely by cleavage at the allylically activated C-8 center. That the m/e 285 and 300 peaks in the taraxerone (XXI) spectrum (Fig. 3) do indeed represent rings A, B and C (for assignment see Fig. 3) is confirmed by the observation that peaks of similar intensity appear at m/e 302 and 287 in the taraxerol (XX) spectrum due to the two-mass unit difference in their molecular weights.

Other types of triterpenoids, notably those without nuclear unsaturation, are currently being examined in our laboratory. However, already the present preliminary results show that mass spectrometry can play an important role in structural studies of polycyclic triterpenoids and when this approach is combined with the earlier derived conclusions from optical rotatory dispersion measurements[10] among this class of natural products, then it can be seen that a very substantial amount of information can be obtained on less than 2 mg. of material by means of these two physical methods.

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[10] C. Djerassi, J. Osiecki and W. Closson, J. Amer. Chem. Soc. 81, 4587 (1959); C. Djerassi, Optical Rotatory Dispersion Chap. 6. McGraw-Hill, New York (1960).