Mass Spectra of Methyl Abscisate and Isotopically Labelled Analogues

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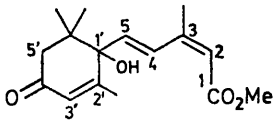
Metastable analysis, isotopic labelling, and high resolution measurements were used to study the cracking patterns of methyl abscisate. Ten labelled analogues of methyl abscisate and methyl 2-*trans*-abscisate were available for this purpose. The principal fragmentation pathway involves ions at *m/e* 222, 190, 162, 134, 106, and 91, and specific labelling of each oxygen atom with oxygen-18 proved especially helpful in defining the structures of the intermediate ions. Other important fragmentations of the molecular ion are the loss of methanol, the loss of water, and the cleavage of the complete side chain to give the abundant ion at *m/e* 125.

MASS SPECTROMETRY (m.s.) aided the original determination of the structure of abscisic acid,[1] and has since been used for the identification of this hormone, especially by the combined g.l.c.–m.s. technique.[2,3] The mass spectra of abscisic acid,[4] its methyl ester (I),[3] and a deuteriated analogue [parent acid of (III)] [5] are on record, but no detailed study of the fragmentations has been published (apart from some improbable recent suggestions [6]), nor has a systematic attempt been made to use labelled analogues or metastable analysis to elucidate the spectra.

To define the cracking patterns of abscisic acid derivatives under electron impact, several analogues of



(I)

methyl abscisate [(II)—(VIII)] and methyl 2-*trans*-abscisate [(X)—(XII)] labelled with deuterium and oxygen-18 were prepared. A sample of ethyl abscisate (IX) was also made for a comparison of its fragmentation characteristics. By comparing the *m/e* values of the principal fragment ions from (II)—(XII) with those of the parent compound (Table 1) and using metastable analysis, we can now explain the major features of the mass spectrum of methyl abscisate (I). The oxygen-18 labelling was especially valuable, permitting the fate of each atom to be accurately traced, until its expulsion from the charged species in question.

Metastable analysis of the mass spectrum of (I) suggests that the fragment ions at *m/e* 222, 190, 162, 134, 106, and 91 occur on the same fragmentation pathway and, as seen in Figure 1, they comprise most of the important ions formed from (I). The fragmentation sequence is clearly defined by the labelling data and is shown in Scheme 1. It is initiated by cleavage of the 4',5'-bond to give species *a* (*cf.* abscisic acid [1]), followed by ejection of a molecule of isobutene and cyclization to give ion *b*, *m/e* 222. The peak due to the corresponding ion from (III) appears cleanly at *m/e* 226, whereas in the parent ion region there are peaks for [$^2H_4$], [$^2H_5$], and [$^2H_6$] species. Therefore in the synthesis of (III) there

TABLE 1

Principal fragment ions (*m/e* values; relative abundance in parentheses) in the normalised mass spectrum of methyl abscisate and its labelled analogues at 70 eV

Compound:	(I)	(II)	(III)	(IV)	(V)	(VI)	(VII)	(VIII)	(IX)	(X)	(XI)	(XII)	(I)
M^+ [a]	278 (3)	279 (15) 278 (9)	284 (35) 283 (32) 282 (15)	282 (4) 280 (1)	280 (4) 278 (1)	280 (3) 278 (1)	280 (6) 278 (15)	280 (7) 279 (22)	292 (6)	278 (7)	280 (15) 278 (1)	280 (8) 278 (20)	278 (3)
$M - H_2O$ [a]	260 (17)	261 (16) 260 (26)	266 (20) 265 (10)	264 (18) 262 (13)	262 (18) 260 (3)	262 (13) 260 (5)	262 (15) 260 (40)	262 (3) 261 (17)	274 (33)	260 (16)	262 (15) 260 (12)	262 (15) 260 (35)	260 (11)
M – MeOH [a]	246 (15)	247 (25) [b] 246 (25)	252 (40) [b]	250 (13) 248 (15)	248 (10) 246 (13)	248 (12)	248 (15) 246 (25)	246 (30)	M – EtOH 246 (15)	246 (12)	248 (4)	248 (15) 246 (20)	246 (11)
$M - 56$ [a]	222 (13)	223 (23) 222 (15)	226 (40)	226 (15)	224 (15)	224 (10)	224 (15) 222 (25)	224 (3) 223 (18)	236 (10)	222 (40)	224 (12)	224 (30) 222 (50)	222 (10)
$M - 59$ [a]	219 (10)	220 (6) 219 (6)	225 (15)	221 (15)	219 (13)	221 (7)	221 (15) 219 (25)	219 (7)	$M - 73$ 219 (10)	219 (7)	221 (2)	221 (10) 219 (25)	219 (8)
$M - 88$	190 (100)	191 (50) 190 (100)	194 (100)	194 (73) 192 (100)	192 (80) 190 (100)	192 (100) 190 (15)	192 (62) 190 (100)	190 (100)	$M - 102$ 190 (100)	190 (100)	192 (100) 190 (10)	192 (68) 190 (100)	190 (100)
$M - 116$	162 (41)	163 (16) 162 (24)	166 (70)	164 (50)	162 (40)	164 (22)	164 (13) 162 (23)	162 (28)	$M - 130$ 162 (21)	162 (47)	164 (30)	164 (11) 162 (19)	162 (70)
$M - 117$	161 (14)	161 (6)	165 (20)	163 (19)	161 (16)	163 (12)	163 (13) 161 (12)	161 (11)	$M - 131$ 161 (8)	161 (12)	163 (10)	163 (15) 161 (11)	161 (22)
$M - 144$	134 (42)	134 (30)	138 (90)	136 (37) 134 (28)	134 (45)	136 (17) 134 (17)	136 (15) 134 (33)	134 (30)	$M - 158$ 134 (20)	134 (72)	136 (27) 134 (25)	136 (10) 134 (33)	134 (90)
$M - 153$	125 (34)	125 (60)	125 (98)	127 (47)	127 (52)	125 (43)	125 (58)	127 (9) 126 (30)	$M - 167$ 139 (27)	125 (12)	125 (13)	125 (20)	125 (50)
$M - 166$	112 (12)	112 (12)	112 (20)	114(17)	114 (20)	112 (18)	112 (19)	113 (10)	126 (6)	112 (7)	112 (8)	112 (13)	112 (14)
$M - 172$	106 (8)	106 (5)	110 (25)	106 (12)	106 (10)	106 (7)	106 (10)	106 (7)	$M - 186$ 106 (3)	106 (16)	106 (10)	106 (10)	106 (26)
$M - 188$	91 (19)	91 (8)	95—91 [b]	91 (28)	91 (26)	91 (20)	91 (24)	91 (23)	$M - 201$ 91 (10)	91 (45)	91 (29)	91 (30)	91 (78)
Instrument:	g.l.c.–MS30	MS902	MS902	g.l.c.–MS30	g.l.c.–MS30	g.l.c.–MS30	g.l.c.–MS30	MS902	g.l.c.–MS30	MS902	MS902	g.l.c.–MS30	MS902

[a] Relative abundances ×5. [b] Ambiguous value due to multiple overlap of isotopic species in the peak group.

[1] K. Ohkuma, F. T. Addicott, O. E. Smith, and W. E. Thiessen, *Tetrahedron Letters*, 1965, 2529.
[2] P. Gaskin and J. MacMillan, *Phytochemistry*, 1968, **7**, 1699.
[3] B. H. Most, P. Gaskin, and J. MacMillan, *Planta*, 1970, **92**, 41.
[4] J. W. Cornforth, B. V. Milborrow, G. Ryback, and P. F. Wareing, *Tetrahedron*, 1966, Suppl. 8, Part II, 603.
[5] J.-C. Bonnafous, L. Fonzes, and M. Mousseron-Canet, *Bull. Soc. chim. France*, 1971, 4552.
[6] A. Rapp and A. Ziegler, *Vitis*, 1971, **10**, 111.

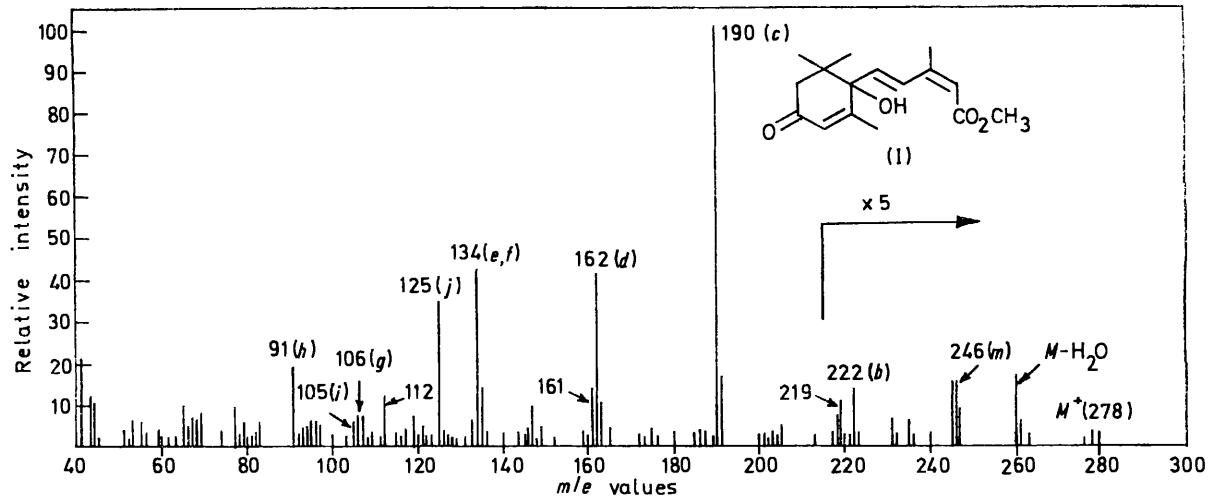
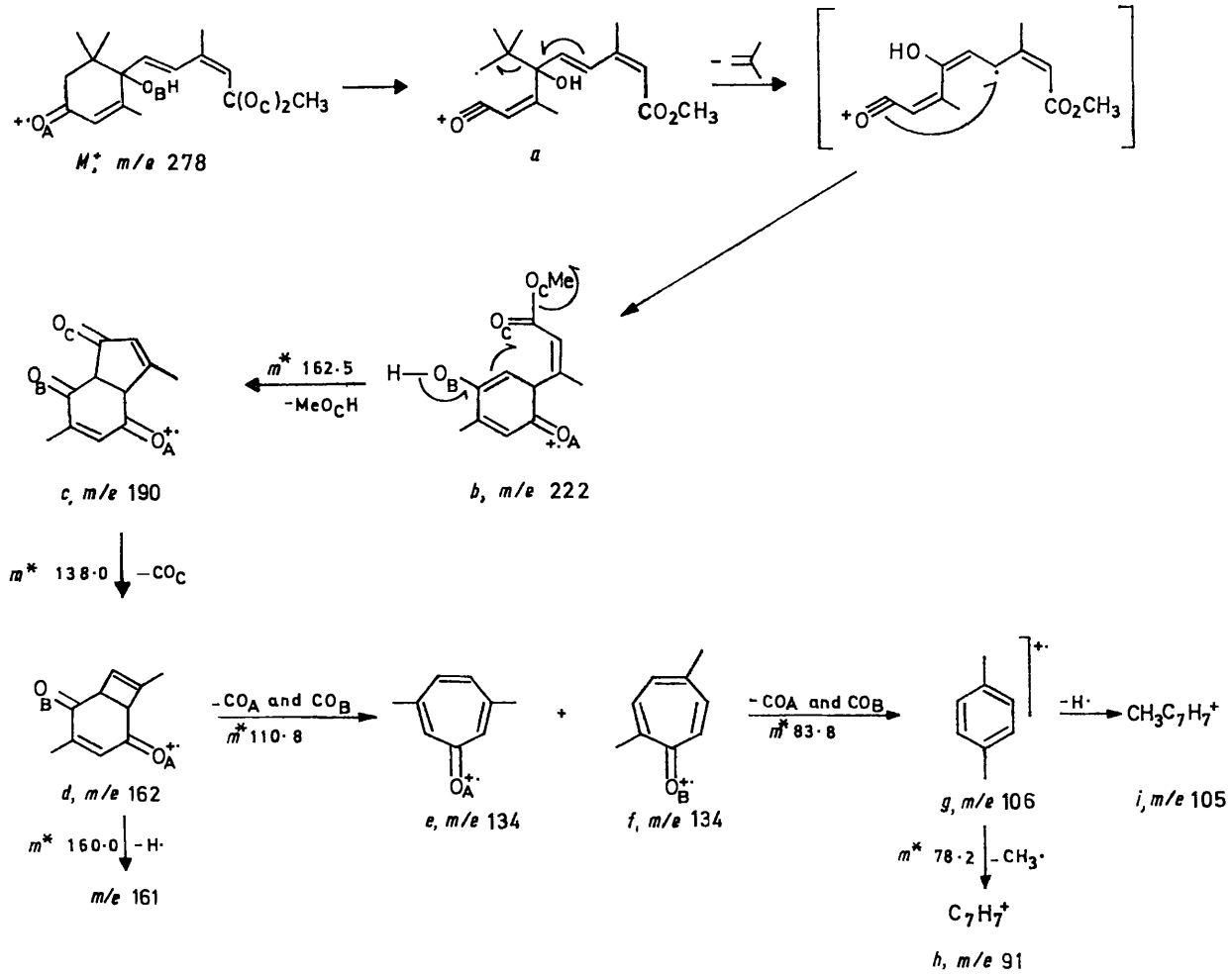


FIGURE 1 Mass spectrum of methyl abscisate (I)



SCHEME 1 Major fragmentation pathway of methyl abscisate

had been complete replacement of the protons at C-3′ and on the C-2′ methyl group by deuterium, but incomplete exchange at C-5′.

Fragment ion *b* decomposes further with loss of a molecule of methanol, the oxygen eliminated originating exclusively from the ester methoxy-group (O_0 in Scheme 1), as shown by the retention of only half the oxygen-18 label residing at C-1 in the corresponding ions from (IV) (Figure 2) and (V) (Figure 3). These two

The subsequent cracking pattern of the ion at *m/e* 190 (*c*), which represents the base peak of the spectrum, involves the consecutive loss of three molecules of carbon monoxide. For the identical fragmentation observed with the free acid of (I), this was confirmed by high-resolution measurements of the masses of ions at *m/e* 190 ($C_{11}H_{10}O_3$), 162 ($C_{10}H_{10}O_2$), and 134 ($C_9H_{10}O$). An abundant metastable ion at *m/e* 138·0 is observed for the first elimination, *m/e* 190 ⟶ 162 (*d*), which involves

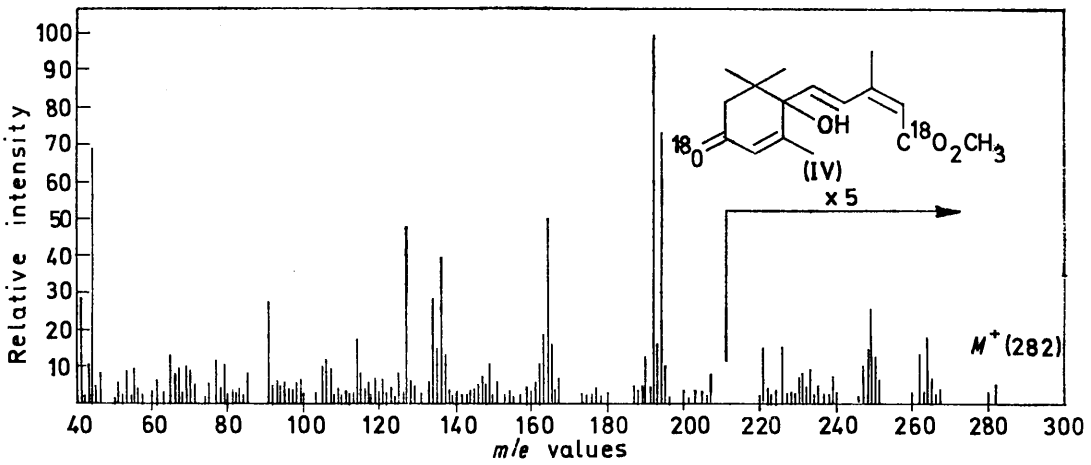


FIGURE 2 Mass spectrum of methyl [1,4′-$^{18}O_2$]abscisate (IV)

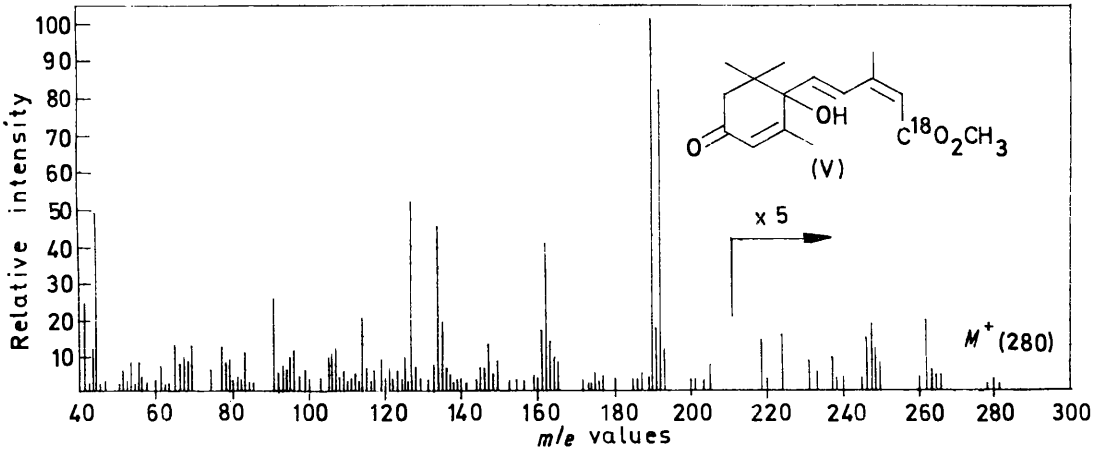


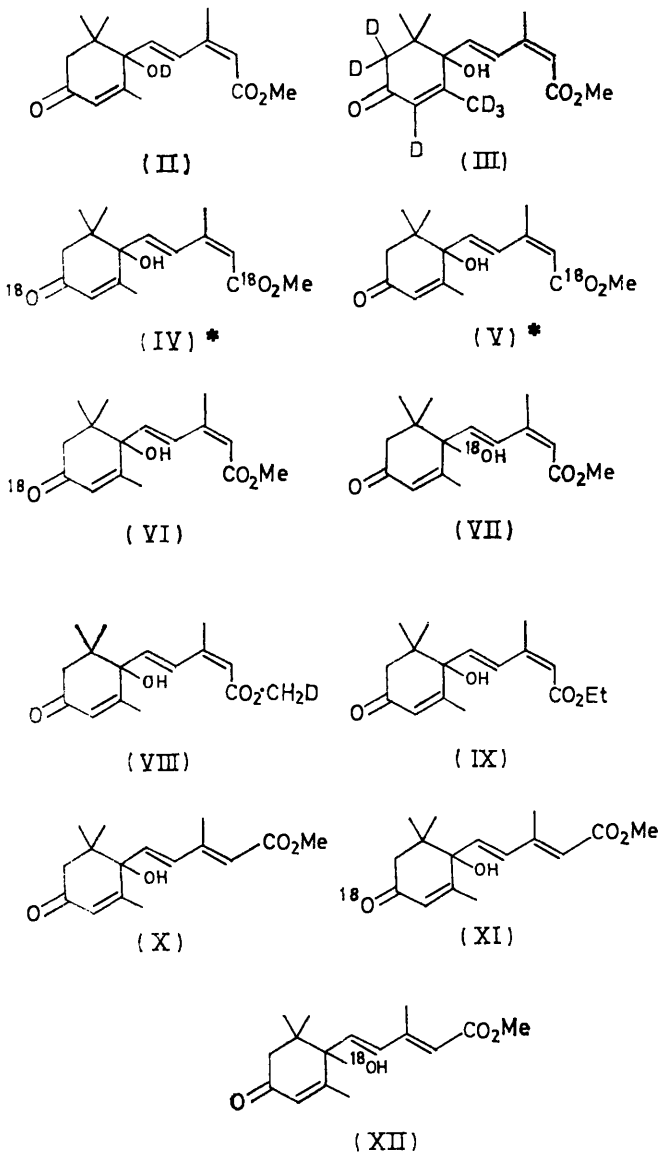
FIGURE 3 Mass spectrum of methyl [1-^{18}O]abscisate (V)

compounds were obtained by methylating the labelled free acids, in which the ^{18}O label was distributed equally between the two oxygen atoms of the carboxyl group. The origin of the additional hydrogen atom is slightly ambiguous, although it is probably lost exclusively from the C-1′ hydroxy-group as shown in Scheme 1. Partial retention of the deuterium label in the corresponding fragment ion from (II) is most probably the result of keto–enol tautomerisation in *b* before fragmentation (Scheme 2). This hypothesis is supported by the appearance of additional deuteriated ions at *m/e* 163, 135, and 92 in the spectrum of (II), which necessarily means deuterium originally on oxygen has at some stage migrated to carbon.

the oxygen atom originally at the ester carbonyl function in (I). The specificity of this elimination is clearly demonstrated by the appearance of the corresponding ion from (IV) (Figure 2), (VI) (Figure 4), and (VII) (Figure 5) at *m/e* 164 whereas that from (V) (Figure 3) occurs only at *m/e* 162.

Two metastable ions are observed for the decomposition of ion *d*, at *m/e* 160·0 and 110·8, the first resulting from loss of a hydrogen atom to give a relatively insignificant fragment ion at *m/e* 161. The second metastable ion originates from loss of another molecule of carbon monoxide giving a fragment ion at *m/e* 134. This ion may be conveniently represented by the ionized dimethyltropone structures *e* and *f*. As might

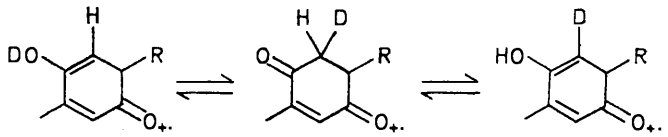
be expected from the near-symmetrical nature of *d*, the remaining oxygen atoms (O_A and O_B) contribute in about equal proportions to the molecule of carbon



* The ^{18}O in the ester groups of (IV) and (V) is distributed equally between the carbonyl and alkoxy-oxygen atoms.

monoxide lost in the transition *m/e* 162 ⟶ 134. Thus the equivalent ions from (IV), (VI), and (VII) are distributed fairly evenly between *m/e* 134 and 136, indicating that the oxygen atoms remaining in *e* and *f* originated equally from the ketone carbonyl group and the tertiary hydroxy-group in the molecular ion.

Following a cracking pattern similar to that described for other alkylated tropones,[7] both *e* and *f* decompose with loss of a further molecule of carbon monoxide to give the *p*-xylene ion *g*, *m/e* 106, for which a metastable ion at *m/e* 83·8 is observed. Another metastable ion, at *m/e* 78·2, arises from cleavage of a methyl group from *g* to give the tropylium ion *h*, *m/e* 91. The ion *g* also has the propensity for loss of a hydrogen atom to give the methyltropylium ion *i* (*m/e* 105), although a metastable ion for this transition is not observed. Similar

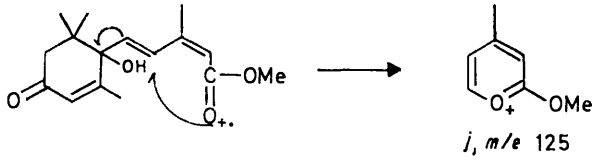


b, R = CMe:CH·CO_2Me

SCHEME 2

cracking patterns have been extensively reported for the corresponding parent aromatic hydrocarbons, whose mass spectra may now be predicted with high accuracy.[8]

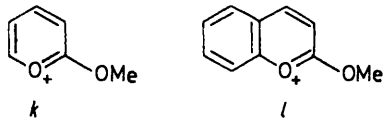
Apart from the principal cracking pattern described above, methyl abscisate undergoes several other less prominent fragmentations. The most important of these involves simple cleavage of the intact side chain, which results in the ion *j*, *m/e* 125. This is equivalent to that at *m/e* 111 in the mass spectrum of the free acid. The mechanism of formation of *j* (Scheme 3) is confirmed



j, *m/e* 125

SCHEME 3

by the labelling data. Thus the corresponding ion from (IV) (Figure 2) and (V) (Figure 3) occurs exclusively at *m/e* 127, and when the ester methyl group is replaced by monodeuteriomethyl (VIII) or ethyl (IX) the equivalent ions are found at *m/e* 126 and 139, respectively. Ions with structures similar to *j* have been reported from the cracking patterns of simple unsaturated esters (*e.g.* *k* from methyl sorbate [9]) and aromatic esters (*e.g.* *l* from



methyl cinnamate [10]). The ion current carried by this ion in the spectrum of methyl 2-*trans*-abscisate (X) is considerably smaller than that from the *cis*-isomer, as might be expected from the stereochemistry of the transition state required for its formation.

The mechanism of several other minor fragmentation pathways from (I) may be defined by using the labelling data at hand. Two of these, resulting in ions at *m/e* 112 and 219, involve the side chain of methyl abscisate, and although no metastable ions are observed, the *m/e* values

[7] J. M. Wilson, M. Ohashi, H. Budzikiewicz, C. Djerassi, S. Ito, and T. Nozoe, *Tetrahedron*, 1963, **19**, 2247.

[8] H. M. Grubb and S. Meyerson in 'Mass Spectrometry of Organic Ions,' ed. F. W. McLafferty, Academic Press, New York, 1963, ch. 10 and references therein.

[9] W. K. Rohwedder, A. F. Mabrouk, and E. Selke, *J. Phys. Chem.*, 1965, **69**, 1711.

[10] J. Ronayne, D. H. Williams, and J. H. Bowie, *J. Amer. Chem. Soc.*, 1966, **88**, 4980.

of the corresponding ions from the labelled compounds clearly define their origins. The ion at *m/e* 112 comprises carbons 1 to 4 of the side chain and must be formed somehow by cleavage of the 4,5-double bond. That at *m/e* 219 results from cleavage of the ester group. The formation of this latter ion is probably accompanied by a hydrogen transfer from the C-3 methyl group to ing cleavage in the spectra of (IV) and (VI) is accompanied by a slight loss of the oxygen-18 label, and the ratio of intensities of $[M - H_2O]^+$ and $[M - HDO]^+$ in the spectrum of (II) is larger than that expected from the isotopic composition of the molecular ion. The mechanism of this dehydration is unclear.

Loss of methanol from the molecular ion also occurs

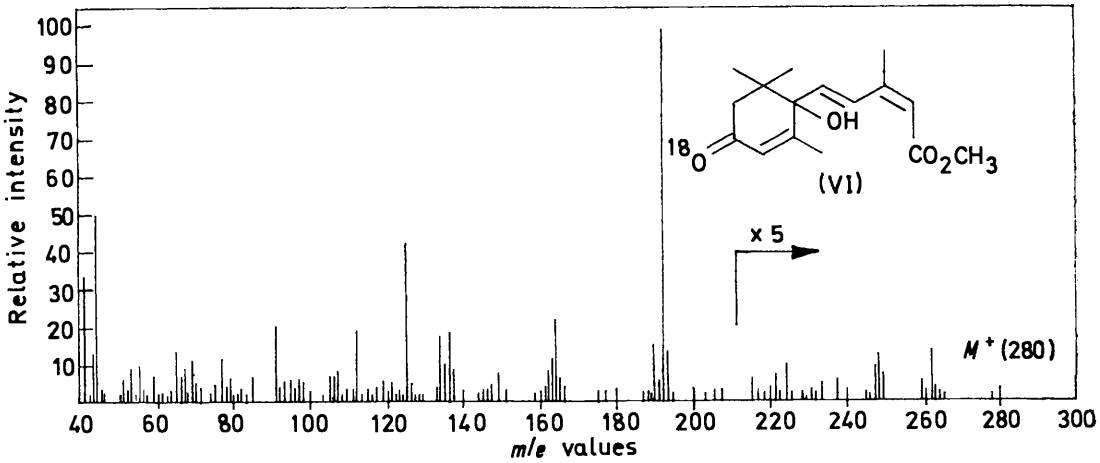


FIGURE 4 Mass spectrum of methyl [4′-^{18}O]abscisate (VI)

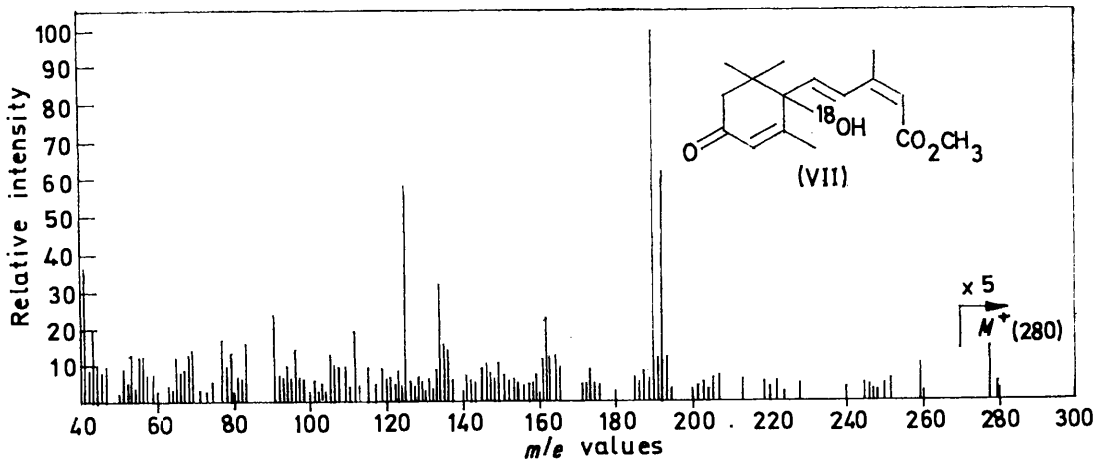
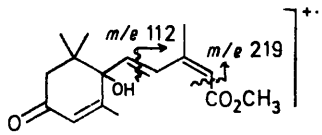
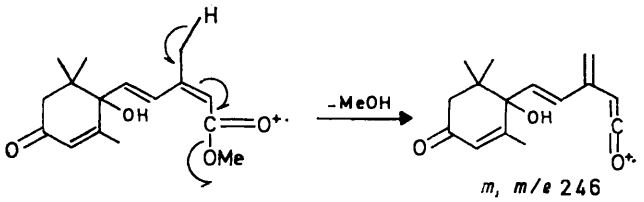


FIGURE 5 Mass spectrum of methyl [1′-^{18}O]abscisate (VII)

C-2, although the mechanism is not clear from the available labelling data.



In the direct dehydration of the molecular ion (*m/e* 278 ⟶ 260) the oxygen atom involved comes predominantly from the tertiary hydroxy-group; this loss may be partly thermal in nature. In addition, however, there is some evidence that the ketone function at C-4′ also plays a part in this elimination, since the correspond- to an appreciable extent, and involves the C-1 methoxy-function. Although the origin of the additional hydrogen atom is not known, a convenient mechanism involving the C-3 methyl group can be invoked (Scheme 4),



SCHEME 4

in which an ion such as *m*, *m/e* 246, is generated. Certainly the cleavage cannot be similar to that in certain other methyl esters,[11] for which it has been found

[11] S. Meyerson and L. C. Leitch, *J. Amer. Chem. Soc.*, 1966, **88**, 56.

that elimination of methanol may be facilitated by activation of a hydrogen atom attached to C-6 of the adjoining alkyl chain.

EXPERIMENTAL

Low resolution mass spectra were obtained with either an A.E.I. MS 902 instrument, by the direct-insertion probe method, or an A.E.I. MS 30 instrument, in the g.l.c.–m.s. mode, as indicated in Table 1. A column of 3% SE-30 on Chromosorb W (1·5 m × 1·5 mm i.d.) was used in the latter system with an oven temperature of 200° and a helium flow rate of 50 ml min^{-1}. The source temperature was 120° (MS 902) or 220° (MS 30), and the inlet probe of the MS 902 instrument was maintained at room temperature. All spectra were measured at 70 eV. Each compound was racemic and was checked for purity by g.l.c. on a column similar to that just described. The deuteriated compounds (III) and (VIII) were kindly donated by Dr. B. V. Milborrow. The isotopic compositions of the labelled compounds, calculated from data from the molecular ion region, are given in Table 2.

TABLE 2

Compound	Isotopic composition
(II)	38·8% [$^2H_0$], 59·2% [$^2H_1$], 2·0% [$^2H_2$]
(III)	4·7% [$^2H_3$], 16·5% [$^2H_4$], 33·0% [$^2H_5$], 45·8% [$^2H_6$]
(IV)	3·4% [$^{18}O_0$], 20·0% [$^{18}O_1$], 76·6% [$^{18}O_2$]
(V)	15·0% [$^{18}O_0$], 85·0% [$^{18}O_1$]
(VI)	13·0% [$^{18}O_0$], 87·0% [$^{18}O_1$]
(VII)	59·5% [$^{18}O_0$], 40·5% [$^{18}O_1$]
(VIII)	10·3% [$^2H_0$], 70·5% [$^2H_1$], 19·2% [$^2H_2$]
(XI)	10·8% [$^{18}O_0$], 89·2% [$^{18}O_1$]
(XII)	59·5% [$^{18}O_0$], 40·5% [$^{18}O_1$]

Methyl [1′-O^{2}H]*Abscisate* (II).—A solution of methyl abscisate (0·02 mmol) in methan[^{2}H]ol (0·25 ml) and deuterium oxide (1 ml) was kept at room temperature for 15 min, then freeze-dried; the procedure was repeated twice. The resulting methyl [1′-*O*^{2}H]abscisate (II) was dissolved in methan[^{2}H]ol and the mass spectrum was obtained by direct insertion. Attempts to increase the deuterium incorporation at C-1′ resulted in material containing varying amounts of [$^2H_2$], [$^2H_3$], and [$^2H_4$]species.

Methyl [1,4′-$^{18}O_2$]*Abscisate* (IV) *and Methyl* [1-^{18}O]-*Abscisate* (V).—A solution of 1,1-carbonyldi-imidazole (0·86 mmol) in dry tetrahydrofuran (5 ml) was added to a solution of abscisic acid (0·76 mmol) in dry tetrahydrofuran (2 ml) and the resulting mixture was stirred under nitrogen at room temperature for 1 h. After evaporation, methanol (2 ml) and water (5 ml) were added and the solution was set aside to crystallize at 0°. The pale cream imidazolide (201 mg, 84%) was filtered off, washed with ice–water, and dried; m.p. 178—181° (decomp.), >95% pure by n.m.r.

A mixture of the imidazolide (0·075 mmol), $H_2{}^{18}O$ (81·8% nominal ^{18}O; 0·25 ml), and sodium (0·12 mmol) was heated at 90° under a drying tube for 15 min. Acidification (conc. H_2SO_4) and conventional work-up gave [1,4′-$^{18}O_2$]-abscisic acid (97%), m.p. 184—187°. A solution of this material (0·036 mmol) in 2N-sodium hydroxide (2 ml) was heated at 85° for 30 min. Following acidification (conc. H_2SO_4) and extraction into ether, [1-^{18}O]abscisic acid (m.p. 183—186°) was recovered in 75% yield. The two labelled abscisic acids were methylated with diazomethane.

Ethyl Abscisate (IX).—A solution of abscisic acid imidazolide (0·1 mmol), prepared as before, in 1% sodium ethoxide (0·5 ml) was left at room temperature for 30 min. Ether (10 ml) was then added, and the solution was extracted with concentrated hydrochloric acid (3 ml). The ethereal phase was washed [N-HCl (5 ml) and saturated aqueous $NaHCO_3$ (5 ml)], dried ($MgSO_4$), and evaporated giving (IX) as an oil (58%) which was used directly for mass spectral analysis.

Methyl [4′-^{18}O]*Abscisate* (VI).—A solution of abscisic acid (0·045 mmol) in 0·25N-Na^{18}OH (0·25 ml; prepared from $H_2{}^{18}O$ of nominal 81·8% ^{18}O content) was heated at 90° under a drying tube for 20 min. After cooling, the mixture was acidified (conc. H_2SO_4) and extracted with ether to give [4′-^{18}O]abscisic acid (83%), m.p. 179—183°, which was then methylated with diazomethane.

Methyl 2-trans-[4′-^{18}O]*Abscisate* (XI).—This was prepared in similar fashion from 2-*trans*-abscisic acid.

Methyl [1′-^{18}O]*Abscisate* (VII) *and Methyl* 2-trans-[1′-^{18}O]*Abscisate* (XII).—A mixture of 2-*trans*-dehydro-β-ionylideneacetic acid [12] (130 mg), eosin (3 mg), dry ethanol (25·0 ml), and dry benzene (25·0 ml) was cooled and stirred under oxygen-18 gas (nominal 98% $^{18}O_2$; *ca.* 20 ml) while illuminated by four 32 W fluorescent lamps. After 3 h the absorption band at 340 nm due to the starting material had disappeared. The solution was evaporated under reduced pressure and the residue was dissolved in ethanol (10 ml) and aqueous 10N-sodium hydroxide (5 ml). After 30 min at room temperature, the solution was diluted with water and extracted with ether. The 2-*trans*-[1′-^{18}O]abscisic acid was isolated from the mixture so obtained by preparative t.l.c. and recrystallised (CH_2Cl_2–CCl_4) to give crystals (55 mg), m.p. 149—151°. Methylation with diazomethane afforded (XII).

By exposing a solution of (XII) in acetone to sunlight for several hours, a mixture of the *cis*- (VII) and *trans*- (XII) isomers was obtained which was used directly for g.l.c.–m.s. analysis.

We thank Mr. D. T. Green, Mr. A. G. Wilkinson, and Mrs. J. P. Tucker for the mass spectrometry.

[4/010 *Received*, 3*rd January*, 1974]

[12] V. Schwieter, C. V. Planta, R. Rüegg, and O. Isler, *Helv. Chim. Acta*, 1962, **45**, 528.