A Stereoselective Elimination in the Mass Spectra of Hexahydrofluorene Derivatives related to Gibberellins

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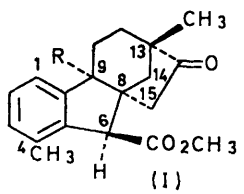
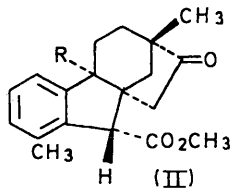
The mass spectra of methyl gibberate and methyl allogibberate and their 9- and 6-epimers were examined. Fragmentation pathways are proposed on the basis of specific deuterium labelling and measurements at high resolution. In particular, when the C-9 hydrogen and C-6 methoxycarbonyl substituents were *cis* to each other, elimination of methyl formate was much preferred over simple methoxycarbonyl cleavage, which became of comparable or greater importance only when the substituents were *trans*. Knowledge of these stereoselective mass spectral fragmentations has been applied to the solution of the relative stereochemistry of two isomers of 1,2,3,4,4a,9a-hexahydrofluorene-9-carboxylic acid.

MASS spectrometry has been applied successfully to the structural identification of gibberellin derivatives. Following the early studies of Zaretskii[1,2] and Takahasha *et al.*[3] for which pure samples were needed for the direct-insertion probe method of determination, more recently combined gas chromatography–mass spectrometry (g.l.c.–m.s.) has become very useful in the identification of known and new gibberellins in plant extracts.[4,5] The early work concentrated entirely on the natural gibberellins and their simple functional derivatives, and although quite detailed fragmentation patterns were proposed, the cracking patterns were presented with little high resolution or labelling data. More recently, Evans *et al.*[6] have proposed certain fragmentation mechanisms for the gibberellic acid degradation product methyl gibberate (Ia), based on evidence obtained from the spectra of certain deuterium-labelled compounds.

To date, no stereochemical assignment of a natural gibberellin or a derivative thereof has been made on the basis of its mass spectrum alone, although several electron-impact induced elimination reactions from compounds of different stereochemistry have been discussed. In particular the common characteristic peaks corresponding to the losses of water, methanol, and methyl formate from the molecular ions of the methyl esters of several gibberellins have been noted.[2] The pattern of further fragmentation of the resulting daughter ions depends on the stereochemical and structural characteristics of each compound. Fragment ions formed by the loss of methyl formate and the methoxycarbonyl radical from methyl 6-*epi*-gibberate and methyl gibberate respectively have been discussed,[6] although no conclusions were made about the stereochemical consequences of such fragmentations. We now report a more detailed study of the cracking patterns of several aromatic derivatives of gibberellic acid of known stereochemistry. Although several fragmentation pathways will be discussed, a knowledge of the stereochemical requirements for the loss of methyl formate and the methoxycarbonyl radical from gibberellins of known stereochemistry has been found to be of particular value in the assignment of stereochemistry to similar compounds of uncertain configuration. There has been considerable interest over the past few years in the effects of stereoisomerism on mass spectra.[7] Several attempts have been made to utilize mass spectrometry for stereochemical assignments in cyclic systems,[7-9] and in the determination of specificity for electron-impact induced loss of water from cyclic alcohols[10] and acetic acid from acyclic acetates.[11] The present assignments are supported by high resolution measurements and some relevant deuterium labelling data.



(I)
a; R = H
b; R = ^{2}H



(II)
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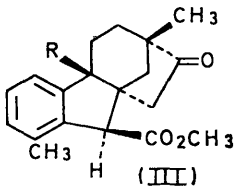
b; R = ^{2}H



(III)
a; R = H
b; R = ^{2}H

Gibberic Acid Derivatives.—The main fragment ions from the mass spectra of the gibberic acid methyl esters (Ia)—(IIIa), together with the corresponding

[1] N. S. Wulfson, V. I. Zaretskii, I. B. Papernaja, E. P. Serebryakov, and V. F. Kucherov, *Tetrahedron Letters*, 1965, 4209.

[2] V. I. Zaretskii, N. S. Wulfson, I. B. Papernaja, I. A. Gurvich, V. F. Kucherov, I. M. Milstein, E. P. Serebryakov, and A. V. Simolin, *Tetrahedron*, 1968, **24**, 2327.

[3] (*a*) N. Takahashi, N. Murofushi, S. Tamura, N. Wasada, H. Hoshino, T. Tsuchiya, T. Aoyama, and H. Morita, *Tetrahedron Letters*, 1967, 895; (*b*) N. Takahashi, N. Murofushi, S. Tamura, N. Wasada, H. Hoshino, T. Tsuchiya, S. Sasaki, T. Aoyama, and E. Watanabe, *Org. Mass Spectrometry*, 1969, **2**, 711.

[4] R. C. Durley, J. MacMillan, and R. J. Pryce, *Phytochemistry*, 1971, **10**, 1891.

[5] R. Binks, J. MacMillan, and R. J. Pryce, *Phytochemistry*, 1969, **8**, 271.

[6] R. Evans, J. R. Hanson, and L. J. Mulheirn, *J.C.S. Perkin I*, 1973, 753.

[7] S. Meyerson and A. W. Weitkamp, *Org. Mass Spectrometry*, 1968, **1**, 659.

[8] R. Hodges, R. C. Cambie, and K. N. Joblin, *Org. Mass Spectrometry*, 1970, **3**, 1473.

[9] J. K. MacLeod and R. J. Wells, *J. Amer. Chem. Soc.*, 1973, **95**, 2387.

[10] M. M. Green and R. B. Roy, *J. Amer. Chem. Soc.*, 1970, **92**, 6368 and references therein.

[11] M. M. Green, J. M. Moldowan, D. J. Hart, and J. M. Krakower, *J. Amer. Chem. Soc.*, 1970, **92**, 3481.

9-deuteriated derivatives (Ib)—(IIIb) are listed in Table 1.

Each compound gives a substantial molecular ion (M^+) upon electron impact (Figures 1—3), which may be used for the calculation of isotopic purity of the deuterium-labelled analogues (see Experimental section).

TABLE 1

Principal fragment ions in the mass spectrum of methyl gibberate and related compounds at 70 eV

Compound	M^+	$M - CO$	$M - C_2H_4O$	$M - CO_2CH_3$	$M - HCO_2CH_3$	$M - (CO + CO_2CH_3)$	$M - (CO + HCO_2CH_3)$	$M - (C_2H_4O + CO_2CH_3)$	$M - C_7H_{11}O_3$
(Ia)	298 (37)	270 (18)	254 (52)	239 (40)	238 (7)	211(64)	210 (40)	195 (39)	155 (100)
(Ib)	299 (58)	271 (30)	255 (41)	240 (58)	239 (10)	212 (90)	211 (60)	196 (44)	156 (61)
	298 (5)		254 (49)		238 (6)		210 (12)	195 (36)	155 (62)
(IIa)	298 (13)	270 (7)	254 (19)	239 (17)	238 (100)	211 (26)	210 (6)	195 (31)	155 (77)
(IIb)	299 (17)	271 (15)	255 (4)	240 (24)	239 (10)	212 (35)	211 (8)	196 (23)	156 (42)
	298 (2)		254 (26)		238 (100)		210 (12)	195 (27)	155 (40)
(IIIa)	298 (17)			239 (11)	238 (100)	211 (19)	210 (13)	195 (31)	155 (48)
(IIIb)	299 (12)			240 (10)	239 (21)	212 (20)	211 (11)	196 (21)	156 (23)
	298 (8)				238 (100)		210 (13)	195 (25)	155 (43)

Loss of carbon monoxide from M^+ provides the first major fragment ion at *m/e* 270 ($C_{18}H_{22}O_2$ by high resolution) in the spectra of (Ia) and (IIa). This elimination may be represented as shown in Scheme 1, resulting in the formation of ion *a* following a hydrogen migration from C-14 to C-15. The loss of CO seems to be dependent on the configuration at C-9 since an equivalent ion is not observed in the spectrum of methyl 9-*epi*-gibberate [(IIIa) Figure 3]. Ion *a* further decomposes with loss of a methoxycarbonyl radical and methyl formate to give ions at *m/e* 211 (*b*) and 210 respectively. The partial retention of deuterium in the equivalent ions from the deuteriated analogues (Ib)—(IIIb) suggests that the additional hydrogen atom that is lost to form the ion at *m/e* 210 possibly derives in part from the adjacent C-8 methyl group (to give *c*), as well as from C-9 (to give *d*), although the mechanism is uncertain from the available labelling data.

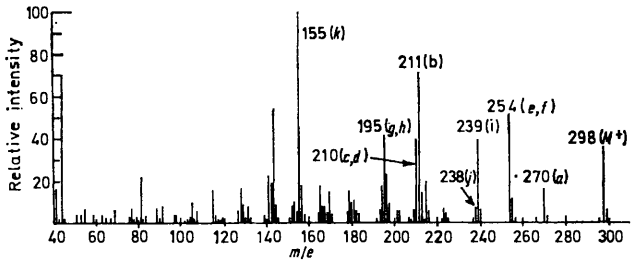


FIGURE 1 Mass spectrum of methyl gibberate (Ia)

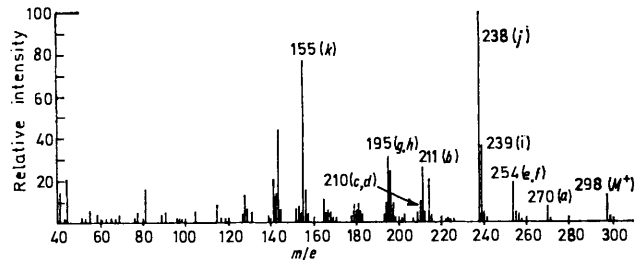


FIGURE 2 Mass spectrum of methyl 6-*epi*-gibberate (IIa)

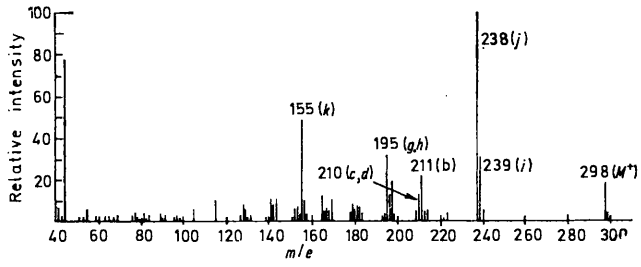


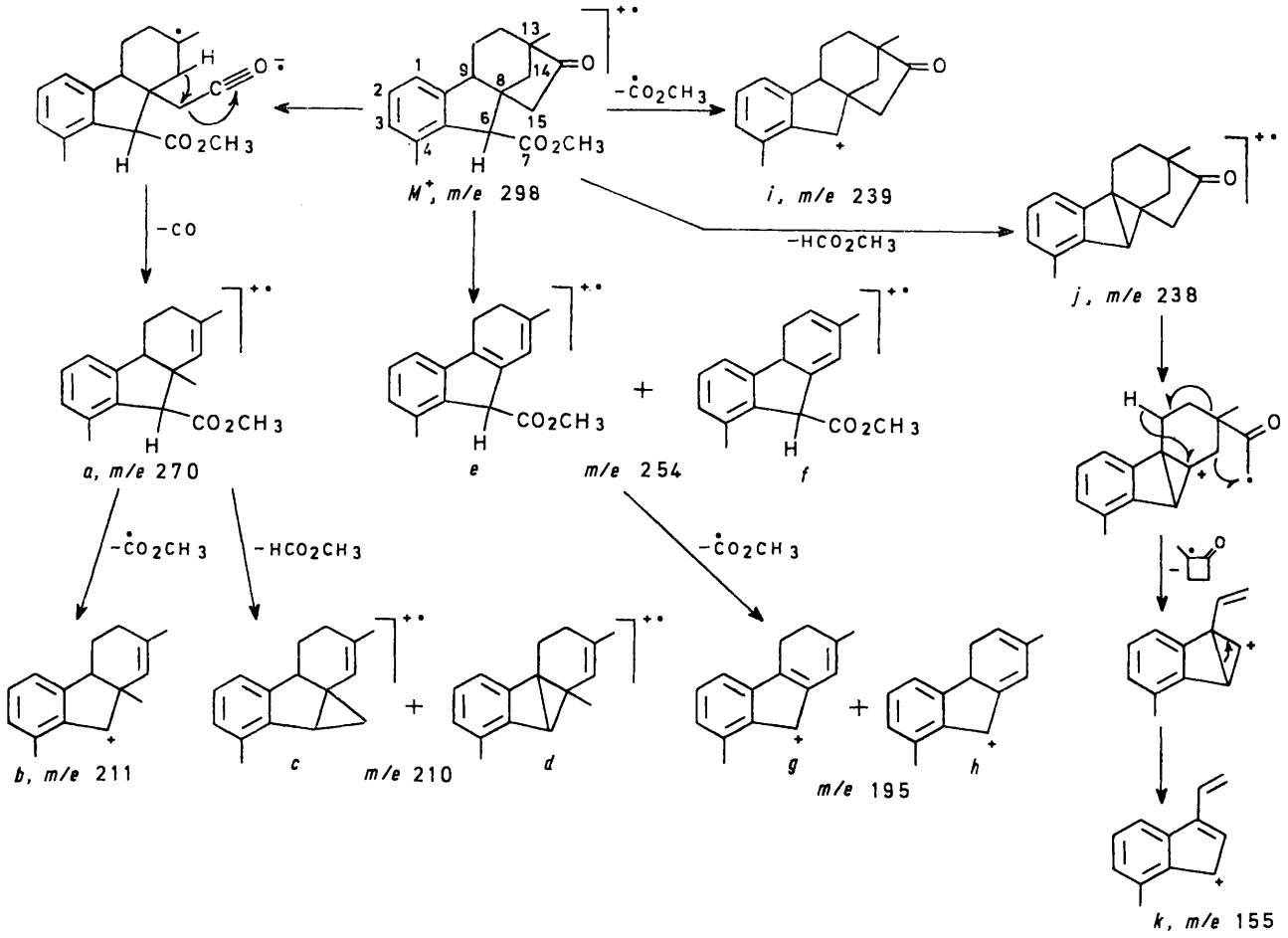
FIGURE 3 Mass spectrum of methyl 9-*epi*-gibberate (IIIa)

A dual mechanism operates for the formation of the ion at *m/e* 254 ($C_{17}H_{18}O_2$). As shown in Scheme 1 this fragmentation involves loss of the D ring together with two additional hydrogen atoms.[6] According to the *m/e* values of the corresponding ions from (Ib) and (IIb), these hydrogen atoms originate in part from C-9, suggesting *e* and *f* as probable structures for the resulting fragment ions. The 9-*epi*-isomer (III) does not fragment in this way, indicating a stereochemical or conformational requirement for the particular fragmentation. Further cleavage of *e* and *f* with loss of a methoxycarbonyl radical results in the stabilized ions *g* and *h* at *m/e* 195.

The most important and useful fragmentation of this series of compounds involves the direct elimination of methyl formate and/or a methoxycarbonyl radical from the molecular ions. As seen in Figures 1—3 and Table 1, the relative intensities of the resultant fragment ions are heavily dependent on the stereochemistry of the substituents at C-6 and C-9. If the methoxycarbonyl group at C-6 and the hydrogen at C-9 have a *trans*-orientation, as in methyl gibberate (I), then the loss of the methoxycarbonyl radical is the uniquely preferred cleavage, resulting in an ion *i* at *m/e* 239 ($C_{17}H_{19}O$). However, if the two groups have a *cis*-orientation, as in the case of the 6-*epi*- (II) and the 9-*epi*-isomers (III), then simple cleavage of the methoxycarbonyl group is considerably suppressed and the much preferred fragmentation involves the concerted elimination of methyl formate to give *m/e* 238 ($C_{17}H_{18}O$). That the stereochemical requirement for this loss of methyl formate is a *cis*-orientation of the two groups is demonstrated from a comparison of the *m/e* values of the corresponding ions from (IIb) and (IIIb). In each case the equivalent ion occurs predominantly at *m/e* 238, demonstrating that the

additional hydrogen atom originates almost completely from C-9, and results in an ion *j* as shown in Scheme 1.

However, a small percentage of the equivalent ions from (IIb) and (IIIb) do retain the original label (9 and 16% respectively). The origin of the additional hydrogen atom for the formation of these ions is unknown. An abundant ion *k* appears at *m/e* 155 ($C_{12}H_{11}$) which derives from *m/e* 238 through loss of C_5H_7O, and a mechanism for the formation of *k* from *j* is shown in Scheme 1. However, such a fragmentation pathway ($M^+ \longrightarrow j \longrightarrow k$) precludes retention of the original C-9 hydrogen atom, and as seen in Table 1, partial retention of label occurs in the corresponding ion from each of the labelled compounds. It is possible that such an ion could result from *i* (*m/e* 239) through loss of C_5H_8O, but it is difficult to write a simple mechanism for such a transformation.



SCHEME 1

Allogibberic Acid Derivatives.—The mass spectra of the methyl esters of compounds in the allogibberic acid series are considerably more complicated than those with similar stereochemistry at C-6 and C-9 in the gibberic acid series. The spectra of compounds (IVa)—(VIa) are shown in Figures 4—6, and are of general use only for fingerprinting of the pure compounds. However, the cracking pattern $M^+ \longrightarrow m/e$ 238 ($C_{17}H_{18}O$) and/or *m/e* 239 ($C_{17}H_{19}O$) $\longrightarrow m/e$ 155 ($C_{12}H_{11}$) is again the most important pathway and provides information regarding the stereochemical requirements for the loss of methyl formate and the methoxycarbonyl radical from this class of compounds. The relative intensities of the relevant ions are given in Table 2, from which it can be seen that the different

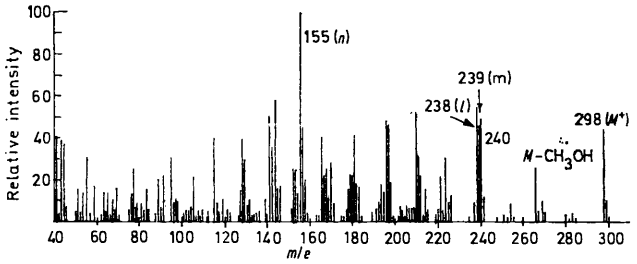


FIGURE 4 Mass spectrum of methyl allogibberate (IVa)

eliminations occur in a similar fashion to those in the gibberic acid series. When the C-9 hydrogen atom and the C-6 methoxycarbonyl group have a *cis*-relationship to one another, the much preferred fragmentation

is loss of methyl formate with some simple methoxycarbonyl cleavage also taking place [30—35% of peak group from (V) and (VI)]. However, when the two groups have a *trans*-orientation [as in (IV)], both types

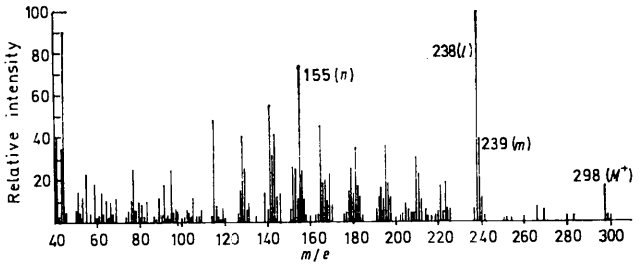


FIGURE 5 Mass spectrum of methyl 6-*epi*-allogibberate (Va)

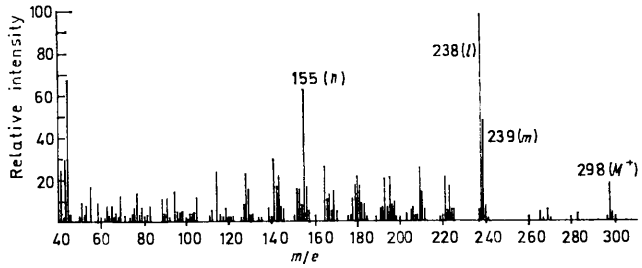
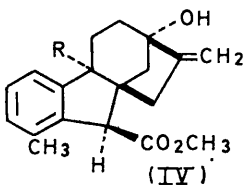
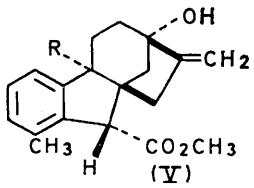


FIGURE 6 Mass spectrum of methyl 9-*epi*-allogibberate (VIa)

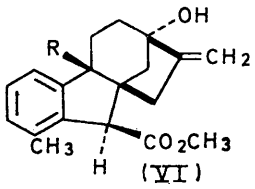
of fragmentation occur to a similar extent (Figure 4), and an additional ion occurs at *m/e* 240, corresponding to the loss of C_3H_6O from the molecular ion.



a; R = H
b; R = ^{2}H



a; R = H
b; R = ^{2}H



a; R = H
b; R = ^{2}H

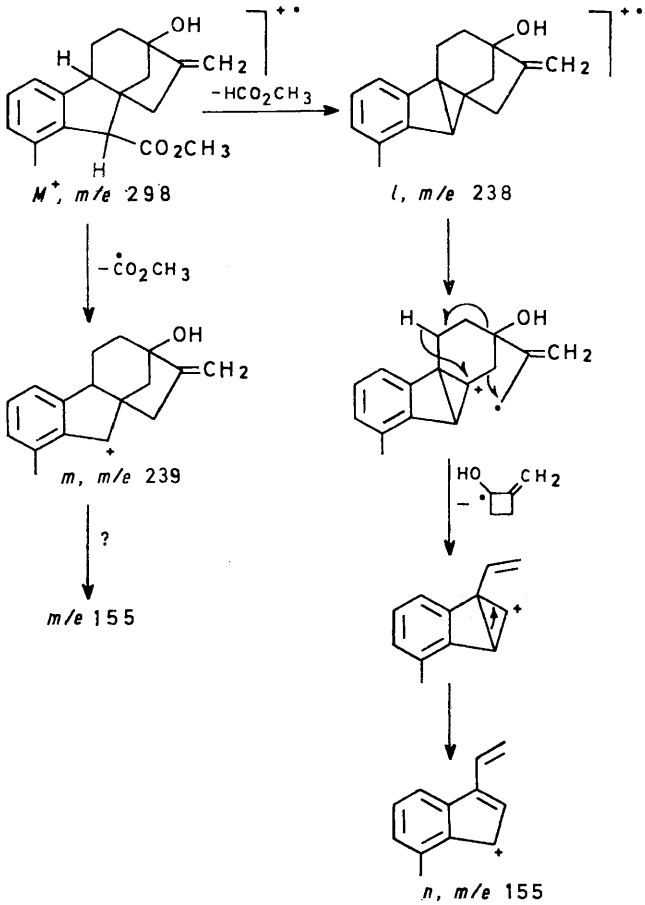
The stereospecific nature of the methyl formate elimination is again clear from the labelling data, the hydrogen atom at C-9 being lost preferentially together with the methoxycarbonyl group in a concerted mechanism, resulting in ion *l* at *m/e* 238 (Scheme 2). The ion at *m/e* 239 (*m*) is the result of simple cleavage of the methoxycarbonyl group from the gibberellin skeleton. Each or both of these species fragments further to the important species at *m/e* 155 (*n*; Table 2). However, as discussed above for the methyl gibberate analogues, the total mechanistic picture for the formation of this ion is uncertain, since the original label is only partially retained in the corresponding ion from each labelled compound. The probable mechanism is presented in Scheme 2 for the formation of *n*, which has lost C-9 hydrogen; we are unable to write a simple mechanism for the other transformation.

TABLE 2

Some important ions in the mass spectrum of methyl allogibberate and related compounds at 70 eV

Compound	M^+	*m/e* 238	*m/e* 239	*m/e* 240	*m/e* 155	*m/e* 156
(IVa)	298 (46)	56	36	43	100	31
(IVb)	299 (34) 298 (3)	20	70	77	66	92
(Va)	298 (16)	100	21		74	15
(Vb)	299 (35) 298 (3)	100	33	44	57	53
(VIa)	298 (17)	100	29		63	10
(VIb)	299 (13) 298 (5)	100	31	25	57	35

Hexahydrofluorene-9-carboxylic Acid Derivatives.—Two isomeric compounds in the 1,2,3,4,4a,9a-hexahydrofluorene-9-carboxylic acid series had been made by catalytic hydrogenation of fluorene-9-carboxylic acid in

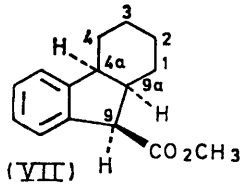


SCHEME 2

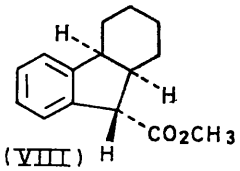
connection with another study. Their synthesis will be described elsewhere.[12] It was assumed that the method of synthesis would result in a *cis*-fused ring

[12] R. J. Pryce, *Phytochemistry*, in the press.

system, but the relative stereochemistry at C-9 remained uncertain. However, by comparison of the mass spectral cracking patterns of the methyl esters of these two compounds with those of the gibberic and allogibberic acid derivatives described above we are now able to assign the relative stereochemistries as shown in (VII) and (VIII).



(VII)



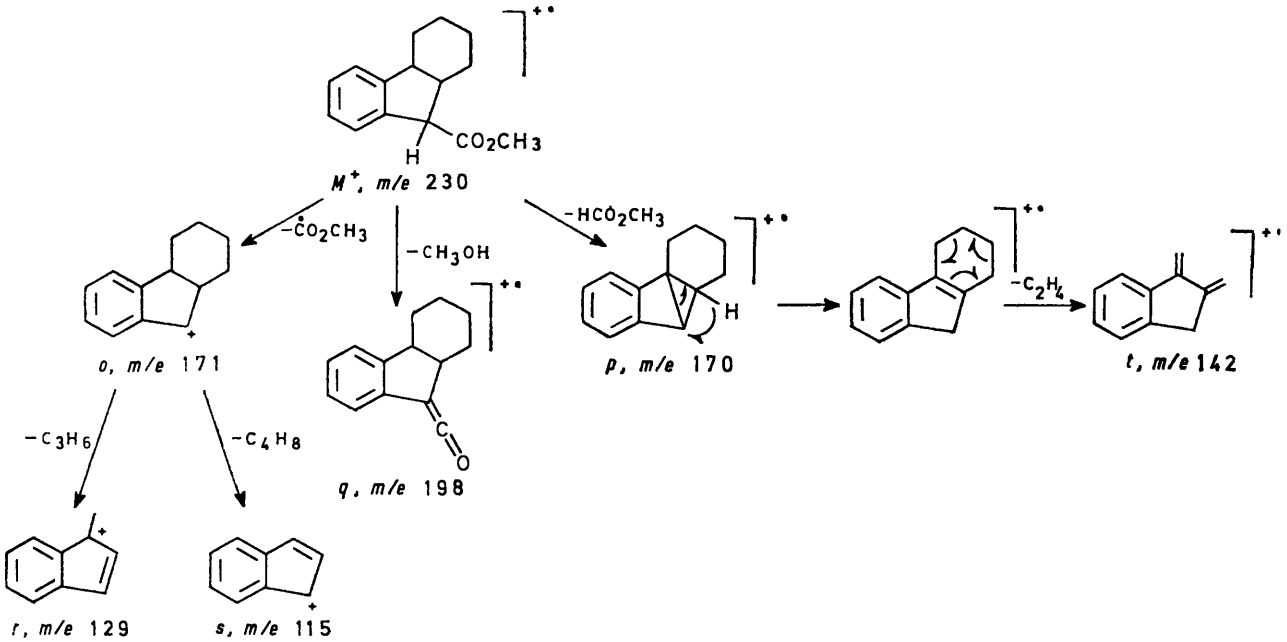
(VIII)

This assignment is consistent with the acid corresponding to (VII) being the major product from catalytic hydrogenation, with hydrogenation occurring to the elimination of the methoxycarbonyl radical and methyl formate respectively from the molecular ion. Since one isomer preferentially loses the methoxycarbonyl radical we assign it the *trans*-4a-H, 9-CO_2CH_3 configuration [as in (VII)], in line with the observed fragmentation pathway in methyl gibberate (Ia) and methyl allogibberate (IVa). This results in the formation of ion *o* at *m/e* 171 as shown in Scheme 3. Similarly, a *cis*-4a-H, 9-CO_2CH_3 configuration [as (VIII)] is assigned to the other isomer because the preferred cleavage involves loss of methyl formate (to give *p*) from the molecular ion, as was the case with the fragmentation of the 9-*epi*- and 6-*epi*-gibberates and allogibberates above. In the simpler hexahydrofluorene ring system of (VIII), the stereochemical control for elimination of methyl formate is not absolute, a large fragment ion at *m/e* 171 (*o*) being observed for this

TABLE 3

Principal fragment ions in the mass spectrum of methyl hexahydrofluorene-9-carboxylates (VII) and (VIII) at 70 eV

Compound	M^+	$M - CH_3OH$	$M - \dot{C}O_2CH_3$	$M - HCO_2CH_3$	$M - (HCO_2CH_3 + C_2H_4)$	$M - (\dot{C}O_2CH_3 + C_3H_6)$	$M - (\dot{C}O_2CH_3 + C_4H_8)$	$C_7H_7^+$
(VII)	230 (19)	198 (10)	171 (96)	170 (26)	142 (7)	129 (44)	115 (8)	91 (15)
(VIII)	230 (22)	198 (3)	171 (79)	170 (100)	142 (15)	129 (59)	115 (28)	91 (24)



SCHEME 3

predominantly *anti* to the carboxy-group. That the acid corresponding to the minor hydrogenation product (VIII) is a 9-epimer of acid from (VII) has been confirmed by alkaline epimerization of the acid from (VII) to that from (VIII).

The mass spectra of (VII) and (VIII) are quite simple, and the relative intensities of the prominent ions are given in Table 3. The only major difference between the two spectra occurs in the relative intensities of the ions at *m/e* 171 ($C_{13}H_{15}$) and 170 ($C_{13}H_{14}$), which correspond compound also. At low electron voltage loss of methyl formate from (VIII) is more pronounced. The relative intensities of the *m/e* 170 and 171 ions from (VIII) at 12 eV are 100 and 61%, while those of the corresponding ion from (VII) remain at 26 and 96% respectively.

Otherwise the spectra of (VII) and (VIII) are quite similar, and the cracking patterns may be rationalized as shown in Scheme 3. Apart from the small peak at *m/e* 198 (*q*) due to loss of methanol, the ions at lower *m/e* values are due to hydrocarbon species *r*—*t* being

formed by further cleavage of the primary fragmentation ions at *m*/e 170 and 171.

EXPERIMENTAL

Low resolution spectra were obtained using an A.E.I. MS30 instrument in the g.l.c.–m.s. mode. A 1·5 m × 1·5 mm i.d. silanized glass column of 1% OV-17 on Gas Chrom Q (100—120 mesh) was used with an oven temperature program of 180—230° at 7° min^{-1} and a helium flow of 45 ml min^{-1}. The source temperature of the mass spectrometer was 250° and each spectrum was obtained at 70 eV. Accurate mass measurements were calculated directly from the u.v. trace of the MS30 and in general had an accuracy of ±20 p.p.m. against a standard sample.[13]

Synthesis of the gibberellin derivatives has been described in a previous paper.[14] The isotopic composition of each deuterium-labelled compound was calculated by summing and averaging spectra obtained by multiple scanning of the molecular ion region, and is shown in Table 4 as a percentage of the molecular ion peak group.

TABLE 4

Compound	Isotopic composition (%)
(Ib)	92·1 $^2H_1$,7·9 $^2H_0$
(IIb)	93·7 $^2H_1$,6·3 $^2H_0$
(IIIb)	61·4 $^2H_1$,38·6 $^2H_0$
(IVb)	92·3 $^2H_1$,7·7 $^2H_0$
(Vb)	93·7 $^2H_1$,6·3 $^2H_0$
(VIb)	69·4 $^2H_1$,30·6 $^2H_0$

We thank Mr. D. T. Green and Mrs. J. P. Tucker for obtaining the high and low resolution spectra.

[4/014 *Received, 4th January,* 1974]

[13] A.E.I. Publication No. 2033-41d EdA 2500 0571.
[14] R. J. Pryce, *J.C.S. Perkin I,* 1974, 1179.