room temperature for 1.5 hr. Dry ether (300 ml.) was added to the clear solution and the solid hydrobromide was collected and washed with dry ether. The dry hydrobromide was dissolved in dimethylformamide (20 ml.), neutralized with triethylamine, and coupled with *p*-nitrophenyl N-carbobenzoxy-S-benzyl-D-cysteinate (0.6 g.). The mixture was stirred for 3 days. Methanol (300 ml.) was added, the solid collected, washed with methanol (150 ml.) and ethyl acetate (150 ml.), and dried in a vacuum desiccator over P_2O_5. Further purification was effected by solution in dimethylformamide, filtration, and reprecipitation with ethyl acetate; wt. 0.5 g., m.p. 237–238° dec., sintering at 230°, $[\alpha]^{20}D$ −32° (*c* 1, dimethylformamide). For analysis the compound was dried *in vacuo* at 100° for 20 hr.

Anal. Calcd. for $C_{65}H_{86}O_{14}N_{12}S_2$: C, 59.0; H, 6.55; N, 12.7. Found: C, 58.8; H, 6.55; N, 12.5.

1-Hemi-D-cystine-oxytocin Isolated by Countercurrent Distribution after Admixture with Tritium-Labeled Oxytocin.—The dry protected nonapeptide intermediate (156 mg.) was dissolved in liquid ammonia (300 ml.), freshly distilled from sodium, and treated with sodium after removal of the acetone–Dry Ice bath until a persistent blue color appeared in the solution. After 5 min. glacial acetic acid (0.5 ml.) was added and the volume of ammonia was reduced to between 15 and 20 ml. in a stream of dry, oxygen-free nitrogen, the remaining ammonia being removed by lyophilization. The residue was taken up in distilled water (250 ml.). This solution, containing 800 units of avian depressor activity, was aerated at pH 6.8 for 5 hr. The nitroprusside test for thiols showed that the hemi-D-cystine-oxytoceine was incompletely oxidized and this process was completed by adding potassium ferricyanide solution until a yellow color appeared in the solution (10.3 ml. of 0.011 *N* $K_3Fe(CN)_6$). The ferrocyanide and ferricyanide ions were removed on a column (0.9 cm. × 15 cm.) of ion-exchange resin AG 3X4 in chloride form as previously described. The eluates and washings from this column were combined to give a solution containing 810 units of avian depressor activity. This solution was evaporated *in vacuo* below room temperature to a volume of 10 ml. and mixed with 10 ml. of a solution containing radioactive oxytocin (370 units of avian depressor activity, with a specific activity of 96.5 × 10^3 counts per minute per unit).[5,17] The combined solution containing 1180 units of avian depressor activity and a specific activity of 30.6 × 10^3 counts per minute per unit was placed in the first 10 tubes of a 6-ml. 400-tube Craig countercurrent machine and subjected to a total of 1,200 transfers in the solvent system butanol–propanol–0.05% acetic acid (3:2:5) at 4°. After 800 transfers a separation into three peaks with *K* values of 0.22, 0.35, and 0.52 had been accomplished, as detected by the Folin–Lowry color reaction.[18] The oxytocin formed a distinct peak with a *K* value of 0.48 as detected by determination of radioactivity. Apart from a trace of radioactive material traveling with a *K* value of 0.21, the peak due to oxytocin accounted for almost all the radioactivity and biological activity. After 1,200 transfers (with recycling) a small shoulder could be detected with the Folin–Lowry reaction on the following edge of the fast-moving peak corresponding in position to the peak in the distribution of radioactivity and of biological activity (*K* = 0.46) presumably due to oxytocin. A fraction (tubes 360–400) was taken from this area to include as much as possible of the radioactive and biologically active material. Similarly, the contents of tubes 0–40 (after recycling) containing the hemi-D-cystine-oxytocin (K = 0.50) were collected and combined. These two fractions were evaporated *in vacuo* and lyophilized to give 9.75 and 16.3 mg. of solid material, respectively. On assay the oxytocin-containing fraction showed 60 units of avian depressor activity per mg. The specific activity on a unit basis was 32.2 × 10^3 counts per minute per unit compared with 30.6 × 10^3 counts per minute per unit initially.

An amino acid analysis of a sample of the hemi-D-cystine-oxytocin after acid hydrolysis was performed on a Beckman–Spinco amino acid analyzer according to the procedure of Spackman, Stein, and Moore[14] using the 30–50° system. The following amino acid molar ratios (isoleucine taken as 1) were obtained: aspartic acid 1.0, glutamic acid 1.0, proline 1.0, glycine 1.0, half-cystine 0.8, half-mesocystine 0.7, leucine 1.0, isoleucine 1.0, tyrosine 0.6, and ammonia 3.0.

The molecular weight of the hemi-D-cystine-oxytocin was kindly determined by Dr. David Yphantis of the Rockefeller Institute, by use of short column equilibrium centrifugation. At concentrations ranging from 0.25 to 1% in 0.15 *M* ammonium acetate at pH 5.57 the average molecular weight was 1070 ± 70, assuming a partial specific volume of 0.71.

The hemi-D-cystine-oxytocin on bioassay for avian depressor activity according to the method of Munsick, Sawyer, and van Dyke[19] showed 2.0 units of avian depressor activity per mg. (average of three four-point assays). The material also showed a very low level of radioactivity; the specific activity was 4.21 × 10^3 counts per minute per unit of avian depressor activity.

Acknowledgments.—The authors wish to thank Mr. Joseph Albert for the microanalyses, Mrs. Lorraine Abrash for the amino acid analyses, and Miss Maureen O'Connell, Miss Lenore McAteer, and Miss Shirley R. Pomeroy for the bioassays.

(17) Liquid scintillation counting was carried out in a Tracerlab scintillation counter using the dioxane–xylene phosphor of Jacobsen, Gupta, and Fernandez (*Arch. Biochem. Biophys.*, **86**, 89 (1960)); 10 ml. of phosphor solution was added to 5 ml. of dioxane solution containing the active sample (usually 100 μl. of an aqueous solution). The efficiency was between 12 and 14% and was controlled with tritiated toluene (New England Nuclear Corp.) as an internal standard.

(18) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

(19) R. A. Munsick, W. H. Sawyer, and H. B. van Dyke, *Endocrinol.*, **66**, 860 (1960).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STANFORD UNIVERSITY, STANFORD, CALIF.]

Mass Spectrometry in Structural and Stereochemical Problems. XXXII.[1] Pentacyclic Triterpenes

BY H. BUDZIKIEWICZ, J. M. WILSON, AND CARL DJERASSI

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Mass spectra were measured of saturated and unsaturated members of the α- and β-amyrin group as well as of representatives of the taraxerol, bauerene, friedelane, and lupane series. Assignments have been made to the principal fragments and, in most instances, plausible mechanisms are proposed to rationalize the formation of these ions. In general, the presence of a nuclear double bond controls the fragmentation behavior, and characteristic mass spectral features have been noted which frequently allow assignment of membership of a given triterpene in one of the major classes by this criterion. In addition, the location of functional groups can often be narrowed down by consideration of the fragmentation pattern. Mass spectrometry thus constitutes an extremely useful physical method in the triterpene field and, when combined with rotatory dispersion measurements of derived ketones, can lead to structure elucidation with a minimum quantity of material.

Mass spectrometry has been used during the last few years to an increasing extent for the structure elucidation of complex polycyclic natural products, especially alkaloids[2,3] and steroids.[4] Very little work has been done so far in the pentacyclic triterpene field[5a] because of the low volatility of these compounds which requires

(1) For paper XXXI see M. Ohashi, J. M. Wilson, H. Budzikiewicz, M. Shamma, W. A. Slusarchyk, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 2807 (1963).

(2) (a) K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., Chapter 8; (b) C. Djerassi, *Pure Appl. Chem.*, **6**, 575 (1963).

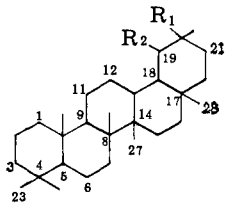
(3) C. Djerassi, H. Budzikiewicz, R. J. Owellen, J. M. Wilson, W. G. Kump, D. J. Le Count, A. R. Battersby, and H. Schmid, *Helv. Chim. Acta*, **46**, 742 (1963), and previous papers cited therein.

(4) D. H. Williams, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 2091 (1963), and previous papers cited therein.

(5) (a) P. de Mayo and R. I. Reed, *Chem. Ind.* (London), 1481 (1956); R. I. Reed, *J. Chem. Soc.*, 3432 (1958). (b) Very recently, J. L. Courtney, and J. S. Shannon, *Tetrahedron Letters*, 13, 173 (1963), have reported a detailed mass spectrometric study of friedelane derivatives. (c) NOTE

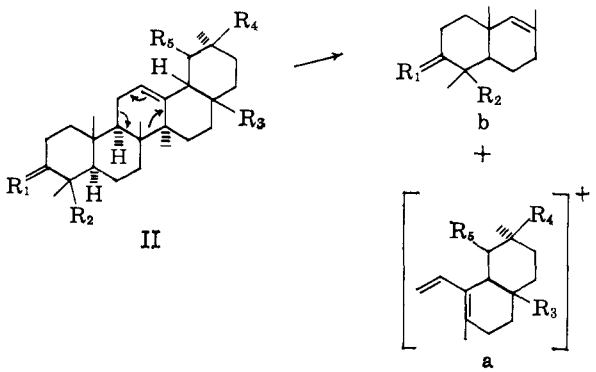
the use of a heated all-glass inlet system or in some cases[5b,6] even a direct inlet system. In a preliminary communication[7] we have reported on the fragmentation behavior of certain unsaturated triterpenes of the α- and β-amyrin group. The aim of the present paper is to give a more detailed discussion of the mass spectra of saturated as well as unsaturated pentacyclic triterpenes and to point out the possibilities and limitations of this method as a diagnostic tool for unknown members of this class of natural products.

(1) Oleanane, Ursane, and Related Triterpenes.—These two series possess the carbon skeleton I and differ only in the position of one methyl group (Ia and Ib). The mass spectra of analogous derivatives of Ia and Ib are very similar, differing in most cases only slightly in the relative intensity of some fragments. Therefore, the behavior upon electron impact of these two classes will be discussed together.



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

(a) Δ^{12}-Unsaturated Oleanenes and Ursenes.—The most characteristic fragmentation of all compounds of this class can be described best by a retro-Diels–Alder reaction[8] in ring C as indicated by the arrows in II, the charge remaining with the diene portion (species a).



II

	R_1	R_2	R_3	R_4	R_5	
IIa,	H_2	Me	Me	Me	H	
b,	O	CO_2Me	Me	H	Me	
c,	O	Me	CO_2Me	Me	H	
d,	(H)OH	Me	Me	CO_2Me	H	
e,	(H)OAc	Me	CH_2OAc	Me	H	
f,	(H)OAc	Me	Me	CH_2OAc	H	
g,	O	Me	CO_2 to C-21	Me	H	
h,	(H)OAc	Me	CO_2Me	Me	H	C-21 OAc

Charge retention with the other fragment (b) can be observed to some extent, but is usually of minor importance. The correctness of this assignment is shown by the fact that substitution in ring A and B does not change the mass of fragment a, while alterations in ring D and E result in the appropriate mass shifts. Thus the unsubstituted parent hydrocarbon β-amyrene (IIa) yields fragment a of *m/e* 218. Methyl β-boswellonate (IIb, Fig. 2) gives fragment a with the same mass (*m/e* 218) as IIa while the isomeric ester methyl oleanonate (IIc) which carries the carbomethoxy group at C-17, exhibits (Fig. 1) species a at *m/e* 262, as does methyl 11-deoxyglycyrrhetate (IId). In the case of erythrodiol diacetate (IIe, Fig. 3) and the isomeric diacetate IIf (Fig. 4), derived from 11-deoxyglycyrrhetic acid, fragment a appears, as expected, at *m/e* 276.

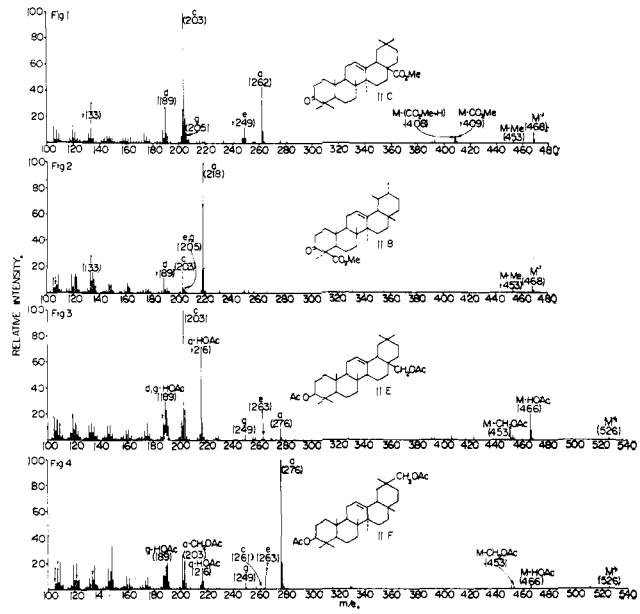


Fig. 1.—Mass spectrum of methyl oleanonate (IIc).

Fig. 2.—Mass spectrum of methyl β-boswellonate (IIb).

Fig. 3.—Mass spectrum of erythrodiol diacetate (IIe).

Fig. 4.—Mass spectrum of 30-hydroxy-β-amyrin diacetate (IIf).

This typical retro-Diels–Alder fragmentation leading to species a can thus be employed as a characteristic diagnostic tool for the presence of a 12–13 double bond in triterpenes of the α- and β-amyrin class. A good illustration of the utility of this mass spectrometric criterion is afforded by the *Stryphnodendron* sapogenins,[9] where the initial chemical evidence tended to exclude the presence of a Δ^{12}-double bond, the presence of which was first indicated by consideration of the mass spectral fragmentation behavior.

Ion a is subject to further fragmentation. Thus, in methyl oleanoate (IIc, Fig. 1), 59 mass units are lost (CO_2CH_3) to yield species c, while 15 mass units are involved in ring D and E unsubstituted substances (*e.g.*, IIa or IIb, Fig. 2). Whether the loss of methyl originates exclusively from the angular C-17 position, as is the case with the carbomethoxy analog IIc, remains an open question.

Similarly, in the case of erythrodiol diacetate IIe, Fig. 3) 73 mass units (CH_2OAc) are lost (loss of acetic acid also occurs: *m/e* 276 → *m/e* 216 in Fig. 3), while the lactone IIg gives rise to the loss of 45 mass units (COOH). In several cases a metastable ion could be observed in connection with the expulsion of the C-17 substituent from species a (*e.g.*, *m/e* 158, calcd. 157.2 for *m/e* 262 → *m/e* 203 in Fig. 1 for methyl oleanonate, IIc).

If the C-17 substituent is a methyl group (*e.g.*, IIa), its loss from species a is not very pronounced (less than

ADDED IN PROOF.—In the meantime a paper by J. S. Shannon (*Australian J. Chem.*, **16**, 683 (1963)) on ifflaionic acid appeared, where the main fragments of several α-amyrine derivatives are discussed.

(6) J. F. Lynch, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *Experientia*, **19**, 211 (1963).

(7) C. Djerassi, H. Budzikiewicz, and J. M. Wilson, *Tetrahedron Letters*, 263 (1962).

(8) This type of fragmentation has first been described by F. H. Field and J. L. Franklin ("Electron Impact Phenomena," Academic Press, Inc., New York, N. Y., 1957, p. 92) as an energetically very favored process and has actually been observed in many cases; *cf. e.g.*, API Catalog of Mass Spectral Data, Spectrum No. 209 (cyclohexene) or α-ionone (K. Biemann, *Angew. Chem.*, **74**, 102 (1962)).

(9) B. Tursch, E. Tursch, I. T. Harrison, G. B. Silva, H. J. Monteiro, B. Gilbert, W. B. Mors, and C. Djerassi, *J. Org. Chem.*, **28**, 2390 (1963).

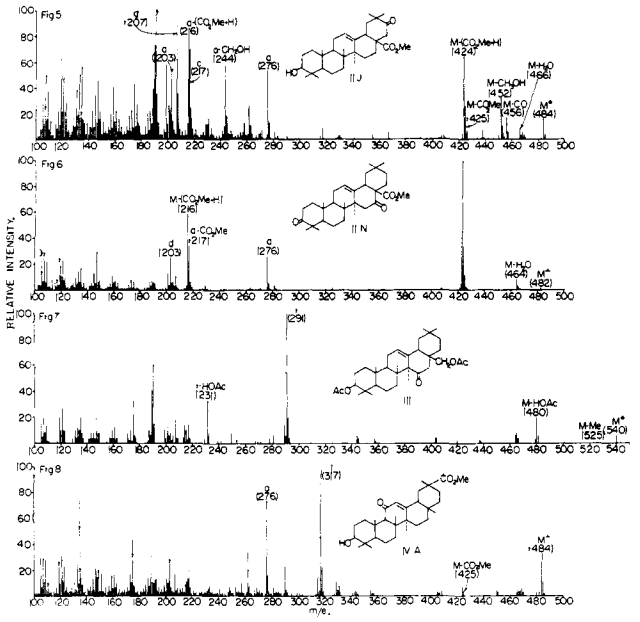


Fig. 5.—Mass spectrum of methyl machaerate (IIj).

Fig. 6.—Mass spectrum of methyl dioxoechinocystate (IIn).

Fig. 7.—Mass spectrum of 15-oxoerythrodiol diacetate (III).

Fig. 8.—Mass spectrum of methyl glycyrrhetate (IVa).

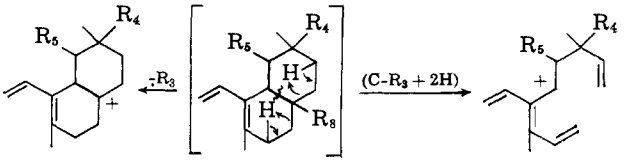
30% of the intensity of ion a). However, in the case of a carbomethoxy (IIc) or lactone (IIg) grouping the intensity of species c equals or slightly exceeds that of a, while fragment c is several times more intense than a when the angular substituent represents CH_2OAc (Fig. 3). The release of steric hindrance may, therefore, play an important role.

Removal of other substituents from species a is much less pronounced. In the spectrum (Fig. 1) of methyl oleanonate (IIc), the loss of methyl from species a is hardly recognizable and the loss of COOMe from ion a derived from methyl 11-deoxyglycyrrhetate (IId) amounts to about 10% of the intensity of a. Similarly, in IIf (Fig. 4) the loss of CH_2OAc from a does not exceed 25% of the intensity of a, while the abundance of species c derived from the isomeric erythrodiol diacetate IIe (Fig. 3) is about ten times that of species a. Hence the relative intensities of fragments a and c offer an important indication about the attachment of a substituent at C-17. If a hydroxy or acetate substituent is attached to rings D or E, species a may also be observed at 18 or 60 mass units lower. This loss of water or acetic acid may well occur prior to ionization by thermal decomposition at the glass and metal surfaces of the heated inlet system.

Species c suffers further decomposition by the loss of 70 mass units yielding a fragment which is not very abundant in most cases but whose genesis can easily be determined by the presence of the appropriate metastable ion (*e.g.*, 87.7, calcd 87.1 for *m/e* 203 → *m/e* 133 in Fig. 1 for methyl oleanonate, IIc). This cleavage is probably due to the partial loss of ring E yielding the highly stabilized ion f.

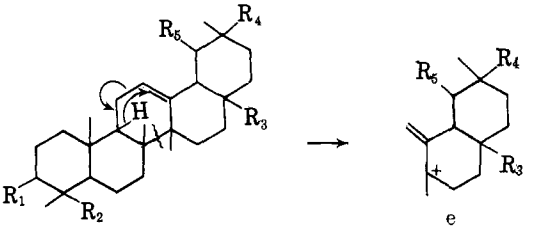
c *m/e* 203 f *m/e* 133

Species c is always accompanied by a less intense ion 14 mass units lower and the presence of a metastable ion in some cases (*e.g.*, 164.3, calcd. 163.8 for *m/e* 218 → *m/e* 189 in Fig. 2 for IIb) indicates that this fragment is formed by a one-step process from species a. Since this fragmentation presumably involves the loss of the C-17 substituent, the additional carbon atom lost is most probably C-17. This cleavage necessitates a double hydrogen transfer and we would like to propose the following mechanism yielding a highly conjugated allylic cation (d).



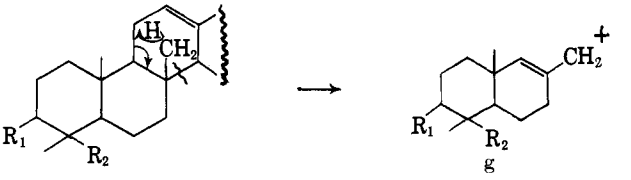
c a d

Species a is accompanied by a fragment (*e.g.*, *m/e* 249 in Fig. 1) of relatively low abundance and containing 13 mass units less. For its genesis the following process seems probably involving one hydrogen transfer and cleavage of an allylically activated bond (yielding species e).



e

There is one more fragment (g) that can be identified very clearly, although it is not too abundant in the spectra of Δ^{12}-unsaturated triterpenes. It contains rings A and B as can be determined by the appropriate shifts upon substitution in these rings. In the hydrocarbon β-amyrene (IIa) it is found at *m/e* 191, while in the 3-ketone methyl oleanonate (IIc) it is shifted to *m/e* 205 (Fig. 1). A 3-hydroxy (IId) and a 3-acetoxy group (IIe) cause shifts to *m/e* 207 and 249, respectively; in both cases, an *m/e* 189 fragment due to the further loss of water or acetic acid from species g can be observed. The formation of g must involve transfer of one hydrogen atom and the following mechanism can be proposed



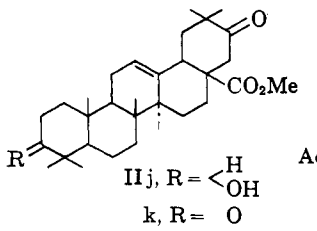
g

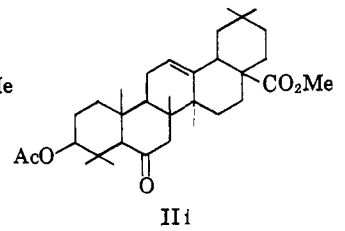
Species g is one of the most characteristic fragmentation products of saturated pentacyclic triterpenes and will, therefore, be discussed more thoroughly below.

The following substituents did not change the general fragmentation pattern as described above, other than in the relative intensities of certain fragments: 3-keto (IIc), 3-hydroxy (IId), 3-acetoxy (IIe),[10] and 4-carbomethoxy groups (IIb) in ring A as well as a keto function at C-6 as in acetyl methyl sumaresinonate (IIi); the same is true for substituents at C-17 (IIc, IIe, IIg) or in ring E (IId, IIf, IIh).

Introduction of a keto group at C-21 adds several characteristic features to the general fragmentation pattern. The mass spectra of all compounds discussed so far show in the high mass range the expected peaks

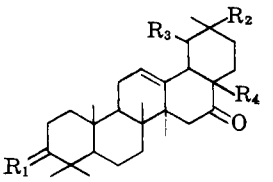
(10) It may be noted here that triterpenoid 3-acetates exhibit a rather small loss of acetic acid from the molecular ion in contrast to steroidal 3-acetates (*cf.* ref. 12), while a very abundant M − 60 fragment is observed with 28-acetates (IIe), thus offering a further possibility to localize certain substituents.



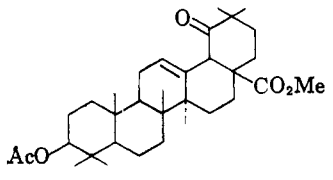


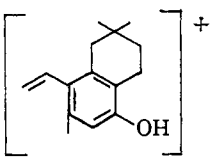
due to the loss of methyl, water (in hydroxy-containing compounds), and acetic acid (in acetates). If a carbomethoxy group is present, usually an M − 59 peak (and if the carbomethoxy group is at C-17 also an M − 60 peak (see Fig. 1)) of comparable size to the molecular ion can be observed. In methyl machaerate (IIj, Fig. 5) as well as its 3-ketone IIk the M − 60 peaks are about four times more abundant than the molecular ion and in addition significantly abundant fragments due to the loss of 28 (probably CO) and 32 (MeOH) mass units can be observed.[11] Similar losses of the elements of MeOH, CO_2Me, and CO_2Me + H (32, 59, and 60 mass units) from species a can be observed in Fig. 5. By contrast, the γ-keto ester IIo, derived from siaresinolic acid, does not exhibit all the additional cleavages characteristic for IIj and IIk, but rather behaves as if the carbonyl group at C-19 exerted no major influence.

While the triterpene 16-ketones, 16-oxobreinol acetate (IIl) and acetyl nor-16-oxoechinocystic acid (IIm), give the expected fragmentation pattern, methyl 3,16-dioxoechinocystate (IIn, Fig. 6) exhibits several additional fragments. Most noteworthy is a very pronounced M − 59 peak[11b] (exceeding more than a hundredfold the molecular ion) as well as an M − 18 fragment[12] of about ten times the intensity of the molecular ion. Furthermore, as in the above discussed 21-ketones IIj and IIk, an a − 60, in addition to the a − 59, peak can be observed in Fig. 6 and the ring D aromatic species h may be ascribed to this fragment.



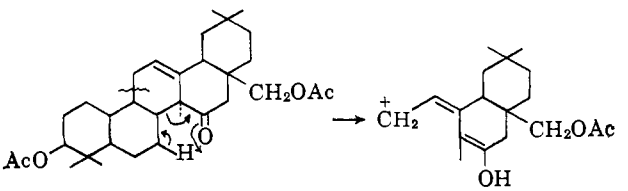
	R_1	R_2	R_3	R_4
III	(H)OAc	H	Me	Me
m	(H)OAc	Me	H	H
n	O	Me	H	CO_2Me





In summary, introduction of a keto group at C-6, C-16, or C-21 does not affect the general fragmentation path of the Δ^{12}-unsaturated oleanenes and ursenes, and only causes additional fragmentations involving a carbomethoxy group when this is present at C-17. Yet the existence of a carbonyl function close to the centers of principal fragmentation can alter drastically the mode of cleavage.

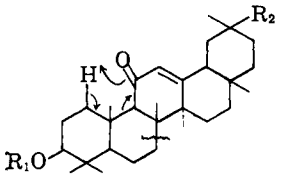
Thus, 15-ketoerythrodiol diacetate (III, Fig. 7) does not show the expected fragment at *m/e* 290 (due to the anticipated retro-Diels–Alder reaction involving the Δ^{12}-double bond), but its most abundant ion (except for the *m/e* 43 acetyl ion originating from the acetate) occurs at *m/e* 291 (i). A hydrogen transfer process similar to that observed in 15-keto steroids[13] seems to occur. The most likely formulation would be as indicated by the arrows in III, but the result obtained in a similar case[4] suggests caution about involving specific hydrogen transfers where no deuterated analogs are available for confirmation.

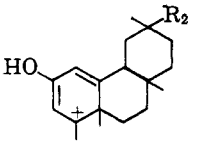


III i, *m/e* 291

It is noteworthy that an i − 60 (CH_3COOH), but no i − 73 (CH_2OAc) fragment can be observed in Fig. 7.

11-Keto groups exhibit a similar influence on the fragmentation pattern. Methyl glycyrrhetate (IVa, Fig. 8) shows the expected fragment a (*m/e* 276), but in addition a somewhat more abundant fragment at *m/e* 317 (j) can be found whose genesis may be formulated as indicated in IV. The same reservations concerning the hydrogen transfer apply also here as in the case of the 15-ketone III.



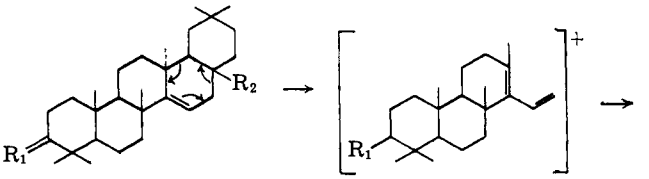


IVa, R_1 = H; R_2 = CO_2Me
b, R_1 = Ac; R_2 = CH_3

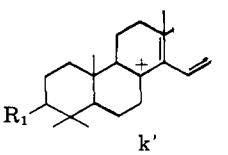
j, *m/e* 317; R = CO_2Me

In 11-oxo-18α-β-amyrin acetate (IVb) the intensity ratio a *vs.* j is changed in favor of j due to the altered stereochemistry at C-18, j being now by far the most intense fragment of the spectrum. In this connection, it is pertinent to note that 18α-β-amyrone exhibits a fragment a about 70% less intense (relative to the molecular ion) than does β-amyrone.

(b) **Δ^{14}-Taraxerenes.**—The spectra of three compounds of this class have been measured, namely taraxerone (Va), taraxerol (Vb), and myricadiol diace-



Va, R_1 = O, R_2 = Me
b, R_1 = (H)OH, R_2 = Me
c, R_1 = (H)OAc, R_2 = CH_2OAc



(11) (a) It is pertinent to mention that analogous cleavages have not been noted in γ-keto fatty acid esters (*cf.* R. Ryhage and E. Stenhagen, *Arkiv Kemi*, **15**, 545 (1960)). (b) Aliphatic β-keto acid esters exhibit only an M-58 fragment (R. Ryhage and E. Stenhagen[11a]). The *m/e* 424 peak in Fig. 6 does not represent an M − 58 ion, but rather is due to the isotope peak of the adjacent M − 59 species.

(12) Loss of water from cyclic ketones has been observed in many instances; *cf.* E. Lund, H. Budzikiewicz, J. M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 941 (1963).

(13) H. Budzikiewicz and C. Djerassi, *ibid.*, **84**, 1430 (1962)

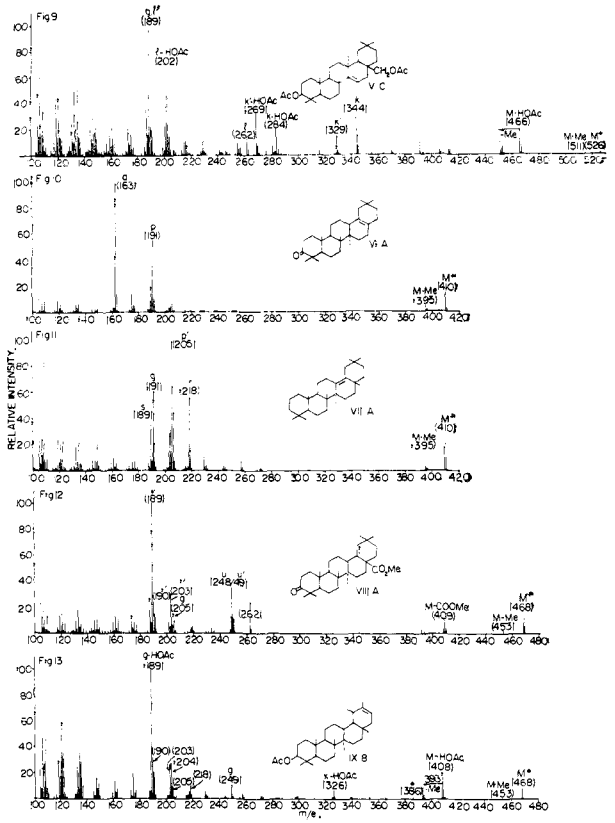


Fig. 9.—Mass spectrum of myricadiol diacetate (Vc).

Fig. 10.—Mass spectrum of 28-nor-Δ^{17}-oleanen-3-one (VIa).

Fig. 11.—Mass spectrum of $\Delta^{13(18)}$-oleanene (VIIa).

Fig. 12.—Mass spectrum of methyl moronate (VIIIa).

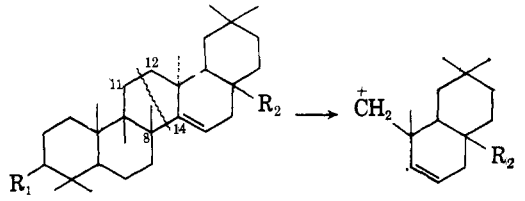
Fig. 13.—Mass spectrum of ψ-taraxasterol acetate (IXb).

tate (Vc) (Fig. 9), thus offering the necessary labels for assigning structures to the major fragments.

In these molecules a similar retro-Diels–Alder decomposition would be expected to operate as has been observed with Δ^{12}-unsaturated derivatives, except that collapse of ring D rather than ring C should occur. This cleavage process is actually observed (see arrows in V), the charge remaining again with the diene portion, which now comprises rings A, B, and C. The resulting fragment k exhibits a mass of *m/e* 300 in the case of Va, 302 for Vb, and 344 (Fig. 9) for Vc, due to the alterations in the C-3 substituent. Ion k is accompanied by a satellite 15 mass units lower, which is formed by the loss of a methyl group, probably the allylically activated one at C-8 (k′). The spectrum of Vb exhibits in addition peaks due to the loss of H_2O and of $H_2O + CH_3$ from species k, while that of Vc contains $k - CH_3CO_2H$ and $k' - CH_3CO_2H$ ions.

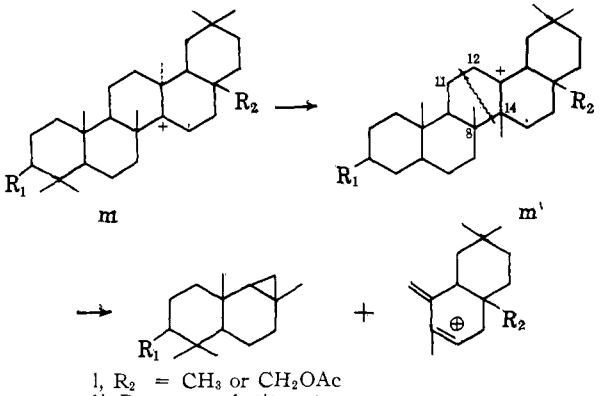
In addition to species k and its further decomposition products, the spectra of Va and Vb show a very abundant fragment (the strongest above *m/e* 60) at *m/e* 204 (l). This cleavage product, therefore, cannot contain ring A but must rather be derived from rings D and E. This assumption is verified by the spectrum of Vc, which contains a rather small peak at *m/e* 262 (l), but an abundant one at *m/e* 202 ($l - CH_3CO_2H$). The latter is either derived from the $M - CH_3CO_2H$ fragment or formed by direct loss of acetic acid from l. Furthermore, fragment l loses the substituent at C-17 (*cf.* the further decomposition of species a in section 1a) giving rise to a fragment $l - CH_3$ for Va and Vb and $l - CH_2OAc$ (Fig. 9) for Vc (*m/e* 189 = 1′ in all cases). The ease of the loss of the C-17 substituent (CH_3 *vs.* CH_2OAc) is comparable to the results obtained for species a (*vide supra*).

The question of the genesis of this fragment (l) is somewhat difficult to answer. Formally it corresponds to a cleavage of 11–12 and 8–14 bonds, as indicated by the wavy line



But it is difficult to visualize the driving force of this fragmentation as this would involve rupture of a bond next to a double bond and cleavage of a bond between two secondary carbon atoms rather than next to the quaternary C-13 center. Furthermore, the resulting ion would not be a very favorable species, since it contains a primary carbonium ion and a radical on a double bond.

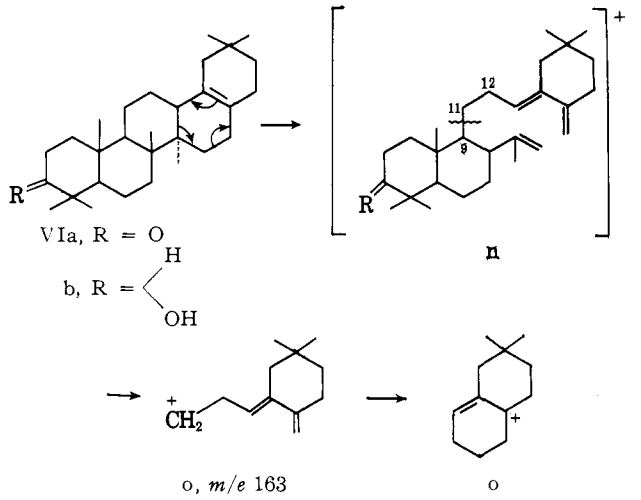
A more palatable mechanism can be proposed by assuming that in the molecular ion the missing electron is preferentially removed from the carbon–carbon double bond (m), migration of the C-13 methyl group, then yielding the radical ion m′. Fission of the 11–12 and 8–14 bonds now gives the stable diene l. There is certainly no experimental proof available for this mechanism, but it may be recalled that many factors influence the formation of a fragment ion, release of strain and stability of the final product being among the important ones.



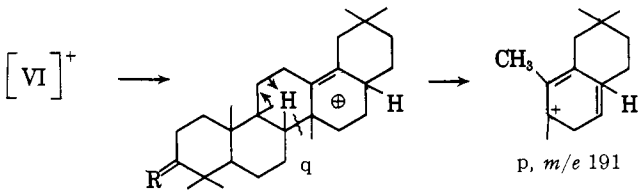
l, R_2 = CH_3 or CH_2OAc
l′, R_2 = no substituent
(*m/e* 189)

(c) **28-Nor-$\Delta^{17(18)}$-oleanenes.**—28-Nor-$\Delta^{17(18)}$-oleanen-3-one (VIa) and the corresponding alcohol VIb exhibit their most abundant fragment ion at *m/e* 163. Assuming again a retro-Diels–Alder reaction involving the opening of ring D, intermediate n will be obtained. Two bonds may be considered now as likely candidates for a further cleavage, *viz.*, 9–11 (next to a tertiary C-atom) and 11–12 (allylically activated). Rupture of the former one is actually observed (Fig. 10), possibly because the resulting ion o (*m/e* 163) may rearrange to the more stable species o′.

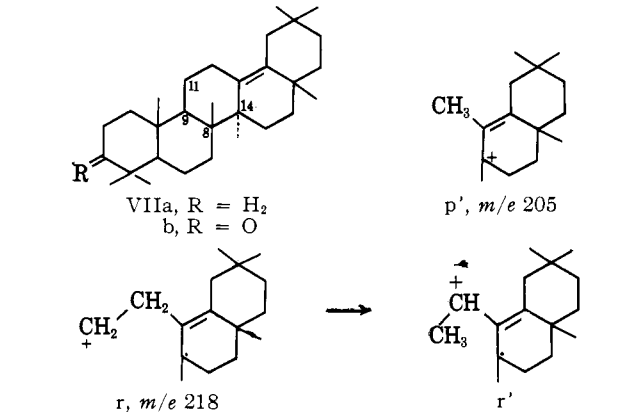
The second strongest peak in the spectra of VIa (Fig. 10) and VIb appears at *m/e* 191 (p). According to its mass, it comprises most likely rings D and E, formed by rupture of the 11–12 and 8–14 bonds. This type of fragmentation is somewhat unexpected, since the 12–13 bond would be allylically activated and therefore more likely to break. However, fragments corresponding in mass to the *m/e* 191 ion of VI (p) are the most important cleavage products (*vide infra*) of triterpenes with a 13–18 double bond and the genesis of p may then be readily explained by assuming a migration of the double bond to form ion q which decomposes



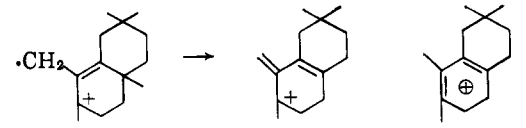
further in the indicated way. Bond migrations in the molecular ion of pentacyclic triterpenes seem to occur occasionally and further examples will be cited below. A more thorough discussion of ion p and of its satellites will be given in the next section.



(d) $\Delta^{13(18)}$-**Oleanenes.**—The most important fragments in the spectrum of $\Delta^{13(18)}$-oleanene (VIIa) (Fig. 11) as well as of $\Delta^{13(18)}$-oleanen-3-one (VIIb) corresponds to *m/e* 205 (p′) and its formation can be assumed to be analogous to that of p from the ion q. It is surrounded by several less intense peaks, the genesis of which demands different hydrogen rearrangement processes, but in the absence of suitable deuterium-labeled analogs, it would be mere speculation to write mechanisms for the formation of all these minor ions. More important is the presence of a satellite (r) 13 units higher, whose production can be assumed to involve cleavage of the 8–14 and 9–11 bonds, shift of one hydrogen, then leading to the allylic cation r′.

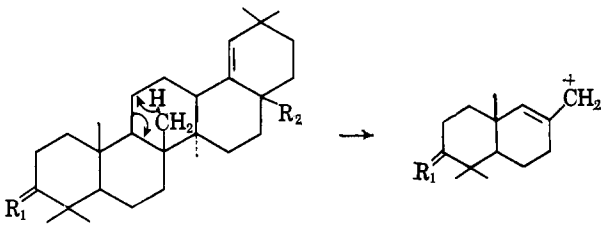


Another satellite of p′ exhibits a mass 16 units lower (*m/e* 189 in Fig. 11). Structure s is proposed for this species, which may be formed by loss of the C-17 substituent from an ion analogous to p′, but without involving a hydrogen rearrangement (p″). That the C-17 substituent is lost from p″ rather than from p′ can be rationalized through the lesser stability of p″, compared with p′ and the higher stability of s when contrasted with a species (s′) containing one more hydrogen atom (even electron *vs.* odd electron ions)

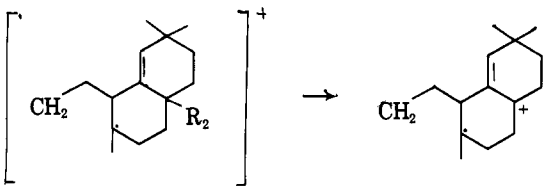


In addition, an ion containing rings A and B can be observed (g), the formation of which has been discussed in section 1 (a). In the spectrum of VIIb, g coincides with p′ (*m/e* 205), but in VIIa it can be clearly recognized at *m/e* 191 (Fig. 11). Finally, in the high mass-range, it should be noted (see Fig. 11) that the $\Delta^{13(18)}$-oleanenes exhibit a substantial M − CH_3 peak, evidently due to expulsion of one of the allylically activated methyl groups (C-27 or C-28).

(e) Δ^{18}-**Oleanenes.**—Methyl moronate (VIIIa, Fig. 12), methyl morolate (VIIIb), Δ^{18}-oleanene (VIIIc), and germanicol acetate (VIIId) offer the necessary labels to identify the more important fragments.



	R_1	R_2
VIIIa,	O	CO_2Me
b,	(H)OH	CO_2Me
c,	H_2	Me
d,	(H)OAc	Me



All four members of this class exhibit a rather pronounced loss of the C–17 substituent[14] as expected by the allylic activation due to the 18–19 double bond. But this center of unsaturation is too remote to influence the major breakdown of the ring system and the general fragmentation pattern resembles strongly that of the saturated compounds discussed below. On the other hand, the double bond assists in the stabilization of the ions still containing rings D and E.

Cleavage (as described in section 1(a)) of ring C yields fragment g (*m/e* 205 for VIIIa in Fig. 12, 207 for VIIIb, 191 for VIIIc, and 189—due to the loss of acetic acid—for VIIId), while an ion comprising the right-hand portion of the molecule (t) (*m/e* 262 for VIIIa and b, *m/e* 218 for VIIIc and d) is formed by the same type of cleavage without hydrogen rearrangement. Species t is indicated as an energetically unlikely radical ion which probably undergoes rearrangement with formation of another double bond or ring. Fragment t suffers further loss of the C-17 substituent giving t′ (*m/e* 203 in all cases—see Fig. 12).

Alternate fission of the 11–12 bond (with and without hydrogen rearrangement) yields species u and u′ (*m/e* 204/205 for VIIIc and d, *m/e* 248/249 for VIIIa and b), which again lose the C-17 substituent giving v and v′ (*m/e* 189/190). It is likely that u rearranges to a more stable ion such as u″.

(14) Where the C-17 substituent is a methyl group it is not established whether it rather than the other allylically activated methyl groups (attached to C-20) are eliminated.

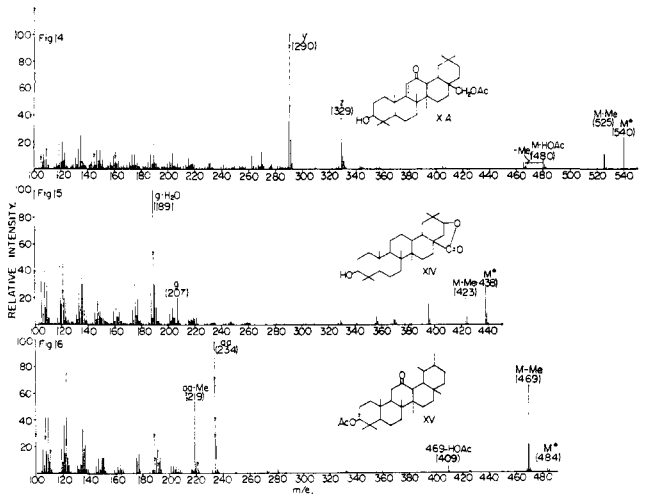
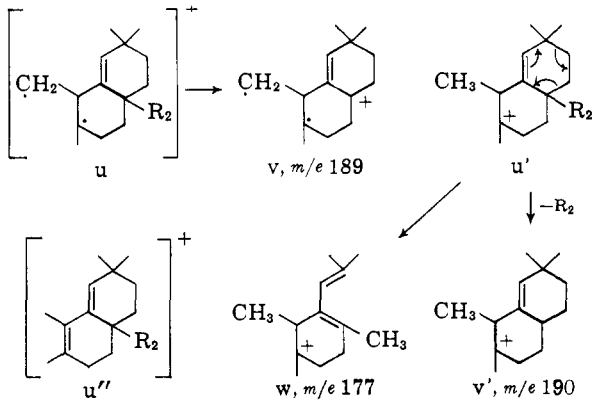


Fig. 14.—Mass spectrum of $\Delta^{9(11)}$-oleanene-3,28-diol-12-one diacetate (Xa).

Fig. 15.—Mass spectrum of dihydromachaerinic acid lactone (XIV).

Fig. 16.—Mass spectrum of 3β-acetoxy-12-oxoursane (XV).

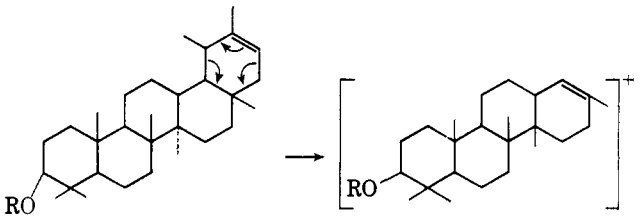
The mass spectra of Δ^{18}-oleanene (VIIIc) and germanicol acetate (VIIId) both exhibit an *m/e* 177 peak, which, therefore, cannot comprise ring A; but a peak at *m/e* 177 or one at *m/e* 211 (expected if the angular carbomethoxy substituent were present) does not occur in



the spectra of VIIIa and VIIIb. The *m/e* 177 peak must, therefore, represent a $C_{13}H_{21}$ ion and its formation must be influenced strongly by the nature of the C-17 substituent. A possible genesis of this fragment would be a retro-Diels–Alder decomposition of species u' leading to w. The inhibition of a retro-Diels–Alder reaction (or the lack of formation of the diene cation) due to the presence of a nearby oxo-function has been observed in Δ^6-α-decalone systems (in contrast to the corresponding hydrocarbon) and may be invoked to rationalize the absence of an appropriate fragment (*m/e* 221) in the spectra of VIIIa (Fig. 12) and VIIIb.

(f) ψ-Taraxasterol.—The only pentacyclic triterpenes with a $\Delta^{20(21)}$-double bond available were ψ-taraxasterol (IXa) and its acetate IXb. Their fragmentation patterns resemble even more closely those of the saturated compounds, species g (*m/e* 207 in IXa and *m/e* 249 in IXb—see Fig. 13) and its decomposition product (*m/e* 189 due to loss of water or of acetic acid) being the most abundant fragments in the spectra. Ions corresponding to t (*m/e* 218), t' (203), u and u' (204, 205), and v and v' (189 and 190; the former coinciding with the g − HOAc peak) occur to a much lesser extent than in $\Delta^{18(19)}$-unsaturated triterpenes.

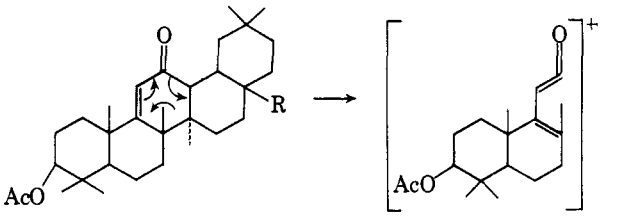
One fragment which deserves further comment is the one formed by the loss of 82 mass units from the molecular ions of IXa and IX band especially from the M − 60 fragment in IXb (see Fig. 13). Its genesis can be assumed to be a retro-Diels–Alder decomposition of ring E leading to species x.



IXa, R = H
b, R = Ac

x

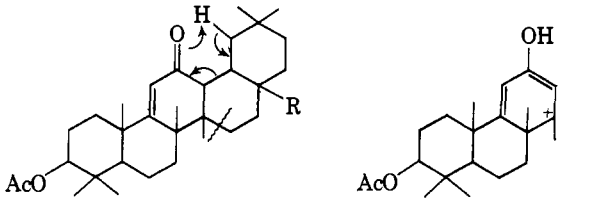
(g) $\Delta^{9(11)}$-Oleanenes.—Only the 12-keto derivatives of this class were available. The main fragment in the spectra of both $\Delta^{9(11)}$-oleanene-3β,28-diol-12-one diacetate and $\Delta^{9(11)}$-oleanen-12-on-3β-ol acetate Xb is evidently formed by retro-Diels–Alder decomposition of ring C yielding species y (*m/e* 290 in Fig. 14).



Xa, R = CH_2OAc
b, R = CH_3

v, *m/e* 290

But as noted already in the case of Δ^{12}-11-ketones IV, a second type of fragmentation occurs, triggered by the keto function. It involves rearrangement of one hydrogen atom and the following mechanism—with the reservation mentioned above—may be proposed, leading to species z (*m/e* 329).



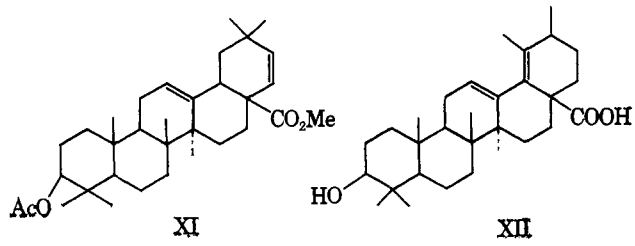
X

z, *m/e* 329

(h) Δ^{12}-Oleanenes Containing a Second Double Bond.—Generally, it can be stated that the fragmentation behavior is determined by the presence of the 12–13 double bond, additional centers of unsaturation exhibiting only secondary effects. This is especially true for Δ^2-unsaturated triterpenes (*e.g.*, methyl 21-acetoxy-Δ^2-oleanen-28-oate) which do not even show the expected retro-Diels–Alder decomposition of ring A. Methyl 21-dehydro-oleanolate 3-acetate (XI) exhibits, compared to methyl oleanolate 3-acetate (acetate of IId), a more pronounced loss of CO_2Me, both from the molecular ion (M − 59 = *m/e* 451) and from species a (a − 59 = *m/e* 201), because of the allylic activation. The same is true for the loss of methyl from species a, but the overall fragmentation of ring C (see II → a) is not altered by the presence of the additional double bond. The same behavior is observed with Δ^{18}-compounds (*e.g.*, vanguerolic acid (XII)) in that the general fragmentation pattern of Δ^{12}-triterpenes (II) is retained, but pronounced loss of CH_3 and CO_2H from species a is noted due to allylic activation.

A Δ^{15}-double bond has no influence on the expulsion of the C-17 substituent as demonstrated by the spectrum[2b] of anhydroechinocystic acid methyl ester acetate (XIIIa). The only additional outstanding frag-

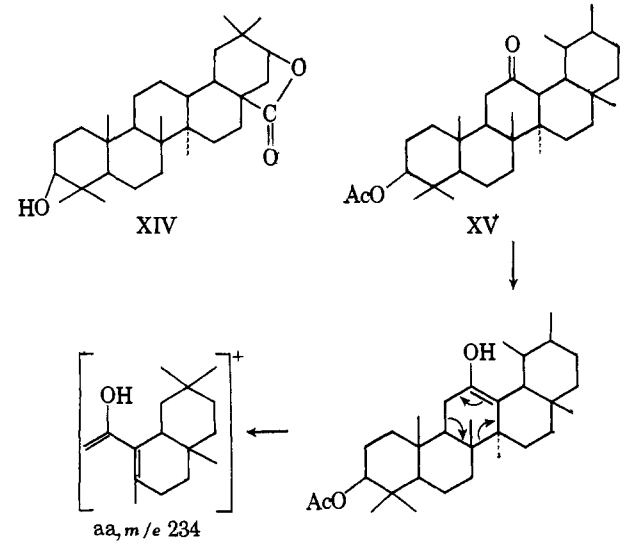
ment is one of *m/e* 131, found both in XIIIa and XIIIb, which clearly represents the aromatic analog of ion f.



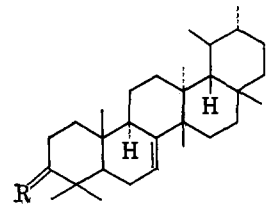
XIIIa, R = CO_2Me
b, R = CH_2OAc

(i) **Saturated Oleananes and Ursanes.**—Members of this class, bearing substituents only in rings A and B, are characterized by spectra which are virtually void of any pronounced fragments from the molecular ion region (with the exception of M^+ and the peaks due to the loss of functional groups such as $M - CH_3$, $M - 60$ in the case of an acetate, and combinations thereof) down to fragment g (which in the case of an alcohol or an acetate undergoes further loss of water or acetic acid), a typical example being dihydromachaerinic acid lactone (XIV)[9] (Fig. 15).

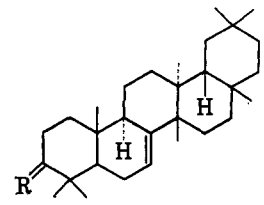
Introduction of a keto group at C-12, as in 3β-acetoxy-12-oxoursane (XV), leads to an abundant fragment of *m/e* 234 (Fig. 16), the formation of which can be explained *formally* by a retro-Diels–Alder decomposition of the enol form yielding the ion aa analogous to the breakdown of Δ^{12}-unsaturated ursanes. Fragment aa shows further decomposition by loss of a methyl substituent.



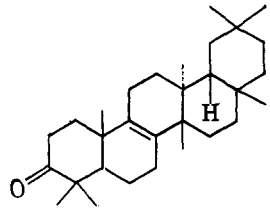
(2) **Bauerene and Related Triterpenes.**—The representatives of this class possess methyl groups at positions 13 and 14, the presence of which influences characteristically the breakdown pattern, the position of the double bond (Δ^7, Δ^8, $\Delta^{9(11)}$) changing only the relative abundance of certain fragments. The triterpenes measured by us are bauerene (XVIa) and its 3-ketone, XVIb, multiflorene XVIIa and its 3-ketone XVIIb, isomultiflorenone (XVIII), and several derivatives of arborinol, for which structure XIXa has been suggested recently.[15]



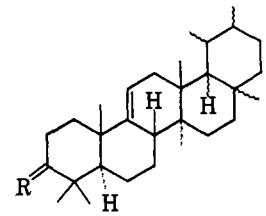
XVIa, R = H_2
b, R = O



XVIIa, R = H_2
b, R = O



XVIII



XIXa, R = (H)OH
b, R = H_2
c, R = O

It has been pointed out in section 1(a) that differences in the stereochemistry of the D/E ring juncture (β-amyrone *vs.* its 18α-isomer), probably due to the reduced steric strain in the 18α-isomer, influence very much the relative intensity of fragment a. Similarly, increase in the steric hindrance in the compounds discussed here fosters cleavage in ring D and E and the pair bauerene (XVIa)–multiflorene (XVIIa) (as well as the corresponding ketones XVIb *vs.* XVIIb) offer the only example encountered so far where the position of the methyl groups in ring E (α- *vs.* β-amyrin type) exerts considerable influence on the general fragmentation behavior.

As is to be anticipated due to the enhanced strain imposed by the C-13 methyl group, most of the fragmentation occurs in the environment of the C/D ring juncture. Only one principal ion comprises the right-hand part of the molecule; it can be found with a mass of *m/e* 205 (Fig. 19) as the base peak in XVIII, still very abundant (about 50% of the base peak) in XVIIa and XVIIb (Fig. 18), less intense (about 25% of the base peak) in XVIa and XVIb (Fig. 17), but virtually absent in XIXc (Fig. 20) and XIXb. Since it occurs, as indicated, both in hydrocarbons XVIa and XVIIa as well as in the corresponding 3-ketones XVIb, XVIIb, and XVIII (Fig. 17–19), it must comprise rings D and E. A plausible mode of formation for this fragment (bb) derived from isomultifluorenone (XVIII) involves one hydrogen transfer (arrows in XVIII), followed by cleavage of the allylically activated 12–13 bond. *Mutatis mutandis*, the same mechanism can be applied to XVI and XVII. It is conceivable that the steric interaction of the 19β-methyl group is responsible for the lesser abundance of fragment bb in the bauerene series (XVI).

All the other major fragments comprise rings A and B of the molecule (as indicated by the appropriate shifts between hydrocarbons and ketones) and it is the relative abundance of certain ions which characterizes the four triterpene types under consideration.

Multiflorene (XVIIa) exhibits its most abundant fragment (cc) at *m/e* 204, which is shifted to *m/e* 218 in the ketone XVIIb (Fig. 18). Corresponding fragments are highly reduced in XVI and XVIII and absent

(15) H. Vorbrüggen, S. C. Pakrashi, and C. Djerassi, *Liebig's Ann.*, in press, where mass spectra are reproduced of the hydrocarbons XVIa, XVIIa, as well as of the dienes XXa and b.

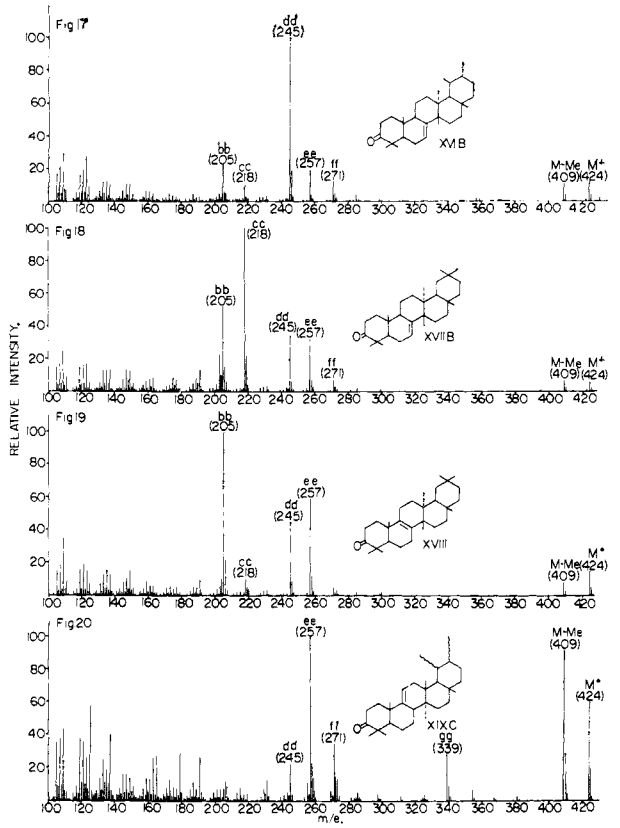


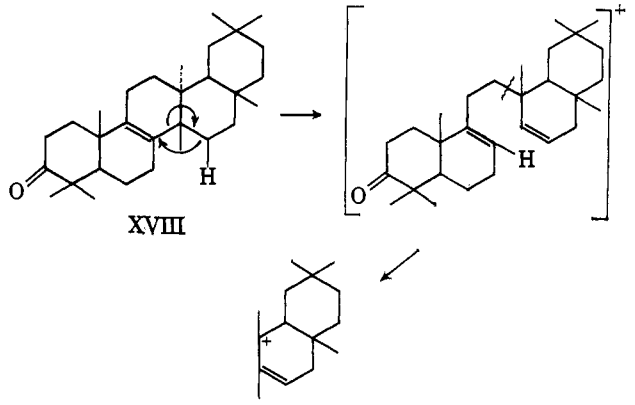
Fig. 17.—Mass spectrum of bauerenone (XVIb).

Fig. 18.—Mass spectrum of multiflorenone (XVIIb).

Fig. 19.—Mass spectrum of isomultiflorenone (XVIII).

Fig. 20.—Mass spectrum of arborenone (XIXc).

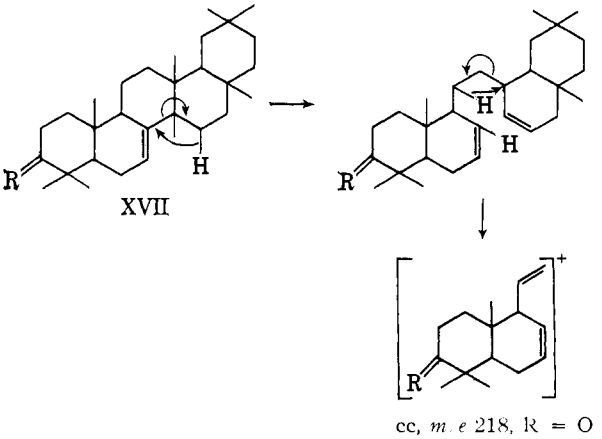
in XIX. The formation of ion cc corresponds formally to a cleavage of the 8–14 and 12–13 bonds, but it seems more reasonable to invoke a double hydrogen transfer



bb, *m/e* 205

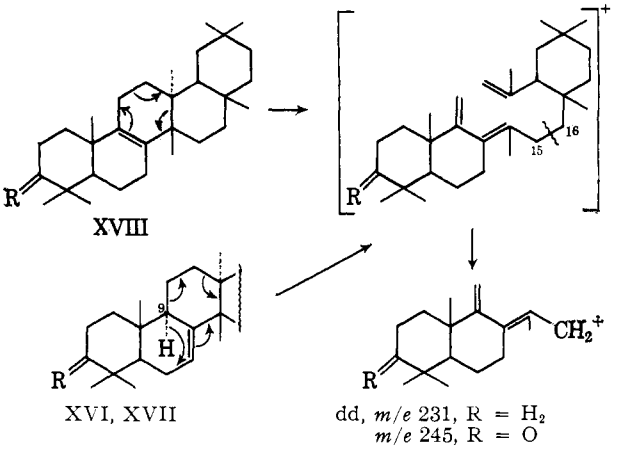
(as indicated for the formation of bb coupled with back transfer of one of the C-11 hydrogens) to avoid the unfavorable cleavage of a bond next to a double bond.

Here it must be the location of the double bond that favors in isomultiflorenone (XVIII) the formation of bb (Fig. 19) and in multiflorenone (XVIIb) (Fig. 18) that of cc, but further discussion is not warranted in absence of deuterated analogs, which would permit a precise elucidation of the origin of the hydrogen transfers. Two further fragments of considerable interest are dd (*m/e* 231 in XVIIa,[15] *m/e* 245 in XVIIb and XVIII—see Fig. 18 and 19) and ee (*m/e* 243 in XVIIa, *m/e* 257 in XVIIb and XVIII—see Fig. 18 and 19). Ion dd, which represents the base peak in the spectra of bauerene (XVIa) and bauerenone (XVIb) (Fig. 16) but



cc, *m/e* 218, R = O

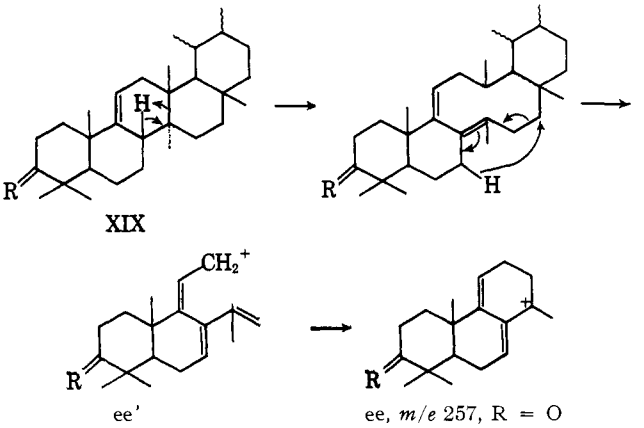
is of rather reduced intensity in XIX (Fig. 20), must comprise two more carbon atoms than cc. A reasonable mechanism starting from isomultifluorenone (XVIII) (Fig. 19) would be a retro-Diels–Alder decomposition of ring C followed by cleavage of the activated 15–16 bond. For the Δ^7-isomers of the bauerene (XVI) and multiflorene (XVII) series one would then have to assume either a double bond migration, prior to this special fragmentation process, or a rearrangement of the C-9 hydrogen atom to yield the same intermediate.



XVI, XVII

dd, *m/e* 231, R = H_2
m/e 245, R = O

The fragment ee which can be observed in XVIa and XVIIa at *m/e* 243, in XVIb, XVIIb, and XVIII at *m/e* 257 (Fig. 17–19), becomes the most important fragment in XIXc (Fig. 20). Due to its mass (*m/e* 257) it must comprise one more carbon atom than dd (*m/e* 245). Formation of this species must be accompanied by a double hydrogen transfer to the neutral moiety in order to account for the low hydrogen content of ee. A possible mechanism is suggested below, but it should be pointed out that in the absence of appropriate deuterium labeling it is highly speculative. The ini-

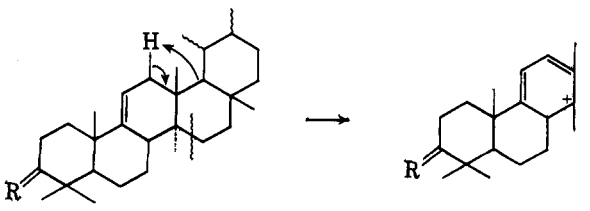


ee'

ee, *m/e* 257, R = O

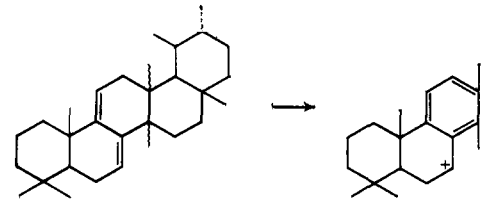
tially formed species ee′ may well rearrange further to a more stable cyclic structure (ee). For XVI, XVII, and XVIII double bond migration prior to this cleavage may be involved. This would explain the gradually decreasing abundance of this fragment (XIX *vs.* XVIII *vs.* XVII and XVI).

The mass spectrum[15] of arborene (XIXb) exhibits an additional fragment at *m/e* 257 (shifted to *m/e* 271 in the ketone XIXc, Fig. 20). This ion (ff) is of lesser importance in the spectra of the double bond isomers XVIb and XVIIb (Fig. 17 and 18) and its genesis may be visualized as



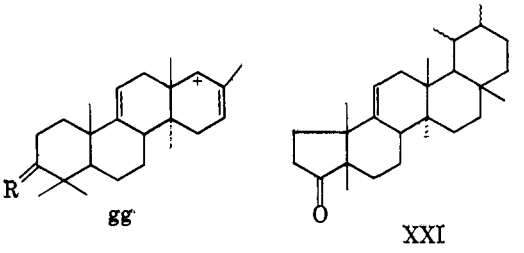
XIX ff, *m/e* 271, R = O

Fragment ff (*m/e* 255) becomes the most important one in the mass spectra[15] of baueradiene (XXa) and arboradiene (XXb), because the additional double bond can render ring C in ff′ completely aromatic. The only additional feature of these diene spectra worth mentioning is the pronounced loss of methyl from the molecular ion.



XXa, 13α, 14β
b, 13β, 14α ff′, *m/e* 255

Arborinone (XIXc), besides a very pronounced loss of methyl, yields one more rather abundant fragment (gg − *m/e* 339 in Fig. 20) involving the loss of 85 mass units from the molecular ion. Its formation can be explained by decomposition of ring E accompanied by rearrangement of one hydrogen atom.



gg XXI

A further proof for the correct assignments of the fragment ions in the spectra of XIXa–c is offered by the ring-contracted ketone XXI which exhibits exactly the same fragmentation pattern, except for the obvious mass shifts.

In summary, the fragmentation pattern of 13,14-dimethylated triterpenes of type XVI–XIX is very much governed by the differences in steric strain, the influence of the position of the double bond apparently being of lesser importance. Nevertheless, the mass spectral features are characteristic enough to make a clear distinction possible.

(3) **Derivatives of Friedelane.**—Derivatives of friedelan-3-one (XXIIa) and friedelan-x-one as well as friedelan-y-one, including deuterated analogs, have been studied by Courtney and Shannon[5b] who concluded

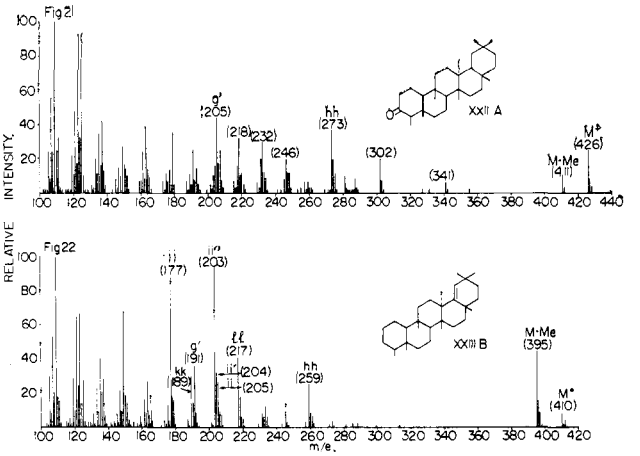
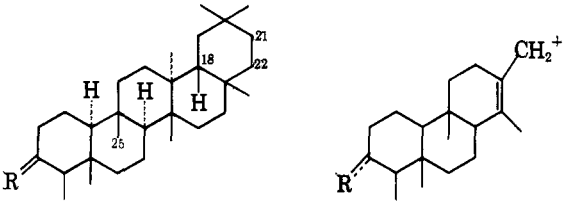
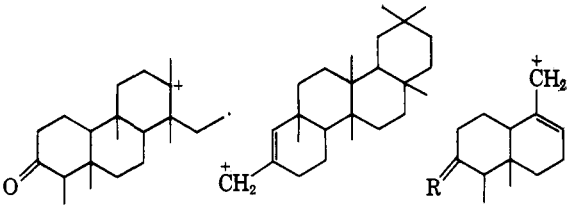


Fig. 21.—Mass spectrum of friedelan-3-one (XXIIa).
Fig. 22.—Mass spectrum of Δ^{18}-friedelene (XXIIIb).

that the keto group in the latter compounds are located at C-21 or C-22 and C-25, respectively. Further, they showed that the fragment characteristic for saturated friedelanes stubstituted only in ring A or E is species hh. Two additional ions (*m/e* 302 and 341) in the spectrum (Fig. 21) of friedelan-3-one (XXIIa) were identified by the Australian investigators.[5b]



XXIIa, R = O
b, R = H_2 hh, *m/e* 273, R = O



m/e 302 *m/e* 341 q′, *m/e* 205, R = O

Comparison of the spectra of friedelane (XXIIb) and its 3-ketone XXIIa (Fig. 21) allows recognition of the analog g′ (*m/e* 191 in XXIIb, *m/e* 205 in XXIIa) of species g mentioned earlier, as well as of three other peak groups (*m/e* 218, 232, 246) which most likely represent species g′ with one, two, and three additional carbon atoms.

The Δ^{18}-unsaturated analogs XXIIIa and b exhibit (see Fig. 22) a very pronounced loss of methyl as is to be expected by the allylic activation of four quaternary methyl groups. Fragment g is recognizable (*m/e* 191 in XXIIIb (Fig. 22 and *m/e* 205 in XXIIIa), while a very abundant fragment occurs in the spectra of both compounds at *m/e* 177 (jj). This is reminiscent of Δ^{18}-oleanene (VIIIc, section 1(e)) and a mechanism similar to the one yielding species w may be invoked here, the postulated precursor being the *m/e* 205 ion (ii, analog to u′).[16]

Cleavage without hydrogen transfer yields species is (*cf.* u), which, by losing methyl, gives kk (*m/e* 189 in Fig. 22), while hydrogen transfer in the opposite direction to that indicated in XXIII (from C-15 to C-8) gives rise to the most abundant ion in this group ii′′

(16) The presence of a weak metastable ion at *m/e* 154 (calcd. 153 for the transition *m/e* 205 → 177) seems to confirm this fragmentation process.

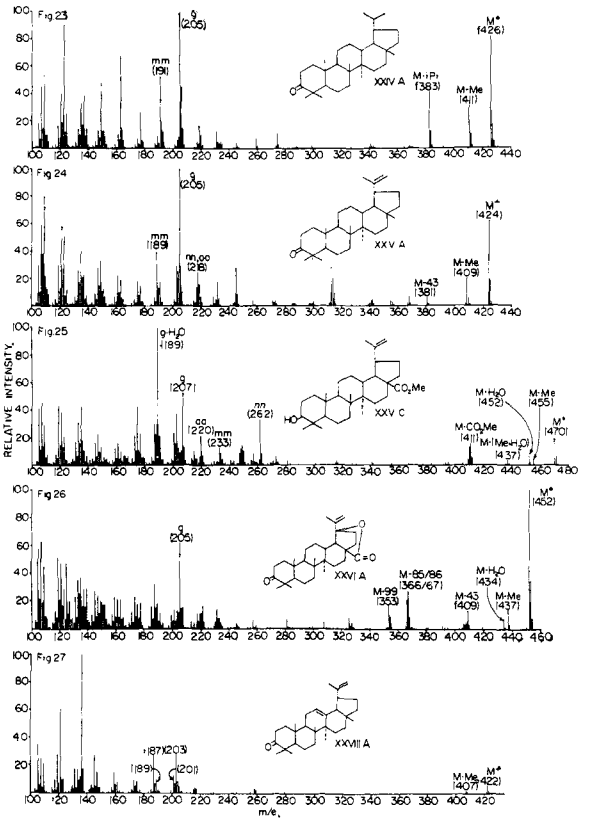


Fig. 23.—Mass spectrum of lupan-3-one (XXIVa).

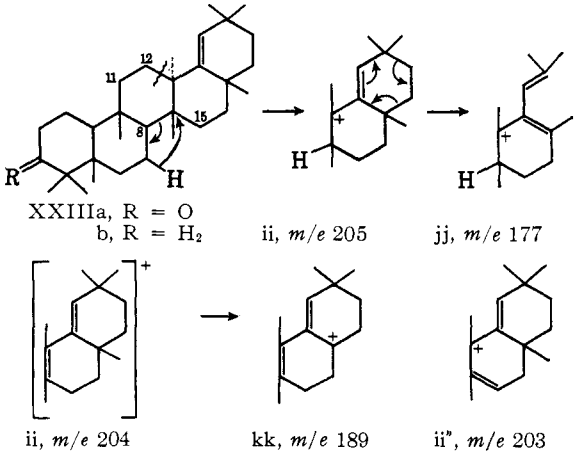
Fig. 24.—Mass spectrum of lupen-3-one (XXVa).

Fig. 25.—Mass spectrum of methyl betulinate (XXVc).

Fig. 26.—Mass spectrum of thurberogenone (XXVIa).

Fig. 27.—Mass spectrum of Δ^{12}-dehydrolupen-3-one (XXVIIIa).

(*m/e* 203). An ion of mass *m/e* 217 (ll) has to be derived from the right-hand part of the molecule and comprises ii'' with an additional CH_2 group. Fragment hh can be observed at *m/e* 259 (XXIIIb, Fig. 22) and 273 (XXIIIa), respectively.

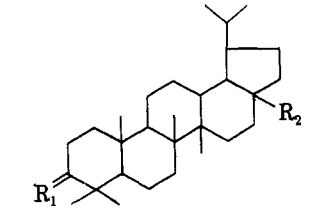


XXIIIa, R = O
b, R = H_2 ii, *m/e* 205 jj, *m/e* 177

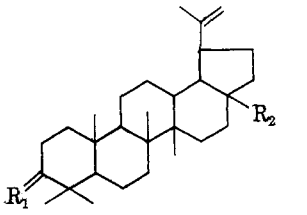
ii, *m/e* 204 kk, *m/e* 189 ii", *m/e* 203

(4) **Lupane Derivatives.**—This series is characterized by contraction of ring E to a five-membered ring to which an isopropyl or isopropenyl group is attached. The loss of 43 mass units (C_3H_7) is very pronounced in certain members, but becomes minimal in highly substituted derivatives or in the presence of an isopropenyl function.

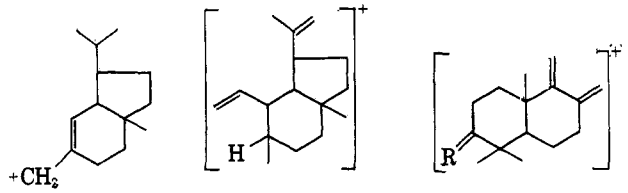
(a) **Saturated Lupanes.**—Lupane-3-one (XXIVa, Fig. 23) exhibits loss of methyl (*m/e* 411) and isopropyl (*m/e* 383). The most abundant fragment, however, occurs at *m/e* 205 and corresponds to species g, observed to some extent in the spectra of all pentacyclic triterpenes. This is the only peak in the spectrum, except for the molecular ion and the M − 15 and M − 43 species, which is shifted upon deuteration at C-2, and therefore fragment g is the only one comprising ring A. The *m/e* 191 ion can be allocated structure mm, since the major part of it shifts to *m/e* 189 in lupen-3-one (XXVa). Most of the other characteristic peaks, however, are made up of at least two different components, as shown by a comparison of the spectra (Fig. 23 and 24) of XXIVa and XXVa. These peaks become split in the lupen-3-one (XXVa) spectrum (Fig. 24), indicating the existence of ions with and without the isopropenyl grouping. In the parent hydrocarbon lupane (XXIVb), species g and mm coincide so that *m/e* 191 is by far the most abundant peak in the upper part of the spectrum. The pattern below *m/e* 191 is nearly identical with that of XXIVa, indicating again that virtually all of these fragments lack ring A.



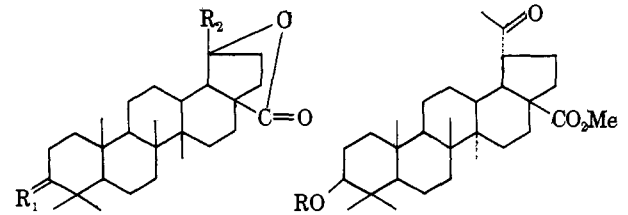
XXIVa, R_1 = O; R_2 = CH_3
b, R_1 = H_2; R_2 = CH_3
c, R_1 = (H)OAc; R_2 = CH_2OAc



XXVa,	R_1	R_2
	O	CH_3
b,	(H)OH	COOH
c,	(H)OH	COOMe
d,	(H)OAc	CH_2OAc



mm, *m/e* 191 nn, *m/e* 218 oo, *m/e* 220, R = OH

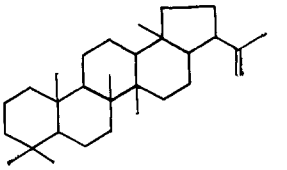


XXVIa, R_1 = O; R_2 = C_3H_5
b, R_1 = O; R_2 = C_3H_7
c, R_1 = (H)OH; R_2 = C_3H_5

XXVIIa, R = H
b, R = Ac

Nevertheless, it is worthwhile to identify two additional fragments in the spectrum (Fig. 24)[17] of XXVa, since they are found in several related compounds and may serve for identification purposes. They coincide at *m/e* 218 and may be represented by nn and oo (both of these fragments are virtually absent in the spectrum (Fig. 23) of lupan-3-one (XXIVa)). In betulinic acid and its methyl ester XXIVb and c, species g (*m/e* 207) and its dehydrated form (*m/e* 199) are the most important fragments (see Fig. 25), but the peaks at *m/e* 233

(17) Hopene II exhibits a rather similar spectrum, with peaks at g (*m/e* 191), nn (*m/e* 204), *m/e* 205, and mm (*m/e* 218), and a very intense molecular ion.



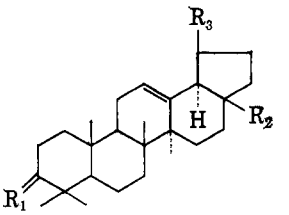
(mm), *m/e* 262 (nn), and *m/e* 220 (oo) can also be identified. In the spectra of betulin diacetate (XXVd) and its dihydro derivative XXIVc, species g − HOAc (*m/e* 189) dominates the spectrum and this is the only fragment that can be determined with certainty.

Thurberogenone (XXVIa, Fig. 26) and its dihydro derivative XXVIb exhibit the fragment g at *m/e* 205, but their most characteristic feature is a very pronounced molecular ion as well as cleavage in ring A (loss of 85/86 and 99 mass units).

This type of fragmentation has been observed with 4,4-dimethyl-3-keto steroids as well as with bicyclic-analogs.[18] The corresponding alcohol, thurberogenin (XXVIc), exhibits corresponding fragments of the mass M − 87 and M − 100/101.

The 30-norketone XXVIIa, derived from methyl betulinate (XXVc), and its acetate XXVIIb exhibit, besides the obvious loss of Me, $COCH_3$, and COOMe, again species g as the only fragment of any importance in the upper part of the spectrum.

(b) **Δ^{12}-Lupenes.**—In the first part of this article, it was shown that the presence of a Δ^{12}-double bond in α- and β-armyrin derivatives gives rise to a very characteristic type of fragmentation, *viz.* retro-Diels–Alder decomposition of ring C. Surprisingly, the members of the lupane series investigated by us (XXVIIIa–XXVIIIe) exhibited this type of fragmentation only to a very minor extent (*m/e* 216, XXVIIIa—see Fig. 27). The most characteristic peaks occur at *m/e* 187, 189, 201, and 203 in both 12,13-dehydrolupenone (XXVIIIa, Fig. 27) and the corresponding 3-acetate XXVIIIb, while in 12,13-dehydro-20,30-dihydrolupeol (XXVIIIc)



	R_1	R_2	R_3
XXVIIIa,	O	Me	C_3H_5
b,	(H)OAc	Me	C_3H_5
c,	(H)OH	Me	C_3H_7
d,	(H)OAc	CH_2OAc	C_3H_5
e,	(H)OAc	CH_2OAc	C_3H_7

peaks at *m/e* 189, 191, and 204 are noticed. The formation of species g was not observed in either case. The only noteworthy feature of 12,13-dehydrobetulin diacetate (XXVIIId) and its 20,30-dihydro derivative XXVIIIe is the very pronounced loss of CH_2OAc, much more abundant than in the ring C-saturated parents XXIVc and XXVd. Evidently, the change of size of ring E, coupled with the altered (18α) stereochemistry of the D/E junction,[19] modifies the steric strain of the system to such an extent that ring C does not become any more the most vulnerable point, even in the presence of the retro-Diels–Alder prone 12–13 double bond.

(5) **General Conclusions.**—It can be seen from the foregoing discussion that the mass spectra of pentacyclic triterpenes based on an actual or modified oleanene or ursene skeleton offer valuable information and that in many cases an unknown compound can be assigned to a certain structural type. In addition the location of substituents can be narrowed down considerably. However, it should be emphasized that the spectra of at least two derivatives of an unknown compound should be measured. In this manner, it can be seen which peaks shift, thus uncovering incidental overlap of peaks with equal mass as well as indicating from which part of the molecule a certain fragment is derived. In general, the fewer functional groups are present in the molecule, the clearer the fragmentation pattern will be, because alternate bond fission, not typical for a specific skeleton, will be less frequent. This should be kept in mind when dealing with an unknown triterpene.

The mass spectra of lupane derivatives seem to be much less characteristic and only in the simplest cases are a few fragments outstanding enough to offer useful information. Therefore, only the molecular weight and information about the presence of certain functional groups can be derived in the majority of members of this class. It should be recalled that the optical rotatory dispersion curves of carbonyl-containing triterpenes[20] have proved very useful for locating certain substituents on the pentacyclic framework. A combination of mass spectrometry and optical rotatory dispersion thus can often give extremely valuable structural information among this group of natural products with approximately 1–2 mg. of substrate.

Experimental

Most of the samples could be run using an all-glass inlet system (heated to 250°) and only in the case of free acids was it necessary to employ a direct inlet system.[6] For polyhydroxy and acetoxy compounds, the use of a direct inlet system[5b,6] may be of advantage, since the thermal decomposition of such substances (*i.e.*, loss of water or of acetic acid) is very much reduced and the actual molecular ion (rather than an M − 18 or M − 60 peak) can be observed.

The spectra were measured using a CEC-21-103C mass spectrometer. The ionization energy was 70 e.v. and the ionizing current 50 μa. The samples came largely from our own collection or represented specimens obtained in connection with our earlier rotatory dispersion studies[20] of triterpenes.

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(18) E. Lund, H. Budzikiewicz, J. M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 1528 (1963).

(19) This change of 18β- to 18α-stereochemistry was shown in the β-amyrin series to cause a major reduction in the occurrence of this retro-Diels–Alder fragmentation.

(20) C. Djerassi, J. Osiecki, and W. Closson, *J. Am. Chem. Soc.*, **81**, 4587 (1959).