Phytochemistry, 1975, Vol. 14, pp. 1045–1053. Pergamon Press. Printed in England.

THE ABSOLUTE CONFIGURATION OF PHASEIC AND DIHYDROPHASEIC ACIDS

B. V. MILBORROW

Shell Research Limited, Milstead Laboratory of Chemical Enzymology, Sittingbourne Research Centre, Sittingbourne, Kent ME9 8AG, England

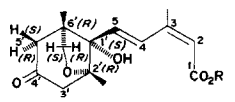
(*Received* 31 *July* 1974)

Key Word Index—Absolute configuration; phaseic acid; dihydrophaseic acid; methyl *O*-acetyl dihydrophaseate; *epi*-dihydrophaseic acid; NMR; H-bonding; deuterium exchange.

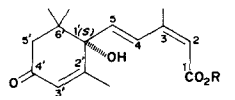
Abstract—A sample of phaseic acid methyl ester (5 mg), isolated from tomato plants fed (±)-abscisic acid, was reduced to a mixture of the epimeric dihydrophaseates which were separated by TLC. The more polar epimer was identical with the dihydrophaseate isolated from beans by Walton *et al.* [14]. Comparison of the NMR and IR spectra (H-bonding) of the two epimers shows the secondary hydroxyl of the less polar epimer is *cis* to the oxymethylene group, which is *cis* to the tertiary hydroxyl group. The absolute configuration of this centre is known so the absolute configuration of phaseic acid can be deduced. Phaseic acid is (−)-3-methyl-5{8[1(*R*),5(*R*)-dimethyl-8(*S*)-hydroxy-3-oxo-6-oxabicyclo-(3,2,1)-octane]}2-*cis*-4-*trans*-pentadienoic acid and both it and the reduction products exist in chair conformations. The more polar epimer isolated by Walton *et al.* is (−)-3-methyl-5{8[3(*S*),8(*S*)-dihydroxy-1(*R*),5(*R*)-dimethyl-6-oxabicyclo-(3,2,1)-octane]}2-*cis*-4-*trans*-pentadienoic acid. It is suggested that the less polar epimer should be referred to as *epi*-dihydrophaseic acid.

INTRODUCTION

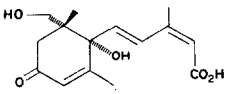
The revised structure of phaseic acid [1] (**1**), first isolated from beans [2], was confirmed by NMR analysis of material biosynthesized from deuteriated abscisic acid (**3**). The stereochemistry of the tertiary hydroxyl group of (**1**) was based on the then accepted absolute configuration of (+)-abscisic acid because phaseic acid was formed by the rearrangement *in vitro* of the unstable Metabolite C (**5**) which has a characteristic ORD spectrum closely similar to that of (+)-ABA [1]. The excess of the (−)-enantiomer of ABA and the β-D-glucopyranoside of (−)-ABA present in tomato plants fed with (±)-ABA [3] also suggested that the (+)-enantiomer only was converted into phaseic acid. This has been confirmed by Sondheimer *et al.* [4] who found that (−)-[2-^{14}C]ABA was not incorporated into phaseic acid by bean seedlings. Consequently the recent revision of the absolute configuration of (+)-ABA [5–9] also reversed the absolute configuration of the tertiary hydroxyl



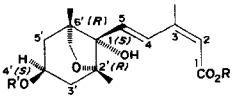
(**1**) Phaseic acid, R = H
(**2**) Methyl phaseate, R = Me



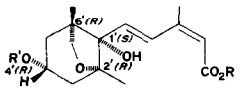
(**3**) (+)-(*S*)-Abscisic acid, R = H (revised absolute configuration)
(**4**) (+)-(*S*)-Methyl abscisate, R = Me



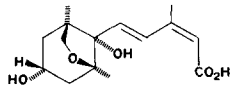
(**5**) Metabolite C



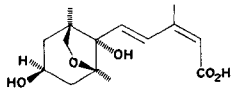
(**6**) Dihydrophaseic acid, R = H, R′ = H
(**7**) Methyl dihydrophaseate, R = Me, R′ = H
(**10**) Methyl 4′-*O*-acetyl dihydrophaseate, R = Me, R′ = MeCO



(**8**) *epi*-Dihydrophaseic acid, R = H, R′ = H
(**9**) Methyl *epi*-dihydrophaseate, R = Me, R′ = H
(**11**) Methyl *epi*-4′-*O*-acetyl dihydrophaseate, R = Me, R′ = MeCO



(**12**) Isomeric dihydrophaseic acid, R = H



(**13**) Isomeric *epi*-dihydrophaseic acid

group of phaseic acid which is now known to be as shown in (**1**). The outstanding questions of the stereochemistry of the oxymethylene bridge of phaseic acid and also the stereochemistry of the secondary hydroxyl groups of the epimeric dihydrophaseic acids (**6**) and (**8**), produced by reduction of the keto group of phaseic acid, remained to be answered.

The only structural evidence for the stereochemistry of the oxymethylene bridge of phaseic acid was the absence of intra-molecular H-bonding between the oxymethylene oxygen and the hydrogen of the tertiary hydroxyl group in CS_2; on this basis the oxymethylene bridge was tentatively assigned a *trans* position in relation to the tertiary hydroxyl group [10], i.e. on the opposite side of the cyclohexane ring. The recent synthesis of four isomers of phaseic acid by Isoe [11] showed that no intra-molecular H-bonding occurred with an oxymethylene bridge either *cis* or *trans* to the tertiary hydroxyl group, so the configuration of the bridge could not be decided on this negative evidence.*

The original numbering of phaseic acid [1] as a 6-oxabicyclo[3,2,1]-octane, while strictly correct, is confusing when referring to the derivation of the atoms of phaseic acid from those of abscisic acid. The numbering of the atoms in ABA has already been applied [12,13] to phaseic and dihydrophaseic acids and is adopted here.

Tinelli *et al.* [12] found that methyl phaseate (**2**) was reduced to a 1:6 mixture [14] of the two epimeric dihydrophaseic methyl esters by methanolic borohydride and suggested that the unequal ratio was caused by steric hindrance between the oxymethylene bridge and the borohydride molecules. The more polar epimer that they had isolated from bean seeds was formed in smaller amounts; consequently the oxymethylene bridge and the 4′-hydroxyl group of the less polar epimer were believed to project from the same face of the cyclohexane ring. They suggest [14] an axial position for the 4′ proton (whose complex signal occurs between 4·48 and 3·85 δ in the NMR spectrum of the more polar dihydrophaseate [12]).

* *Note added in proof.* Since this paper was prepared it has been learnt that Professor S. Isoe has independently confirmed these results using similar methods on synthetic materials and this work will be reported in *Tetrahedron*.

The elucidation of the direction of cyclisation of abscisic acid [15] depends on the absolute configuration of phaseic acid being known, so an attempt was made to relate unambiguously the known stereochemistry of the tertiary hydroxyl group of phaseic acid to the stereochemistry of the oxymethylene ring. The NMR and IR spectra of the two epimeric methyl dihydrophaseates permit the deduction of these relative configurations and consequently the absolute configuration is now known.

RESULTS AND DISCUSSION

General considerations

The methyl esters of the epimeric dihydrophaseic acids were prepared by reduction of methyl phaseate, and the epimer that had previously been isolated from bean seeds was identified by comparison of its NMR spectrum with the data given by Tinelli *et al.* [12]. It also co-chromatographed on silica gel TLC plates with the methyl ester of the more polar and abundant epimer of dihydrophaseic acid formed from (+)-[2-^{14}C]ABA by avocado fruit and bean plants. This epimer chromatographed at a slightly lower R_f in TLC system II than methyl *epi*-dihydrophaseate which appeared to be formed in slightly larger amounts during reduction with borohydride. Equal quantities of the crystalline materials were obtained (2·0 mg each); possibly the use of ice-cold aqueous methanol as solvent for the reduction gives a more equal ratio of products than the procedure used by Tinelli *et al.* [12].

The two dihydrophaseic esters were both prepared from the same sample of methyl phaseate and both, therefore, have the same absolute configuration as phaseic acid at C-1′, C-2′ and C-6′. Given that the absolute configuration at C-1′ is known, only two pairs of structures are possible for the two dihydrophaseic acids: **6**–**8** and **12**–**13**. Thus any evidence assigning a structure to one epimer automatically assigns a structure to the other; conversely, any evidence excluding a structure for one epimer excludes the corresponding structure for the other.

These stereochemical questions have to be answered in assigning structures to phaseic acid and its dihydro derivatives: (1) is the cyclohexane ring

Table 1. NMR spectra of methyl phaseate, methyl dihydrophaseate and methyl *O*-acetyl dihydrophaseate at 100 MHz

	Methyl phaseate			Methyl dihydrophaseate (more polar epimer)					Methyl *O*-acetyl dihydrophaseate	
	$CDCl_3$ δ*		*J* Hz	$CDCl_3$ δ	*J* Hz	C_6D_6 δ		*J* Hz	$CDCl_3$ δ	*J* Hz
C-1 O methyl	*s*	3·74		*s* 3·72		*s* 3·40			*s* 3·72	
C-2 H	*m*	5·82		*m* 5·76		*m* 5·70			*m* 5·76	
C-2 methyl	*d*	2·02	2	*d* 2·04	1·5	*d* 1·62		1·5	*d* 2·06	1·5
C-4 H	*d*	8·19	16	*d* 8·04	17	*d* 8·46		17	*d* 8·07	16
C-5 H	*d*	6·25	16	*d* 6·42	17	*d* 6·38		17	*d* 6·39	16
C-1′ OH	*s*	2·09		*s* 1·88		*s* 1·47			*s* 1·86	
C-2′ methyl	*s*	1·25		*s* 1·17		*s* 1·12			*s* 1·16	
C-3′ 2H	*s*	2·64		*c* 2·24 to 1·20		*c* 2·14 to 1·20			*c* 2·14 to 1·20	
C-4′ H		—		*c* 4·48 to 3·85		*c* 4·15 to 3·85			*c* 4·50 to 3·91	
C-4′ OH		—		*s* 3·50		*s* 3·15				
C-5′ HA	*m*	A 2·54	<2†							
HB		B 2·52		*c* 2·24 to 1·20		*c* 2·14 to 1·20			*c* 2·14 to 1·20	
C-6′ methyl	*s*	1·02		*s* 0·95		*s* 0·77			0·94	
C-6′ methylene 2H	*q*	A 3·982	8;2	*s* 3·78 (shoulder 3·80)		*q* A 4·002		8	*s* 3·84 (shoulder 3·80)	
		B 3·788	8			B 3·813		8;2*		
C-4′-*O*-acetyl		—	—	—		—			*s* 2·01	

* Chemical shifts in ppm from TMS.
† Long range coupling.

a chair or a boat (no other conformations are possible because of the rigidity imposed by the bicyclic structure, but the three substances need not have the same conformation)?; (2) which dihydrophaseic epimer has its secondary hydroxyl *cis* to the oxymethylene bridge?; and (3) is the oxymethylene bridge *cis* or *trans* to the tertiary hydroxyl (this relation is the same in all three substances)? These questions are answered in order below.

Conformation of the cyclohexane ring. This appears to be a chair in all three substances. The evidence is based on NMR spectra (see Tables 1 and 2 and Fig. 1).

All three esters show long-range coupling between one of the 5′ hydrogens and one of the hydrogens of the oxymethylene bridge. The "w" arrangement on which this coupling depends is approached more closely in the chair form than in the boat (see Fig. 2). The difference between the chemical shifts of the 4′ hydrogens, and the extent of coupling (c) of these hydrogens with their neighbours at C-3′ and C-5′, are readily explained on the basis of axial and equatorial hydrogens in a cyclohexane chair, but not in a boat. This evidence is discussed further below. Apart from this evidence, a boat conformation leads to severe interaction

Table 2. NMR spectra of methyl *epi*-dihydrophaseate and methyl-*O*-acetyl *epi*-dihydrophaseate at 100 MHz

	Methyl *epi*-dihydrophaseate (less polar epimer)					Methyl *O*-acetyl-*epi*-dihydrophaseate	
	$CDCl_3$ (δ)*	*J* Hz	C_6D_6 δ		*J* Hz	$CDCl_3$	*J* Hz
C-1 O methyl	*s* 3·73		*s* 3·40			*s* 3·71	
C-2 H	*m* 5·76		*m* 5·71			*m* 5·75	
C-3 methyl	*d* 2·01	1·5	*d* 1·57		2	*d* 1·99	1·5
C-4 H	*d* 8·038	16	*d* 8·365		16;2	*d* 8·04	16
C-5 H	*d* 6·172	16	*d* 5·945		16	*d* 6·115	16
C-1′ OH	*s* 1·86		*s* 1·45			*s* 1·88	
C-2′ methyl	*s* 1·21		*s* 1·09			*s* 1·15	
C-3′ 2H	*c* 2·14 to 1·2		*c* 2·14 to 1·20			*c* 2·14 to 1·20	
C-4′ H	*m* 4·01		*m* 3·84			*m* 5·0 to 5·17	
C-4′ OH	*d* 3·18	13	*d* 2·80		15	—	
C-5′ 2H	*c* 2·08 to 1·20		*c* 2·14 to 1·20			*c* 2·14 to 1·20	
C-6′ methyl	*s* 0·91		*s* 0·66			0·92	
C-6′ methylene	*q* A 4·133	8	*q* A 4·002			*q* A 4·125	8
	B 3·917	8;1†	B 3·813		8;2†	B 3·800	8;2†
C-4′-*O*-acetyl	—		—			*s* 2·07	

* Chemical shifts in ppm from TMS.
† Long range coupling.

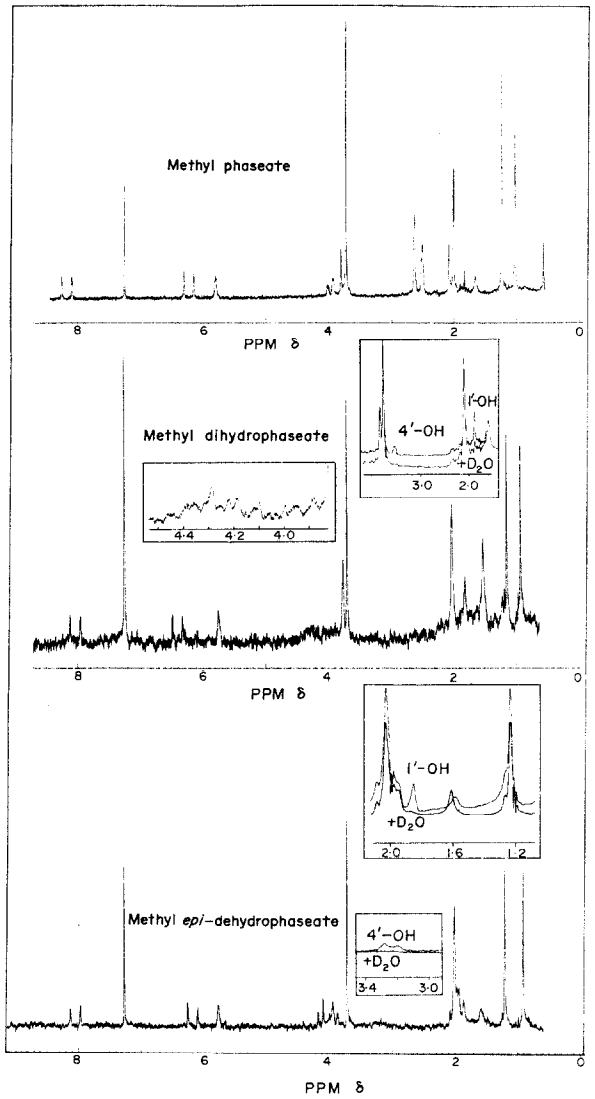


Fig. 1. NMR spectra (100 MHz, $CDCl_3$) of the methyl esters of phaseic and the epimeric 4′-dihydrophaseic acids. The previously isolated more polar epimer (methyl dihydrophaseate) has the structure shown in **7**. Insets show a computer averaged (9291 runs) expanded spectrum of the C-4′ proton signal of **7** and the removal of the 1′- and 4′-hydroxyl hydrogen signals on addition of D_2O.

between C-1′ and C-4′ substituents in one of the dihydrophaseic acid epimers.

Relative configurations of the oxymethylene bridge and the 4′-hydroxyl group. The oxymethylene bridge and the 4′-hydroxyl group of the less polar epimer are on the same site of the cyclohexane ring as shown by NMR and IR data.

Intra-molecular hydrogen bonding, involving the 4′-hydroxyl group, is seen only in the less polar dihydrophaseic ester epimer; it is absent from methyl phaseate, from the more polar dihydrophaseic ester epimer, and from the 4′-*O*-acetates of both epimers. The presence of intra-molecular hydrogen bonding in the less polar dihydrophaseic ester is deduced from both IR and NMR evidence; in chloroform solution its IR hydroxyl absorption showed the same intensity relative to the carbonyl absorption as the solution was diluted whereas the IR absorption of the polar epimer was reduced in intensity on dilution, as expected for inter-molecular H-bonding.

The NMR signal of the 4′-hydroxyl hydrogen of the less polar epimer occurs as a doublet because the H-bonding limits the rotation and the hydroxyl hydrogen signal is split by the 4′-hydrogen. The analogous signal of the other epimer lacks this splitting (Table 2).

The oxymethylene proton of phaseic acid that shows long-range coupling is the more downfield of the pair. The signal of the other oxymethylene proton occurs at approximately the same position in methyl phaseate and the more polar dihydrophaseate but it shows a considerable downfield shift in the less polar epimer. This shift is attributed to the proximity of the pro-(*S*) proton to the 4′-hydroxyl oxygen in this compound.

The C-2′ methyl signals of the less polar dihydrophaseate and its 4′-*O*-acetyl derivative are farther downfield than the respective signals of the more polar compound and its acetate. The larger shift can be explained by mutual deshielding between the 4′-hydroxyl or 4′-*O*-acetyl carbonyl oxygen atoms and the oxygen of the oxymethylene bridge with consequently a greater effect on the signals of protons borne by neighbouring atoms.

Relative stereochemistry of the oxymethylene bridge and the 1′-hydroxyl group. Both are on the

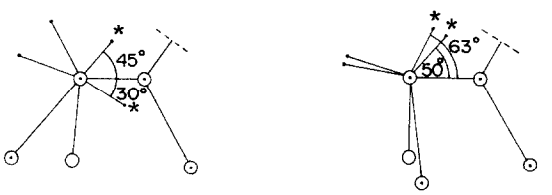


Fig. 2. The angles the *w*-coupled protons (*) of phaseic acid make with the plane of the C-5′:C-6′ and C-6′:oxymethylene carbon bonds are considerably smaller when the cyclohexane ring is in a chair than in a boat conformation.

same side of the cyclohexane ring as shown by NMR data.

The NMR signals of the two oxymethylene protons of the polar epimer are almost exactly superimposed and the protons are almost equidistant from the 1′- and 4′-hydroxyl groups in structure 7. Such identity of position is impossible with the oxymethylene bridge *trans* to the 1′-hydroxyl group (**13**).

In methyl phaseate and the less polar epimer the chemical shifts of the C-5 proton signals are similar, and similar to that in methyl abscisate [10]. In the polar epimer this signal occurs 0·3 δ farther downfield. This is accounted for by the closeness with which the 4′-hydroxyl oxygen can approach the C-5 proton of the side chain. Such proximity is impossible with structures **12** and **13**.

In the 4′-*O*-acetyl derivative of the more polar epimer the C-5 proton signal is moved even farther downfield. The acetyl carbonyl oxygen of the polar epimer can approach closer to the side chain than can the 4′-hydroxyl oxygen and the proximity of the carbonyl oxygen to the C-3 methyl group accounts for the increased downfield shift of this latter group's signal in **10**.

Hereafter, for ease of reference, the more polar epimer, isolated from beans [12, 13], is designated **6** and the less polar epimer as **8**. The experimental evidence will be discussed in more detail to show how alternative formulations of the structures are excluded. For this purpose observations on different parts of the molecules will be taken in order.

The 4′-hydroxyl groups

Intra-molecular H-bonding of the 4′-hydroxyl in the methyl ester of the less polar dihydrophaseate (**9**) was observed both by IR and by NMR spectroscopy. The signal of the 4′-hydroxyl hydrogen of **9** (Fig. 1) occurs as a broad doublet at 3·18 δ (J 13 Hz); this signal disappeared on addition of D_2O and was absent from the spectrum of the *O*-acetate (**11**). The 1′-tertiary hydroxyl signal of **9** occurred as a sharp singlet at 1·86 δ and was present in the 4′-*O*-acetyl derivative at the same position. The tertiary hydroxyl of ABA has not been acetylated and the MS of **10** and **11** shows that only the 4′-hydroxyl groups of the dihydrophaseates are acetylated [12, 16]. The double peak of **9** at 3·18 δ is attributed to splitting between the 4′-proton and 4′-hydroxyl protons which have restricted rotation when H-bonded. The NMR spectrum of **7** shows a broad singlet (3·50 δ), exchangeable with D_2O, which is absent from the *O*-acetyl compound (**10**). *The sharp singlet of the 1′-tertiary hydroxyl of* **10**, exchangeable with D_2O, was at 1·86 δ. These observations demonstrate that the oxymethylene bridge and 4′-hydroxyl group of the less polar epimer (**9**) are in close proximity; this can occur for **9** only and then only when in a chair conformation.

The IR spectra ($CDCl_3$) of **7** and **9** showed OH absorption at 3604 and 3610 cm^{-1} respectively. In addition **7** showed absorption at 3475 cm^{-1}, attributed to inter-molecular H-bonding, and **9** also showed slight absorption in this region (3448 cm^{-1}); these absorptions diminished on dilution in relation to the carboxyl absorption at 1700 cm^{-1}. **9** Shows intra-molecular H-bonding absorption at 3552 cm^{-1} which is unaffected in intensity, relative to the 1700 cm^{-1} peak, in dilution. This absorption is attributed to the 4′-hydroxyl hydrogen bonding to the oxygen of the ether bridge because intra-molecular H-bonding is absent from the IR spectra of methyl phaseate [10], and the 4′-*O*-acetates **10** and **11**. The IR of both 4′-*O*-acetyl derivatives lack absorption at 3552 cm^{-1} which can be attributed to intra-molecular H-bonding; thus the observed absorption of **9** at this wavelength is confirmed as intra-molecular H-bonding between the 4′-hydroxyl hydrogen and the oxymethylene oxygen. Both **10** and **11** show a trace of inter-molecular H-bonding absorption at about 3475 cm^{-1} when in a concentrated solution in $CHCl_3$; this can also be seen in the IR spectra of methyl phaseate and methyl abscisate.

The dienoic acid side chain

The NMR signals of the ester methyl protons of methyl phaseate and the epimeric methyl dihydrophaseates show virtually identical chemical shifts, as do the signals of their C-2 protons (Table 1). In methyl phaseate (**2**) and the methyl ester of the less polar dihydrophaseate (**9**) the C-4 proton signals register as a doublet (16 Hz) at 8·19 and 8·04 δ respectively (this identification of the C-4 and C-5 follows that of the analogous signals in ABA [17, 18] and some related pentadienoic compounds [19], it is confirmed by the long range coupling of J 1 Hz observed between the C-4 proton at 8·19 δ with the C-3 methyl). The C-5 proton

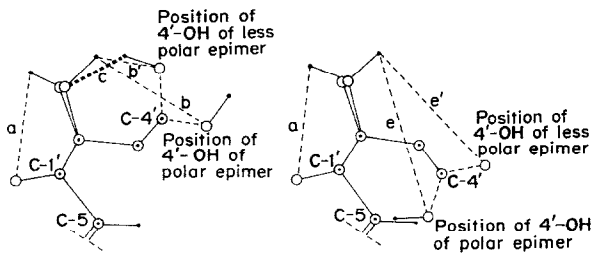


Fig. 3. Diagram showing the steric relationships of the epimeric 4'-hydroxyl groups when the cyclohexane rings are in chair (left) and boat (right) conformations. The distance (a) between the 1'-hydroxyl oxygen and the *pro*-(*R*) oxymethylene proton is similar to that (b) between the 4'-hydroxyl oxygen and the *pro*-(*S*) proton of **7** as a chair but not when in a boat (e), or of **9** in either (b', e'). H-bonding of the 4-hydroxyl hydrogen to the oxymethylene oxygen is possible in **9** in chair conformation only (c).

signal of **7** at 6·42 δ is 0·25 δ farther downfield than that of **9**. This shift is attributed to the closer proximity of the 4'-hydroxyl group to the side chain in dihydrophaseate (**7**) than is possible in *epi*-dihydrophaseate (**9**). No such difference would be expected in the signals of the C-5 protons of **12** and **13**.

The 3-methyl doublet of **7** is farther downfield (by 0·03 δ) than the 3-methyl signal of **9** and **2** (2·01 δ) and the difference is even more marked in D_6 benzene solution than in $CDCl_3$. The interpretation of these observations is that the 4'-hydroxyl group of **7** can approach quite closely to the C-5 proton, and to the C-3 methyl group; this cannot occur with the 4'-hydroxyl of **9** (Fig. 3), the ketone of **2**, or either isomeric compound (**12** and **13**). In (**10**) the 3-methyl group signal occurs at 2·06 δ, 0·05 δ farther downfield in comparison with that of the 4'-*O*-acetyl methyl. In the other epimer (**11**) the 4'-*O*-acetyl methyl signal occurs at 2·07 δ and that of the 3-methyl is upfield at 1·99 δ. This transposition can be accounted for by the closer approach the acetyl carbonyl of **10** can make to the side chain, even in the chair configuration (Fig. 4a). In **11** such proximity is impossible (Fig. 4b) and the 3-methyl signal occurs 0·07 δ farther upfield than the 3-methyl of **10**. The occurrence of the 4'-*O*-acetyl methyl signal at lower field in **11** than in **10** is attributed to mutual deshielding between the acetyl carbonyl and the oxymethylene oxygen atoms in the former compound. In accord with this postulate the oxymethylene and 2'-methyl signals are also farther downfield in **11** relative to those of the unacetylated compound **9** than are the equivalent signals in the 4'-(*S*) pair (**7** and **10**). The C-5 proton of **10** is even farther downfield than the C-5 proton of **11**, which also suggests that the acetyl carbonyl is close to the side chain in **10**. These differences cannot be accommodated by the two isomeric dihydrophaseates **12** and **13**.

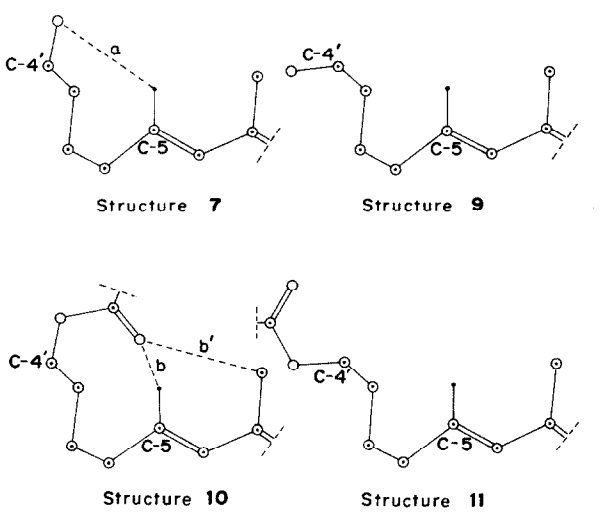


Fig. 4. Projections of **7** and **9**, and their 4'-*O*-acetyl derivatives in chair conformations. The 4'-hydroxyl oxygen of **7** can approach the C-5 proton quite closely (a) but not that of **9**. In the 4'-*O*-acetyl derivative of **7** (**10**) the carbonyl oxygen approaches the C-5 proton (b) and the C-3 methyl (b') even more closely.

The oxymethylene bridge

The signals of the oxymethylene protons of methyl phaseate (**2**) occur as an AB quartet, the signal of the proton at lower field (3·982 δ) shows long range coupling (J 2 Hz), with one of the methylene protons of C-5' (2·54 δ) (Fig. 1). Such coupling is favoured by a planar *w*-arrangement of the protons and their carbon atoms. Figure 2 shows

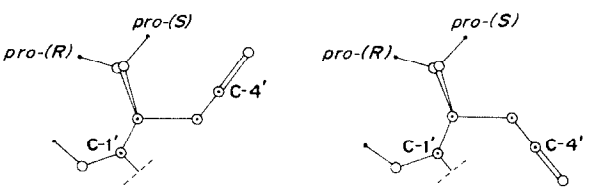


Fig. 5. Projection of phaseic acid showing the position of the oxymethylene protons in relation to the 1'-hydroxyl and 4'-ketone oxygen atoms when in chair (left) or boat (right) conformation.

that in **2** the dihedral angles are small (27° and 45°) with the cyclohexane ring in a chair conformation and large in a boat conformation (55° and 60°).

In methyl phaseate (**2**) the 6′-oxymethylene proton that is not *w*-coupled with a C-5′ proton is closer (Fig. 5) to the 4′-keto oxygen than is its *w*-coupled *pro*-(*S*) partner; nevertheless the latter is farther downfield. Presumably the 1′-tertiary hydroxyl oxygen causes a larger shift downfield of this methylene proton than does the 4′-keto oxygen of its partner.

The downfield shift of the oxymethylene protons in **9** compared with **7** or **2** (Table 3) is attributed to the proximity of the 4′-(*R*)-hydroxyl to the oxymethylene when both project from the same side of the six-membered ring, as in **9**. The position of the 4′-hydroxyl group in **7**, on the opposite side of the ring to the oxymethylene bridge, accounts for the signal of the *pro*-(*R*)-oxymethylene proton being farther upfield than the analogous signal in methyl phaseate.

In the chair configuration the two oxymethylene protons of **7** are almost equidistant from the two hydroxyl groups, hence their signals occur as a single peak, whereas in **9** the 1′-hydroxyl group occupies the same relative position but the 4′-hydroxyl is closer to the *pro*-(*S*) proton of the oxymethylene bridge (Fig. 5). Consequently in the asymmetric environment of **9** the two protons are no longer equivalent. While the signals of the oxymethylene protons of **7** and **10** occur as a sharp singlet in $CDCl_3$ in benzene they occur as an AB quartet (J 8 Hz) and the proton at higher field (3·487 δ) shows *w*-coupling (J 1 Hz). The signals of the upfield proton of the AB quartet ($CDCl_3$) in the less polar compound (**9**) in $CDCl_3$ are slightly narrower and higher than those of its partner, even allowing for the latter's being superimposed to vestigial *w*-coupling and in the 4′-*O*-acetyl-4′-(*R*) derivative (**11**) the 4′ proton signal is at 5·07 δ and the *w*-coupling can be clearly seen in the signal at 3·800 δ.

The 2′-methyl group

The 3-proton singlet of methyl phaseate at 1·25 δ has been identified by deuterium labelling [1] as the C-2′ methyl, the signal at 1·02 δ is attributed to the C-6′ methyl. A similar pattern can be seen in the dihydrophaseates but in methyl 4′-*epi*-dihydrophaseate the signals occur at 1·21 δ (C-2′ methyl) and 0·91 δ (C-6′ methyl). The molecules of methyl phaseate and the dihydrophaseates are all almost symmetrical, they differ across a plain of symmetry, running through C-1′ and C-4′, in the presence of an ether oxygen rather than a methylene group in the ether bridge. Yet one of the epimeric hydroxyl groups (**9**) on the "plane of symmetry" moves the 2′-methyl signal downfield in relation to that in **7** and **2** while the signal of the 6′ methyl group, which is in the equivalent position on the opposite side of the "plane of symmetry" is less affected. The effect on the protons of the C-2′-methyl group can be accounted for by the mutual deshielding of the ether and 4′-(*R*)-*epi*-hydroxyl oxygen atoms in this epimer; they are close together in the chair form of compound **9**, and far apart in **7**.

The 4′-proton signals

Perhaps the most critical difference between the NMR spectra of the two epimers lies in the signals of their 4′-protons. **7**, in the chair form, has its 4′-hydroxyl in an equatorial position and the 4′-hydrogen axially, almost in the same plane as that in which the four protons of C-3′ and C-5′ occur (Fig. 6a). In this arrangement the 4′-proton can couple strongly with the C-3′ and C-5′ protons and this accounts for the highly complex signal between 3·85 and 4·48 δ (i.e. 0·16 δ farther downfield than the equatorial 4′-proton's signal of **9**), attributed to the 4′-proton (Fig. 1) of **7**. In contrast to this the chair form of **9** has its 4′-hydroxyl in an axial position and the 4′-hydrogen, which is equatorial, projects directly away from the plane in which all four of the C-3′ and C-5′ protons occur (Fig. 6b). This arrangement would be expected to allow little coupling between the C-4′ proton and the protons

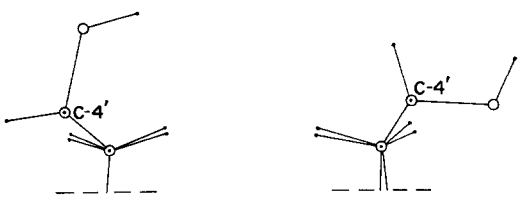


Fig. 6. The steric relationships of the C-3′ and C-5′ protons to the C-4′ proton. When **7** is in a chair (left) conformation the C-4′ proton is axial and its bond is near to planarity with those of the protons of C-3′ and C-5′. When **7** is in a boat (right) conformation the C-4′ proton is equatorial and projects out of the plane in which the C-3′ and C-5′ protons' bonds occur.

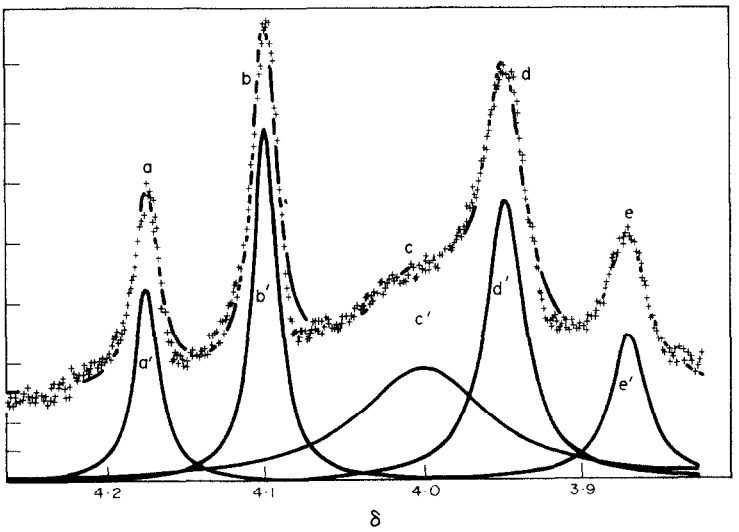


Fig. 7. Digitized NMR data analysed by a curve-fitting computer programme and fitted to five Lorenzian curves. Peaks a and b are attributed to the *pro*-(*S*)-proton of the C-6′ methylene carbon of methyl *epi*-dihydrophaseate, peaks d and e to the *pro*-(*R*)-proton. Shoulder C is attributed to the envelope of the C-4′ proton. The integrated signal showed the presence of three protons and the integrals of the fitted curves a′ + b′; c′; and d′ + e′ were 30·6%, 32·4% and 37·0% of the total respectively. The lower peak height of d′ and e′ compared with a′ and b′ is attributed to vestigial *w*-coupling with the *pro*-(*S*) proton of C-5′.

of C-3′ and C-5′ and this is borne out by the simple envelope (4·01 δ) of the signal of the C-4′ proton of **9** (Fig. 7).

CONCLUSION

The foregoing analysis shows that the 4′-epimer of dihydrophaseic acid isolated from beans by Walton *et al.* [12, 13] has the 4′-hydroxyl group on the opposite side of the cyclohexane ring to the oxymethylene bridge. Other evidence defines the stereochemistry of the oxymethylene bridge in relation to the 1′-tertiary hydroxyl group and as C-1′ has the same stereochemistry in phaseic and (+)-abscisic acids the absolute configuration of phaseic acid can be deduced to be as shown in **1**. All lines of evidence, several of which are mutually supporting, can be interpreted in terms of structure **6** for the methyl ester of the more polar epimer of dihydrophaseate isolated from beans by Walton *et al.* and structure **9** for the major product formed from methyl phaseate by borohydride reduction. Phaseic acid can now be defined as (−)-3-methyl-5(8(1(*R*),5(*R*)-dimethyl-8 (*S*)-hydroxy-3-oxo-6-oxabicyclo (3,2,1)-octane))2-*cis*-4-*trans*-pentadienoic acid. The more polar epimer of dihydrophaseic

Table 3. ε Values of phaseic acid derivatives

Compound	λ_{max} (MeOH)	$\epsilon \times 10^{-2}$	Reference
Phaseic acid	258	145	1
Methyl phaseate	263		18
Methyl dihydrophaseate	267	199	13
Methyl dihydrophaseate	266		Samples used in this investigation
Methyl 4′-*O*-acetyl dihydrophaseate	265·5		
Methyl *epi*-dihydrophaseate	266		
Methyl 4′-*O*-acetyl-*epi*-dihydrophaseate	267		
Methyl phaseate	262		

acid isolated from beans [12, 13] (**6**) is (−)-3-methyl-5{8[3(*S*),8(*S*)-dihydroxy-1(*R*),5(*R*)-dimethyl 6-oxabicyclo-(3,2,1)-octane]}2-*cis*-4-*trans*-pentadienoic acid.

It is suggested that to avoid the confusion of the numbering systems **6** can be referred to as "dihydrophaseic acid" and **8** can be referred to as "*epi*-dihydrophaseic acid".

EXPERIMENTAL

The materials and isolation methods used were as described previously [20]. The phaseic acid was prepared by feeding an aq soln (200 ml) of (±)-ABA (40 mg) to 1 kg of 5-week-old tomato shoots (c. var. Arastor) cut off just above ground level (*ca* 5 g fr. wt) and extracting them with MeOH (3 × 2 l.) after 2 days incubation in dim light. The EtOAc-soluble acid fraction was isolated and chromatographed on Si-gel GF_{254} TLC plates in toluene–EtOAc–HOAc (50:30:4) (system I) containing 2,6-di-*t*-butyl-4-methyl phenol (BHT) (20 mg/l.). The phaseic acid was eluted, methylated with ethereal CH_2N_2 and rechromatographed in hexane–EtOAc (3:1) (system II) using multiple development. The methyl phaseate (5 mg) crystallized after elution with MeOH; it was dissolved in MeOH (0·5 ml) at 0° to which was added H_2O (1·0 ml) and successive small amounts of $NaBH_4$. After 2 hr the MeOH was blown off with N_2 and the products were extracted with EtOAc. The epimeric methyl dihydrophaseates were separated by multiple development on 3 Si gel TLC plates in hexane–EtOAc (2:1) (system III). Both samples crystallized after elution with EtOAc. The previously described material chromatographed at R_f 0·2, the methyl *epi*-dihydrophaseate at R_f 0·27 (system III). GLC was carried out at 200° in a Varian Aerograph series 1400 with a 3 mm i.d. glass column (2 m) packed with XE-60 stationary phase on a 100–120 mesh gas chrom Q support. The two epimers were 4′-*O*-acetylated with Ac_2O in C_6H_5N (1:2) at 40°. The elution profile of the GLC suggested that the **7** and **9** contained less than 2% of the other epimer and no 2-*trans* isomer was detectable.

Spectroscopy. Literature ϵ values vary somewhat between phaseic acid and dihydrophaseic acid methyl ester but were used for the calculation of the amounts of the respective compounds. *Epi*-dihydrophaseate was assumed to have the same ϵ as its 4′-(*S*) counterpart. (Table 4).

The IR spectra of $CHCl_3$ solns were obtained using a 0·2 mm NaCl cell. Methyl dihydrophaseate (**7**) shows OH absorption at 3604 cm^{-1} and a broad peak at 3475 cm^{-1} (from 3559 to 3310) which was reduced in intensity in comparison with the 1700 peak on dilution. Methyl *epi*-dihydrophaseate (**9**) shows OH absorption at 3610 cm^{-1} and a broad peak at 3552 cm^{-1} (from 3571 to 3226) which was unaffected by dilution (intra-molecularly bonded hydrogen). A slight shoulder at 3448 cm^{-1} was reduced in intensity by dilution (inter-molecularly bonded hydrogen). In addition **9** showed absorption at 2952, *m*; 2933, *m*; **6** at 2954, *m*; 2937 cm^{-1}. Both **7** and **9** exhibited absorption at 1700, *vs*; 1620, *m*; 1600, *s*; 1440, *w*; 1415, *w*; 1376, *m*; 1164, *m*; 1110, *w*; 1095, *w*; 1000, *w*; 885, *w* cm^{-1}. Thereafter **9** showed 1048, *s*; 1170, *w*; 885, *w* and **7** 1065, *w*; 1045, *m*; 1030, *m* and 1010 cm^{-1}, *w*. The 4′-*O*-acetyl derivative (**10**) of **7** showed additional absorptions at 1730, *s*; 1445, *m*; 1360, *m* and 908, *s*; **9** 1725, *s*; 1440, *m*; 1360, *m* and 908 cm^{-1}, *w*.

The NMR spectral analysis was carried out with a Varian HA 100 using $CDCl_3$ and $D_6C_6H_6$ incorporating tetramethyl silane as an internal standard; δ values are given as chemical shifts from this.

Acknowledgements—My thanks are due to Professors MacMillan and Walton whose preliminary work and discussions stimulated this investigation and whose tentative assignment of the absolute configuration is now confirmed. Messrs. R. A. G. Carrinton and D. Barnet obtained the many nmr spectra and their help is gratefully acknowledged. Thanks are also due to Mrs. Janet Tucker for the MS, Professor J. A. D. Zeevaart for MS and GLC data, and Dr. S. Isoe for permission to cite his unpublished work on the synthesis of phaseic acid.

REFERENCES

1. Milborrow, B. V. (1969) *Chem. Commun.* 966.
2. MacMillan, J. and Pryce, R. J. (1968) *Chem. Commun.* 124.
3. Milborrow, B. V. (1970) *J. Exp. Botany* **21**, 17.
4. Sondheimer, E., Galson, E. C., Chang, Y. P. and Walton, D. C. (1971) *Science* **174**, 829.
5. Harada, N. (1973). *J. Am. Chem. Soc.* **95**, 240.
6. Koreeda, M., Weiss, G. and Nakanishi, K. (1973) *J. Am. Chem. Soc.* **95**, 239.
7. Mori, K. (1973) *Tetrahedron Letters*, 2635.
8. Oritani, T., Yamashita, K. and Meguro, H. (1972) *Agr. Biol. Chem.* **36**, 885.
9. Ryback, G. (1972) *Chem. Commun.*, 1190.
10. Milborrow, B. V. (1971) in *Aspects of Terpenoid Chemistry and Biochemistry*. Goodwin, T. W. ed. p. 137. Academic Press, London.
11. Isoe, S. Personal Communication.
12. Tinelli, E. T., Sondheimer, E., Walton, D. C., Gaskin, P. and MacMillan, J. (1973) *Tetrahedron Letters*, 139.
13. Walton, D. C., Dorn, B. and Fey, J. (1973) *Planta* **112**, 87.
14. MacMillan, J. and Walton, D. C. (Personal Communication).
15. Milborrow, B. V. *Phytochemistry* (In Press).
16. Zeevaart, J. A. D. (Unpublished work).
17. Koshimitzu, K., Inui, M., Fukui, H. and Mitsui, T. (1968) *Agr. Biol. Chem.* **32**, 789.
18. MacMillan, J. and Pryce, R. J. (1969) *Tetrahedron* **25**, 5893.
19. Mallaby, R. (1974). Ph.D. Thesis, University of London.
20. Milborrow, B. V. (1972) *Biochem. J.* **128**, 1135.