

# PLANT HORMONES—IX<sup>1</sup>

## PHASEIC ACID, A RELATIVE OF ABSCISIC ACID FROM SEED OF *PHASEOLUS MULTIFLORUS*. POSSIBLE STRUCTURES

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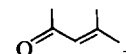
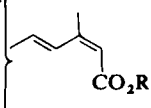
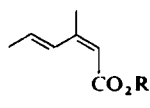
(Received in the UK 8 August 1969; Accepted for publication 20 August 1969)

**Abstract**—IR, UV, NMR and MS have been used to investigate the structure of phaseic acid, a C<sub>15</sub>-acid isolated from immature seed of *Phaseolus multiflorus*. Of the seven structures considered, the spectroscopic data is best interpreted in terms of the epoxide (I) or the oxetane (XV). Phaseic acid appears to be a close structural relative of the natural plant growth inhibitor, (S)-(+)-abscisic acid.

The previously uncharacterized acid, m.p. 207–209°, obtained from immature seed of *Phaseolus multiflorus* by MacMillan *et al.*<sup>2</sup> has been re-isolated and named phaseic acid.<sup>3</sup> Detection and isolation of the acid was greatly aided by GLC after methylation with diazomethane. With limited amounts of phaseic acid (6 mg from 25 Kg seed) our information on its structure is derived mainly from spectroscopic data. In a preliminary communication<sup>3</sup> we briefly described this data which obliged us to advance structure (I) depicting phaseic acid as a close structural relative of (S)-(+)-abscisic acid (II).<sup>4–6</sup> Since several doubts remain\* over the structure of phaseic acid we present our evidence in full in this and the following paper.<sup>7</sup>

Phaseic acid was shown to be a monocarboxylic acid, C<sub>15</sub>H<sub>20</sub>O<sub>5</sub>, by high resolution MS of the acid and its monomethyl ester, m.p. 155–158°. IR and NMR data for phaseic acid and methyl phaseate are presented in Tables 1, 2 and 3 and compared with the

TABLE 1. IR SPECTRA OF PHASEIC AND ABSCISIC ACIDS AND THEIR METHYL ESTERS

Phaseic acid (Nujol mull) cm <sup>-1</sup>	Assignment	Methyl phaseate (CHCl <sub>3</sub> ) cm <sup>-1</sup>	Absciscic acid (KBr disc)* cm <sup>-1</sup>	Assignment	Methyl abscisate (CHCl <sub>3</sub> ) cm <sup>-1</sup>
3480	— OH —	3588	3405	— OH —	3610
1718	— sat C=O —	1723	1650		1667
ca. 2700 vbr		ca. 1680 sh	3405–2300 vbr		
1684					
ca. 1670 sh					
1637					
1610		1711	1674		1710
		1639	1623		1637
		1606	1600		1604

Abbreviations: sat = saturated, br = broad, vbr = very broad, sh = shoulder

\* Added in Proof. See also B. V. Milborrow, *Chem. Comm.* 1969, 966. We thank Dr. Milborrow for his courtesy in sending us a copy of his manuscript before submission.

TABLE 2. NMR SPECTRA OF ABSCISIC AND PHASEIC ACIDS AT 100 MHz

Absciscic acid (D <sub>6</sub> -acetone)			Phaseic acid (D <sub>6</sub> -acetone)		
$\tau$	Integral	Assignment (see II)	$\tau$	Integral	Assignment (see III→VII)
9.00	3H	a,b	9.01	3H	A
8.96	3H		8.84	3H	B
8.15 db $J$ 2Hz	3H	c,d	7.95 br $\Delta\nu\frac{1}{2} J$ 2.5Hz	3H	C
8.00 db $J$ 2Hz	3H				
7.89	1H	e,f	7.71	1H	D,E F,G
7.54	1H		7.33	1H	
qt $J_{ef}$ 16Hz			7.64	1H	
			7.25	1H	
8.00 (ca.)	1H	—OH	7.80 (ca.)	1H	—OH
			6.42 db $J_{HI}$ 7.5Hz	1H	H
			6.11	1H	I
			qt $J_{HI}$ 7.5Hz and $J$ 3.5Hz		
5.80 (ca.) vbr	1H	—CO <sub>2</sub> H	6.15 (ca.) vbr	1H	—CO <sub>2</sub> H
4.33 br	1H	g,h	4.28 br	1H	J
4.26 br	1H		3.42	1H	K
3.71	1H	i	qt $J_{KL}$ 16Hz	1H	L
2.16	1H	j		1H	

db = doublet, qt = quartet(AB), br = broad, vbr = very broad, and all the other peaks are sharp singlets.  
 $\Delta\nu\frac{1}{2}$  = line width at half height.

corresponding data for abscisic acid and methyl abscisate. Analysis of this data indicate many similarities between the structures of phaseic acid and abscisic acids and, together with UV and MS data discussed below, suggests the presence of the structural units (III to VII) in phaseic acid and methyl phaseate.

The presence of the  $\beta$ -methyl-2-*cis*-4-*trans*-dienoic acid grouping (III, R = H) has been amply confirmed. While the IR data on the acid and methyl ester do not distinguish between the *cis*, *trans*-, *trans*, *trans*-, and *trans*, *cis*-dienoic acids,<sup>8</sup> the UV spectrum of phaseic acid [ $\lambda_{\max}$  (MeOH) 258 nm ( $\epsilon$  14,500)] is closest to that expected for a *cis*, *trans*-geometry.<sup>4, 9-11</sup> Strong evidence for the *cis*, *trans*-geometry (III) is also provided by the NMR spectra (Tables 2 and 3) of phaseic acid and methyl phaseate. The chemical shift of the  $\beta$ -Me protons (C), allylically coupled to the  $\alpha$ -proton (J), is similar to that in abscisic acid and methyl abscisate and supports the assignment<sup>4</sup> of the *cis*-stereochemistry to the  $\alpha,\beta$ -double bond. A *trans*-double bond would be expected to shift the  $\beta$ -Me protons downfield by ca. 0.25 ppm.<sup>12</sup> The  $\gamma$ (L)- and  $\delta$ (K)-protons are similar in coupling constants and chemical shifts to the corresponding protons in abscisic acid and methyl abscisate. Only one OMe signal is observed in the NMR spectrum of methyl phaseate confirming that phaseic acid is a monocarboxylic acid.

Further evidence for the 2,4-dienoic acid grouping (III) in phaseic acid and methyl

TABLE 3. NMR SPECTRA OF METHYL ABSICATE AND METHYL PHASEATE

Methyl abscisate (CCl <sub>4</sub> ) 60MHz* τ		Methyl phaseate (CDCl <sub>3</sub> ) 100MHz τ		Methyl phaseate [(CD <sub>3</sub> ) <sub>2</sub> SO] 100MHz τ	
Integral	Assignment (see II)	Integral	Assignment (see III→VII)	Integral	Assignment (see III→VII)
8.99 8.91	3H } 3H }	9.00 8.80	A B	9.16 8.97	3H A 3H B
8.89 7.99	3H } 3H }	8.04 br (db J 1.5Hz)†	C	8.02 br	3H C
6.92	1H —OH	7.80 (ca.) br	—OH	7.78	2H } Two AB systems
7.73 } 7.71 }	2H e,f	7.55 7.43	D,E F,G	7.40 } 7.40τ J <sub>DE</sub> 18Hz qt at 7.40τ J <sub>FG</sub> 18Hz and J 3Hz	1H D,E 1H F,G
6.37	3H —OCH <sub>3</sub>	6.36	—OCH <sub>3</sub>	6.45	3H —OCH <sub>3</sub>
		6.32 db J <sub>HI</sub> 8Hz	H	6.55 } 6.29 } db J <sub>HI</sub> 8Hz qt J <sub>HI</sub> 8Hz and J 3Hz	1H H 1H I
4.35 4.29	1H } 1H }	6.10 4.28 br (qt J 1.5Hz)	I J	4.23 br	1H J
3.86 } 2.86 }	1H } 1H }	3.82 } 1.89 } qt J <sub>KL</sub> 16Hz	K L	3.54 } 2.10 } qt J <sub>KL</sub> 16Hz	1H K 1H L

\* Obtained by J. W. Cornforth with (RS)-methyl abscisate, private communication.

† Refers to a 60MHz spectrum of methyl phaseate in CDCl<sub>3</sub>.

ester is provided by mass spectroscopy.<sup>7</sup> Phaseic acid and methyl phaseate show significant peaks at  $m/e$  111 and  $m/e$  125 respectively which can be assigned to the rearrangement ions (IX, R = H and R = Me). The appropriate metastable for the transition  $M^+ \rightarrow m/e$  125 ( $C_7H_9O_2$ ) was observed for the methyl ester. The formation of aromatic oxonium ions such as IX by 5,6-vinylic cleavage of 2,4-dienoic acids is well-documented,<sup>13,14</sup> and may be represented by X. We have also observed the ions (IX, R = H and R = Me) in the respective mass spectra of abscisic acid and methyl abscisate. In accord with the presence of a 2-*cis*-4-*trans*-dienoic acid grouping (III) in phaseic acid it is found that solutions of both methyl phaseate and methyl abscisate, on exposure to light, produce similar ratios of the *cis*, *trans*- and *trans*, *trans*-isomers.<sup>15</sup>

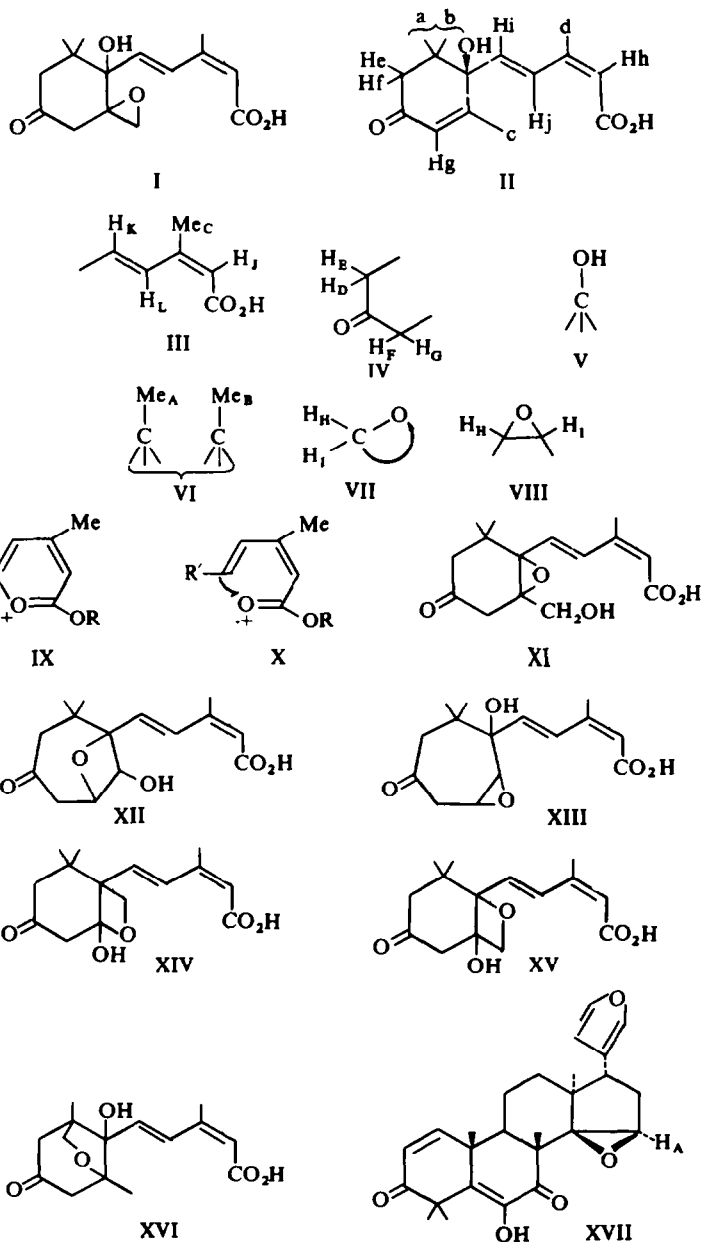
The IR spectra of phaseic acid and methyl phaseate (Table 1) indicate the presence of a saturated CO function which cannot be in a 5- (or smaller) membered ring. The NMR spectrum of phaseic acid (Table 2) strongly indicates that this CO function has four  $\alpha$ -methylene protons (D,E,F,G) which appear as two AB-quartets having chemical shifts and coupling constants consistent with their assignment to the protons in the grouping (IV). In methyl phaseate in  $CDCl_3$  solution, the pairs of protons D,E and F,G have the same chemical shift and appear as two 2-proton singlets.

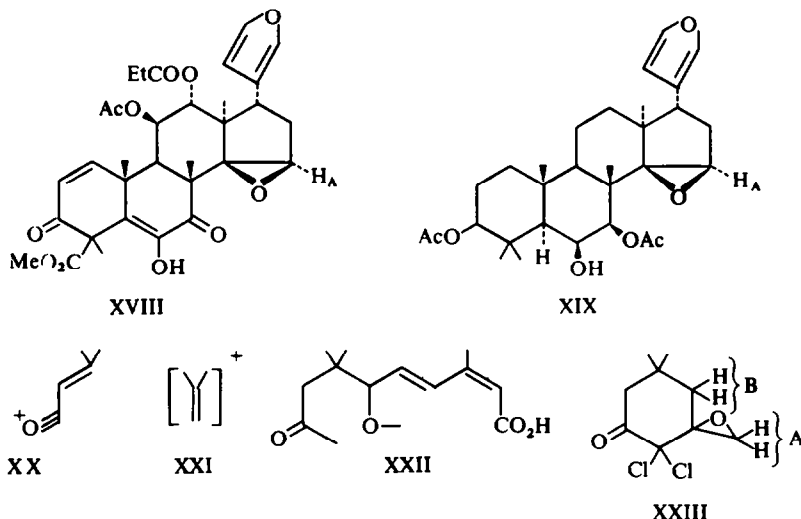
The presence of a tertiary OH group (V) in phaseic acid is indicated by the IR spectra of the acid and its methyl ester and by the NMR spectrum of the methyl ester in hexadeuterodimethyl sulphoxide which showed to additional couplings<sup>16</sup> compared with spectra in deuteriochloroform solution where no C-H—O-H coupling could be detected. We also found that methyl phaseate, like methyl abscisate, did not form a trimethylsilyl ether with hexamethyldisilazane and trimethylsilyl chloride in pyridine.<sup>3</sup> Although, since then, trimethylsilyl ethers of methyl abscisate<sup>17</sup> and methyl phaseate<sup>18</sup> have been prepared with bis-(trimethylsilyl)acetamide, we consider that the lack of formation of these derivatives with the hexamethyldisilazane reagent is indicative of a hindered tertiary OH group in phaseic acid as in abscisic acid. Similar non-formation of trimethylsilyl ethers with hexamethyldisilazane has been observed<sup>19</sup> in the case of hindered tertiary OH's of some carotenoids where the OH's were in a similar hindered environment to that in abscisic acid.

The presence of two tertiary Me groups (VI) in phaseic acid is revealed by the occurrence of two sharp 3-proton signals (A and B) in NMR spectra of phaseic acid and methyl phaseate.

The function of the fifth oxygen and of the two hydrogens (H,I) which are not accounted for in the expressions (III and VI) are crucial in defining the structure of phaseic acid. The two hydrogens (H,I) appear as an AB-quartet in the NMR spectra of phaseic acid (Table 2) and methyl phaseate (Table 3). Their chemical shifts indicate that they are attached to C atom(s) bearing the fifth oxygen function which is concluded to be ethereal from the absence of spectroscopic evidence for any other type of oxygen function. The coupling constant  $J_{HI}$  7.5 Hz and the chemical shift of protons H and I are discussed in detail later with reference to specific structures for phaseic acid but the NMR data indicate the grouping VII rather than the alternative VIII. Another fact to be accounted for in defining the structure of phaseic acid is that the proton I in the grouping VII is further coupled to proton D in the system IV; this coupling, shown by spin decoupling, is most clearly seen in the spectra of the methyl ester in hexadeuterodimethylsulphoxide.

The functions in groupings (III–VII) indicate that phaseic acid,  $C_{15}H_{20}O_5$ , is bicyclic with one carbocyclic ring and one oxygen-containing ring. Seven such structures were considered for phaseic acid. Of these, structures XI and XII were eliminated on the evidence, cited above, that phaseic acid contained a tertiary hydroxyl group; and structure XIII seemed improbable from the coupling constant,  $J_{HI}$  7.5 Hz, which is much larger than that found<sup>20a</sup> for *trans*-(2.3 to 3.1 Hz) or *cis*-(3.0 to 4.5 Hz)-vicinal coupling in 1,2-epoxides.





A decision between the four remaining structures—the terminal epoxide (I), the oxetanes (XIV and XV), and the 5-ring ether (XVI)—cannot easily be made on the NMR data. None of these structures provides an obvious explanation for the observed  $J$  3.5 Hz between proton I in VII and proton D in IV. Nor can any of these structures be conclusively excluded by comparing the NMR data for the protons H and I in phaseic acid with the  $\tau$ - and  $J_{gem}$ -values, reported in the literature, for the  $\alpha$ -protons in cyclic ethers (Table 4). Although the  $J_{HI}$  in phaseic acid is higher, and the  $\tau$ -values

TABLE 4. COMPARISON OF THE  $\tau$ -VALUES AND  $J_{gem}$  OF  $\alpha$ -PROTONS IN CYCLIC ETHERS AND OF PROTONS H AND I IN PHASEIC ACID

	Phaseic acid	Tetrahydrofurans	Oxetanes	Terminal epoxides
$\tau$	6.11, 6.42	6.0–6.7 <sup>21a-d</sup>	5.0–6.0 <sup>23a-c</sup>	6.5–7.7 <sup>20a-d</sup>
$J_{gem}$ (Hz)	7.5	6.7–9.9 <sup>22</sup>	5.0–7.3 <sup>22, 23e</sup>	4.0–6.3 <sup>22</sup>

for these protons a little lower, than the values found for terminal epoxides the effect of the cyclohexanone CO function is difficult to assess. For example, the epoxide protons  $H_A$  in cedrelone (XVII)<sup>24</sup> and hirtin (XVIII)<sup>25</sup> have chemical shifts of 6.22 and 6.07  $\tau$  respectively; they are probably deshielded by 7-CO group since proton  $H_A$  in the diacetate (XIX), derived from cedrelone, resonates at 6.78  $\tau$ .<sup>24</sup> The oxetane structures (XIV and XV) provide the least best fit; although  $J_{HI}$  seems a reasonable fit, the observed  $\tau$ -values for protons H and I are higher than normally found for oxetanes. Indeed the CO group in the cyclohexanone ring might be expected to deshield the  $\alpha$ -protons in these oxetanes (*vide supra*). Although the  $\alpha$ -methylene protons in the tetrahydrofuran (XVI) provide an excellent fit for the  $\tau$ - and  $J_{HI}$ -values in phaseic acid, this structure and the oxetane structure (XIV) were excluded on the following evidence.

High resolution MS of phaseic acid and methyl phaseate, discussed in detail in the following paper,<sup>7</sup> contained prominent peaks with appropriate metastable ions for

the sequence  $M^+ \rightarrow m/e$  83 ( $C_5H_7O$ )  $\rightarrow m/e$  55 ( $C_4H_9$ ). These fragment ions were formulated as XX and XXI respectively and it was concluded that phaseic acid, like abscisic acid, possesses a *gem*-dimethyl grouping on carbon,  $\beta$ - to the CO group. This conclusion seemed in harmony with the similar chemical shifts of the two tertiary Me groups (A,B) in phaseic acid and methyl phaseate with those (a,b) of abscisic acid and its methyl ester (Tables 2 and 3). This similarity together with the similar chemical shifts of the  $\delta$ -protons (K and i) in the dienoid side chain in both series further suggested the presence of an oxygen function between the dienoid acid grouping (III) and the *gem*-dimethyl grouping as in partial structure XXII. Additional evidence for the sequence XXII is adduced from high resolution MS and is presented in the following paper.<sup>7</sup> The  $\gamma$ -proton (L) in the dienoid acid (IV) occurs at lower field in phaseic acid and methyl phaseate than in abscisic acid and its methyl ester and is presumably deshielded by the fifth oxygen function in phaseic acid.

The presence of the grouping XXII in phaseic acid would rule out the tetrahydrofuran (XVI) and the oxetane (XIV) structures for phaseic acid, leaving the epoxides (I) and the oxetane (XV). A decision between these two structures, in favour of the epoxide (I), is made in the following paper.<sup>7</sup> Structure I requires a long-range coupling of 3.5 Hz between one of the epoxide protons and one of the methylene protons,  $\alpha$ - to the CO group (*vid supra*). Long-range coupling through epoxides has been observed but of a lower magnitude, the largest being 1.1 Hz in indene oxide.<sup>26</sup> A long-range coupling of 0.7 Hz has been observed<sup>27</sup> in the epoxide (XXIII) between the high-field signal of protons B and the lower field proton of protons A.

Although abscisic acid (II) exhibits a very intense positive Cotton effect with extrema at 287 nm ( $[\alpha] + 24,000^\circ$ ) and 245 nm ( $[\alpha] - 69,000^\circ$ ) in acidified ethanol, phaseic acid shows only a plain negative curve. It would appear therefore that the enhanced Cotton effect in abscisic acid may be due to interaction between the chromophores of the dienoid acid and the  $\alpha,\beta$ -unsaturated ketone. This Cotton effect has been used to detect very low concentrations of (*S*)-(+)-abscisic acid in several plant extracts<sup>28, 29</sup> but it could not be detected in the crude acidic fraction from extracts of seed of *P. multiflorus* by this method.

## EXPERIMENTAL

M.ps are corrected. GLC was carried out with a Pye 104 dual column instrument fitted with flame ionization detectors. Silanized glass columns,  $5' \times 5/32''$  i.d. were packed with 2% QF-1 or 2% SE-33 on demineralized and silanized Gaschrom A. Trimethylsilyl ethers were prepared as previously described.<sup>30</sup> For TLC silica layers (0.3 mm) were developed in the solvent systems described and sprayed with 4%  $Ce(SO_4)_2$  in 10%  $H_2SO_4$  aq. UV spectra were obtained with a Cary 14 spectrophotometer. IR spectra were obtained with a Perkin Elmer 257 spectrophotometer. NMR spectra were obtained with a Varian HA100 or A-60 spectrometer for solns as described with TMS as internal standard. Mass spectra were obtained with an A.E.I. M.S.9 instrument. Light petroleum had b.p. 60–80°.

*Isolation of phaseic acid.* Immature seeds of *P. multiflorus* (25.8 Kg) were extracted and the crude acid extract (16.1 g) chromatographed as previously described.<sup>30</sup> Phaseic acid was isolated from fraction 40, eluted with 47% acetone in water, from charcoal–celite chromatography of the crude acid extract. Phaseic acid and gibberellin A<sub>1</sub> had previously been detected in this fraction as their methyl esters by GLC. Methyl phaseate had retention times of 6.3 and 3.8 min (methyl abscisate 5.0 and 3.0 min) on 2% QF-1 and 2% SE-33 columns respectively (column temp 180°; carrier gas,  $N_2$ , 60 ml/min in all cases). Crude phaseic acid (16 mg) was eluted from a celite–silicic acid column with 5 to 15% EtOAc in  $CHCl_3$ . Repeated crystallization from EtOAc–light petroleum gave phaseic acid (6.4 mg) in prisms m.p. 205–207.5° (cf. 207–209° ref 2) parent ion measured 208.132,  $C_{15}H_{20}O_5$  requires 280.131; TLC  $R_f$  value, 0.55 in EtOAc/ $CHCl_3$ /AcOH (15:5:1) (abscisic acid had  $R_f$  value, 0.65 under the same conditions).

**Methyl phaseate.** A methanolic soln of phaseic acid (5.4 mg) was treated with excess ethereal diazomethane. After filtration and evaporation, crystallization from benzene-light petroleum afforded methyl phaseate (5 mg) in needles m.p. 155–158°; parent ion measured 294.145,  $C_{16}H_{22}O_3$  requires 294.147; TLC  $R_f$  value, 0.23 in EtOAc/benzene (3:7) (methyl abscisate had  $R_f$  value 0.40 under the same conditions).

**ORD determination of abscisic acid in the crude acid extract of *P. multiflorus*.** A portion of the crude acid extract (26 mg) of immature seed of *P. multiflorus* was chromatographed on a thin layer of pre-eluted (EtOAc) silica gel (20 × 20 cm) eluting with n-propanol/n-butanol/ammonia/water (6:2:1:2, v/v).<sup>28</sup> An authentic sample of (RS)-abscisic acid had  $R_f$  value, 0.85 on a similarly eluted analytical plate. Bands of  $R_f$  values, 0.80 to 0.90 (0.4 mg), 0.70 to 0.80 (2.5 mg), and 0.60 to 0.70 (3.5 mg) were extracted with MeOH and examined by ORD for abscisic acid. In none of these fractions could any abscisic acid be detected.

**Acknowledgements**—For gifts of synthetic (RS)-abscisic acid we are indebted to Dr. J. W. Cornforth, F.R.S. and Dr. D. Yeo of Shell Ltd. Mass spectra was determined mainly by Dr. R. Binks, but we are grateful to B. E. Webster, I.C.I. Ltd. for some of the initial spectra. Some of the NMR spectroscopy was carried out by Mr. D. Greatbanks, I.C.I. Ltd. The ORD determinations on phaseic acid were carried out by Dr. J. W. Cornforth, F.R.S. and G. Ryback, and the ORD determination of abscisic acid in *P. multiflorus* extracts was by Dr. J. R. Hanson.

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