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PLANT HORMONES—X[1]
THE CONSTITUTION OF PHASEIC ACID; A RELATIVE OF ABSCISIC ACID FROM *PHASEOLUS MULTIFLORUS*. AN INTERPRETATION OF THE MASS SPECTRUM OF PHASEIC ACID AND A PROBABLE STRUCTURE

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Abstract—After a detailed examination and interpretation of the MS of phaseic acid and methyl phaseate structure (I) is preferred for this putative relative of abscisic acid from *Phaseolus multiflorus*. Two facts, more difficult to reconcile with this structure are an abnormally large long-range coupling and the apparent inability of phaseic acid to undergo an alkali-induced β-elimination of the epoxide oxygen to the γ-hydroxy-αβ-unsaturated ketone. Attempted one step conversion of methyl phaseate into methyl abscisate produced a complex but apparently identical reaction mixture to that obtained from methyl abscisate when treated under the same conditions.

IN THE preceding communication[1] we presented an analysis of spectroscopic data which led us to favour the epoxide (I) and the oxetane (II) structures for phaseic acid, a C_{15}-acid from immature seed of *Phaseolus multiflorus*. With both these structures, which show phaseic acid as a close relative of the endogenous plant growth inhibitor (*S*)-(+)-abscisic acid (III), there remains the question of the magnitude of the long-range NMR coupling (2 to 3·5 Hz) between one of the α-methylene CO protons and one of the epoxide or oxetane protons. While we remain unable to explain this fact we find that a detailed examination of the mass spectra of phaseic acid and methyl phaseate favours the epoxide structure (I) for phaseic acid. Other data of a more chemical nature to be presented here are more difficult to reconcile with this proposed structure (I).

The mass spectra of phaseic acid and methyl phaseate are shown in Fig. 1. The interpretation that follows (Schemes 1 to 8) is justified from high resolution measurements mainly with the spectrum of methyl phaseate. The similar relative abundances of corresponding fragment ions in the mass spectra of both phaseic acid and methyl phaseate, together with some high resolution measurements from phaseic acid, support the interpretation below for both acid and ester. Many metastable ions were observed which support the interpretation of the spectra, they are indicated in the Schemes 1 to 8 where the structures are intended to be illustrative only.

Both the spectra of phaseic acid and methyl phaseate (Fig. 1) show a base peak at *m/e* 43 and in the case of the ester this has been shown to be a 7:1 mixture of the acetylium cation (C_2H_3O), and propyl cation, $(C_3H_7)^+$. In contrast the base peaks of the mass spectra of abscisic acid (III) and methyl abscisate are at *m/e* 190;[2] this is apparently the ion formed by loss of water and isobutylene or methanol and isobutylene from their respective parent ions by fragmentation of the isophorone ring.[3,4] Phaseic

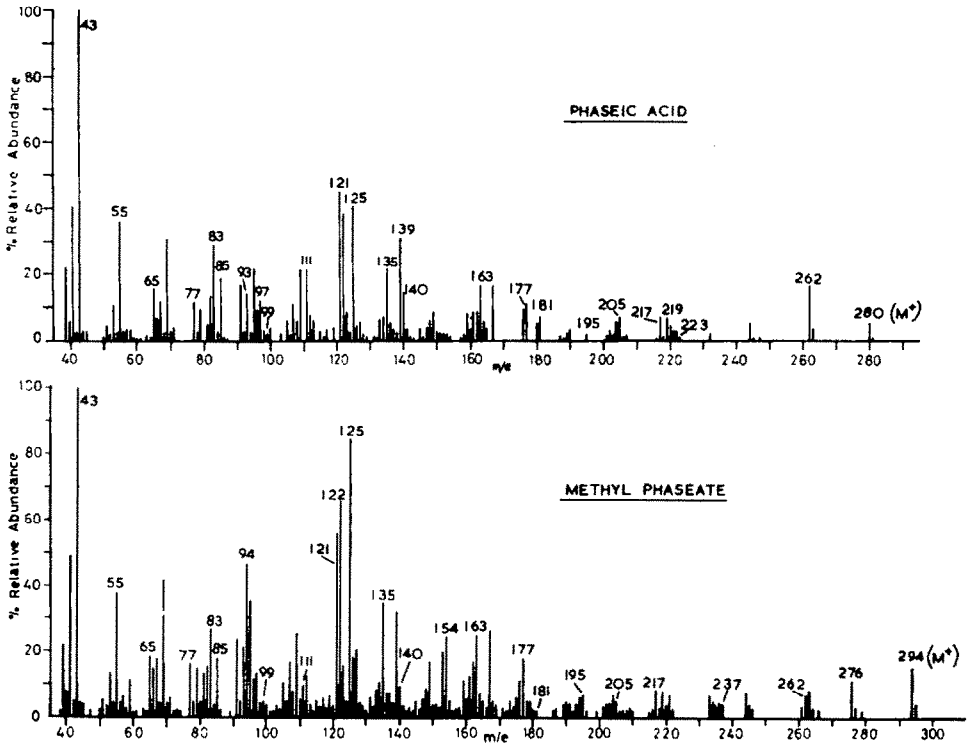


FIG. 1 Mass spectra of phaseic acid and methyl phaseate.

acid and methyl phaseate show sequential losses of two molecules of water or methanol and water respectively; appropriate metastable ions being found in both cases.

The important fragmentation shown in Scheme 1 involves complete extrusion of the side chain together with the tertiary OH and the carbon bearing it by a double allylic cleavage. This particular fragmentation is only possible with the epoxide structure (I) for phaseic acid. The oxetane (II) has no terminal OH on the side chain. Further fragmentations of the side chain fragment ion (Scheme 1) are shown in Scheme 2. The *m/e* 125 and *m/e* 111 ions, which occur as fragment ions in Scheme 2 can also arise by direct vinylic cleavage of the 2,4-dienoic acid side chain in the parent ion and this has been referred to previously.[1] Subsequent fragmentations of the other fragment ion resulting from Scheme 1, *m/e* 140 for acid and methyl ester, are shown in Scheme 3. This ion, the residue of the 6-membered ring in phaseic acid, can undergo a McLafferty-type rearrangement involving an epoxide hydrogen and the CO group. Subsequent α-cleavage of the rearranged ion (*m/e* 99) provides one of the several routes to the most abundant ion, *m/e* 43. Simple α-cleavage of the *m/e* 140 ion is also depicted in Scheme 3 though it is of less significance.

The fragmentations discussed above (Schemes 1, 2, and 3), together with the UV, IR, and NMR evidence previously presented,[1] provide the main grounds for favouring the epoxide structure (I) for phaseic acid. The remainder of the mass spectral inter-

pretation is discussed in terms of the epoxide structure (I) although most of what follows could probably be equally well interpreted in terms of the oxetane structure (II).

Scheme 4 shows perhaps the most fundamental fragmentations of phaseic acid. By hydrogen transfer and ring opening of the epoxide in a 6-membered "transition state" the equivalent of a linear triketone ion is produced from the parent ion or the parent ion less H_2O or MeOH. These triketone ions then undergo fragmentation mainly by α-cleavage as shown in Scheme 4. As expected, fragment ions containing the side chain are more abundant in the fragmentation of the dehydrated or demethanolated rearranged parent ions. Several possible routes to the base peak at *m/e* 43 are included in Scheme 4.

Some further fragmentations of the *m/e* 141 and *m/e* 262 ions from Scheme 4 are shown in Scheme 5. Both these ions provide additional possible routes to the base peak at *m/e* 43 by McLafferty rearrangement and α-cleavage, analogous to that previously discussed for the *m/e* 140 ion in Scheme 3. The *m/e* 141 ion can, however, give rise to the base peak by α-cleavage alone.

In Scheme 6 two particular rearrangements of phaseic acid and methyl phaseate under electron impact are shown. These involve opening of the epoxide ring and recyclising on to the side chain in a similar manner to schemes proposed for some carotenoid epoxides.[5] In this way 6-membered (*m/e* 177) and 8-membered (*m/e* 217) oxygen heterocyclic ions are formed. Subsequent fragmentation of the *m/e* 177 ion by a retro-Diels Alder mechanism gives rise to the relatively abundant *m/e* 135 ion and thence the *m/e* 55 ion. The *m/e* 55 can, however, arise by the fragmentation shown in Scheme 7 which also shows the probable origin of the *m/e* 83 fragment ion. This fragmentation (Scheme 7) has been referred to previously[1] and was very important in restricting the choice of possible structures of phaseic acid.[1] From the data given in Schemes 6 and 7 only 25% of the *m/e* 55 peak in the mass spectrum of methyl phaseate can arise *via* the epoxide-side chain rearrangement (Scheme 6) and therefore the major route is probably that shown in Scheme 7.

Scheme 8 shows how, from lactonised forms of the parent ions, the *m/e* 181 and *m/e* 163 fragment ions could arise. The loss of water by the *m/e* 181 ion to give the *m/e* 163 ion probably occurs from the epoxide. Loss of water from epoxides, and cyclic ethers in general, under electron impact is well documented.[5, 6]

The mass spectral data and the interpretation, presented in Schemes 1 to 8, strongly support the structural information previously derived from UV, IR, and NMR spectral data;[1] and provide evidence in favour of the epoxide structure (I) for phaseic acid. Two chemical tests have been applied to the proposed epoxide structure (I) for phaseic acid. The first was an attempted conversion to abscisic acid (III) *via* the βγ-unsaturated ketone (IV) by Cornforth's[7] mild method for reduction of epoxides to olefins. When a small amount of methyl phaseate was subjected to this procedure a complex reaction product was obtained which contained no methyl abscisate by TLC or GLC. However, when (*RS*)-methyl abscisate was subjected to the same reductive procedure an apparently identical (TLC and GLC) complex reaction product was obtained. This result, although inconclusive, is consistent with the epoxide structure (I) for phaseic acid, suggesting that both methyl phaseate and methyl abscisate reacted *via* the same intermediate or intermediates and that methyl phaseate may have been first converted into methyl abscisate.

A second, less specific, test of the proposed structure (I) of phaseic acid was the

attempted alkali-induced β-elimination of the epoxide oxygen to the γ-hydroxy-αβ-unsaturated ketone (V). However, when a few drops of 2N-NaOMe were added to an ethanolic solution of phaseic acid [λ_{max} 258 nm (ε, 14500)] or to a methanolic solution of methyl phaseate (λ_{max} 263 nm) no significant change was observed in their UV spectra. Shifts of UV absorption maxima to lower wavelength with an increase in extinction coefficients would have been expected if the new isophorone chromophore[3] [λ_{max} (MeOH) 236 nm (ε, 12600)] in V had been produced. Indeed the spectra should have changed to practically those of abscisic acid[2, 3] [λ_{max} (alkaline EtOH) 244 nm; λ_{max} (MeOH) 246 nm (ε, 25200)] and methyl abscisate[2] [λ_{max} (EtOH) 264 and 238 (shoulder) nm].

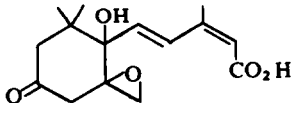
This apparent stability of phaseic acid and its methyl ester to alkali raises doubts about the proposed epoxide structure (I). However, a possible explanation for this stability can be offered by considering the conformations of the two possible epoxides (VII and VIII), corresponding to (*S*)-(+)-abscisic acid. The following arguments also apply to the enantiomers of VII and VIII.

From Dreiding models, it was deduced that the preferred conformations of the epoxides VII and VIII are VIIa and VIIIa respectively in which the cyclohexanone ring is in a chair conformation with the dienoic acid side-chain in an equatorial configuration; the flexible boat and skew boat conformations appear to be less stable due to interaction of the pendant groups. Concerted β-elimination of the epoxide oxygen with base would be expected to occur readily in the epoxide (VIII) with the conformation (VIIIa) where the departing groups are *trans*-diaxial. This *trans*-diaxial relationship does not exist in the preferred conformation (VIIa) of the epoxide (VII) and concerted β-elimination of the epoxide oxygen would be expected to occur only with difficulty. Similar conclusions are reached if alkali-induced β-elimination is considered as a non-concerted process *via* the enolate anion (VI). Inspection of models using sp^3 carbon at the epoxide carbon (see VI) to indicate the orientation of bonding orbitals shows that good overlap can be attained between the enolate anion β-orbitals and the C—O bonding orbitals in the epoxide VIII, VIIIa but not in the epoxide VII, VIIa. If these conformational arguments are valid, they provide a reason for the stability of phaseic acid to alkali in terms of the epoxide structure (VI) or its enantiomer and, consequently, fix the relative *cis*-stereochemistry of the epoxide and OH oxygens.

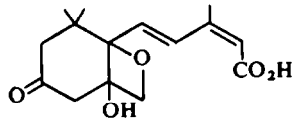
Like abscisic acid phaseic acid has been found to possess inhibitory properties on plant growth but of a much lower order of magnitude in assays tried to date. In the excised wheat embryo test phaseic acid shows about 1% of the activity of abscisic acid and in the cotton abscission assay about 10% that of abscisic acid. It shows little or no activity in the *Rumex*, oat mesocotyl, lettuce hypocotyl, or oat leaf base bioassays in the presence or absence of gibberellin A_3.

Abscisic and phaseic acids could either be photolytic products of carotenoids[8] or true sesquiterpenes derived directly from farnesyl pyrophosphate. While the idea that the endogenous (*S*)-(+)-abscisic acid could arise *in vivo* by photolysis of carotenoids remains an unproved but interesting biosynthetic possibility,[8] recent incorporations of (2-^{14}C) mevalonic acid into abscisic acid by intact fruit similarly in the absence or presence of light[9] might imply that abscisic acid, and therefore probably phaseic acid, are indeed true sesquiterpenes. There is so far little evidence for the co-occurrence of abscisic and phaseic acids in plants. Abscisic acid could not be detected along with phaseic acid in our extracts[1] of *P. multiflorus* and phaseic acid could not

be detected in apple juice extracts which contained abscisic acid.[10] There is, however, some indication of the co-occurrence of abscisic and phaseic acids in cotton fruit.[11]



I



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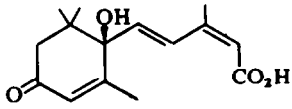

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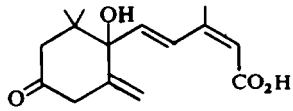

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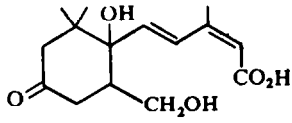
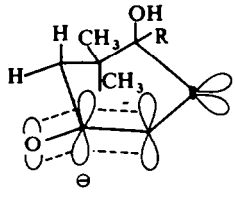
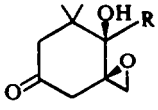


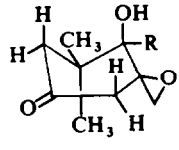
V



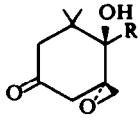
VI



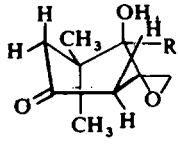
VII



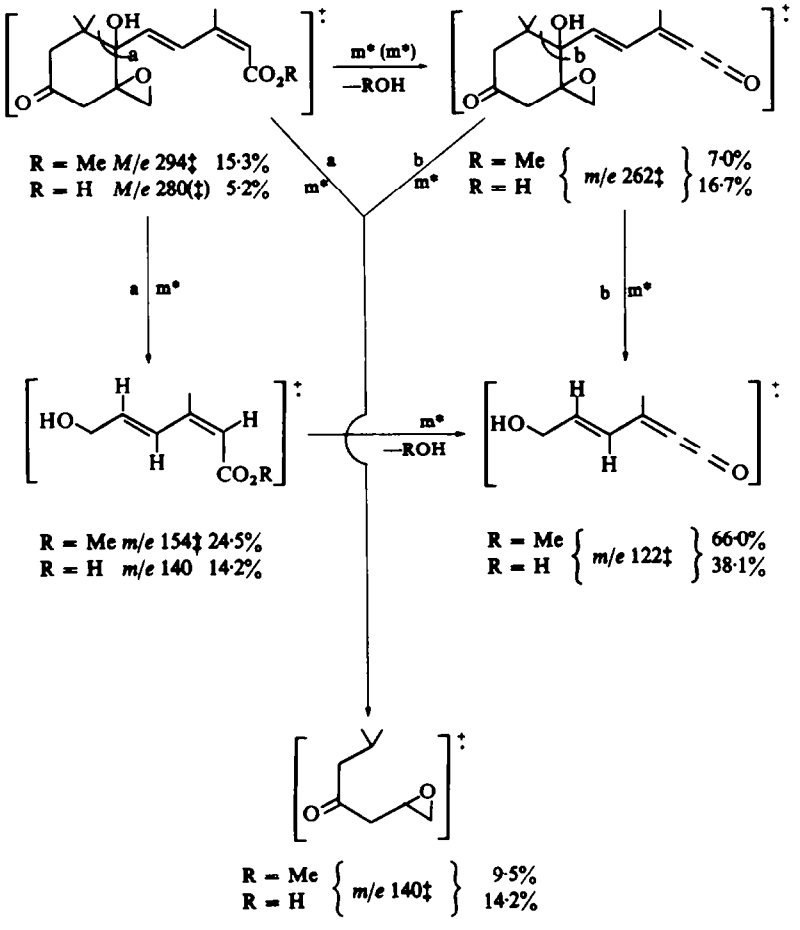
VIIa



VIII



VIIIa



SCHEME 1

Legend for Schemes 1–8

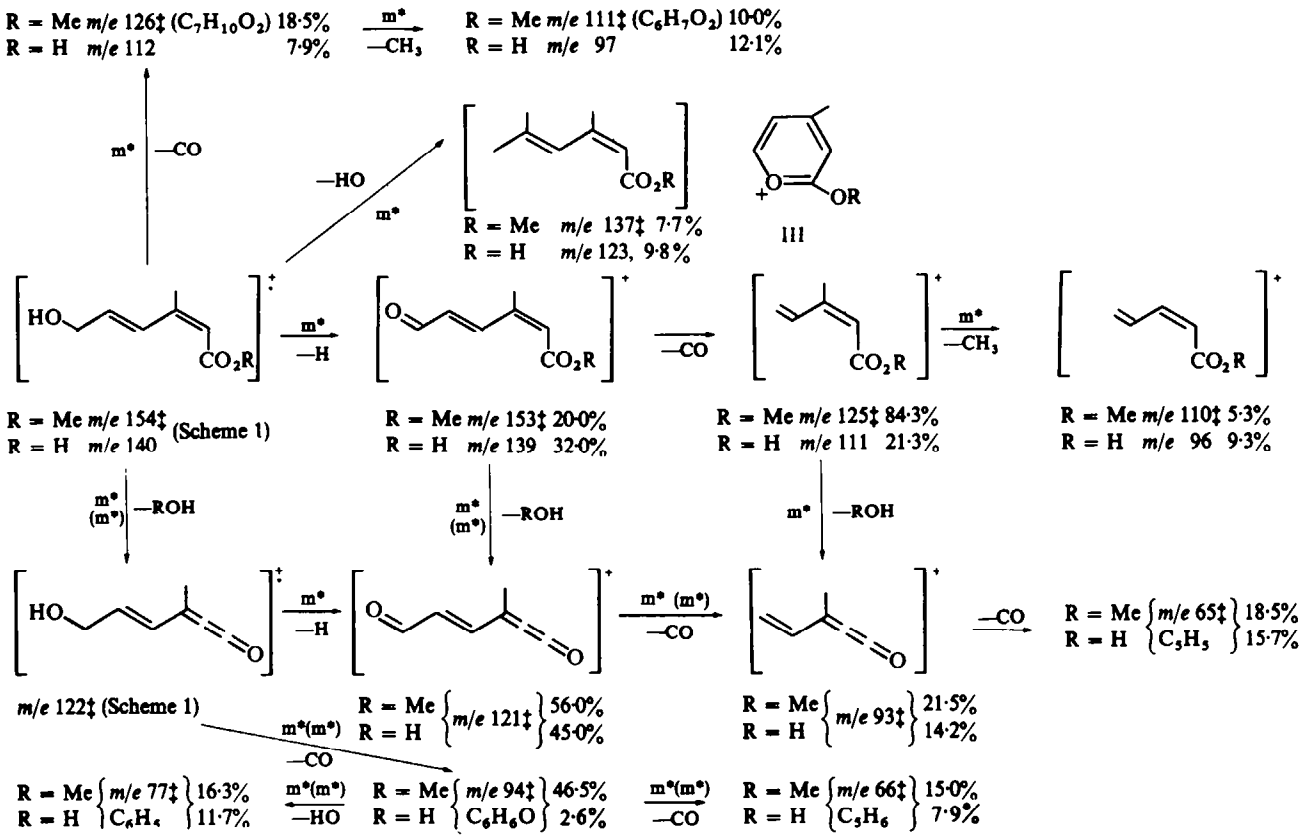
m* = metastable from the methyl ester spectrum.

(m*) = metastable from the acid spectrum.

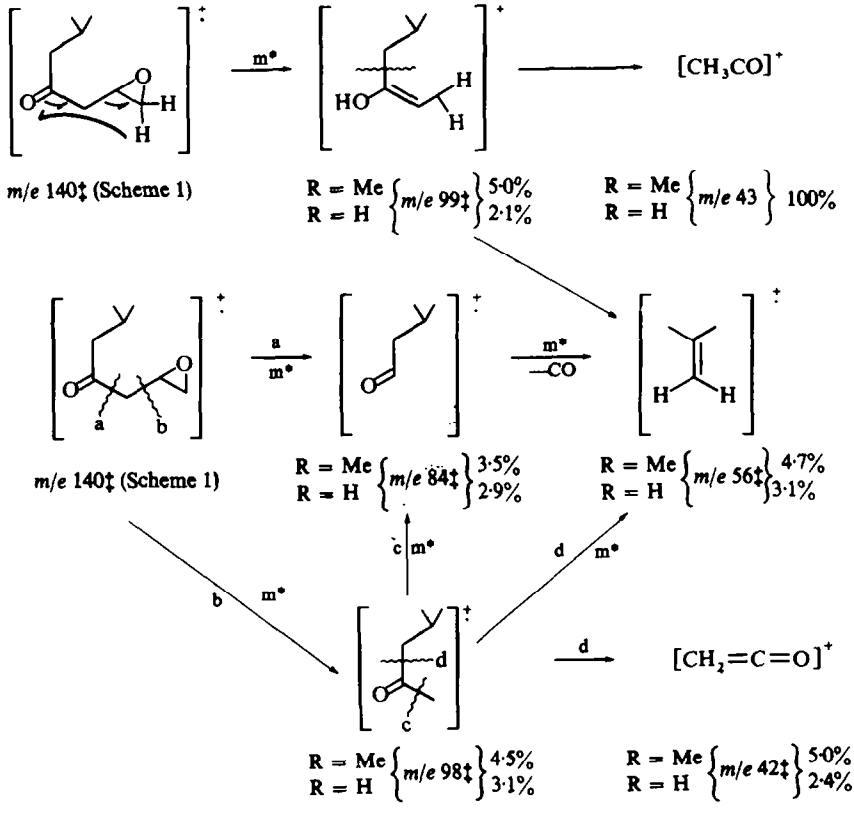
‡ = composition determined by high resolution measurements for the methyl ester.

(‡) = composition determined by high resolution measurements for the acid.

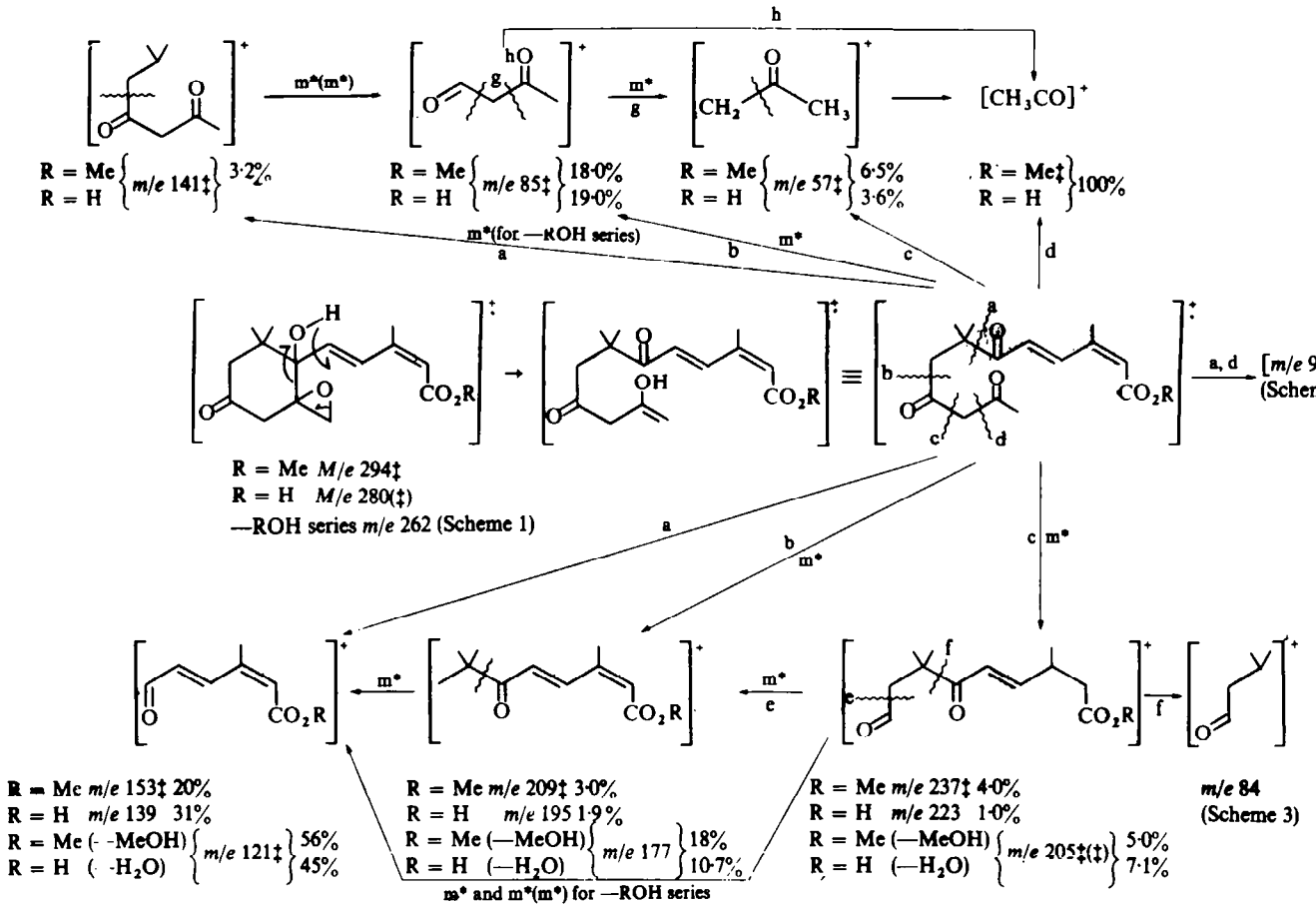
Percentages represent abundances relative to the base peak at *m/e* 43 (100%) for both acid and ester.


R = Me m/e 126‡ (C7H10O2) 18·5%
R = H m/e 112 7·9%
m* —CO
R = Me m/e 111‡ (C6H7O2) 10·0%
R = H m/e 97 12·1%
m* —CO
—HO m*
R = Me m/e 137‡ 7·7%
R = H m/e 123, 9·8%
111
HO
CO2R
m* —H
R = Me m/e 154‡
R = H m/e 140
(Scheme 1)
R = Me m/e 153‡ 20·0%
R = H m/e 139 32·0%
—CO
R = Me m/e 125‡ 84·3%
R = H m/e 111 21·3%
m* —CH3
R = Me m/e 110‡ 5·3%
R = H m/e 96 9·3%
m* (m*) —ROH
m* —ROH
m* —H
m* (m*) —CO
—CO
R = Me m/e 65‡ C5H5 18·5%
R = H C5H5 15·7%
m/e 122‡ (Scheme 1)
m*(m*) —CO
R = Me m/e 121‡ 56·0%
R = H 45·0%
R = Me m/e 93‡ 21·5%
R = H 14·2%
R = Me m/e 77‡ C6H5 16·3%
R = H C6H5 11·7%
m*(m*) —HO
R = Me m/e 94‡ C6H6O 46·5%
R = H C6H6O 2·6%
m*(m*) —CO
R = Me m/e 66‡ C5H6 15·0%
R = H C5H6 7·9%

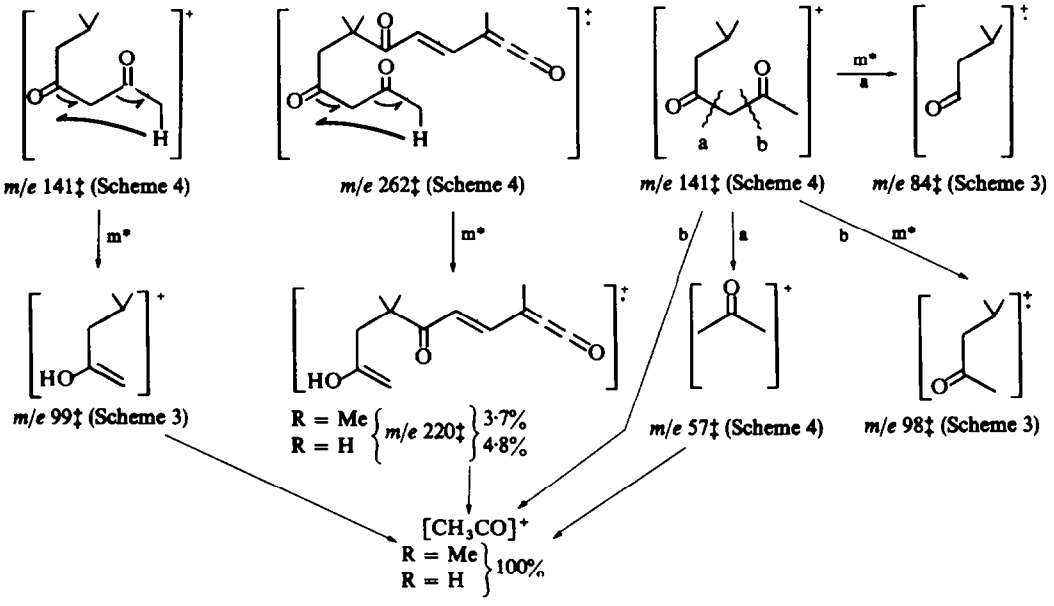
SCHEME 3


m*
[CH3CO]+
m/e 140‡ (Scheme 1)
R = Me m/e 99‡ 5·0%
R = H m/e 99‡ 2·1%
R = Me m/e 43 100%
R = H m/e 43 100%
a
m*
m*
–CO
m/e 140‡ (Scheme 1)
R = Me m/e 84‡ 3·5%
R = H m/e 84‡ 2·9%
R = Me m/e 56‡ 4·7%
R = H m/e 56‡ 3·1%
c m*
d m*
b m*
d
[CH2=C=O]+
R = Me m/e 98‡ 4·5%
R = H m/e 98‡ 3·1%
R = Me m/e 42‡ 5·0%
R = H m/e 42‡ 2·4%

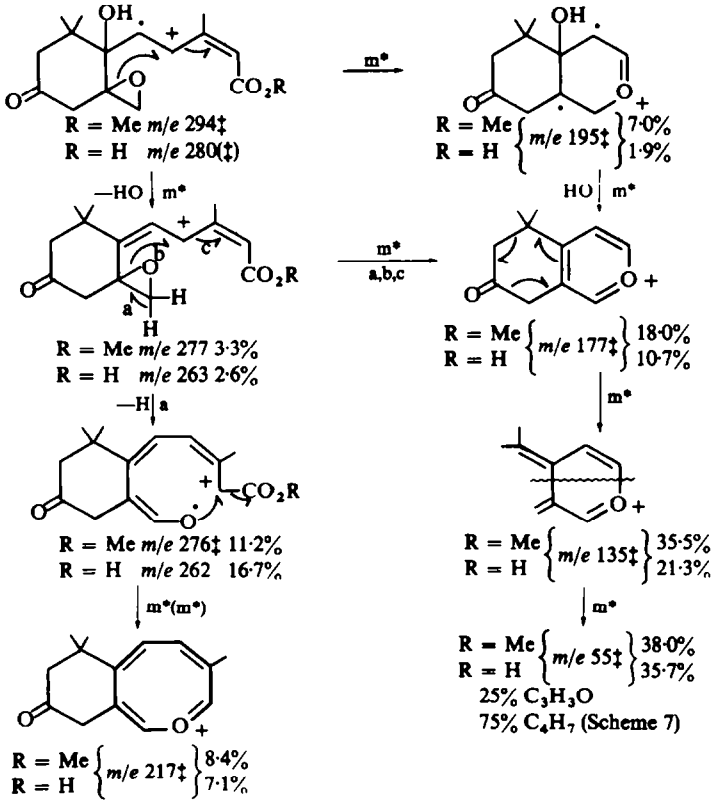
SCHEME 4


h
m*(m*)
m*
g
[CH3CO]+
R = Me { m/e 141‡ } 3·2%
R = H
R = Me { m/e 85‡ } 18·0%
R = H 19·0%
R = Me { m/e 57‡ } 6·5%
R = H 3·6%
R = Me‡ } 100%
R = H
m*(for —ROH series)
a
b
m*
c
d
R = Me M/e 294‡
R = H M/e 280(‡)
—ROH series m/e 262 (Scheme 1)
a, d
[m/e 98]‡
(Scheme 3)
a
b
m*
c m*
m*
m*
e
f
R = Me 153‡ 20%
R = H m/e 139 31%
R = Me (— -MeOH) { m/e 121‡ } 56%
R = H (— -H2O) } 45%
R = Me m/e 209‡ 3·0%
R = H m/e 195 1·9%
R = Me (—MeOH) { m/e 177 } 18%
R = H (—H2O) } 10·7%
R = Me m/e 237‡ 4·0%
R = H m/e 223 1·0%
R = Me (—MeOH) { m/e 205‡(‡) } 5·0%
R = H (—H2O) } 7·1%
m/e 84
(Scheme 3)
m* and m*(m*) for —ROH series

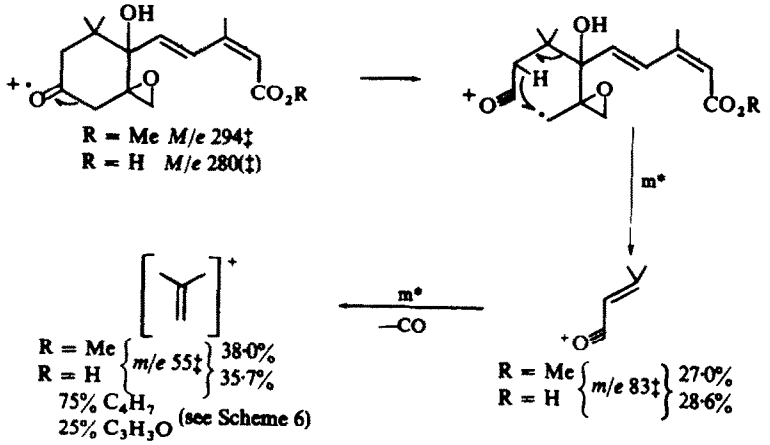
SCHEME 5



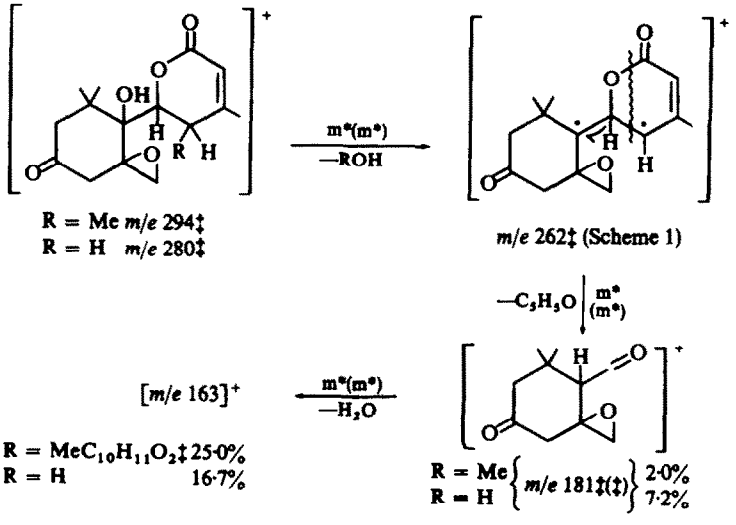
SCHEME 6



SCHEME 7



SCHEME 8



EXPERIMENTAL

Instrumentation and materials used here were exactly as previously described.[1]

Treatment of methyl phaseate and (RS)-methyl abscisate with Cornforth's[7] reagent for mild reduction of epoxides to olefins. NaI (525 mg) and anhyd NaOAc (175 mg) were dissolved in AcOH (1 ml) and water (0·075 ml). To this ice cold, stirred mixture was added Zn dust (600 mg) and then methyl phaseate (ca. 250 μg) [or (*RS*)-methyl abscisate (ca. 300 μg) in a minimum quantity of acetate buffer [AcOH (4·2 ml), NaOAc (700 mg), and water (0·2 ml)]. The reaction was continued for 1 hr then Zn dust was removed by filtration and washed with AcOH. The filtrate and washings were poured into water (10 ml) and extracted with ether (4 × 7 ml). The combined ether extract was washed with $NaHSO_3$aq and then water. After drying over $NaSO_4$, evaporation gave a gum (12 mg) (5·5 mg from methyl abscisate). TLC [eluting solvent, EtOAc/benzene (3:7)] of the reaction products from methyl phaseate and methyl abscisate gave an identical series of spots none of which corresponded to methyl abscisate. After identical fractionation of both of these reaction products by preparative TLC the identity of the fractions from both reaction products was confirmed by GLC.

Acknowledgements—For gifts of (*RS*)-abscisic acid we are indebted to Dr. J. W. Cornforth, F.R.S. and Dr. D. Yeo of Shell Ltd. Mass spectrometry was kindly carried out by Drs. R. Binks and B. E. Webster and bioassays by Prof. F. T. Addicott, Dr. L. C. Luckwill and Dr. B. V. Milborrow.

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