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MASS SPECTROMETRY OF GIBBERELLINS—II*

THE LOCATION OF THE DOUBLE BOND IN THE GIBBANE SYSTEM

V. I. Zaretskiĭ, N. S. Wulfson and I. B. Papernaǰa
Institute for Chemistry of Natural Products, USSR Academy of Sciences, Moscow†

and

I. A. Gurvich, V. F. Kucherov, I. M. Milstein, E. P. Serebryakov
and A. V. Simolin
Zelinsky Institute of Organic Chemistry, USSR Academy of Sciences, Moscow

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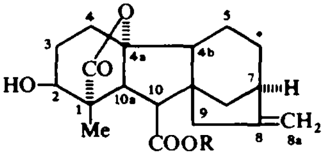
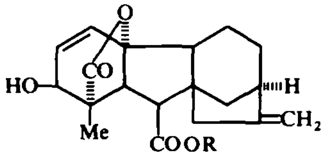
Abstract—Fragmentation patterns of gibberellin A_4 methyl ester (I) and its 2-keto-7α-hydroxy-8,8a-dihydro analog (X) as well as mass spectra of some Δ^3- and Δ^4-unsaturated compounds of the gibberellin series (including gibberellin A_7 methyl ester, III) have been investigated. Gibberellin derivatives, unsaturated in the ring A, give different mass spectra, depending on the position of the double bond. Therefore, these compounds can be the assigned Δ^3- or Δ^4-structure on the basis of mass-spectrometric evidence.

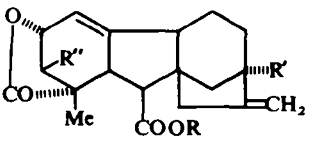
As shown earlier,[1] the method of mass spectrometry can be used to identify gibberellins A_1 and A_3 (in form of their alkyl esters) as individual compounds or as mixtures. We report now the mass-spectra of alkyl esters derived from gibberellin A_4 (I, II), gibberellin A_7 (III, IV), "Δ^4-iso-gibberellin A_3" (V, Va, VI) and "Δ^4-iso-gibberellin A_7 (VII). In addition the mass spectra of several 2-keto analogs, (VIII, VIIIa, IX) and (X, Xa, Xb, XI) have been investigated.



I: R = Me
II: R = Et



III: R = Me
IV: R = Et



V: R = Me, R' = R'' = OH
Va: R = Me, R' = R'' = OD
VI: R = Et, R' = R'' = OH
VII; R = Me, R' = H, R'' = OH

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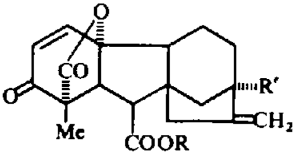
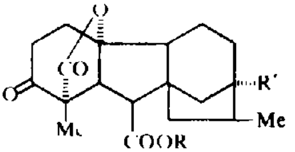
* Part I, see [1].

† The Laboratory of Mass Spectrometry of this Institute.



VIII: R = Me, R′ = OH
VIIIa: R = Me, R′ = OD
IX: R = Et, R′ = OH



X: R = Me, R′ = OH
Xa: R = Me, R′ = OD
Xb: R = Me, R′ = OH, 3,4,8,8a-d_4
XI: R = Et, R′ = OH

Analysis of the mass spectra reveals that a change in the position of the double bond in the gibbane system brings about a remarkable change in the pattern of fragmentation induced by electron impact. Consequently, derivatives of gibberellins A_3 and A_7 can be distinguished from their Δ^4-isomers.

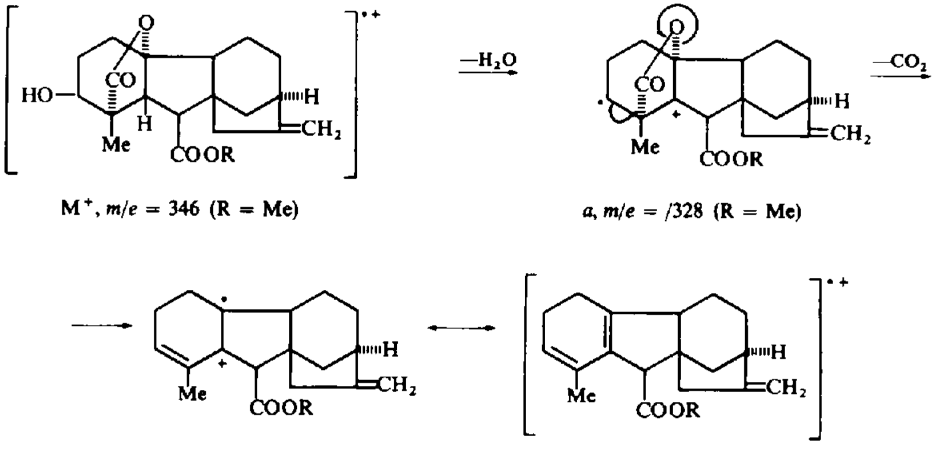
A comparison of the mass spectra of the alkyl esters of gibberellins A_1 and A_3[1] with those of compounds I–XI reveals the common characteristic peaks corresponding to the elimination of water, ROH and HCOOR from the molecular ion (Figs 1, 2, 3). The pattern of further fragmentation of the M-H_2O, M-ROH and M-HCOOR ions depends on the structural features of each compound.

(A) *Alkyl esters of gibberellins* A_4 *and* A_7

A comparison of the mass spectrum of I with that of III shows that the main characteristic peaks differ by two mass units (Figs 1a and 1b), and corresponds to the difference in molecular weight of these compounds. Therefore, a detailed analysis of their mass spectra can be carried out on the example of gibberellin A_4 methyl ester (I).

Peak at m/e 284 (*ion* a_1). The formation of ion a_1 ($m/e = 284$ when R = Me or $m/e = 298$ when R = Et) is the result of elimination of water and CO_2 from the molecular ion (Scheme 1).

SCHEME 1



M^+, $m/e = 346$ (R = Me)

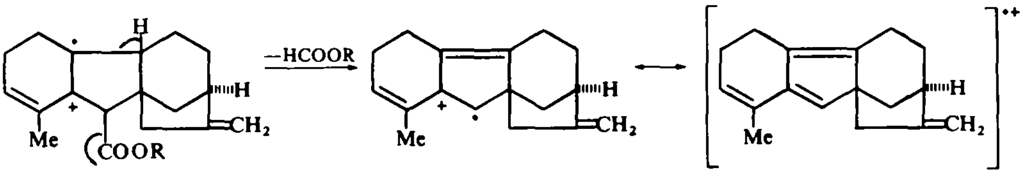
a, m/e = /328 (R = Me)

a_1, $m/e = 284$ (R = Me)

This mechanism of formation of ion a_1 is supported by the spectrum of gibberellin A_7 ethyl ester (IV) which shows a metastable peak, at m/e 257 ($296^2/340 = 257{\cdot}5$), corresponding to the transformation of ion $M\text{-}H_2O$ (m/e = 340) into $M\text{-}H_2O{-}CO_2$ ion (m/e = 296).

Peak at m/e 224 (*ion* a_2). This is the most intense peak in the spectrum of I. The corresponding ion contains no alkoxy group since the spectrum of the ethyl ester II displays the same highly intense peak. Ion a_2 may originate from the molecular ion either by consecutive loss of water (ion a), CO_2 (ion a_1) and $HCOOCH_3$ (ion a_2) or by elimination of $HCOOCH_3$ and then H_2O and CO_2. Since the mass spectrum of II reveals the presence of a metastable peak at m/e 169 ($224^2/298 = 168{\cdot}5$) the former sequence ($a \rightarrow a_1 \rightarrow a_2$) seems to be prevailing. The preponderance of this pattern of fragmentation may be due to the formation of a system with conjugated double bonds (Scheme 2).

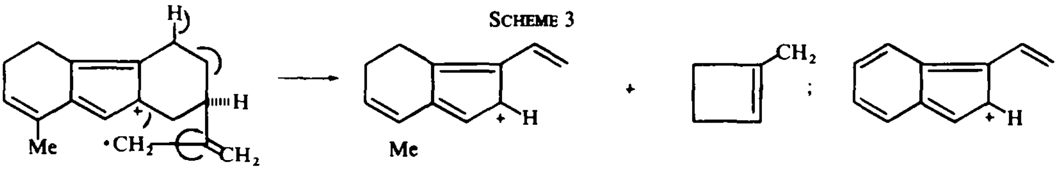
SCHEME 2



a_1, m/e = 284 (R = Me) a_2, m/e = 224

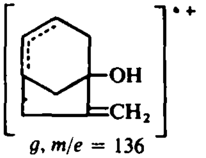
Peak at m/e 155 *and* 157 (*ion* a'_3 *and* a_3). The mass spectra of the gibberellin A_7 derivatives (III, IV) differ from those of their more saturated analogs (I, II) by the presence of an isolated peak at m/e 155 (ion a'_3). The spectra of I and II contain in the corresponding region (m/e 157–155) three peaks of comparable intensity (Figs 1b and 1a). The predominant formation of a fragment of mass 155 in the case of III and IV may be explained by a greater degree of conjugation in ion a'_3 as compared with the corresponding ion a_3 (m/e 157) in gibberellin A_4 series. The latter must arise from I and II by the same type of cleavage as ion a'_3. Both fragments are likely to contain ring A, for the mass numbers of ions a_3 and a'_3 differ by two mass units (Scheme 3).

SCHEME 3



a_2, m/e = 224 a_3, m/e = 157 (from I or II) a'_3, m/e = 155 (from III or IV)

In contrast with alkyl esters of gibberellins A_1 and A_3 which possess an additional OH at C-7, the alkyl esters of gibberellins A_4 and A_7 produce no abundant ions of type g with m/e 120 upon electron impact (in the case of gibberellin A_3 alkyl esters ion g had m/e 136[1]).



g, m/e = 136
(from gibberellin A_3 alkyl esters)

It is noteworthy that, unlike the mass spectrum of gibberellin A_1 methyl ester which contains a noticeable peak at *m/e* 275,[1] the mass spectrum of I does not exhibit analogous peaks at *m/e* 259.

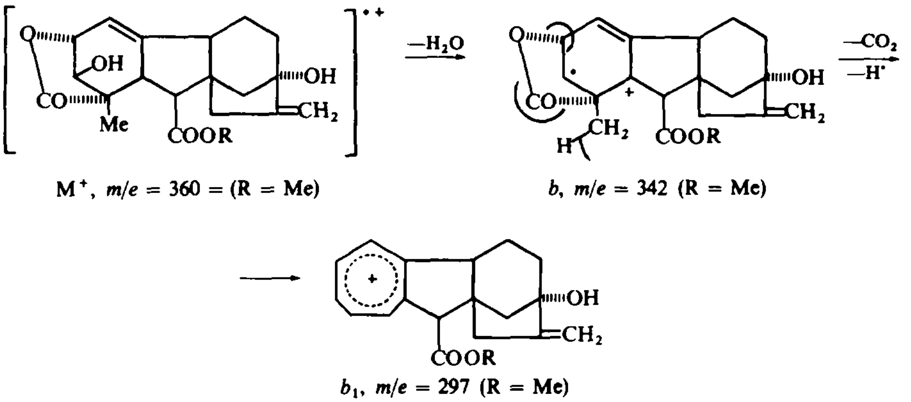
(B) *Alkyl esters of "Δ^4-isogibberellins"*

The shift of the double bond from Δ^3- to Δ^4-position in the gibbane system involves considerable changes in the pattern of fragmentation upon electron impact for compounds of this group. Thus, in the case of esters V, VI and VII the main pathway is a rearrangement process in which one molecule of water, the lactonic bridge and one hydrogen atom are eliminated.

Since the esters of "Δ^4-isogibberellin A_3" (V–VI) and "Δ^4-isogibberellin A_7" (VII) virtually follow the same path of fragmentation (Figs 2a and 2b), the mass numbers of the main characteristic peaks in the spectra of V and VII differing by 16 mass units, the course of fragmentation can be studied in detail *via* "Δ^4-isogibberellin A_3" esters (V–VI).

Peak at m/e 297 (*ion* b_1). This peak, which is the most intense in the spectrum of V, must be due to the elimination of the lactonic bridge together with one hydrogen atom and one molecule of water from the molecular ion. Ion b_1 may originate from the molecular ion by two alternative pathways: either M-H_2O (ion *b*) → M-H_2O—(CO_2 + $H^\cdot$) or M-(CO_2 + $H^\cdot$) (ion *c*) → M-(CO_2 + $H^\cdot$)—H_2O. Which of these actually takes place is not known since no metastable peak could be detected in the spectra of V and VII. Tentatively one may consider the formation of a tropilium ion (Scheme 4).

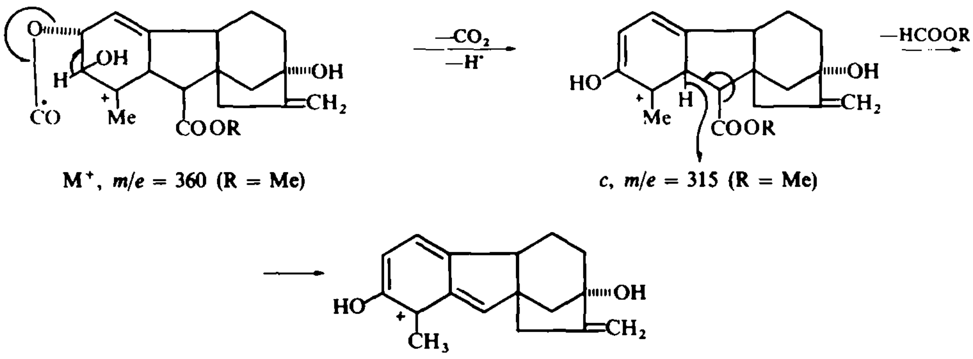
SCHEME 4



Ion b_1 contains the ring C and carbalkoxy group, for in the mass spectra of VI and VII the mass numbers of this ion are shifted by fourteen (to *m/e* 311) and sixteen (to *m/e* 281) mass units, respectively. In the case of the dideutero compound Va the mass number of ion b_1 is displaced only to *m/e* 298. This fact, together with the observation that the mass number of the most intense peaks in the spectra of V and VII differ by 16 mass units, implies that ion b_1 no longer contains the OH group at C-2 whereas the centre at C-7 remains unaffected.

Peak at m/e 255 (*ion* c_1). Ion c_1 originates from the molecular ion either by successive elimination of the lactonic bridge together with one hydrogen (ion c) and one molecule of alkyl formiate (ion c_1) (Scheme 5) or by an alternate pathway: M—HCOOR → M—HCOOR—(CO$_2$ + H$^\cdot$).

Scheme 5



M$^+$, m/e = 360 (R = Me)

c, m/e = 315 (R = Me)

c_1, m/e = 255

The mechanism of formation of this ion is substantiated by the mass spectra of the 7-desoxy analog VII, dideutero compound Va and ethyl ester VI. In the first of these, the mass number of ion c_1 is shifted by 16 mass units to m/e 239, in the spectrum of Va it is shifted by two mass units to m/e 257 and remains unaffected (m/e = 255) in the spectrum of VI.

Peak at m/e 237 (*ion* b$_2$). The formation of ion b_2 (m/e 237) may proceed either by elimination of HCOOR from ion b_1 or by dehydration of ion c_1. The presence of a metastable peak at m/e 190 in the spectrum of V shows that the former pathway (i.e. the transition from m/e 297 to m/e 237) is more likely to occur. The mechanism of formation of ion b_2 follows from the inspection of the mass spectra of VII, Va and VI, as in the case of ion c_1.

Peak at m/e 209 (*ion* b$_3$). The mass number of this peak remains unaffected in the spectrum of VI, whereas in the case of Va and VII it is shifted by one (to m/e 210) or sixteen (to m/e 193) mass units, respectively. One may assume that ion b_3 is formed from ion b_2 upon the loss of C-5 and C-6 in form of ethylene.

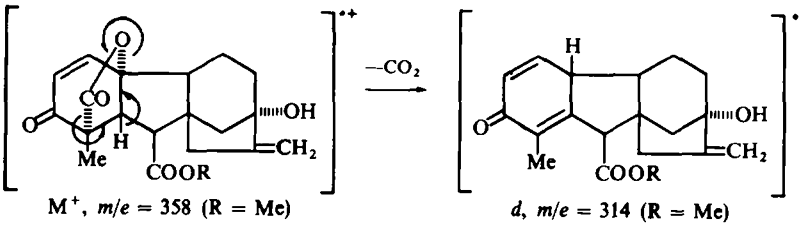
Peak at m/e 155. This peak, revealing the presence of a double bond in the ring A of gibberellin A$_7$, is also present in the spectrum of VII and, to a smaller degree, in the spectra of V and VI. The formation of a fragment with m/e 155 from VII is probably analogous to the formation of ion a'_3 from III and IV.

(C) 2-*Keto analogs of gibberellin* A$_1$ *and* A_3 *alkyl esters*

Unsaturated ketones VIII, IX. In the spectra of ketones VIII and IX, derived from gibberellin A$_3$, the peaks of molecular ions are more prominent than in the spectra of their 2-hydroxy analogs. At the same time the contribution of the M-H$_2$O peaks (m/e 340 or 354) and M-ROH peaks (m/e 326) decreases, and the intensity of the peaks corresponding to the elimination of the lactonic bridge (m/e 314, 286, 254 etc.) becomes much higher.

(a) *Peak at* m/e 314 (*ion* d). Ion d (M-CO$_2$) is formed by loss of the lactonic bridge from the molecular ion. It must retain the carbalkoxy group and the OH at C-7 since in the mass spectra of the ethyl ester IX and dideutero compound VIIIa the

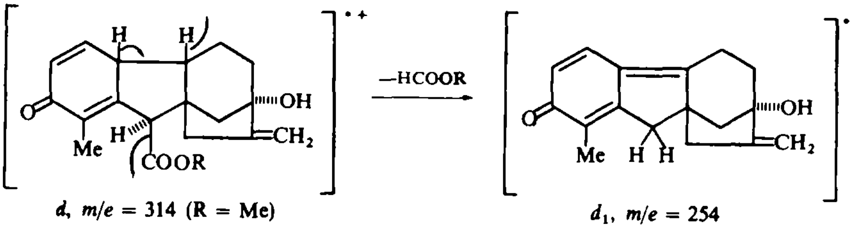
Scheme 6



mass number of ion *d* peak is shifted to *m/e* 328 or to *m/e* 315, respectively (Scheme 6).

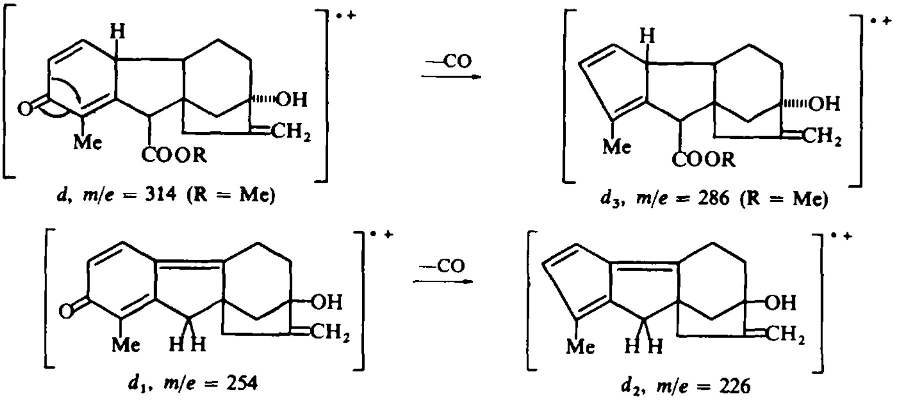
(b) *Peak at* m/e 254 (*ion* d_1). This fragment originates from the molecular ion either by the sequence M-CO_2 → M-CO_2—HCOOR or by the alternatife pathway M-HCOOR → M-HCOOR—CO_2. The mechanism of its formation is substantiated by the mass spectra of IX and VIIIa. In the first case the mass number of ion d_1 peak remains unaffected while in the case of VIIIa a displacement by one mass unit (to *m/e* 255) is observed. The formation of ion d_1 from ion *d* is tentatively shown on Scheme 7.

Scheme 7



(c) *Peaks at* m/e 226 (*ion* d_2) *and* 286 (*ion* d_3). Ions d_2 and d_3 (*m/e* 226 and *m/e* 286) seem to arise from ions d_1 and *d*, respectively, by expulsion of 28 mass units (in form of CO or ethylene). Their origin can be deduced from the fact that in the spectrum of the ethyl ester IX the peak of d_2 remains undisturbed (*m/e* 226) while the peak corresponding to d_3 is displaced to *m/e* 300. In the spectrum of VIIIa both peaks are shifted by one mass unit each to *m/e* 227 and *m/e* 287, respectively (Scheme 8).

Scheme 8

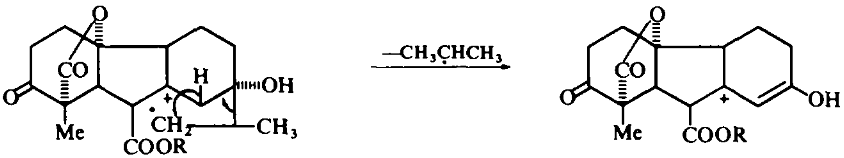


(d) *Peak at* m/e 136 (*ion* g). It is interesting to note that the spectra of VIII and IX display an intense peak at *m/e* 136 which in the case of gibberellin A_3 alkyl esters represents the most abundant ion.[1] The appearance of this peak in the spectra of VIII and IX seems to be due to the presence of a double bond in Δ^3-position. On the other hand, the fact that the mass number of ion *g* remains unaffected in the case of VIII and IX provides additional support to the mechanism of its formation discussed earlier.[1]

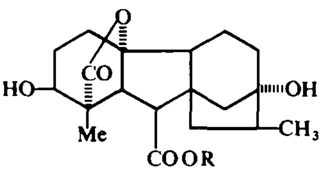
Saturated ketones X *and* XI. The spectra of ketones X and XI contain well pronounced peaks of a molecular ion which is reminiscent of the mass spectra of their unsaturated analogs, VIII and IX. However, the electron impact-induced fragmentation of the former differs considerably from all pathways discussed previously[1] and above. Thus, in the spectra of X and XI all peaks corresponding to the expulsion of the lactonic bridge and to the elimination of H_2O,ROH and HCOOR from the molecular ion are of moderate or low intensity. At the same time, the intensities of those peaks which correspond to the fragment M-43 (*m/e* 319 or 333) and to the products of its further decomposition are greatly increased.

(a) *Peak at* m/e 319 (*ion* f) is the most intense peak in the spectrum of X. Its origin from the molecular ion may be explained either by the ejection of C-2 and C-3 in form of CH_3CO or by elimination of C-8, C-8a and C-9 in form of an isopropyl radical (Scheme 9). Recognition of a metastable ion at *m/e* 281 in the spectrum of X is consistent with the transition from M^+ to M-43 ($319^2/362 = 281$). The mass spectrum of the 3,4,8,8a-d_4-ketone (Xb) in which the mass number of ion *f* is shifted by two mass units (to *m/e* 321) also corroborates the mechanism of formation of this ion, although it is difficult to decide which of the two possibilities occur. The ejection of a CH_3CO radical from the molecular ions of some angularly substituted β-decalones has been reported by Djerassi *et al.*[2] On the other hand, the mass spectrum of the compound XII exhibits a very intense peak M-43. This seems to imply that the destruction of ring D is operating, at least, to some extent, in the case of X.

SCHEME 9



M^+, *m/e* = 362 (R = Me) *f*, *m/e* = 319 (R = Me)



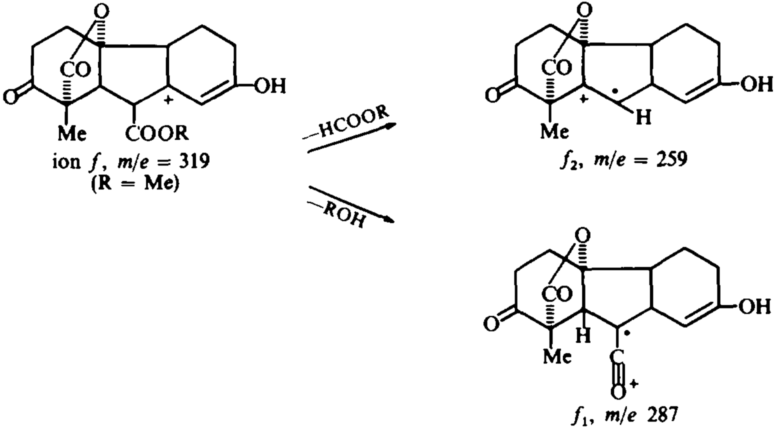
XII: R = Me

The presence of a carbalkoxy and OH groups in ion *f* is substantiated by the mass spectra of the ethyl ester XI and dideutero-compound Xa. In the first case, the mass number of ion *f* is shifted by 14 mass units (to *m/e* 333), in the second, by one mass unit (to *m/e* 320).

(b) *Peaks at* m/e 287 (*ion* f_1) *and* m/e 259 (*ion* f_2). Fragment f_1 is formed from ion f upon the loss of MeOH; the passage from *m/e* 319 to *m/e* 287 is supported by the appearance of a metastable peak at *m/e* 258 ($287^2/319 = 258$). The mechanism of formation of ion f_1 follows from the inspection of mass spectra of XI, Xa and Xb. In the spectrum of XI the mass number of ion f_1 remains unaltered while in the spectrum of Xa it is shifted by one mass unit (to *m/e* 288) and in the spectrum of the tetradeutero analog Xb, by two mass units (to *m/e* 289).

The peak at *m/e* 259 is due to the expulsion of 28 mass units from ion f_1 or to the elimination of HCOOR from ion f (Scheme 10). In the first case two alternative routes are possible: either the loss of C-5 and C-6 in form of ethylene or the cleavage of the bond between C-10 and the positively charged CO group. The origin of fragment f_2 is corroborated by a comparison of the mass spectra of XI, Xa and Xb as in the case of ion f_1.

SCHEME 10



(c) *Peaks at* m/e 301 (*ion* f_3) *and* m/e 273 (*ion* f_4) arise from ion f by successive expulsion of 18 and 28 mass units (H_2O and C_2H_4 or CO, respectively). The mechanism of formation of ions f_3 and f_4 is substantiated by the mass spectra of XI, Xa and Xb. The mass numbers of peaks f_3 and f_4 in the spectra of XI and Xb are displaced by fourteen mass units (to *m/e* 315 and 287) and two mass units (to *m/e* 303 and 275) respectively whereas in the spectrum of Xa both peaks remain unaffected.

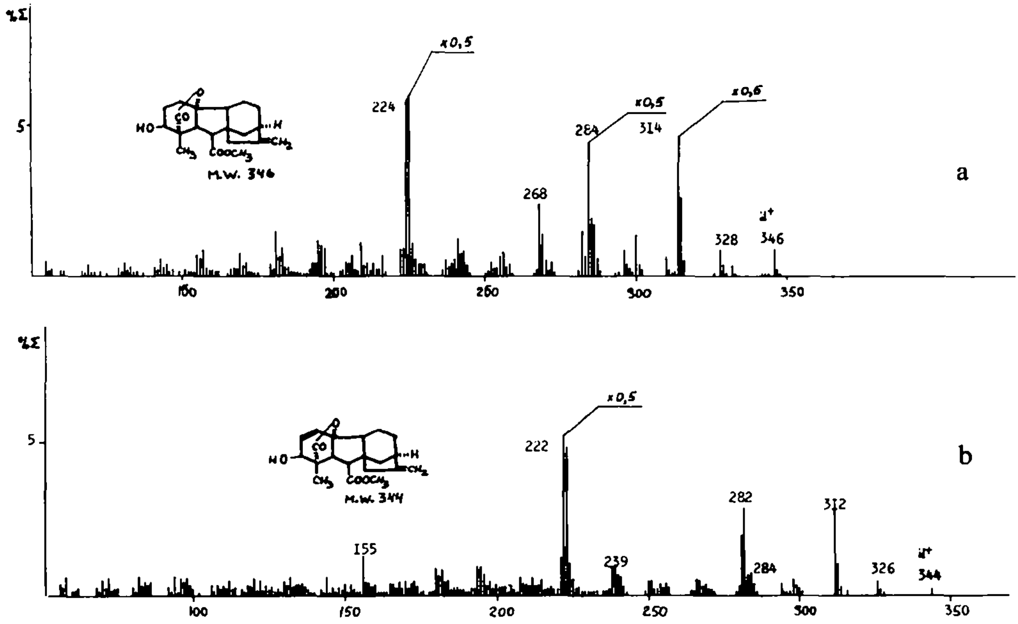


FIG. 1 Mass spectra of: (a) gibberellin A_4 methyl ester; (b) gibberellin A_7 methyl ester.

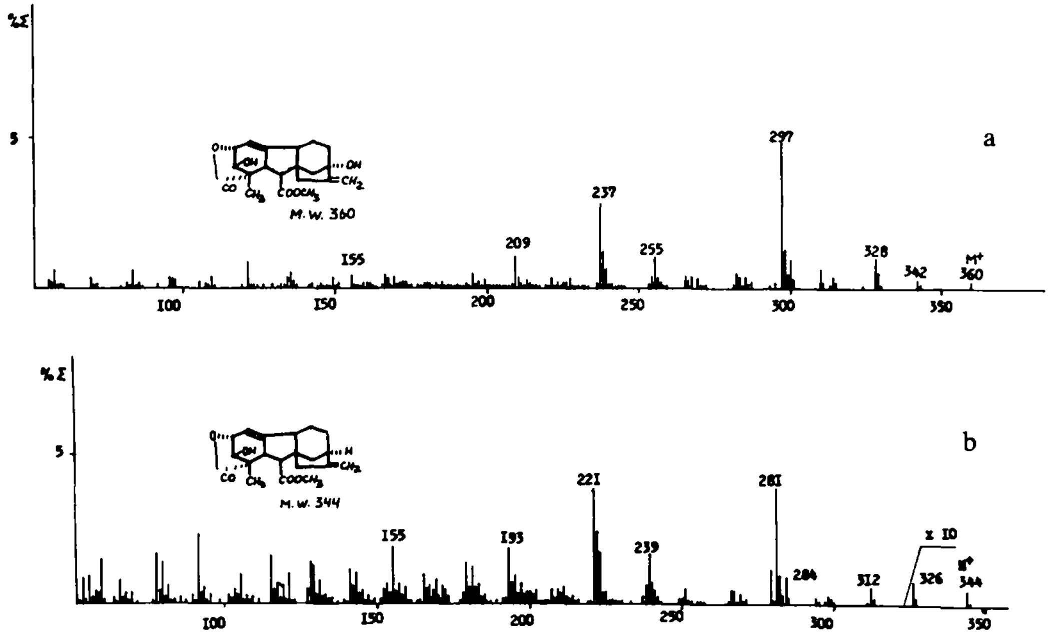


FIG. 2 Mass spectra of (a) Isogibberellin A_3 methyl ester; (b) Isogibberellin A_7 methyl ester.

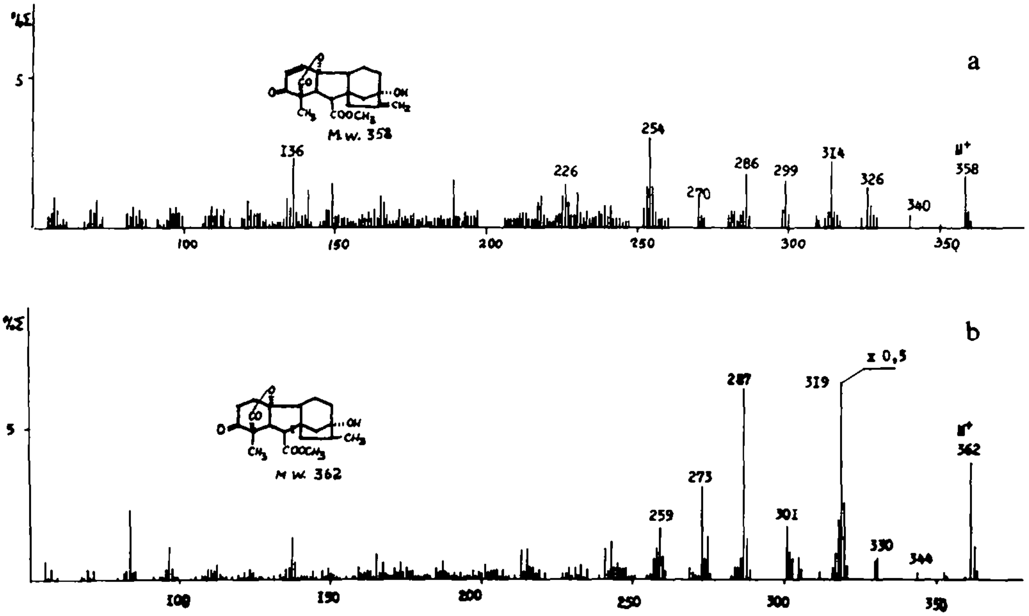


Fig. 3 Mass spectra of: (a) unsaturated ketone VIII methyl ester; (b) saturated ketone X methyl ester.

EXPERIMENTAL

All mass spectra were determined with a commercial MX-1303 mass spectrometer, equipped with an all-glass inlet system for the vaporization of samples directly into the ion source. Ionization energy was 24 — 30 ev and temp range 130 to 150° (±1°, temp stabilization).

Compounds I–X were prepared according to the known procedures and had the following constants (Table 1).

Table 1

Compound	M.p.	Compound	M.p.	Compound	M.p.
I[a]	175–179°	VI[a]	amorphous powder	VIII[a]	181–183°
II[a]	142–145°	Va[b]	103–108°	X[d]	131–132°
III[a]	105–168°	VII[a]	218–219°	XI[d]	76–78°
IV[a]	186–189°	VIII[c]	184–185°	Xa[b]	124–128°
V[a]	106–109°	IX[c]	gum	Xb[e]	126–127°

[a] Obtained by treatment of the corresponding pure acids with diazoalkane solutions. For I, III, V and VII the value of $[\alpha]_D^{20}$ was −6, 7, +34°, +112·5° and +72°, respectively.

[b] Obtained on repeated refluxing of V, VIII and X in MeOD (3 × 3 hr).

[c] Obtained from the corresponding esters of gibberellin A_3 according to Gurvich *et al.*[3]

[d] Obtained on hydrogenation of VIII and IX over Pd–C in ethyl acetate.

[e] Obtained as above, H_2 being replaced by D_2.

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